Volume 39 | Issue 3 | July 2022

Journal of Experimental & Clinical Medicine

JECM https://dergipark.org.tr/omujecm

ONDOKUZ MAYIS UNIVERSITY
FACULTY OF MEDICINE

e-ISSBN 1309-5129

JOURNAL OF
EXPERIMENTAL & CLINICAL MEDICINE
Volume 39 - Issue 3 - 2022
ISSN 1309-4483 e-ISSN 1309-5129

Owner
On Behalf of Ondokuz Mayis University
Yavuz ÜNAL

Director in Charge
Cengiz ÇOKLUK

Publisher Administration Office
Ondokuz Mayıs University Faculty of Medicine Atakum / Samsun, Turkey

Publish Type
Periodical

Online Published Date
30/08/2022

Indexed: Scopus, EBSCO, Google Scholar, Crossref, EMBASE, TurkMedline

Cover Design
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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 597-603
doi: 10.52142/omujecm.39.3.1

Stereological examination of the effect on the hippocampal dentate gyrus granule cells number of rats subjected to wireless internet during prenatal period: A preliminary study

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Received: 05.04.2022 • **Accepted/Published Online:** 06.06.2022 • **Final Version:** 30.08.2022

Abstract

As a result of industrialisation and the many recent advances in technology, we are being intensely subjected to non-ionised radiation sources. As such, the effect of non-ionised radiation on human tissue is now being researched. In this study, the effects of Wi-Fi waves found in some third-generation mobile phones to facilitate internet connection on the brain hippocampal dentate gyrus granule cell quantity of rats during the prenatal period was stereologically examined. During the study, mated albino rats were subjected to Wi-Fi throughout pregnancy. A month after giving birth, six rats from each group, for a total of 12 rats, was sacrificed under perfusion and anaesthesia. Their skulls were opened and brains were removed to undergo routine checks in which the granule cells were counted. Strategic sections were defined, and every 45th section couple (with a thickness of 5 µm) was taken and dyed with haematoxylin and eosin (H&E) and cresyl violet stain. The granule cells were counted using a combination of the stereological disector method and Cavalieri's principle. Then, the results were analysed using the Mann–Whitney U test. No statistically significant difference has been observed between the groups ($p > 0.05$). The findings were discussed in light of the relevant literature, and it was determined that throughout pregnancy, Wi-Fi modem device did not result in any changes in the dentate gyrus granule cell count of the hippocampus of postnatal rats' brains.

Keywords: number of granule cells, pregnancy, prenatal rat, stereology, wi-fi

1. Introduction

In recent years, studies on human health in relation to electromagnetic fields (EMF) have investigated the health effects that may arise from low-level, long-term EMF exposure throughout a human's lifetime. Radiofrequency (RF) waves have two types of effect mechanisms: thermal and non-thermal. If there is a level of RF application that can cause a heat increase in biological structures, this first leads to heat increase in the tissue and then to biological changes due to this heat increase. Currently, the number of studies investigating the effects of RF radiation on the nervous system is consistently increasing. In particular, the question of whether the effect of exposure to EMFs on the blood–brain barrier is thermally induced is being discussed by researchers. Exposure to high levels of RF energy causes damage to the nervous system's structure and function. In studies conducted on isolated brain tissue, it has been found that there are different causes, other than thermal mechanisms, influencing neurological electroencephalography (EEG) changes, blood–brain barrier permeability, and calcium flow changes (1). Exposure to RF radiation sources has been reported to cause various pathological conditions such as headaches, insomnia and memory loss (2). Today, with the rapid development of technology, exposure to RF radiation sources has become inevitable. These sources include radars, wireless communication systems, microwave ovens, base stations, mobile phones, televisions and radios (3). There is increasing number of studies stating that radiofrequency waves emitted from EMF sources, which have become an indispensable part of our lives today, have harmful effects on human health (4-7).

Studies analyzing the role of RF in the development of leukemia, lymphoma, other cancers, chromosomal disturbances, behavior disorders, learning difficulties in children, decreased reproduction, blood–brain barrier permeability, cell and DNA synthesis, and increased brain electrical activity (i.e., EEG) have also gained prevalence in recent years. It has also been observed that hormones are affected; carbohydrate, nucleic acid and protein metabolism changes occur; cellular respiration is decreased; structural changes occur; tissue and cell hormonal response is changed; and immune response to different antigens is affected (3).

Increased basal corticosteroid levels, decreased locomotor activity and neuronal damage occurs, with abnormal brain

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functioning (8), as well as increased sympathetic activity (9) have been shown in rat brains exposed to EMFs of different intensities (10). However, other studies contend that EMFs have no negative effect on tissues (11). Alzheimer's and Parkinson's diseases, as well as various neurological disorders, have been found to be more common in radio operators exposed to EMF and others who work with data processing devices, telephone lines, substations and power stations (12). Most studies have focused on the development of brain tumors through the use of mobile telephones. A significant increase in the risk of brain tumors has been observed in mobile and cordless telephone users over the past 10 years, as well as an increase in glioma (especially astrocytoma), acoustic neuroma and meningioma tumors (13-19).

It has been reported that EMF affects fertility in rats, causes genetic disorders, causes cellular and molecular changes in rat brains, affects neuroendocrine system, is associated with cancer formation, affects brain functions and may cause many neurological effects (20). Another study concluded that EMF decreased melatonin hormone production and could lead to depression by decreasing melatonin levels. In addition, the study noted that the EMF emitted by household appliances affects the secretion of neuroendocrine products and causes a deterioration of sleep phases (21). In 1997, Lai et al. reported that exposure to RF waves produces DNA fractures in rat brain cells (22).

Prenatal irradiation may also increase the risk of cancer in childhood. It is for this reason that pregnant women should avoid having diagnostic X-ray tests in the abdomen until delivery. Should an embryo or fetus be exposed to high doses, serious deformities or death may occur. The threshold for these effects is between 0.1 and 1 Sievert (Sv), or higher, depending on the pregnancy period (23). It has been shown that when female rats are exposed to an EMA field while pregnant, this exposure can affect the development of Purkinje cells in the cerebellum of the fetus; the results of this pathological effect persist after the postnatal period (24).

There was a significant decrease in glutathione peroxidase (GSH-Px), glutathione (GSH) and antioxidant vitamin concentrations in the brain and liver of rat pups exposed to 2.45 GHz of Wi-Fi during pregnancy 1 hour, 5 days a week. It has been reported that oxidative damage due to EMF radiation is more likely to occur than is to be (25). In a study on the determination of oxidative DNA damage and malondialdehyde (MDA) levels in brain tissue in rats exposed to a 2100 Hz EM field at intervals of between 10 and 40 days, an increase in DNA damage and a decrease in MDA levels were observed after 10 days. At the end of 40 days, there was a decrease in DNA damage, and this result was attributed to the adaptation of the repair mechanism (26).

According to our review of the relevant research, no studies have been found to satisfactorily investigate the effect of Wi-Fi on dentate granule cells using a stereological method. Therefore, in this preliminary study, we planned to evaluate the effects of Wi-Fi, which is found in some third-generation mobile phones, on the number of dentate gyrus granule cells in rat brain hippocampus in the prenatal period.

2. Material and Methods

2.1. Specimens

Ethical permission required for the study was obtained by Ethic Committee from Van Yüzüncü Yıl University, with the decision numbers 2016/04 and 05/05/2016. Experimental materials were obtained from the Experimental Animals Unit of Van Yüzüncü Yıl University.

The study was conducted in the stereology laboratory of the Department of Medical Histology and Embryology, Faculty of Medicine, Yüzüncü Yıl University. Nine healthy female and three healthy male Wistar albino rats weighing an average of 220 g were randomly used during the reproductive period. Appropriate room temperature and humidity, normal tap water and standard rat feed were given in a light/dark environment. Three female rats and one male rat were taken into separate plexiglass (polypropylene) cages for mating. Male rats were kept in cages for 48 hours for mating and were then removed from the cages. The female rats were weighed daily for one week. Those with an increase in their weight were accepted as pregnant; only rats that were pregnant were included in the study. In the first and second cages (Group A), three rats were exposed to Wi-Fi for 1 hour/day in a lead-coated private room completely isolated from external EMF radiation; the control (Group B) groups were not exposed to Wi-Fi. After four weeks, six of the new-born pups were selected as the control group and six were used as the experimental group (Table 1).

2.2. Methods

For Wi-Fi exposure during pregnancy, for 1 hour per day, the pregnant rats were placed in the middle of a 33 x 60 x 20 cm3 standard rat cage (Fig. 1). TP-link, TD-W8961N model with a multi-directional fixed antenna system, a 2.45 GHz radio frequency, an operating voltage of 9V and a working current of 0.85A; the exposure to Wi-Fi modem devices occurred at a maximum bandwidth of 300 Mbps (Fig. 2). Throughout the operating time, the frequency verification of the RF waves from the Wi-Fi system was performed using the Good Will GSP-730 150 KHz ~ 3 GHz Spectrum Analyser (Fig. 3). The frequency stabilization of the EMF waves was checked.

After birth, the pups were fed with breast milk until they were approximately one month old. On postpartum day 30, to all rats with 50 mg/kg of Ketamin was administered to six randomly selected male and female rats, which were anesthetised using deep anaesthesia. Transcardiac perfusion was then performed from the left heart via a cannula. For perfusion, general anaesthetics were provided to the rats by intraperitoneal ketamine injection, and the rats were fixed to the operating table in the supine position. The rats were

divided into two by a mid-sagittal incision of the sternum, the diaphragm was released and the rib cages were opened. A three-cannulated syringe needle was inserted 2–3 mm into the left ventricle. Then, 0.5 mg heparin was administered, and a 2–3 mm incision was made in the right atrium to drain the blood. When the blood started to discharge, saline was given; specifically, 100 ml of 0.9% NaCl solution was administered until the fluid coming out of the right atrium became clear (within 5–8 min). After clarification, 10% neutral buffered formaldehyde solution was administered. Muscle twitching was observed with the introduction of fixation fluid so that the fixative reached the tissues and perfusion was maintained by the neutral buffered formaldehyde. After perfusion, the cervical dislocation method was used, and the rats were decapitated. With the help of clamps, the head bones were broken from the superstructure in small dimensions, and their brains were made visible. Brains were excised of the brainstem level (Fig. 4).

Fig. 1. Wi-Fi application setup

Fig. 2. 300Mbps Wireless N ADSL2 + Modem Router

Fig. 3. Spectrum Analyzer

The brains obtained from the experimental and control groups were kept in 10% buffered formaldehyde for at least 72 hours, and the left hemispheres were separated for routine fixation, follow-up and embedding using histological methods. The frontal sections were taken using a rotary microtome (Leica RM 2125, Leica instrument, Nussloch, Germany), and each 45th section of the paraffin blocks (5 µm thick) was placed on a slide and then in an oven. Sections stained with haematoxylin and eosin (H&E) and cresyl violet were dropped onto the coverslip with entellan and allowed to dry. Stereological, the dentate gyrus granule cell count used a combination of the disector method and Cavalieri's principle. The Shtereom 1.5 software as used for volume measurement (Fig. 5). The results were compared statistically.

Fig. 4. Whole rat brain for one month

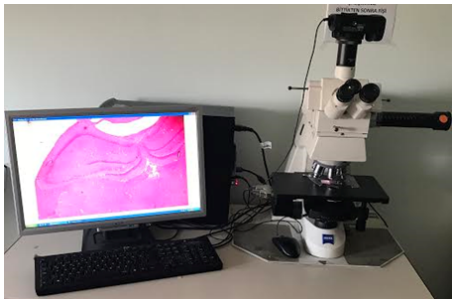

Fig. 5. Counting light microscope with camera and computer

2.3. Stereology for light microscopy

Brain tissue samples were first detected in 10% formaldehyde solution using light microscopy. After fixation, the tissue samples were placed in cassettes and washed under under a stream of water. In the follow-up process, tissues were passed through an increasing series of alcohols to remove water, passed through a xylene series for clarification and embedded in molten paraffin. Sections of 5 µm thickness obtained from prepared paraffin blocks were stained with H&E and cresyl violet. The sections were evaluated and photographed using the ZEISS (Göttingen, GERMANY) computer-assisted imaging system AxioVision 4.8 (Figs. 5–7). The approximate brain weights (g) of the control and experimental group animals were measured. Sections taken from the all experimental groups were kept in an oven at 80°C for about 30 minutes; then, xylene was taken for 5 minutes three times and paraffin-free. The tissues were then passed through a decreasing series of alcohols for 5 minutes three times. After washing in the stream of water, and stained in Harris haematoxylin for 1–3 minutes and the slides were washed in the stream of water. The Slides were then immersed in

ammonia solution three times and washed until turbidity disappeared. The slides were kept in eosin for 2–5 minutes, washed again in the stream of water and then passed through the alcohol and xylene for 5 minutes; they were sealed by dropping them into Entellan. The combination of the disector method and Cavalieri's principle was used for the calculation of the number of granule cells (27, 28). In particular, the physical dissector counting method was used in our study (29).

Our formula as: $N = \Sigma p \times \bar{Q}^{-} \times k \times \frac{(a/p)}{a(frame)}$

N = Total number of particles; ∑ p = Total number of points; $\bar{Q}^{-}$ = Average numbers of disector granule neuron counts; k = Section thickness: 5µm; a/p = Area represented by a point: 5625 µm²; a frame = Unbiased counting frame area: 1.525,68 µm²

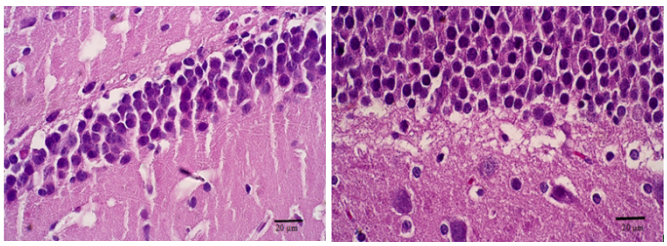

Fig. 6. Dentate gyrus granule cells (Wi-Fi) (H&E) (x63)

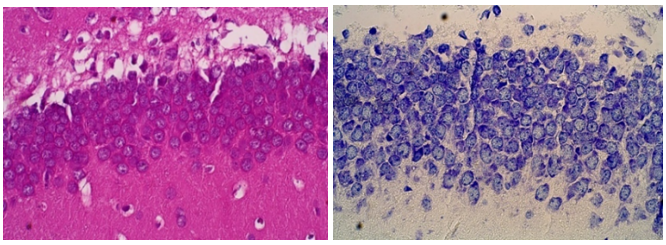

Fig. 7. Dentate gyrus granule cells (Control) (H&E) (x63) and (Cresyl Viole) (x63)

2.4. Calculation of error and coefficient variance

According to the standard stereological approach, the coefficient of variation (CV) and coefficient of error (CE) are taken into consideration to determine the optimal sample size in each group. The following formula was used to calculate the CE (29, 30). The stereological estimation values based on our results are given in Table 1.

Table 1. Calculation of error coefficient (CE) in a sample

Section	Q	Qi.Qi	Qi.Qi+1	Qi.Qi+2
1	25	625	950	250
2	38	1444	380	532
3	10	100	140	180
4	14	196	252	98
5	18	324	126	270
6	7	49	105	0
7	15	225	0	0

NOISE:127 VAR (srs):8.441666667 Total Var:135.4416667
CE:0.091637329 % NOISE: 93.7673045

2.5. Statistical analysis

Data were compared non-parametrically using the Mann–Whitney U test (Table 2) ($p > 0.05$). The statistical significance level was set at 5%, and the SPSS statistical package program was used for the calculations.

3. Results

In this study, the dentate gyrus granule cells of one-month-old offspring of both control rats and subject rats exposed to Wi-Fi during pregnancy were counted (Fig. 1–2). The mean brain weights of the control group were 1.08 ± 0.12 g and of the experimental group rats were 1.15 ± 0.18 g. The difference in the total number of granule cells between the subjects and controls was not statistically significant. Therefore, the one-sample Kolmogorov–Smirnov test was not required for the distribution of the data. Data were compared non-parametrically using the Mann–Whitney U test. There was no statistically significant difference between the two groups in terms of N values ($p > 0.05$) (Table 2) or granule cell numbers (CE and CV values) (Table 3).

Table 2. Descriptive statistics about the total number of granule neurons

	N	Medyan	Mean	SEM	Min.	Max.	P
Control	6	1246173	1541285,17	835819,357	654868	2890018	
Wi-Fi	6	1941799	1861036,67	1019873,304	727315	3435868	0.566
Total	12		1701160,92	904554,176	654868	3435868	

4. Discussion

Wireless Internet is widely used in schools, hospitals, libraries, cafeterias and homes. There are a limited number of studies reporting the effects of this radiation interval on the human system (31). However, there is no detailed study investigating the effects of wireless Internet-induced EMFs on foetal development. This study was conducted to investigate the prenatal effects of EMF exposure associated with wireless Internet use in pregnant rats using a stereological method. In our study, the number of dentate gyrus granule cells in the brain hippocampus of postnatal rat pups exposed to Wi-Fi as foetuses was calculated using the stereological method.

The limbic system is the action system of the brain, which consists of a group of nuclei connected to each other for the regulation of memory and mood. This neuron system evaluates rewards and has an important place in causing motivation. The limbic system plays a role in the autonomic functions of the brainstem, the combination of conscious and unconscious behaviours, the facilitation of the retrieval of information and the biological rhythm. The hippocampus, which is one of the most important parts of the limbic system,

takes part in organizing and recalling memories (32, 33). The hippocampus is seen to be more related to long-term memory and is responsible for the transformation of short-term memory into a long-term state (34). Many stimuli from the cortical center are introduced through the entorhinal cortex; the stimuli are transmitted to the dentate gyrus—which is a thin, scalloped cortical strip—and from there to the hippocampus. Thus, the dentate gyrus serves as a station for the transfer of information to the hippocampus (35).

Table 3. Control and Wi-Fi granule cells number, CE and CV values

Control	N	N-CE	Wi-Fi	N	N-CE
1- Control	1.012.140	0.08	1- Wi-Fi	3.435.868	0.05
2- Control	1.279.756	0.07	2- Wi-Fi	1.748.736	0.06
3- Control	1.212.589	0.07	3- Wi-Fi	727.315	0.07
4- Control	2.198.340	0.07	4- Wi-Fi	2.323.301	0.06
5- Control	2.890.018	0.07	5- Wi-Fi	796.138	0.07
6- Control	654.868	0.09	6- Wi-Fi	2.134.862	0.05
Mean	1.541.285	0.07	Mean	1.861.036	0.06

CV Control groups: 0.49 CV Wi-Fi groups: 0.5

It has been reported that prolonged exposure to a low-intensity EM field has effects on cancer formation, biomolecule synthesis and cell division in both physiological and biochemical cells and tissues. It has been observed that as a result of such exposure, hormones are affected; carbohydrate, nucleic acid and protein metabolism are different; cellular respiration is decreased; structural changes occur; tissues' and cells' hormonal responses are changed; and immune responses to different antigens are affected (3).

Radioactive waves can adversely affect brain structure and physiology by damaging cell membranes and organelles at both the cellular and molecular levels. Daniels et al. (2009) exposed rat brains to EMF resonance waves, observing that locomotor activity decreased, basal corticosterone levels increased and neuronal damage and abnormal brain functioning occurred (8). Wilen et al. (2006) showed that increased sympathetic activity occurred with exposure to EM waves (9). In a study of rat astrocytes and capillary endothelial cells in porcine brains conducted by Schirmacher et al. (2000), cells were exposed to a 1.86 GHz EMF (4). Another study found that the increase in blood–brain barrier permeability was significantly higher in cells exposed to EMF compared to the control group (10). As our study was not conducted at the molecular level, the findings cannot be compared. Odacı et al. (2008) showed that EMFs inhibit the formation and differentiation of neural stem cells during embryonic development and showed a statistically significant decrease in the number of dentate gyrus granule cells of prenatal rats exposed to an EMF (900 MHz) ($p < 0.01$) (36). Rağbetli et al. (2010) and Sonmez et al. (2010) exposed female rats to a 900 MHz wave frequency EMF and evaluated the number of Purkinje cells in the cerebellum that was it found that the number of Purkinje cells was decreased in rats exposed to an EMF (37-40). This may be due to the fact that the distance between the antenna and the subjects was as small as 1 cm and due to the difficult of keeping the subject's distance from the antenna constant in a narrow area.

Schmitz et al. (2002) observed a decrease in the number of hippocampal granule cells due to prenatal stress in female rat hippocampus (41). Alchalabi et al. (2016) reported that prolonged exposure to EMFs during pregnancy leads to chronic stress and has adverse effects on prenatal and postnatal development (42). These probable effects were not observed in our study because there was no long-term exposure. In addition, the stress factor was not relevant because the animals were free to roam about in our experimental setup.

Sangun et al. (2015) observed postnatal growth restriction and puberty delay in rats—especially female rats—exposed to an EM field of 2450 MHz in the prenatal period (5). They also found that total oxidant state and oxidative stress index values increased in brain and ovarian tissues. Tök et al. (2014) reported that melatonin has beneficial effects on lipid peroxidation and the regulation of TSH values in rats exposed to Wi-Fi (2.45 GHz; 60 min/day) (41). In our study, we could not compare with biochemical results because only the number of dentate gyrus granule cells of the hippocampus was calculated.

In contrast to the mentioned effects of EMFs, some studies have reported that EMFs have no harmful effects on biological systems and tissues (11). Takahashi et al. (2010), for example, reported that whole-body exposure to a 2.14 GHz EMF during pregnancy and lactation did not have any negative effects on the development of the pregnancy or of the rat pups themselves (42). Ohtani et al. (2015) observed that long-term exposure to RF EMFs in developing rats had no adverse effects on the number and activation of T cells (43). Rağbetli et al., (2010) observed no change in cerebellum granule cell count in postnatal offspring of mice exposed to cell phones (890–915 MHz) during pregnancy (37). Sambucci et al. (2010) did not observe any effect on prenatal effects in mice exposed to Wi-Fi signals during pregnancy (44). Rağbetli et al. (2009) observed no change in the number of hippocampal pyramidal cells in prenatal mice exposed to cell phones (890–915 MHz) (45). Our study results are similar to these results. However, in our study, the prenatal effect was examined differently.

In humans, granule cells begin to develop in the sixth week of the embryonic period (46). In rats, the primary critical period occurs from the last week of pregnancy to the

tenth day after birth (47). In this study, the application of the EMF was based on this period. Prenatal administration was performed, and the rats were expected to be one month old at the time of their evaluation. Therefore, there was no loss or error in terms of application time. According to our results, no statistically significant difference was observed in the number of hippocampus dentate gyrus granule cells.

In the prenatal period, it was observed that Wi-Fi increased the number of granule cells, but this was not statistically significant. Therefore, the wireless modem had no anyone affect. The distance of the Wi-Fi modem device to the subjects was 33 cm in diameter, and no significant effect was observed due to the presence of an EMF at this distance. No histopathological effect of Wi-Fi on the hippocampus was observed. It can be said that the Wi-Fi effect decreases as you move away from the modem device. This is especially true for distances greater than 10 cm.

A Wi-Fi device with multi-directional antenna system was used (TP-link, TD-W8961N model, 2.45 GHz radio frequency, 9V operating voltage, 0.85A operating current, 300 Mbps maximum bandwidth and values conforming to international standards). It is possible that no negative effects were observed because the Wi-Fi system used complies with certain requirements and standards. As such, the results of the current study, and those of other genetic, biochemical and physiological studies, should be supported with further research. Another reason why Wi-Fi has no numerical effect on the hippocampus dentate gyrus granule cells in stereological and statistical terms may be due to the fact that the RF radiation from Wi-Fi cannot directly lead to ionization. The RF waves used in this study are non-ionizing frequencies.

Conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

Acknowledgments
The authors are grateful to Yuzuncu Yil University Department of Scientific Research Projects (2009-TFU-U075).

Ethical Approval

Ethical permission required for the study was obtained by Ethic Committee from Van Yüzüncü Yıl University, with the decision numbers 2016/04 and 05/05/2016. Experimental materials were obtained from the Experimental Animals Unit of Van Yüzüncü Yıl University.

This research, which taken from Buşranur Özalper's master's thesis, was supported by Van Yüzüncü Yıl University Scientific Research Projects Unit as TYL-2016-5331.

Authors' contributions

Concept: M.Ç.R., T.Ç., Design: M.Ç.R., T.Ç., Data Collection or Processing: B.Ö., Analysis or Interpretation: B.Ö., Literature Search:B.Ö., Writing: M.Ç.R., B.Ö., T.Ç.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 604-610
doi: 10.52142/omujecm.39.3.2

Psychological reactions of different affective temperaments to the COVID-19 pandemic

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Received: 11.08.2021 • **Accepted/Published Online:** 29.11.2022 • **Final Version:** 30.08.2022

Abstract

Various studies show that the temperament characteristics of individuals play a decisive role in how much and how they will be affected by traumatic events. However, no research has been found in the literature examining the relationship between affective temperament and trauma. The Covid 19 pandemic process is described as a social trauma. In this context, in this study, it is aimed to examine the reactions of different affective temperaments to social trauma within the framework of the pandemic. Within the scope of the research, the Depression, Anxiety and Stress Scale (DASS-21) and Temperament Evaluation of Memphis, Pisa, Paris, San Diego Autoquestionaire (TEMPS-A) were used. The scales were applied online to a total of 1075 volunteer participants (653 women and 422 men). Multiple regression analysis was conducted to determine the significance of temperament types in the prediction of depression, anxiety, and stress levels of individuals. The method employed was stepwise regression analysis. In the data obtained, it was observed that depression, anxiety, and stress levels and anxious, cyclothymic, depressive, and irritable temperament types showed a positive correlation at different levels and a low level of negative correlation with hyperthymic temperament type during the pandemic process. The four aforementioned affective temperament types were significant predictors for depression, three were predictors for stress, and two for anxiety. Additionally, the results of this study indicated that hyperthymic temperament can be protective against the effects of trauma.

Keywords: depression, anxiety, stress, temperament, COVID-19

1. Introduction

The 2019 coronavirus disease (COVID-19) is a global health threat that originated in China (1). The World Health Organization (WHO) has declared the COVID-19 outbreak a public health emergency of international concern (2). According to WHO data, as of June 30, 2020, the number of people diagnosed with the disease has exceeded 10,000,000 and the number of people who have died from the virus is more than 500,000 (https://covid19.who.int/). The epidemic has not only threatened health and life, but it has also caused economic, social, cultural, and psychological effects (3,4).

If we look at the psychological effects of the pandemic, the research conducted by Wang et al. (2020) (5) in China when the virus first appeared can be a good example. According to the results of this study research, 53.8% of participants were psychologically affected by the epidemic at a moderate or severe level and 16.5% gave depression, 28.8% anxiety, 8.1% stress reactions, and the evaluation undertaken after 4 weeks revealed there has not seen a significant change in these levels (5). As another example, in the study conducted by Mazza et al. (2020) (6) in Italy, it was determined that 22.4% of society experienced high levels of depression, 27.2% stress, and 18.7% anxiety during the pandemic. when considering the pandemic in the light of these findings, it is possible to describe this process as a disaster accompanied by psychological traumas (7, 8).

Disasters can be defined as the consequences of natural, technological, and anthropogenic events that directly affect people and cause physical, economic, social, and environmental losses to human settlements, affecting communities by stopping or interrupting human activities in normal life (9, 10). The stressor factors that occur in the event of a disaster do not affect every individual in the environment at the same level (11). As a matter of fact, different research has been providing different results about the prevalence of post-natural disaster traumatic stress disorder (12). This situation has been indicating that individuals' levels of psychological reactions to disasters may differ (13, 14). Although there may be many reasons for these differences, some studies have revealed that the temperament characteristics of individuals are a significant parameter for the psychological effects of trauma (15, 16). In this sense, findings of research conducted by Strelau and Zawadzki

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(2004) (17) has been showed that one of the best predictors of the risk of developing post-traumatic stress disorder in individuals who had experienced trauma was temperament traits.

In general, temperament is composed of continuous(chronic) emotional, thought, and behavioral traits with a structural, biological, and genetic basis (18). In other words, temperament includes the predominance of various emotional states and a tendency to experience similar emotional responses to similar events. Since the time of Hippocrates, humans have considered there to be different temperamental traits. About 2,500 years ago, Hippocrates developed the "Theory of Temperament," which included four temperaments (liquids) that he labelled blood, phlegm, yellow bile, and black bile. In the last century, Kraepelin defined temperament in terms of four basic affects or types: depressive, hyperthymic, irritable, and cyclothymic (19). Akiskal et al. (1987) (20) introduced the concept of affective temperament by adding anxious temperament to these four temperament types defined by Kraepelin.

Since affective temperament is generally thought to be associated with mood disorders, it has been studied frequently in that context (21-25). However, studies related to affective temperaments are rare in relation to other areas, including trauma. In this study, therefore, we examined the relationship between affective temperaments and reactions to the pandemic, which we define as psychological trauma. We associated our work with the depression, anxiety, and stress concepts, which are described as the most common psychological reactions during the COVID-19 epidemic, presented in the review study by (26).

In general, the aim of this study is to determine the effects of five affective temperament types (anxious, cyclothymic, irritable, depressive, and hypertimic) on depression, anxiety and stress and the importance of these effects during the pandemic.

2. Subject and Methods

In this research, the descriptive method was used in the relational scanning model. The aim of this research model is to determine whether there is a change between two or more variables, and if there is a change, its level (27).

2.1. Sample

The research population consisted of individuals from 18–80 years of age residing in Turkey. According to 2019 data from The Turkey Statistic Institute (TSI), the number of people in this age bracket in Turkey is 56,108,215't. Thus, the number of suitable samples to be taken from this group for research is 1,067, with a 95% confidence interval and ± 3 margins of error. In the research, online forms were delivered to 1800 people within the simple random sampling method. Of those who sent the online form, 1075 participated in the research and filled out the form completely. In this context, a total of 1,075 participants (653 women and 422 men) were included in the study. The sociodemographic details of the sample have presented in Table 1The online forms were disseminated to individuals between in the aforementioned age group via social media, and there were no participation criteria for individuals in this age range other than volunteering. Since the online form used in the research was based on the obligation of the participants to answer all questions without exception, there was no missing data. The details about the sample have presented in Table 1.

Table 1. Sociodemographic variables

Variable		n	%	SD
Gender	Women	653	60.7	±.48
	Men	422	39.3	
Age	18-30	510	47.4	±1.03
	31-40	282	26.2	
	41-50	174	16.2	
	51-60	99	9.2	
	60 and over	10	.9	
Marital status	Married	576	53.6	±.53
	Single	477	44.4	
	Divorced	22	2.0	
Education	Primary	81	7.5	±1.31
	High School	151	14.0	
	Bachelor	682	63.4	
	Postgraduate	161	15.0	

2.2. Data collection tools

Depression, Anxiety and Stress Scale (DASS-21): This scale was developed by Lovibond and Lovibond (1995) (28) and Turkish adaptation studies were carried out by (29). Validity and reliability studies showed that the Cronbach's alpha internal consistency reliability coefficient is $\alpha = 0.89$ for the depression subscale, $\alpha = 0.87$ for the anxiety subscale, and $\alpha = 0.81$ for the stress subscale. In addition, the scale was found to differ for the patient population and normal individuals in terms of depression, anxiety, and stress: major depression (mean depression score = 13.27; anxiety mean score = 09.03; mean stress score = 12.10), anxiety disorders (mean depression score = 11.23; anxiety mean score = 10.70; mean stress score = 12.37) and normal individuals (mean depression score = 3.23; anxiety mean score = 2.41; mean stress score = 3.99) ($F = 2.306$, $p = 0.00$). Thus, the obtained psychometric properties show that the DASS-21 is a valid and reliable measurement tool to determine levels of depression, anxiety, and stress. In this study, the Cronbach alpha coefficients obtained for depression, anxiety and stress subscales were .84, .80, and .86, respectively.

Temperament Evaluation of Memphis, Pisa, Paris, San Diego Autoquestionaire (TEMPS-A): This questionnaire, which was developed by Akiskal et al. (2005) (30), is a Likert-type scale that consists of 5 sub-dimensions that determine depressive, cyclothymic, hyperthymic, nervous, and anxious temperaments. The Turkish adaptation study of the scale was conducted by (31). In the Turkish form of the scale, the test-retest reliability ranged from 0.73 to 0.91 and the internal consistency was from 0.77 to 0.85. In this context,

it was confirmed in the adaptation study that the 5-factor structure should be preserved and the scale had a valid and reliable structure. The Cronbach's alpha values obtained with the data in this study were .78 in the depressive subscale, .88 in the cyclothymic subscale, .80 in the hyperthymic subscale, .84 in the irritable subscale, and .88 in the anxious subscale.

2.3. Data collection

Data were collected via online forms in accordance with the measures recommended due to the COVID-19 outbreak. In the statement introducing the data collection tools, it was made clear that the research was voluntary, personal information would not be requested, the participants' only responsibility was to fill out the forms completely and truthfully, and the data would only be evaluated collectively, meaning no individual's responses would be singled out.

In this way, the participants were fully informed about their rights and what would be expected of them during the process.

2.4. Data analysis

In this study, correlation analysis was used to understand the relationship between the valuables, and multiple regression analysis was conducted to determine how important temperament types are in predicting the depression, anxiety, and stress levels of individuals. The stepwise regression method was used in this research. The model assumptions were examined, and the suitability of the model for regression analysis was tested. First, no autocorrelation was found in the model (Durbin-Watson = 2.0). Second, there was no problem related to multiple connections between the explanatory variables (VIF < 5). Standardized residuals and Cooks values were checked, and contrary observations were found (13 piece observation was not included in the analysis). The multiple correlation coefficient for the model was determined to be R = 0.714 and corrected to R2 = 50.6%. The estimated regression model was found to be generally significant (P = 0.000 < 0.001).

3. Results

First, in order to provide a general perspective in the study, the correlation results between affective temperament types, depression, anxiety, and stress scores are presented below.

Table 2. Correlation between temperament types, depression, anxiety, and stress

Dimensions	Depressive	Cyclothymic	Hyperthymic	Irritable	Anxious	Depression	Anxiety	Stress
Depressive	1							
Cyclothymic	.585**	1						
Hyperthymic	-.373**	-.075*	1					
Irritable	.435**	.622**	.002	1				
Anxious	.590**	.675**	-.118**	.591**	1			
Depression	.477**	.533**	-.159**	.457**	.536**	1		
Anxiety	.383**	.465**	-.080**	.396**	.575**	.674**	1	
Stress	.372**	.503**	-.086**	.467**	.536**	.774**	.748**	1

**p<.001

When the values in Table 2 are examined, it is evident that depressive temperament has a moderate positive correlation with depression (r = .47, p < .01), anxiety (r = .38, p < .01), and stress (r = .37, p < .01). Similarly, cyclothymic temperament is positively correlated with depression (r = .53, p < .01), anxiety (r = .46, p < .01), and stress (r = .50, p <.01). Looking at the hyperthymic temperament, there is a low level of negative correlation with depression (r = -.15, p < .01), anxiety (r = -.08, p < .01), and stress (r = -.08, p < .01). Irritable temperament type is positively correlated with depression (r = .45, p < .01), anxiety (r = .39, p < .01), and stress (r = .46, p < .01). Finally, anxious temperament, which is the fifth temperament type considered in the study, showed a positive and moderate correlation with depression (r = .53, p < .01), anxiety (r = .57, p < .01), and stress (r = .53, p < .01).

Table 3. Hierarchical regression analysis to determine the temperament types that predict depression

Predictors	β	Sβ	t	p	R^2	R^2_{adj}
Anxious	.157	.230	6.495	.000**	.406	.403
Cyclothymic	.163	.227	6.245	.000**		
Depressive	.131	.125	3.712	.000**		
Irritable	.162	.164	5.168	.000**		
Hyperthymic	-.046	-.053	-2.023	.043*		

**p< .001, *p< .05

In Table 3, affective temperament types are given in order of their depression-explanation power. Accordingly, the temperament type with the most power to explain depression is anxious temperament. Affective temperament types explain 40% of depression in total (R_adj^2=.403). Anxious temperament, depression (33%), cyclothymic temperament (31%), irritable temperament (21%), depressive temperament (11%), and hyperthymic temperament contribute .3% to this explanation power. In general, it was observed that four temperament types (excluding hyperthymic temperament)

were positive for depression while hyperthymic temperament was a negative predictor. Finally, the predictive power of the five temperament types was significant.

Table 4. Hierarchical regression analysis to determine temperament types that predict anxiety

Predictors	β	Sβ	t	p	R^2	R^2_{adj}
Anxious	.248	.508	15.383	.000**	.377	.376
Cyclothymic	.074	.143	4.327	.000**		

**p< .001

The values in Table 4 show that anxious and cyclothymic temperaments have significant effects in explaining anxiety, and the power of predicting anxiety in the other three affective temperament types is not statistically significant. The two affective temperament types together explain about 37% of anxiety (R_adj^2=.376). When the levels of contribution to this explanatory power are examined, it is revealed that anxious temperament contributes 91% and cyclothymic temperament 9%.

Table 5: Hierarchical regression analysis to determine temperament types that predict stress

Predictors	β	Sβ	t	p	R^2	R^2_{adj}
Anxious	.210	.307	8.785	.000**		
Cyclothymic	.140	.194	5.683	.000**	.359	.357
Irritable	.185	.187	5.390	.000**		

**p<.001

When Table 5 is examined, it is seen that anxious, cyclothymic, and irritable temperament types have important effects in explaining stress. These three affective temperament types together explain about 36% of stress (R_adj^2=.357). When examined in terms of their contribution to the explanatory power, it was revealed that anxious temperament contributed 54%, irritable temperament 25%, and cyclothymic temperament 21%.

4. Discussion

The COVID-19 pandemic has psychologically affected individuals, as well as directly and indirectly affected humanity as a social trauma (32). During the pandemic, many studies have been carried out on depression, anxiety, and stress levels that have led to significant findings (6, 33,5,1). The affective temperament types defined by Akiskal (1987) (19) were used in this study to examine the relationship between temperament types, which are predictors of psychological responses to trauma (16, 17), depression-anxiety, and stress responses. Although Akiskal (1987) (20) has defined these temperament types, he suggested that mood disorders should be viewed within a spectrum (34). In particular, Akiskal et al. (1980) (35) described subthreshold temperament traits that did not require treatment at one end and pathological processes up to psychosis at the other.

In the data obtained in this study, depression anxiety-stress levels and all temperament types (anxious, cyclothymic, depressive, irritable, and hyperthymic) showed a significant correlation at different levels during the pandemic process. If we examine these findings with Akiskal's spectrum approach, we can say that as the affective temperament features become clear, the person approaches the pathological end. In psychiatry, pathology is generally expressed as the deterioration of an individual's adjustment. Since we define the COVID-19 pandemic as a traumatic period in which many adaptations are required, it was expected that adaptation would deteriorate with the prominence of affective temperament features, thus increasing depression-anxiety and stress responses in a correlated way.

While our normal expectation is that depressive temperament should show a higher correlation with depression compared to other temperament types, in our study, it was observed that anxious and cyclothymic temperaments were more correlated with depression. This may actually be an indication that the study was conducted during a traumatic period rather than under normal conditions. The fact that trauma-related disorders fall under the heading of anxiety disorders up to DSM-IV (American Psychiatric Association 2000) supports the relationship of trauma to anxiety and, therefore, to anxious temperament.

The concept of intolerance to uncertainty, which is directly related to anxious temperament, is accepted as the main component underlying anxiety disorders (36). In the study conducted by Satici et al. (2020) (4) during the COVID-19 pandemic, it was shown that psychological wellbeing was negatively affected by the increase in levels of uncertainty and intolerance. This finding supports the higher correlation of anxious temperament in all the parameters (depression, anxiety, and stress) compared to all other temperaments, and its prediction of all three parameters.

Another temperament type that was predictive for all three parameters was cyclothymic temperament. Powers et al. (2015) (15) found that emotional dysregulation associated with cyclothymic temperament predicted dissociative symptoms as a result of post-traumatic stress disorder (PTSD) (14). Additionally, variable self-esteem and emotional instability are also considered to be components of cyclothymia (37). Similarly, Kashdan et al. (2006) (38) found in their study that variable self-esteem and emotional

instability were significantly more intense in the group that developed PTSD among those exposed to the same trauma compared to the group that did not develop PTSD (38). Both studies support our findings by showing that processes associated with cyclothymic temperament increase responses to trauma.

Strelau (1996) (39) describes six temperament traits in the regulative theory of temperament: briskness, perseverance, sensory sensitivity, emotional reactivity, endurance, and activity. In the study of Oniszczenko et al. (2017) (40) comparing the regulative temperament types with the affective temperament types, it was observed that there was a positive correlation between emotional reactivity and persistence from regulative temperament theory and anxious, cyclothymic, irritable, and depressive temperaments from the affective temperament model. In Oniszczenko's (2014) (41) study, emotional reactivity and post-traumatic reactions were found to increase in firefighters. In the study conducted by Oniszczenko and Laskowska (2014), (42) high emotional reactivity was shown to intensify cancer-related trauma symptoms in adult patients. Strelau and Zawadzki (2005) (16) found that persistence and emotional reactivity increase the effects of trauma, and emotional reactivity in all samples is the best predictor of the intensity of PTSD symptoms. In another similar study, the authors' results showed that emotional reactivity increased trauma symptom intensity in HIV positive participants (43). Finally, Zawadzki and Popiel (2012) (44) discovered that emotional reactivity increased the symptoms of PTSD together with the severity of the trauma.

In all these studies related to regulative temperament, there are significant relationships among emotional reactivity and persistence and traumatic reactions. In our affective temperament study, a positive correlation was found between anxious, cyclothymic, irritable, and depressive temperament types and post-traumatic reactions on depression, anxiety, and stress scales. In addition, all the above-mentioned affective temperament types were significant predictors for depression, three temperament types (anxious, cyclothymic, irritable) for stress, and two temperament types (anxious, cyclothymic) for anxiety. Considering the relationship between affective temperament types and regulatory temperament traits, it can be said that related research supports the findings of our study. It has been observed that vitality, emotional sensitivity, and resilience characteristics, especially activity in regulatory temperament theory, are related to the hyperthymic temperament type in affective temperament theory (40). In Oniszczenko's (2014) (41) study, vitality associated with hyperthymic temperament showed a negative correlation with post-traumatic symptoms in soldiers. Another similar study found that vitality temperament traits can protect against the development of PTSD (44). Temperament characteristics, such as vitality and endurance, have also been seen to function as buffers that reduce the effect of traumatic events (16). In our study, hyperthymic temperament was negatively correlated with each of the variables of depression, anxiety, and stress. In addition, it was found to be a negative low but significant predictor for depression. In other words, it is understood that certain hyperthymic tendencies are preventive for depression. When considered as a whole, these studies suggest that hyperthymic temperament can be protective against the effects of trauma.

In this study, it was understood that there is a relationship between affective temperament types and depression, anxiety, and stress during the Covid-19 pandemic process. When the details of the research are examined, it is understood that while hyperthymic temperament is a negative predictor for depression, anxious, cyclothymic, depressive, and irritable temperaments are positive predictors for depression. Additionally, the results revealed that anxious, cyclothymic, and irritable temperament types were significant predictors for stress. Anxious and cyclothymic temperament types were significant predictors for anxiety. Hyperthymic temperament was negatively correlated with depression, anxiety, and stress. Considering these results, it can be stated that hyperthymic temperament is protective against the effects of trauma.

It should be noted that this research has some limitations. Due to the pandemic conditions, online data collection method was used in the study. The general limitations of the online data collection method are also valid for this research. Secondly, the measurement tool used in the research is generally aimed at determining the levels of depression, anxiety, and stress. It is not a measuring tool that directly addresses the pandemic. This may be a limitation. In order to eliminate this limitation, the participants were asked to evaluate each question under pandemic conditions. Finally, this study examined the effects of directly affective temperament types on depression, anxiety, and stress. There may be some demographic intermediary variables that can affect this relationship. In this study, direct effect was examined and mediator effects were not considered. This study drew attention to the scarcity of studies examining the relationship between affective temperament and trauma. However, studying affective temperament types in relation to different areas in the field of trauma should provide significant benefits.

In this study, it was clear that the anxious temperament was the most affected by the pandemic. Therefore, a more detailed examination of the relationship between the anxiety spectrum and the pandemic process is important in terms of identifying groups at risk and providing more effective biopsychosocial support.

Conflict of Interest

The authors declare that they have no conflict of interest.

Funding

None.

Ethical Approval

This study was approved by the Ethical Committee of Erzincan Binali Yildirim University. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration.

Data Availability Statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 611-615
doi: 10.52142/omujecm.39.3.3

Investigating permanent first molars of a Turkish pediatric sample in the eastern mediterranean region: A radiagraphic study

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Received: 24.09.2021 • **Accepted/Published Online:** 29.11.2022 • **Final Version:** 30.08.2022

Abstract

The early loss of first permanent molars (FPMs) may cause various problems in the dentofacial region. In the present study, it was aimed to evaluate the conditions of FPMs of a Turkish pediatric sample living in the Eastern Mediterranean Region. Dental panoramic radiographs (DPR) with good diagnostic quality and demographic characteristics of patients aged 7-17 years were retrospectively reviewed. Accordingly, affected teeth were evaluated by caries, filling, root canal treatment, retained root, apical lesion, and extraction. FPMs were compared by mandible, maxilla, and right-left jaw positions. The study was carried out with a total of 929 children, 442 (47.60) females and 487 (52.40) males. The participants were between the ages of 7-17 (M=12.10 ± 2.92). Among 3,974 FPMs evaluated, 2,018 (50.79%) were determined to be healthy, while 1,956 (49.21%) were affected. DC1 and F1 were the most common conditions of all evaluated FPMs. Regarding mandibular and maxillary FPMs, 1.137 of the FPMs1+FPMs2 and 881 of the FPMs3+FPMs4 were discovered to be healthy, and the difference was statistically significant ($p<0.001$). However, any significant differences were not found between right and left FPMs by treatment. While FPMs remained healthy significantly more in the age group of 7-9 years (Group-I), extraction, root canal treatment, and apical treatment were significantly higher in the age group of 16-17 years (Group-IV) ($p< 0.001$). Ultimately, it was concluded that FPMs start to be affected from early ages and that incidences of root canal treatment, extraction, and apical lesions become more prevalent with advancing age. This situation emphasizes the importance of applying preventive therapies as soon as the eruption of these teeth in children and attempting to increase relevant awareness among families.

Keywords: dental caries, FPMs, dental panoramic radiography, child, adolescent, oral health

1. Introduction

First permanent molars (FPMs) are the first erupted permanent teeth in the posterior (1). Yet, these teeth are frequently affected because they are susceptible to caries attacks (2, 3). Among other permanent teeth, there are multiple factors causing FPMs to have the highest prevalence of caries and/or extractions: the anatomical form of teeth (pit and fissure structure), their earlier eruption compared to other permanent teeth, exposure to the oral cavity, the neglect of their care due to their co-existence with the deciduous teeth, and knowledge gap in parents (e.g., mistaking these teeth with deciduous teeth) (4, 5). On the other hand, it was previously reported that early caries might occur in FPMs in cases such as early childhood caries and caries in the second molars (2). FPMs have essential roles in maintaining regular mastication function and dentofacial compliance (3, 6). Some of these roles are to support muscles, hold the vertical axis, and determine the occlusal relation in the vertical plane, and guide force distribution and the eruption of other permanent teeth (6).

Early loss of FPMs may cause many problems such as localized reduction in mastication force, osteoporotic alterations to the trabecular bone, a premature eruption of permanent second and third molars, increased overbite and rotation, tilting and rotation of adjacent teeth, and temporomandibular discomfort (TMD) (3, 7, 8). In the literature, it was reported that fissure sealants, as well as oral hygiene practices and fluoride, are effective in preventing caries in permanent posterior teeth (9, 10). Therefore the premature loss of FPMs should be prevented because it may cause many problems. For this purpose, it is needed to identify the caries risk and treatment needs of FPMs in different populations, which may contribute to planning preventive measures and appropriate distribution of treatment services in the country. Besides, the World Health Organization has recently updated its oral health targets.

These updates emphasize that the recommendations are not equally applicable to all countries and populations and that there should be regional and national oral health targets (11).

The relevant literature hosts research evaluating FPMs

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among different age groups in different geographical regions (12-16).

Ultimately, recruiting dental panoramic radiographs (DPR) from Kahramanmaraş Sütçü İmam University School of Dentistry, to which almost all pediatric patients with various socioeconomic statuses were referred, the present study aimed to evaluate FPMs (condition, caries, filling, extraction by mandible, maxilla, and right-left jaw positions) of a Turkish patient sample aged 7-17 years living in the Eastern Mediterranean region.

2. Materials and Methods

The ethical approval to the present study was granted by the Ethics Committee of Kahramanmaraş Sütçü İmam University Non-Interventional Clinical Research (Session No: 2021/16; Decision No: 06). DPRs with good diagnostic quality of healthy patients aged 7-17 years, who applied to Kahramanmaraş Sütçü İmam University School of Dentistry for dental treatments between 2019-2021, were systemically selected and retrospectively analyzed along with the demographic characteristics of the patients. No additional radiographs were required from the patients for this study. Children with a systemic, congenital disease, syndromic children, and children who did not have erupted permanent first molars, and those receiving orthodontic treatment were excluded from the study. Although the eruption of these teeth starts about at the age of 6, the minimum age limit was determined to be 7 in this study to exclude those without fully erupted teeth better. While the radiographs were evaluated by the oral maxillofacial radiologist, the pediatric dentist was consulted in cases where she was in dilemma.

DPRs were taken on GENDEX GDP -700 device (Kavo Kerr, Biberach, Germany) at 66 kVp, 6.3 mA and 14 sec. DPRs of a total of 929 patients (3,974 FPMs) were reviewed. FPMs were investigated by dividing the patients into four age groups: 7-9 years (Group-I), 10-12 years (Group-II), 13-15 years (Group-III), and 16-17 years (Group-IV).

Right permanent maxillary first molars (FPMs1), left permanent maxillary first molars (FPMs2), left permanent mandibular first molars (FPMs3), and right permanent mandibular first molars (FPMs4) were considered in DPRs. All permanent first molars in the maxilla and mandible were categorized by their conditions. Healthy teeth were denoted by (H), extracted (E), with dentin caries (DC1, DC2, DC3), while affected teeth were categorized as those filled (F1, F2, F3), with root canal treatment (RCT), with an apical lesion (AL), and with a retained root (R) (12, 13). In addition, FPMs were evaluated by segment, sex, and age.

2.1. Statistical analysis

The data were analyzed using SPSS 16.0 (IBM, Chicago, USA) software. The Chi-square test and Pearson's correlation coefficients were considered to compare the variables. The descriptive statistics were presented in percentage, mean (±), and standard deviation. In all analyses, $p<0.05$ were accepted as statistically significant.

3. Results

A total of 929 patients, 442 females and 487 males, were included in the study (Table1). The individuals included in the study were between the ages of 7-17 years, and the mean age was 12.0 ± 2.92.

Table 1. Percentage of gender of the study

Gender	Percent (%)	Noun (n)
Female	47.60	442
Male	52.40	487

Of 3,974 FPMs evaluated, 2,018 (50.79%) were determined to be healthy, while 1,956 (49.21%) were affected (caries, filling, apical lesion, root canal treatment, extraction, and retained root) (Table2).

The distribution of healthy and affected teeth is presented in Table 3.

DC1 and F1 were the most common conditions of all evaluated FPMs. While the total number of extracted FPMs was 32 (0.80%), the number of those with root canal treatment was 74 (1.86%). The numbers of FPMs with an apical lesion and retained root were calculated to be 168 (4.22%) and 32 (0.80%), respectively (Table3).

It was realized that FPMs1 and FPMs2 remained significantly healthier ($p<0.001$). Yet, FPMs3 were significantly more affected ($p<0.001$). Besides, FPMs4 were highly affected, but a significant difference could not be obtained ($p>0.005$). It was also discovered that 1.137 of the FPMs1+FPMs2 and 881 of the FPMs3+FPMs4 remained healthy, and the differences were statistically significant ($p<0.001$). However, any significant differences were not found in the left and right FPMs by treatment (Table2).

While it was found that FPMs remained healthy significantly more in the age group of 7-9 years (Group-I), extraction, root canal treatment, and apical treatment were significantly higher in the age group of 16-17 years (Group-IV) ($p< 0.001$) (Table 4).

Finally, regarding sex, FPMs1 and FPMs2 significantly remained healthier in males and females. However, FPMs3 and FPMs4 were found to be significantly more affected in both males and females (Table 5).

Table 2. Chi-square test result in comparison of health and affected status of teeth

% (n)	FPMs1	FPMs2	FPMs3	FPMs4	Total
Healthy	59.77[a] (569)	69.43[a] (568)	40.73[b] (426)	43.91[b] (455)	%50,79 (2018)
Affected Status	40.23[b] (383)	39.57[b] (372)	59.27[a] (620)	56.08[a] (581)	% 49,21 (1956)
Chi-Square	4098.06**	4089.94**	1923.89**	2097.08	

Table 3. FPMs1, FPMs2, FPMs3, FPMs4 percentage of teeth in terms of healthy and affected status

	H % (n)	E %(n)	DC1 % (n)	DC2 %(n)	DC3 %(n)	F1 %(n)	F2 %(n)	F3 %(n)	RCT % (n)	AL %n)	R % (n)	Total (n)
FPMs1	59.80 (569)	0.50 (5)	18.60 (177)	4.00 (38)	0.40 (4)	11.90 (113)	1.60 (15)	0.40 (4)	1.40 (13)	0.60 (6)	0.50 (5)	952
FPMs2	60.40 (568)	0.10 (1)	16.60 (156)	7.60 (71)	0.70 (7)	11.40 (107)	1.20 (11)	0.20 (2)	0.40 (4)	0.50 (5)	0.60 (6)	940
FPMs3	40.70 (426)	1.00 (10)	17.10 (179)	6.00 (63)	3.10 (32)	17.10 (179)	2.00 (21)	0.70 (7)	2.80 (29)	7.80 (82)	1.10 (11)	1046
FPMs4	43.90 (455)	1.50 (16)	16.30 (169)	6.30 (65)	3.30 (34)	14.40 (149)	2.00 (21)	1.00 (10)	2.70 (28)	7.20 (75)	1.00 (10)	1036
Total	2018	32	681	237	77	548	68	23	74	168	32	

(H): Healthy teeth, (E): Extracted, (DC1, DC2, DC3): Dentin caries, (F1, F2, F3): Filled tooth, (RCT): Root canal treatment, (AL): Apical lesion, (R): Retained root.

Table 4. Comparison of healthy and affected teeth according to age groups

% (n)	Age Group I (7-9)	Age Group II (10-12)	Age Group III (13-15)	Age Group IV (16-17)
H	83.10 (779)	45.00 (519)	40.80 (489)	33.80 (231)
E	0	0.20 (2)	0.90 (11)	2.80 (19)
DC1	10.60 (99)	20.60 (237)	20.10 (241)	15.20 (104)
DC2	1.60 (15)	5.10 (59)	8.00 (96)	9.80 (67)
DC3	0.10 (1)	3.50 (40)	2.10 (25)	1.60 (11)
F1	4.40 (41)	18.00 (207)	16.30 (196)	15.20 (104)
F2	0	1.00 (12)	2.20 (26)	4.40 (30)
F3	0	0.30 (4)	0.70 (8)	1.60 (11)
RCT	0	1.00 (12)	2.50 (30)	4.70 (32)
AL	0	4.40 (51)	4.80 (57)	8.80 (60)
R	0.10 (1)	0.50 (6)	1.20 (14)	1.60 (11)
Chi-Square	3683.98**	3091.03**	2599.27**	820.32**
Toplam	936	1149	1193	680

(H): Healthy teeth, (E): Extracted, (DC1, DC2, DC3): Dentin caries, (F1, F2, F3): Filled tooth, (RCT): Root canal treatment, (AL): Apical lesion, (R): Retained root.

Table 5. Comparison of healthy and affected teeth according to gender

% (n)								
	Female	Male	Female	Male	Female	Male	Female	Male
Healthy	61.54 (272)	58.24 (297)	61.40 (264)	59.60 (304)	36.40 (182)	44.69 (244)	39.68 (196)	47.79 (259)
Affected Status	38.46 (170)	41.76 (213)	38.60 (166)	40.39 (206)	63.60 (318)	55.31 (302)	60.32 (298)	52.21 (283)
Chi-Square	1270.76**	2084.44**	1556.24**	1349.50**	850.76**	1125.34**	874.58**	1264.99**
Total	442	510	430	510	500	546	494	542

4. Discussion

The literature often reports that oral and dental health awareness is unfortunately low in developing countries. In turn, prevalent caries incidence is not surprising in these countries due to rare follow-up examination habits or visiting a dentist only upon complaints (17-20).

In this study, it was determined that 49.21% of the FPMs of a Turkish pediatric sample (7-17 years) in the Eastern Mediterranean were affected; that is, the sample had at least one of the signs of caries, extraction, filling, root canal treatment, apical lesion, and retained root. In a study evaluating FPMs among children aged 7-12 in Izmir, it was concluded that 44.1% of the participants had one or more FPMs with caries, filling, and extraction (12). In another study with children aged 8-12 years in Malatya, it was reported that 58.6% of their FPMs were affected (13). One more study in Izmir with children in a different age group (12-18 years) discovered that 58.1% of the participants had one or more FPMs with caries, filling, extraction (14). Öter et al. evaluated the treatment needs of FPMs of children aged 6-12 years who applied to clinics of dentistry faculties in

Istanbul and Ankara. They concluded that about 50% of the FPMs needed relevant treatments (21). While the findings in this study were similar to some studies investigating the rates of affected FPMs in different regions in Turkey (12, 17) this study presented lower rates of affected teeth than some other studies (13-15).

In a previous study evaluating FPMs of 12-year-old students in Iran, it was reported that caries incidence was 53.12%-66.04% (15). In another study in Saudi Arabia, caries prevalence in FPMs was reported to be 66.4% (16). Moreover, the researchers explored the conditions of FPMs of 1,538 adolescents aged 12-15 years in Mexico based on clinical examination and reported that 56.4% of the sample had healthy FPMs (22). Variation in the rates of affected FPMs may stem from the differences in the possibilities of accessing treatment by region and socio-cultural and economic differences, as well as the methodological variances in previous research. In this study, the rates of problematic conditions in FPMs were found to be as follows: extraction (0.80%), retained root (0.80%), root canal treatment (1.86%), apical lesion (4.22%). In a similar study evaluating pediatric patients aged 7-12 years in Turkey, the rates of extraction and root canal treatment were determined to be 0.8% and 0.6%, respectively (12). Similarly, in a study with 4,872 Turkish children aged 6-12 years, while the rate of extracted teeth was 0.6%, the rate of teeth with root canal treatment was 0.5% (23). In this study, the rates of extraction and retained root were the same (0.80%). Considering that retained roots also require extraction, it is not prudent to claim that the rate of children needing extraction treatment is 1.6%. Ultimately, the rates of extraction and root canal treatment in this study are similar to other studies in Turkey. On the other hand, the fact that the rate of apical lesion was higher than the other findings in the present study may be related to the wide age range of our sample. Preservation of FPMs becomes essential since these teeth play an important role in maintaining a regular mastication function and dentofacial compliance. Therefore, first, oral health education should raise awareness of the importance of oral hygiene and proper nutrition, especially to limit sugar intake and non-abrasive soft foods. Second, preventive programs should broaden their target groups to include younger children, potentially starting with prenatal education and taking appropriate precautions (3, 6).

In addition, DC1 and F1 were the most prevalent of all FPMs evaluated in this study. In parallel with the present study, many studies with pediatric samples reported occlusal surface caries to be the most prevalent problem in FPMs (14, 24). It was then found that mandibular FPMs were significantly more affected ($p<0.001$), while no significant difference was determined between right and left FPMs. The findings are consistent with many studies in the literature (12, 16, 23, 25). More caries in mandibular FPMs is often explained by their earlier and more exposure to the oral cavity and more pits and grooves in these teeth (16). On the other hand, the close positioning of the palatal salivary gland duct to the maxillary FPMs may allow these teeth to remain cleaner than their mandibular counterparts (14). Although there was no significant difference between right and left FPMs in many studies, it was reported that hand selection in tooth brushing might affect appropriate cleaning of the right or left teeth (12, 21).

It was concluded that FPMs were significantly more affected as age advanced ($p<0.001$), which is a consistent finding with the relevant literature (12, 16). Besides, extraction, root canal treatment, and apical treatment were found to be significantly more prevalent in the group aged 16-17 years (Group-IV), FPMs were significantly more healthy in the group aged 7-9 years (Group-I). In parallel with this study, Duman et al. found the highest extraction rate (34.1%) in the group aged 12-18 years (Group-III) (13). In addition, Öter et al. reported that the rates of filled and missing teeth were significantly higher in the group aged 11-12 years, while this rate was found to be significantly lower in 8-year-olds (21). Similarly, in the study of Dhar et al., the group aged 8-10 years needed more treatment than the group aged 6-7 years (26). Similar results were obtained in the study regarding FPMs in both males and females; there was no significant difference by sex. Similarly, Bulut et al. found no statistical difference between FPMs with caries, extraction, and filling by sex (12). Yet, Zhu et al. reported that the females aged 6-8 years had more caries in their FPMs than their male peers 3. Poureslami et al. also detected a higher caries rate in females (3). It was previously stated that this condition in females might develop due to earlier puberty and tooth eruption in females, anatomical diversity in teeth, sex-related nutrition, anthropological factors, and differences in saliva quality and quantity (15, 27).

This was a retrospective radiological study, which might have caused the inability to detect initial caries in the radiographs. In addition, the results cannot be generalized to the Turkish population, as they were obtained from a specific region. Further studies may adopt both radiographic and clinical examinations in a larger sample to include different regions in Turkey.

In our study, the rates of extraction, root canal treatment, apical lesion were high in direct proportion to age, which highlights the importance of brushing teeth at an early age, regular dental examination, taking preventive measures, and raising parental awareness to retain FPMs in the mouth.

Conflict of interest

The authors declared no conflict of interest.

Funding

No funding was used for the study.

Acknowledgments

None to declare.

Authors' contributions

Concept: K.T.T., A.S.O., Design: K.T.T., A.S.O., Data Collection or Processing: K.T.T., A.S.O., Analysis or Interpretation: K.T.T., Literature Search: K.T.T., A.S.O., Writing: K.T.T., A.S.O.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 616-619
doi: 10.52142/omujecm.39.3.4

The effect of iv tranexamic acid on blood loss during myomectomy

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Received:30.09.2021 • **Accepted/PublishedOnline:**19.07.2022 • **Final Version:** 30.08.2022

Abstract

Uterine leiomyomas are benign gynecological tumors of the uterus which are commonly seen in women under 50 years of age and they are one of the common reasons of abnormal uterine bleeding. Myomectomy is the most common treatment method among women who want to remain fertile and/or to protect the uterus. Approximately 1-4% of myomectomy operations end up with hysterectomy due to uncontrollable bleeding. Many different methods are used in order to reduce the incidence of uncontrollable bleeding and minimize blood loss. In this study, we have examined the effect of intraoperative intravenous tranexamic acid on blood loss on type 3, 4, 5, and 6 myomas during myomectomy according to FIGO grading and its postoperative results.

Keywords: tranexamic acid, myomectomy, blood loss, uterus

1. Introduction

Regarding myomas presenting with clinical symptoms the rate is about 33% according to literature with the remaining population being incidentally detected. These symptoms may range from variable amounts of uterine bleeding to clinically significant anemia, persistant pelvic pressure, pain, urinary disfunction and infertility (1-7).

Symptomatic myomas should be treated or followed according to the individual but when concerning treatment options, there is a wide spectrum available from hormonal or nonhormonal medical alternatives to surgical procedures (8). The suitable treatment method is usually determined by considering several factors such as, patient age, number of myomas, fertility preference and the symptoms of the patient. Myomectomy is the most common treatment method among women who want to remain fertile and/or to protect their uterus. Approximately 1-4% of myomectomy operations end up with hysterectomy due to uncontrollable bleeding (9). Many different methods are used in order to reduce the incidence of uncontrollable bleeding and minimize blood loss (10,11).

The use of intraoperative iv tranexamic acid (TXA) is more common than ever in recent years. The fundamental mechanism of action is to reduce bleeding by inhibiting plasminogen activity (12). There are studies in literature suggesting that the use of TXA reduces blood loss during cardiovascular surgery, orthopedic operations, and organ transplantation (13-15).

In this study, we have examined the effect of intraoperative intravenous tranexamic acid on blood loss on type 3, 4, 5, and 6 myomas during myomectomy according to FIGO grading (16) and its postoperative results. Our secondary purpose was to determine whether TXA use changes the need for blood products.

2. Material and Methods

Eighty patients with type 3, 4, 5, and 6 myomas (FIGO) planned to undergo myomectomy were included in this study. Patients were sequentially enrolled from July 2018 to January 2020 at two tertiary centers. Eighty patients were allocated to study and control groups in a 1:1 ratio design, respectively. The 1st group was injected 10 mg/kg bolus iv TXA within 10 minutes (maximum 1 gr), 15 minutes before the incision. Then infusion was applied continuously for 10 hours within 1 liter of saline as 1 mg/kg/hour. The 2nd group was also given the same amount fluid in the same manner without TXA.

Intraoperative blood loss was calculated in accordance with the blood volume in the aspirator and the weight of the sponge used. Both groups were compared in terms of operation duration, perioperative and postoperative hemoglobin count, hospitalization periods, and intraoperative and postoperative complications.

2.1. Patient selection

Eighty patients with myomectomy indications between 30 and 45 years of age, whose body mass indexes were within the normal limits (BMI: 20-25), who were diagnosed with type 3-6 myomas through trans vaginal ultrasonography (TVUSG) or

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magnetic resonance imaging (MRI) according to FIGO classification and who had 2 and/or 4 myomas, were selected consecutively (1:1). The approximate volume of the myomas were measured by TVUSG using 3-dimensional distance measurements.

Patients with a history of malignant disease, thromboembolic disease, coronary artery disease, cerebrovascular disease and smoking were not included in this study. In addition, patients with a single myoma, or more than 4 myomas were also excluded and also patients included in the study, who regularly use nonsteroid anti-inflammatory drug and acetyl salicylic acid were asked to stop using their medication 10-14 days before the operation. Preoperative hemoglobin, APTT, PT, bleeding time, and coagulation time of the patients were noted. If any kind of blood products were used, preoperatively and/or postoperatively, the number of units used were noted.

2.2. Operation and evaluation

The operations were performed by two experienced surgeons through Pfannenstiel incision and performing by intracapsular myomectomy technique (17). The 1st group was injected 10 mg/kg bolus iv TXA within 10 minutes (maximum 1 gr), 15 minutes before the incision. Then infusion was applied continuously for 10 hours within 1 liter of saline as 1 mg/kg/hour. The 2nd group was also given the same amount fluid in the same manner without TXA.

Total blood loss was calculated by summing up the blood volume in the aspirator, the weight differences between sponges, and the total volume of liquid from the drain. The localizations of myomas were noted postoperatively by the surgeons. Hemoglobin values were noted postoperatively on the 2nd day. The symptomatic patients with hemoglobin values under 8 mg/dl were given blood transfusion. All patients had used antiembolic stockings until they were released from the hospital. All operational complications were noted.

Tablo1. Patients' demographics and preoperative data

Particularities	Operation	Control	P Value
Age[a]	39.5 (30-45)	38.5 (30-45)	0.809
Body Mass Index[a]	23 (20-25)	23 (20-25	0.772
Preoperative PT[b]	12.2 ± 0,9	11.5±0,9	<0.001
Preoperative APTT[a]	32 (36-35)	32 (27-35)	0.505
Preoperative Hb[b]	10.27 ± 0,66	10.11±0,68	0.554
Figo Grade (n, %) [c]			
Type 6	7 (17.5)	9 (22,5)	0.915
Type 3	13 (32.5)	11 (27,5)	
Type 4	9 (22.5)	10 (25)	
Type 5	11 (27.5)	10 (25)	
Myoma'sVolume[a]	295(224-365)	305 (205-320)	0.310

[a]Mann-Whitney U Test was used
[b]T test was used
[c]Chia test was used

2.3. Statistical analysis

SPSS version 26.0 was used for analysis. Kolmogorov-Smirnov test was made in order to evaluate the normality of the distribution of data. T test was used for normally distributed numeric variables; Mann Whitney U test was used for non-normally distributed numeric variables. Chi-square test was used for categorical variables. P<0.05 was considered significant.

3. Results

The pre-operational demographics of the patients such as age, gender, BMI, etc., were given in Table 1. The intraoperative and postoperative complication rates, bleeding amounts, transfusion need rates and postop hemoglobin and hematocrite values of 80 patients were given in Table 2. When intraoperative blood loss amounts were compared, it was found that the TXA group's blood loss amount was less than the control group. This difference was statistically significant (p=0.008). Both groups did not need blood transfusion. On the postoperative 2nd day, it was observed that the Hg values decreased less in TXA group when compared to the control group (p=0.002). There was no significant difference between both groups in terms of operation duration (p=0.131).

Table 2. Patients' intraoperative and postoperative attributes

Particularities	Operation	Control	P Value
OperationPeriod[a]	67 (60-78)	69 (60-75)	0.131
Length of Stay[a]	2 (2-3)	2 (2-3)	0.366
Blood Loss (cc)[a]	155 (115-176)	162.5 (115-180)	0.008
Postoperative Hemoglobin[b]	9.62 ± 0.67	9.35 ± 0.73	0.111
Pre-postop. Hemoglobin Difference [a]	0.65 (0.4-1)	0.75 (1.1-0.45)	0.002

[a]Mann-Whitney U Test was used.
[b]T test was used.

4. Discussion

Uterine leiomyomas are benign gynecological tumors of the uterus which are commonly seen in women under 50 years of age and they are one of the common reasons of abnormal uterine bleeding.

In this study, a comparison of intraoperative bleeding in myomectomy operation was evaluated retrospectively between an intravenous TXA administered group and a placebo group. We have found that the use of intraoperative TXA had a positive impact on reducing the amount of bleeding. There are not many studies in literature regarding the use of TXA in gynecological operations.

In a study, where the amount and duration of TXA use was investigated (18), it was found that the amount of bleeding reduced significantly during operation in patients who were administered TXA before incision when compared to the placebo group and the hemoglobin values of the patients were less affected in the postoperative period.

However, no statistically significant differences were found between the two groups in terms of blood transfusion and operation time rates.

In a study regarding intraoperatively administered TXA's reducing effect on bleeding during myomectomy, the surgical methods employed were all minimally invasive and since the surgical method would affect the incision type and the amount of bleeding, all patients in our study were selected among patients who were operated through Pfannenstiel incision (19).

In another study, injection of oxytocin, TXA, and vasoconstrictive agents into myoma's subcapsular area were mentioned among the options provided to control bleeding throughout myomectomy besides other torniquet methods. However, the difference of the effect of TXA administration prophylactically before the operation and intraoperative TXA administration was not compared as we did in this study (20).

In another study, it was found that the use of TXA acid did not have an impact on blood loss during myomectomy (21). Unlike our study, that study included patients with only a single myoma.

Although the incision and operation types were similar, it should be noted that there were two active surgeons, the fact that locations of myomas of the patients were different even though they were similar in size, and these would impact the duration of the operation.

In this study, the long-term complications of the patients were not recorded. Neither group needed postoperative blood transfusion in our study. Future studies on this matter may include more patients.

Consequently, we have found that the use of intraoperative TXA had a positive impact on reducing the amount of bleeding. The results of a meta-analysis (22) published on the use of TXA during myomectomy support the results of our study. However, only 4 articles were examined in relevant literature regarding this subject, and it shows that more randomized controlled studies are needed on this matter. In addition, new studies should be conducted about the safe TXA dosage range and its effect on intraoperative blood loss.

Conflict of interest

The authors declared no conflict of interest.

Funding

No funding was used for the study.

Acknowledgments

None to declare.

Authors' contributions

Concept: Ü.K., K.B., Design: Ü.K., K.B., Data Collection or Processing: Ü.K., M.Ö., Analysis or Interpretation: Ü.K., M.Ö., Literature Search: Ü.K., M.Ö, Writing: Ü.K., M.Ö., K.B.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 620-627
doi: 10.52142/omujecm.39.3.5

Evaluation of biostatistics knowledge and skills of medical faculty students

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Received: 19.10.2021 • **Accepted/Published Online:** 18.04.2022 • **Final Version:** 30.08.2022

Abstract

Successful implementation of a scientific study and correct analysis of data obtained is possible with advanced biostatistics knowledge. The aim of this study is to find out efficacy of basic biostatistics program given to medical faculty students and to evaluate students' biostatistics knowledge, attitude and behaviour levels. Medical Faculty students in a Turkish university participated in this study. 123 of the respondents (52.6%) were male and 111 (47.4%) were female, with an average age of 20.2 ± 1.7 years. The survey used included items questioning demographic information, biostatistics knowledge, attitude and behaviours of students and 10 multiple choice questions including the subjects learned during the program. The students filled in this survey before and after training and data obtained were evaluated. Students' positive responses to having biostatistics basic knowledge were 68.0% before training and 95.7% after training. The frequency of knowing the purpose of biostatistics was 81.5% before training and 96.6% after training. While the rate of positive response was 60.9% for population and sample, 63.2% for basic principles in summarizing data, 54.7% for central tendency-location measurements, 51.5% for variability measurements before training, they were found as 95% and higher after training. Positive responses of 70.8% for hypothesis and error types, 48.7% for statistical assumptions, 36.5% for parametric hypothesis tests, 33.0% for nonparametric hypothesis tests and 27.4% for statistical package programs before training were 93.6% and higher after training. Total score obtained from responses to multiple choice questions was 2.5±1.4 before training and 7.5±2.1 after training, which was statistically significant ($p<0.001$). In this study, biostatistics knowledge, attitudes and behaviours of medical faculty students were evaluated. Biostatistics training needs changes due to latest developments in information technology. Many medical faculties currently teach basic biostatistics concepts and carry out biostatistics training studies to allow critical evaluation during the process.

Keywords: biostatistics, statistics, education, evidence-based medicine

1. Introduction

Evidence based medicine (EBM) is the application of the best medical approach for the patient as a result of combining the best evidence gathered from studies, clinical experience and patient preferences. It is the conscious, clear and reasonable use of best evidence available while making decisions about the care of the individual patient (1).

In evidence based medicine, the clinician is faced with a large number of articles that address various problems, evaluate treatment methods and investigate the predictive value of various factors on these methods (2). Physicians need to have access to original research reports and to evaluate the design, implementation, analysis and results of each study critically in order to answer a large number of clinical questions. A certain level of expertise is required on the subject for the evaluations to be made in this process (3).

Most physicians feel comfortable while reviewing parts of a research article such as abstract, introduction, methods, results and discussion. Various sources and references provide a solid basis for evaluating the quality of a research article in terms of purpose, logic and conclusion (4, 5). For this reason, it is not difficult for a knowledgeable healthcare professional to identify whether the purpose, methodology and results are compatible with the scientific methodology. However, it is more difficult to evaluate the appropriateness of statistical analysis and to interpret the results of statistical analysis (6).

Since complicated biostatistical methods are reported in medical literature, critical evaluation of original report may be difficult for many physicians. Although there are many statistical course books and articles that may help in interpreting the validity of statistics, most of the time, these references are very detailed for individuals to comprehend these statistical concepts quickly and to apply them to statistical parts of articles (7).

Many physicians have little understanding of statistical tests and therefore they have limited ability to interpret study

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results. This has been clearly shown in studies conducted on the subject (8, 9). Similar studies have shown that even physicians who are more familiar with literature and research principles have limited biostatistics knowledge. Preliminary studies conducted on evidence based medicine have shown that basic statistical concepts are poorly understood by clinicians while evaluating medical literature (10-12). The need for competence in biostatistics results from the increasing quantification of health sciences and many researchers have recommended a better statistical training in health sciences (13, 14).

Since understanding biostatistics in health sciences has a significant effect on evidence based diagnosis and treatment practices, it is indispensable in the management of clinical practice process. Carrying out a scientific study successfully, analysing the data obtained from clinical researches correctly and evaluating complex statistical results is possible with advanced biostatistics knowledge (15, 16).

The aim of this study is to identify the biostatistics knowledge, attitude and behaviour levels of students before and after receiving basic biostatistics training provided in medical faculty, to compare these and to evaluate the efficacy of biostatistics training program.

2. Materials and Methods

2.1. Participants

The present study included medical faculty students in a Turkish university. In total, 123 of the students (52.6%) were male and 111 (47.4%) were female and the average age of the students was 20.2 ± 1.7 years. OMÜ KAEK approved the study and written informed consent was obtained from all individual participants (decision number 2019/86).

2.2. Scale and procedure

Within the scope of this study, a survey was conducted to evaluate the biostatistics training process of the students. The survey consisted of items questioning demographic information, biostatistics knowledge, attitude and behaviours of students and ten multiple choice questions including the subjects students learned during the program. The students filled in this survey before and after they received biostatistics courses and the data obtained were evaluated.

The courses on biostatistics within the four-week block in the second year of Medical Faculty are taught as 20 hours of theory and 12 hours of practice. The block also covers ethics and research techniques.

Topics including the goals of the block related with biostatistics are as follows: the definition of biostatistics and its place of use in the field of health; frequently used terms (statistics, parameter, reliability, validity, systematic error, random error, etc.); population, sample, sample and concepts of sampling error; sampling methods; grouping the data as qualitative (nominal, ordinal)- quantitative (discrete, continuous) and in four basic measurement levels (nominal, ordinal, interval and ratio); frequency table and graphical methods for qualitative and quantitative variables; measures of central tendency (arithmetic mean, median, mode, weighted mean, harmonic mean, geometric mean), measures of location (such as quartile, percentage values) and measures of variability (range of variation, mean absolute deviation, interquartile range, semi interquartile range, variance, standard deviation, standard error, variation of coefficient); the definition of hypothesis in statistics, establishing the null and alternative hypothesis, type 1 error, type 2 error, statistical significance, concepts about the power of test; normality concept in health data, distribution tables and evaluation of assumptions. One proportion-two proportions test, chi-square analyses (Pearson and continuity correction) and Fisher's exact test, parametric tests for the comparison of two independent groups (Student t and Welch tests) and non-parametric tests (Mann Whitney U, Wilcoxon RS), parametric tests for the comparison (paired groups t test) and non-parametric tests (Wilcoxon SR) of two dependent groups, one way variance analysis, Kruskal-Wallis variance analysis, multiple comparison tests, regression and correlation analyses are also other subjects taught.

Applied courses are carried out by using SPSS package program in the block. The application starts with the introduction of SPSS menu and entering a new data set to the program. Descriptive statistics, obtaining and processing graphs suitable for data, hypothesis control and hypothesis tests are carried out by using real health data. In the last part of the application, interpretation of all statistical results obtained and their presentation in a scientific article are explained to students.

2.3. Statistical analysis

Statistical analyses were performed with SPSS 21.0 for Windows (17). Data were presented as mean ± standard deviation (SD), as median (min-max) as frequence (%). The Shapiro–Wilk test was used to analyze normal distribution assumption of the quantitative outcomes. The data of two dependent groups were Wilcoxon Signed Rank test. The frequencies were compared, using the Pearson Chi-square, Continued Corrected Chi-square, Fisher Exact test and Mc-Nemar test. A p value less than 0.05 was considered as statistically significant.

3. Results

Demographic information of the students in the study was shown in Table 1. The students' grade point average for the previous year was 70.7±14.7.

Table 2 shows the pre-training biostatistics related knowledge, attitudes and perspectives of the students studying medical faculty in Turkish and English. Of the items examined, it was found that students who were studying in English answered the question "Have you been educated in Biostatistics (or Statistics) before?" with "yes" at a higher rate than the students who were studying in Turkish, statistical

difference was found between the groups (p=0.002), while no statistical difference was found in terms of the other items (p>0.05).

Table 1. Demographic information of the students

	Mean±SD (Min-Max)*	n (%)
Age	20.2±1.7 (18.0-32.0)	
Average score	70.7 ±14.7 (2.6-86.0)	
Gender		
Female		111 (47.4)
Male		123 (52.6)
Nationality		
T.C		192 (82.1)
Other		42 (17.9)
Education		
Turkish		150 (64.1)
English		84 (35.9)

*Mean± Standard Deviation (Minimum-Maximum)

Table 2. Pre-training evaluation of Turkish and English class students

	Turkish Education	English Education	p
Have you been educated in Biostatistics (or Statistics) before?			0.002
No n(%)	144(96.0)	70(83.3)	
Yes n(%)	6(4.0)	14(16.7)	
Do you consider yourself proficient about biostatistics?			0.074
No n(%)	146(98.0)	78(92.9)	
Yes n(%)	3(2.0)	6(7.1)	
Do you think that you can assess an article statistically?			0.652
No n(%)	133(88.7)	72(85.7)	
Yes n(%)	17(11.3)	12(14.3)	
In which year of medical education do you think Biostatistics education should be given?			0.330
1-3.grade range	127(87.6)	76(92.7)	
4-6.grade range	18(12.4)	6(7.3)	
Do you think that biostatistics lesson will be useful for your future career?			0.133
Disagree	20(13.3)	19(22.6)	
Undecided	48(32.0)	20(23.8)	
Agree	82(54.7)	45(53.6)	
Is Biostatistics important for you?			0.200
Disagree	28(18.7)	24(28.6)	
Undecided	57(38.0)	30(35.7)	
Agree	65(43.3)	30(35.7)	
Should Statistics literacy be one of the important goals of the education in Medicine Faculty?			0.183
	21(14.1)	18(21.4)	
Undecided	49(32.9)	31(36.9)	
Agree	79(53.0)	35(41.7)	

Evaluations of the students before and after biostatistics training are given in Table 3. For all the topics examined, the frequency of "yes" after training was found to increase when compared with before training and this increase was found to be statistically significant (p<0.001).

The answers given by students to 10 multiple choice questions asked randomly from all subjects they learned during the block were compared before and after training (Table 4). The frequency of answering all the questions correctly increased after training and this increase was found to be statistically different (p<0.001).

Table 3. Students' assessments regarding biostatistics before and after training

	Before Training		After Training		p
	No n(%)	Yes n(%)	No n(%)	Yes n(%)	
I have basic information about biostatistics	74(32.0)	157(68.0)	10(4.3)	221(95.7)	<0.001
I know the intended purposes of biostatistics	43(18.5)	190(81.5)	8(3.4)	225(96.6)	<0.001
I have information about population and sample	91(39.1)	142(60.9)	10 (4.3)	223(95.7)	<0.001
I know the basic principles in the organization and summary of data	86(36.8)	148(63.2)	11(4.7)	223(95.3)	<0.001
I have information about central tendency and location measurements and their places of use	105(45.3)	127(54.7)	10(4.3)	222(95.7)	<0.001
I know about dispersion measurements and their places of use	113(48.5)	120(51.5)	12(5.2)	221(94.8)	<0.001
I have information about the definition of hypothesis and types of error	68(29.2)	165(70.8)	12(5.2)	221(94.8)	<0.001
I have information about parametric hypothesis tests	148(63.5)	85(36.5)	13(5.6)	220(94.4)	<0.001
I have information about non-parametric hypothesis tests	156(67.0)	77(33.0)	15(6.4)	218(93.6)	<0.001
I know which assumptions should be checked for hypothesis tests	120(51.3)	114(48.7)	13(5.6)	221(94.4)	<0.001
I have information about statistical package program SPSS	170(72.6)	64(27.4)	15(6.4)	219(93.6)	<0.001

Table 4. Comparison of the answers given by students to multiple choice questions before and after training

Questions	Before Training		After Training		p
	False n(%)	True n(%)	False n(%)	True n(%)	
Which of the following is not one of the basic characteristics of a sample with the ability to represent?	132(56.4)	102(43.6)	57(24.4)	177(75.6)	<0.001
Which of the following is the value obtained by dividing a health event that occurs within a defined period of time (for example 1 year) into the midyear population under risk within that period?	200(85.5)	34(14.5)	152(65.0)	82(35.0)	<0.001
Which of the following is the simplest measurement scale?	138(59.0)	96(41.0)	48(20.5)	186(79.5)	<0.001
Which of the following is not one of the criteria showing central tendency?	124(53.0)	110(47.0)	31(13.2)	203(86.8)	<0.001
Which of the following is not one of the criteria that shows variability?	157(67.1)	77(32.9)	80(34.2)	154(65.8)	<0.001
If Ho is really correct, and if the researcher rejects this correct argument according to the value he/she calculates as a result of the statistical test conducted with the evidence gathered, how can the result be interpreted?	190(81.2)	44(18.8)	32(13.7)	202(86.3)	<0.001
Which of the following is the test method to be used to compare the averages of two independent groups with data which show normal distribution?	207(88.5)	27(11.5)	26(11.1)	208(88.9)	<0.001
Which of the following is the non-parametric equivalent of paired t test?	194(82.9)	40(17.1)	57(24.4)	177(75.6)	<0.001
The researcher wants to test the association between two-category state of smoking (yes,no) and four category educational status (primary education, high school education, undergraduate education, graduate education). Which of the following is the degree of freedom of the Chi-square statistics obtained	210(89.7)	24(10.3)	51(21.8)	183(78.2)	<0.001
Which of the following (in the SPSS menu) is used to check the normal distribution assumption of a data set?	182(77.8)	52(22.2)	59(25.2)	175(74.8)	<0.001

Fig.1 shows the comparison of the scores found by adding the correct answers given by students to multiple choice questions before and after training. Total scores before and after training were found as 2.5±1.4; 2.0 (0-6.0) and 7.5±2.1; 8.0 (0-10.0), p<0.001.

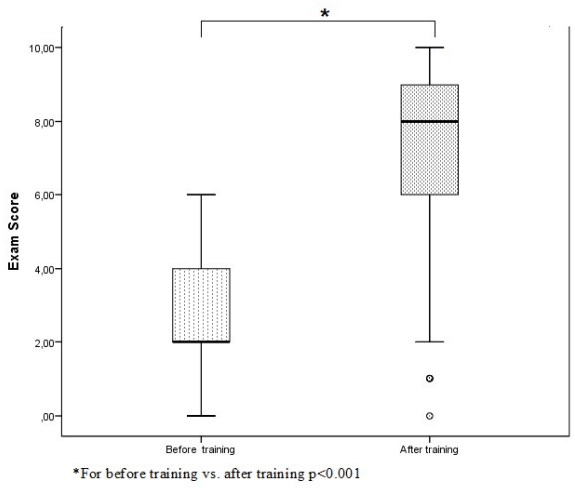

Fig. 1. Exam score comparison before and after training

4. Discussion

This study evaluated the theoretical and applied biostatistics education given to medical faculty students and examined the students' knowledge, attitude and behaviours. 47.4% of the students in the study were female and 52.6% were male; 82.1% were Turkish and 17.9% were from other nations. 64.1% of the students were studying in Turkish and 35.9% were studying in English.

Before statistics training, the students studying in Turkish and English classes were compared in terms of some subjects. 16.7% of the students studying in English and only 4% of the students studying in Turkish stated that they had received education on the subject before and this difference between the groups was statistically significant. More than 90% of the students in both groups did not consider themselves competent in biostatistics and more than 85% students stated that they would not be able to evaluate an article in terms of statistics. Most of the students in both groups preferred the training to be given in the first three years. The students in both groups responded positively to the question of whether biostatistics was important for them and for their career with a rate higher than 50% and 35%, respectively; to the question

of its importance in medical faculty education with a rate higher than 40%; to the question of importance of statistical literacy with a rate higher than 80%. There were no statistical differences between the groups in terms of the topics compared.

In a study conducted on students receiving orthodontic education, 63% of the participants agreed with the statement "I would like to gain more knowledge on biostatistics", 19.9% agreed with the statement "I understand all the statistical terms seen in journal articles" and 22% agreed with the statement "I often use statistical information to formulate decisions in orthodontic treatment" (18). In a study conducted on 277 residents from different branches on residents' understanding biostatistics results in medical literature, it was found that 75% did not understand the statistics in journal articles and 95% thought that in order to be an intelligent literature reader, it was important to understand these concepts (19).

In this study, it was found that most of the students did not consider themselves competent about biostatistics before receiving biostatistics training and they thought they could not evaluate an article statistically. It was found that although the students could not comprehend the importance of biostatistics well, they thought statistical literacy was important. In studies conducted on dentistry and medicine faculty students, similarly the participants stated that they could not understand most of the statistics in articles. However, it was found that the participants believed statistical literacy was important and they wanted to have more statistical knowledge. The common characteristic in this study and other studies is the result that students did not consider themselves competent about biostatistics and they understood the importance of statistical literacy.

All of the students were asked some questions about biostatistics before and after training. While 68.0% of the students answered the question of having basic information about statistics before training positively, this rate was found as 95.7% after training. While the frequency of knowing about the purpose of biostatistics was 81.5%, this rate was found as 96.6% after training. While the frequency of positive answers was found as 60.9% for population and sample, as 63.2% for basic principles in summarizing data, as 54.7% for central tendency-location measurements, as 51.5% for variability measurements, the rates were found as 95% and higher after training. The frequency of positive answers was found as 70.8% for hypothesis and error types, as 48.7% for statistical assumptions, as 36.5% for parametric hypothesis tests, as 33.0% for non-parametric hypothesis tests and as 27.4% for statistics package program use before training and the rates was found as 93.6% and higher after training.

In a study by Polychronopoulou et al. (18), 83.5% evaluated themselves as fairly to highly confident in interpreting p value, while 65.3% evaluated themselves as fairly to highly confident in understanding statistical methods and 78.7% evaluated themselves as fairly to highly confident in interpreting statistical analysis results in articles. In a survey study conducted on 201 clinicians in a research hospital in North Malaysia, it was found that 79.1% could interpret p value and 91.5% could interpret the statistical method used, 87.1% could identify the factors affecting the power of the study, while only 6% could evaluate the correct statistical procedure to be used in the study (20). The most regularly encountered statistical concept was inferential statistics with 63.7%, which was followed by data organization with 58.7%, correlation and dispersion with 53.7%, measures of central tendency with 45.8%, measures of dispersion with 43.3%, and measuring scales with 33.8%. In this sample, nearly 75% of the clinicians stated that they understood biostatistical results (20). In Windish's (21) study, residents rated a mean of 4.2 or greater for the curriculum helping them understand study designs, interpret p value and CI, choose a statistical test to make comparisons, interpret the results of statistical tests and assess if the correct statistical procedure was used to answer a research question. Most of the residents stated that they needed time to understand different statistical tests, 60% stated that they thought some subjects could be taught in more detail and a great majority stated that more examples would be better (21). In this study, it was found that students' levels of knowledge about the basic subjects of biostatistics increased after training and this increase was statistically significant. All these indicators show that the goals and subjects in biostatistics training program were learned and understood by students. In other studies, the rates of correct answers about topics were found to be lower or higher than the initial rates obtained in the present study. The reason for this is the fact that the participants in other studies had received biostatistics training previously. In the present study, higher correct answer rates were found after training, with the application of the survey immediately after training, the rates of remembering the outputs of the training are higher.

10 random multiple choice questions including the goals of the course program were asked to students before and after training and the students' rates of giving correct answers were compared. While the total score obtained from the answers given to multiple choice questions was 2.5±1.4 before training, it was found to increase to 7.5±2.1 after training and this increase was found to be statistically significant. With this training, the rate of correct answers to the question of sample with the ability to represent was found to increase to 75.6% from 43.6%. The frequency of correct answers to the question about basic concepts was found to increase to 35.0% from 14.5%. While the frequency of correct answers to the question about measurement level was found as 41.0% before training, it was found to increase to 79.5% after training. While the frequency of correct answers to the questions of central tendency and variability measurements were found as

47.0% and 32.9%, respectively before training; they were found as 86.8% and 65.8%, respectively after training. While the frequency of correct answers to the questions about error types, samples from parametric and non-parametric tests, Chi-square analysis and package program use were found as 18.8%, 11.5%, 17.1% and 10.3%, respectively before training; they were found as 86.3%, 88.9%, 75.6% and 78.2%, respectively after training.

In a study evaluating the biostatistics knowledge levels of postgraduate orthodontic students, the correct answer rate of the participants was found as 43.8% (18). Correct answer rates for topics were found as 44.8%, 44.0%, 37.8%, 70.0%, 62.2%, 33.0%, 42.5%, 11.8% and 40.1%, respectively for a continuous variable, a nominal variable, standard deviation, null hypothesis, parametric methods, p value, analysis of variance, chi-square test and t-test. It was found that the students' biostatistics knowledge was affected by the related trainings they received previously (18). The rates of correct answers given to multiple choice questions in a study conducted on residents in Connecticut were found as 33-44%, 50%, 30%, 59%, 57%, 58%, 26% and 47 %, respectively for variable types, standard deviation, the relationship between test power-sample size and significance level, interpretation of p value, choosing the appropriate statistical test, t test, Chi-square test and ANOVA (19). Additional advanced degrees, prior biostatistics training and enrolment in a university-based training program were factors associated with higher scores in residents. In this study, most of the residents did not have the required biostatistical knowledge to interpret many results in published clinical research. In order to successfully prepare residents for this important lifelong learning skill, the curricula of residency programs should include more effective biostatistics training şeklindeydi (19). The rate of correct answers for 20 multiple choice questions after statistical training received by 52 residents in Yale Primary Care Internal Medicine Residency Program was found as 58±16. The program included hypothesis test process, sample size, test power, p value, confidence interval, statistical significance, variable types measurement level, statistical methods and Kaplan Meiyer (21). When the answers given to questions were examined, it was found that variable types were answered correctly with a rate of over 71%, variance analysis was answered correctly with a rate of 54%, Chi-square was answered correctly with a rate of 38%, student t test was answered correctly with a rate of 71%, interpretation of p value was answered correctly with a rate of 75%, power, sample size and statistical significance was answered correctly with a rate of 40% (21). In Belgrade University Public Health postgraduate program, it was evaluated whether blended learning was a more effective method than traditional method in students' gaining biostatistical competence (22). Course program for blended learning included statistical definition, parameter, probability, normal distribution, sample and methods, statistical power, point and range estimation, confidence limit, statistical significance and statistical test, parametric and nonparametric statistics, t test, Chi-square test, correlation, regression and linear regression. The program was evaluated according to final score. Both the final statistics score (89.65 ±6.93 vs. 78.21±13.26; $p<0.001$) and knowledge test score (35.89±3.66 vs. 22.56±7.12;$p<0.001$) of the blended learning group were higher than for the on-site group (22). The mean of correct answers given to 10 multiple choice questions in the survey study conducted to evaluate pharmacists' understanding and assessing statistical information in literature was 2.8±2.0 (23). The rate of correct answers given to the questions in the survey which included the most common statistical terms and tests was 77.7% for definition of assumptions related with statistical techniques, 62.5% for statistical test characteristics, 50.8% for statistical and clinical significance, 50.8% for statistical and clinical significance, 22.9% for confidence limit, 18.2% for hypotheses, 13% for p value, 10% for student t test, 17.8% for test power, 5.1% for Chi-square and 2% for ANOVA (23). A six-stage training program was implemented on endodontic first year residents to develop an initiative curriculum in post-graduate health education (24). At the end of the curriculum, all residents were found to show competency. 36.9% pre-test correct answer rate increased to 79.8% in the post-test (24).

In this study, all of the questions had higher correct answer rates after training when compared with before training and the difference was found to be statistically significant; this result shows that training was sufficient to provide knowledge and behaviors to students in basic topics. In other studies conducted on the subject, it was found that basic biostatistics questions had different response rates. Similarly, the rate of answering the questions increased before and after biostatistics training and this was found to be statistically significant. It was also found that having received biostatistics training previously had an effect on the score.

A large number of medical faculties currently teach basic biostatistics concepts and studies are carried on biostatistics training that will allow critical evaluation during the process in medical faculty. Accreditation boards also provide practices to improve education and services to teach biostatistics better (25, 26). It is also important to know the knowledge levels of students to solve the problems that occur during biostatistics training and to plan curriculum for both undergraduate and postgraduate education (27).

As a conclusion, the basic biostatistics training program given in medical faculty was evaluated in addition to biostatistics knowledge, attitude and behaviors of students for this study. It was found that the biostatistical knowledge of students, which was limited before training, increased after training. At the same time, the importance of statistical literacy within the framework of evidence based medicine and analysis of statistical literature were shown. Biostatistics, which is widely used in clinical education for evidence based

medicine, is important to ensure the understanding of biostatistics teaching, to interpret clinical data and to evaluate research evidence. The continuity of evidence based medicine depends on clinicians' commitment to keep the latest clinical information up-to-date.

Conflict of interest

The authors declare no conflict of interest.

Funding

None.

Acknowledgments

None to declare.

Authors' contributions

Concept: LT., Design: LT., HC., Data Collection or Processing: LT., HC., Analysis or Interpretation: LT., Literature Search: LT., Writing: LT.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 628-632
doi: 10.52142/omujecm.39.3.6

Evaluation of the presence of reinfection in patients presenting to the emergency department with COVID-19 symptoms after recovery

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Received: 21.10.2021 • **Accepted/Published Online:** 12.05.2022 • **Final Version:** 30.08.2022

Abstract
This study aimed to evaluate COVID-19 reinfection in patients that presented to the emergency department with similar or different COVID-19 symptoms after recovery from the disease. This retrospective study included patients aged over 18 years presenting to the emergency department between January 1, 2021 and July 1, 2021, who had been previously diagnosed with COVID-19 and received treatment for the disease. Statistical analysis was performed using SPSS version 22.0.A total of 199 patients, 54.3% female, were included in the study. PCR positivity was present in 2.5% of the patients, and it was statistically significantly low($p<0.001$). The most common symptom was fatigue (46.2%), and the least common symptom was loss of taste (3.0%). However, there was a statistically significant correlation between the loss of taste and smell and PCR positivity ($p=0.024$ and $p=0.043$, respectively). The logistic regression analysis revealed that the loss of taste and loss of smell alone did not have an effect on PCR positivity ($p=0.848$, 95% confidence interval: 0.005-71.83 and $p=0.287$, 95% confidence interval: 0.001-9.369). In the Covid-19 management and treatment guide, it is necessary to make changes in terms of symptoms for people who have had Covid-19 disease.

Keywords: Covid-19 reinfection, Covid-19 symptoms, PCR

1. Introduction

A previously unidentified viral pneumonia was detected in Wuhan at the end of December 2019, and by January 7, 2020, researchers identified the novel virus as SARS-CoV-2 and the disease caused by this virus was named COVID-19. The disease quickly spread to other countries within a few months and turned into a pandemic. In Turkey, the first case was seen on March 11, 2020, the first peak occurred toward the end of April, and the number of cases began to decrease by the end of May. From August 30 to December 10, 2020, there was a daily increase of 100-500 cases. After December 10, the number of cases entered a downward trend. End of February 2021, cases started to increase again, reaching 40,000 by the beginning of April 2021. With the measures taken, the number of cases decreased to 6,000 by the end of May. Since then, the gradual increase in the number of cases has continued, with more than 20,000 people testing positive everyday throughout August and September 2021 (1). Determining COVID-19 symptoms is very important for effective triage and early intervention. With growing information about COVID-19, different symptoms and signs have started to facilitate the diagnosis of the disease. As in many countries around the world, in Turkey, the approach to patients has been revised in line with the pandemic conditions. Accordingly, in addition to contact history, patients have begun to be questioned in terms of complaints such as fever, history of fever, cough, shortness of breath, flu symptoms, and loss of taste and/or smell (2). However, although these findings are of guiding nature at the time of first presentation, there are no guidelines concerning whether similar approaches can be effective in patients that have already recovered from COVID-19.

In this study, we aimed to evaluate COVID-19 reinfection in patients that presented to the emergency department with similar or different COVID-19 symptoms after recovery from the disease.

2. Materials and Methods

2.1. Study design

This clinical retrospective study was carried out in the Emergency Department of Ümraniye Education and Research Hospital, which has a COVID-19 outpatient clinic. This department is a comprehensive clinic serving an average of 500,000 patients every year with green, yellow and red zones and a resuscitation unit.

2.2. Patient population

Patients aged over 18 years presenting to the emergency

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department between January 1, 2021 and July 1, 2021, who had been previously diagnosed with COVID-19 and recovered from the disease, were included in the study. Patients with a saturation value of 98% and above, respiratory rate below 30/min, and no 30-day mortality were included in the sample. Patients who previously had symptoms of COVID-19 but had a negative PCR test at that time, those who presented within less than 30 days after recovering from COVID-19, patients under 18 years of age, those without a history of COVID-19, and those with missing data were excluded.

2.3. Data collection

The patients' presentation symptoms, whether the symptoms were the same or different from the first time they had tested positive for COVID-19, severity of symptoms, medical history, hospitalization history, and whether they had been vaccinated were recorded. The polymerase chain reaction test results and three-month mortality status were also noted.

2.4. Statistical analyses

Statistical analysis was performed using SPSS version 22.0. The conformance of variables to the normal distribution was examined with visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov test). The chi-square test was conducted to evaluate the relationship between categorical data. The Mann-Whiney U test was used to compare non-parametric numerical data between two groups. If there were more than two groups, the Kruskal-Wallis test was used to compare non-parametric numerical data. A correlation analysis was performed with the Spearman correlation test, and data were evaluated separately with the binary logistic regression analysis. A p value of <0.05 was accepted as statistically significant.

2.5. Ethical considerations

Ethical approval for the study was obtained from the local clinical research ethics committee of our hospital (date: June 17, 2021, number: B.10.1.TKH.4.34.H.GP.0.01/190).

3. Results

The study included a total of 199 patients, of whom 54.3% were female. The mean age of the whole sample was 37.35±13.87 years, and the mean time from the first COVID-19 diagnosis to referral to our Covid-19 clinic by triage due to COVID-19 symptoms was 140.32±80.49 days. When evaluated according to PCR positivity, the difference was not statistically significant (p =0.681).

The most common symptom of the patients was fatigue (46.2%) and the least common symptom was loss of taste (3.0%). There was a statistically significant correlation between taste loss and PCR positivity (p=0.024). When the patients with more than one symptom were examined, no statistically significant correlation was found between the increase in the number of symptoms and PCR positivity (p=0.331). No statistical significance was found between the patients having more than one comorbidity and PCR positivity (p=0.232) (Table 1).

Of our patients, 2.5% had a positive PCR test, and this was statistically significantly lower than in our entire patient population(p<0.001). While 52.3% of our patients presented with complaints similar to the initial COVID-19infection, 12.1% had more severe symptoms (p<0.001) (Table 2). There was no statistically significant correlation between the severity of symptoms and PCR positivity (p=0.802).

Table 1. Evaluation of the relationship of demographic data, symptoms and comorbidities with second-time PCR positivity

	n (%)	P value
Age (mean ± SD)	37.35 ± 13.87	0.177
Time(day) (mean ± SD)	140.32 ± 80.49	0.681
Gender, n (%)		
Female	108 (54.3%)	0.181
Male	91 (45.7%)	
Symptom severity, n (%)		
Similar	104 (52.3%)	0.802
Milder	71 (35.7%)	
More severe	24 (12.1%)	
Symptoms, n (%)		
Fever	35 (17.6%)	0.295
Enteritis	18 (9.0%)	0.619
Cough	20 (10.1%)	0.415
Myalgia	62 (31.2%)	0.497
Loss of taste	6 (3.0%)	**0.024**
Loss of smell	7 (3.5%)	**0.043**
Difficulty breathing	9 (4.5%)	0.792
Runny nose	21 (10.6%)	0.431
Headache	22 (11.1%)	0.553
Chest pain	9 (4.5%)	0.792
Hospitalization at first infection	16 (8.0%)	0.655
Hospitalization at second infection	1 (0.5%)	0.975
Comorbidities, n (%)		
Asthma	15 (7.5%)	0.673
CAD	5 (2.5%)	0.879
COPD	2 (1.0%)	0.950
CVD	1 (0.5%)	0.975
CRF	2 (1.0%)	0.950
Hypertension	20 (10.1%)	0.585
Depression	5 (2.5%)	0.879
Malignancy	5 (2.5%)	0.879
Single-dose vaccine	36 (18.1%)	0.635
Number of symptoms	1.79 ± 0.824	0.331
Number of comorbidities	0.33 ± 0.718	0.232

Time, the time between the day with covid-19 (+) for the first time and applying to the covid-19 polyclinic for the second time; CAD, coronary artery disease; COPD, chronic obstructive pulmonary disease; CVD, cerebrovascular disease; CRF, chronic renal failure, p < 0.05

Table 2. Evaluation of PCR positivity and symptom severity in the whole sample at the time of second presentation

		n	%	p value
PCR result	Positive	5	2.5	**0.000**
	Negative	194	97.5	
Symptom	Similar	104	52.3	**0.000**
	Milder	71	35.7	
	More severe	24	12.0	

p < 0.05

The correlation analysis revealed that PCR positivity was not correlated with the time elapsed since the initial COVID-19 infection, symptom, or the increase in the number of symptoms (Table 3). In the logistic regression analysis, it was observed that the variables of loss of taste, loss of smell, and symptom severity did not have an effect on PCR positivity when evaluated independently (Table 4).

Table 3. Correlation analysis of the variables (Spearman's correlation test)

		Gender	Age	Time elapsed	Symptom severity	PCR	Number of symptoms	Number of comorbidities
Gender	Correlation coefficient	1	0.082	-0.005	0.025	0.11	-0.087	0.051
	p	.	0.249	0.948	0.722	0.12	0.222	0.477
	r	199	199	199	199	199	199	199
Age	Correlation coefficient	0.082	1	-0.028	-0.004	0.096	0.034	0.398**
	p	0.249	.	0.697	0.956	0.178	0.639	0
	r	199	199	199	199	199	199	199
Time elapsed	Correlation coefficient	-0.005	-0.028	1	0.042	-0.029	0.051	-0.071
	p	0.948	0.697	.	0.559	0.682	0.473	0.318
	r	199	199	199	199	199	199	199
Symptom severity	Correlation coefficient	0.025	-0.004	0.042	1	0.045	-0.039	0.029
	p	0.722	0.956	0.559	.	0.528	0.585	0.689
	r	199	199	199	199	199	199	199
PCR	Correlation coefficient	0.11	0.096	-0.029	0.045	1	0.069	-0.085
	p	0.12	0.178	0.682	0.528	.	0.332	0.233
	r	199	199	199	199	199	199	199
Number of symptoms	Correlation coefficient	-0.087	0.034	0.051	-0.039	0.069	1	0.118
	p	0.222	0.639	0.473	0.585	0.332	.	0.097
	r	199	199	199	199	199	199	199
Number of comorbidities	Correlation coefficient	0.051	0.398**	-0.071	0.029	0.085	0.118	1
	p	0.477	0	0.318	0.689	0.233	0.097	.
	r	199	199	199	199	199	199	199

$p < 0.05$

Table 4. Effect of investigated variables on PCR positivity at the time of second presentation (binary logistic regression analysis)

	p	*OR*	% confidence interval for OR Lower	95% confidence interval for OR Upper
Gender	0.168	0.187	0.017	2.026
Age	0.239	1.043	0.972	1.118
Time elapsed	0.923	1.001	0.989	1.013
Symptom severity (similar)	0.538	-	-	-
Symptom severity (milder)	0.285	0.231	0.016	3.387
Symptom severity (more severe)	0.337	0.256	0.016	4.135
Loss of taste	0.848	0.628	0.005	71.843
Loss of smell	0.287	0.070	0.001	9.369
Constant	0.705	0.454	-	-

$p < 0.05$, OR: odds ratio

4. Discussion

In this study, PCR positivity was found to be statistically significantly low at the second visit of patients with COVID-19-like symptoms. When we evaluated the time from the initial COVID-19 diagnosis to referral to our department by triage due to COVID-19-like symptoms according to PCR positivity, there was no statistical significance (p =0.681). Variability in symptom severity also did not affect PCR positivity when evaluated alone.

In some sources, reinfection is defined as a positive PCR test after 90 days without symptoms (3), while other sources indicate that the interval between two infection episodes should be at least one month in order to evaluate symptoms and compare PCR results in patients who have had two COVID-19 infections. In a multicenter case study, of the total 45 patients confirmed to have had two COVID-19 infections, all 12 that recovered from their first COVID-19 infection through home follow-up and experienced only mild dyspnea only presented with the mild form of the disease for the second time and only four of these patients had dyspnea at the time of the second infection. In contrast, the frequency of headache increased at the second presentation (4). In our study, the time from the first COVID-19 infection to the presentation with COVID-19-like symptoms was at least one month, and 52.3 % of our patients presented with symptoms of similar severity. Similarly, in a case series by Lechien et al. (4), reinfection was not observed in any of the patients who

had been hospitalized and had a relatively more severe form of the disease at the time of the first diagnosis.

In a study conducted in Denmark in which PCR positivity was evaluated for the second time, there was no statistically significant relationship between reinfection and gender, while the rate of reinfection was higher in the elderly population (5). In our study, there was no statistically significant relationship between gender and age and a second positive PCR test. In addition, we observed no statistically significant relationship between the presence and number of comorbidities and PCR positivity.

In Turkey, when patients refer to a hospital with different symptoms, they are referred to COVID-19 outpatient clinics by triage based on the symptoms of the disease in accordance with the guidelines of the Turkish Ministry of Health (2). In our study, loss of taste and loss of smell, which are symptoms specific to COVID-19, had a statistically significant relationship with PCR positivity for the second time, but we found that neither symptom had a significant effect on PCR positivity in the regression analysis. We also detected no statistically significant correlation between other complaints, such as flu symptoms and myalgia and PCR positivity. In a case report published by Jain et al. (6), a 21-year-old female patient with PCR positivity had the recurrence of loss of smell complaint at the end of the first-second week after her complaints had resolved. The authors performed a swab test 30 days after the first test and detected PCR positivity again.

To date, most COVID-19 reinfections have been known to be milder than the first encounter with the virus (7). In our study, the rate of patients with milder symptoms was 35.7 %, and the rate of those presenting with similar symptoms was statistically significantly higher. However, it should not be forgotten that our patients were those did not require intensive care.

In vaccine studies, it has been stated that immune response through vaccination may be stronger than immune response that occurs with COVID-19 transmission (7,8). At the time of our study, a single dose of a vaccine was being administered in the relevant patient group, and the rate of our patients that received a single dose of a vaccine was 18.1%. There was no statistically significant correlation between the second-time PCR positivity and presence/absence of vaccination. Our vaccinated patients did not receive the mRNA vaccine, but they were vaccinated with a subunit vaccine that inhibited T cell activation. It remains unclear whether vaccines that neutralize active T cells provide sustained and sterilizing immunity in the long term (3). In addition to knowing that reinfections can happen, it is also important to note that having a COVID-19 infection significantly increases antibody levels. Despite studies indicating that the antibody level decreased during the follow-up, Alter et al. showed that antibody titers were stable for four months (9).

In our study, the number of PCR positivity for the second time was quite low. In addition, at the time of the study, a single dose of vaccine was administered in our country and the number of patients who had covid was very low compared to the first applications. We aimed to reduce the PCR request in terms of cost and to reduce the density of patients with similar symptoms.

Symptoms specified in the guidelines should be re-evaluated when ordering a PCR test, and changes should be made to the PCR requests and patient referrals to COVID-19 outpatient clinics. Currently, when there are great efforts to achieve immunization against COVID-19, the rise in the number of hospital presentations will cause an increase in the risk of transmission and hospital costs. Current guidelines should be updated in light of the results of vaccination studies.

Conflict of interest

The authors declared no conflict of interest.

Funding

No funding was used for the study.

Acknowledgments

None to declare.

Authors' contributions

Concept: A.H.S., K.T.K.., Design: A.H.S., Ö.S., Data Collection or Processing: A.H.S., K.T.K., Ö.S., Analysis or Interpretation: K.T.K., Ö.S., Literature Search: A.H.S., Ö.S., Writing: A.H.S., K.T.K., Ö.S.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 633-636
doi: 10.52142/omujecm.39.3.7

A study comparing intelligence scores of patients of hypermetropia and myopia in children

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Received: 29.11.2021 • **Accepted/Published Online:** 05.05.2022 • **Final Version:** 30.08.2022

Abstract

This study aimed to evaluate the association between intelligence and refractive error in children. Children with refractive error were consecutively enrolled from the outpatient eye disease clinic at the blinded for peer review. Seventy-six Turkish children were thirty-two female and Forty-four male, aged 6–16 years, participated in this study. Wechsler Intelligence Scale for Children Revised was used for Intelligence quotient. Full Intelligence quotient score and verbal Intelligence quotient, picture completion, similarities comprehension, digit span subgroups in children with myopia were higher than that of hypermetropia group ($p<0.05$). Present study indicated cognitive ability in children with myopia is higher than that of hypermetropia children. The mechanisms of high cognitive ability in children with myopia should be described in further studies.

Keywords: intelligence, children, hypermetropia, myopia

1. Introduction

Vision problems in childhood are frequently seen (1). Myopia, is a kind of vision impairment, is a defect where by rays of light from a distant object come to focus in front of the retina rather than on it and it resulting in the observer seeing a blurred image (2). Also, it is known that, in hypermetropia the light falls behind the retina because of a short eye or insufficiently curved cornea and it is difficulty seeing close objects clearly (2).

Myopia and hypermetropia are seen more frequently than other visual impairments and the prevalence is increasing globally.(3). In Turkey, a study done in year of 2007, it was founded that about one-third of Turkish medical students had myopia (4), while a met-analyse showed that close to half of the world's population may be myopia by 2050 (5).

There are many literatures about the reasons of myopia. The risk factors like environmental factors such as time spent outdoors (6), near work, educational level and parental history, a possible indicator of genetic susceptibility, are most often investigated (7, 8). In another study, it was revealed factors associated with reading may play a part in myopia development (9).

Association between intelligence with visual function in children is being investigated frequently in the recent period (9–15). A review concerning the relation between refractive errors and intelligence quotient (IQ) concluded that children with myopia may have a higher IQ probably identified by genetic and environmental factors (16). Large eyes (as measured by axial length) may lead to myopia and large brains may be more intelligent. This relation may arise because of a single genetically controlled mechanism affecting both brain size and eye size. It was found that no statistically appreciable difference in measured intelligence, thus suggesting that the myopia and high intelligence may be more related to environment than any other factor (17). At the same time, there is uncertainty about whether IQ is associated with myopia; some authors say that near-work activity result children perform better in IQ tests (1). Additionally, in a study which determined whether myopia is associated with cognitive function, it was founded that cognitive function, especially verbal intelligence, was strongly and consistently associated with myopia among adolescents (18). Further, some studies suggested that the most frequent refractive error was mixed astigmatism and followed by hypermetropia, in children with intellectual disabilities (8).

Because of myopia is a leading cause of visual impairment worldwide, we aimed to investigate whether the intelligence test based on the type of visual deficits in children with visual impairment in this study.

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2. Materials and Methods

The study was designed case-control and cross sectional. Children with refractive error were consecutively enrolled from the outpatient Eye Diseases clinic at the blinded for peer review. Seventy-six Turkish children which thirty-two females and forty-four males, aged 6–16 years, participated in this study. All children underwent a detailed ocular examination including ocular history, ocular motility assessment, external inspection of the eyes and lids, vision assessment, fundus and pupil examination. Exclusion criteria's for the study are presence of motor deficits, poor head control, anti-convulsive treatment, previous eye operations and speech problems.

Wechsler Intelligence Scale for Children—Revised (WISC-R) was used for Intelligence quotient (IQ). The WISC-R is an intelligence test prepared by David Wescler in 1939. The test, first appeared for adults, has been redesigned for children. The WISC-R test is suitable for children aged 6 to 16 years. This individually administered test takes 1-1.5 hours. The WISC-R test is divided into two main sections; the Verbal and Performance Sections, each with six subtests (19). The Verbal Section assesses Information, Similarities, Comprehension, Arithmetic, Digit Span and Vocabulary. We used five verbal subtests of the WISC-R: Information, Similarities, Comprehension, Arithmetic and Digit Span. The Performance Scale measures are Picture Arrangement, Picture Completion, Object Assembly, Block Design, Coding and Labyrinths. The WISC-R by Savasir et al was translated and standardized in Turkey in 1997 (20).

We used five efficiency tests of the WISCR: Picture Completion, Arrangement, Block Design, Object Assembly and Coding. The Verbal IQ and Performance IQ acquired from the test are the brief measures of verbal and efficiency skills, and the Full Scale IQ, based on the 10 tests included in the Verbal IQ and Performance IQ scales, is a general measure of intellectual functioning.

Protocol adhered to the tenets of the Declaration of Helsinki and all patients provided written informed consent. This study was approved by the local ethics committee and written informed consent was obtained from the parents of subjects.

2.1. Statistical analysis

Statistical analysis was performed using SPSS software (version 18, SPSS Inc). All clinical variables were evaluated as mean±SD. The categorical variables with the x^2 test and continuous data with student *t* and Mann-Whitney tests were compared. The proportional contribution of confounding factors to IQ to determine was performed multiple stepwise linear regression tests. *p* -value < 0.05 is considered to be statistically significant.

3. Results

Table 1 shows characteristics of the myopia and hypermetropia groups. Subtest scores and composite scores of the myopia and hypermetropia groups are seen in Table 2. Children with myopia had a higher IQ score compared to hypermetropia group at all scales significantly (p =0.03).

Table 1. Characteristics of the myopia and hypermetropia group

Characteristic	Myopia N=20	Hypermetropia N=56	P value
Age	9.0±2.4	9.2±2.5	0.7
Gender (f/m)	11/9	21/35	0.1
Education	1.1±0.5	1.2±0.6	0.4

Table 2. Subtest scores and composite scores of the myopia and hypermetropia group

WISC-R Scores	Myopia N=20	Hypermtropia N=56	*p*
Full Scale IQ	98.4±20.3	86.1±26.4	**0.03**
Verbal IQ	99.6±16.4	89.5±19.7	**0.03**
Performance IQ	48.0±11.3	43.0±14.1	0.10
Similarities	12.0 ± 4.9	9.0 ± 4.6	**0.01**
Information	9.5 ± 3.0	8.3 ± 3.6	0.10
Digit Span	10.6± 2.6	9.1 ± 3.0	**0.04**
Arithmetic	10.1 ± 2.6	9.5 ± 3.8	0.50
Comprehension	8.3 ± 3.0	6.6 ± 3.1	**0.04**
Picture completion	10.3± 3.8	8.1 ± 3.3	**0.01**
Picture arrangement	8.2 ± 3.7	8.1 ± 4.4	0.90
Block design	9.7 ± 3.9	9.0 ± 5.2	0.60
Object assembly	10.4 ± 1.8	9.7 ± 3.3	0.30
Coding	9.3 ± 3.5	8.4 ± 3.2	0.30

Ten subtests scores: each with M=10, SD=3, Three composite scores: verbal, performance, full scale, each with M=100, SD=15

Verbal IQ, similarities, digit span, picture completion and comprehension scores were higher in myopia group compared to hypermetropia group, too. (respectively; p=0.03, p=0.01, p=0.04, p=0.01, p=0.04).

Ten subtests scores: each with M=10, SD=3, Three composite scores: verbal, performance, full scale, each with M=100, SD=15

Table 3 reveals significant relationship between cognitive ability, measured by Full Scale IQ score as dependent variable and myopic group, age, gender as independent variables in multivariate model.

Table 3. Relationship between cognitive ability as dependent variable and age, gender as independent variables in only myopic children in multivariate model

Multivariate model	Full Scale IQ score	
	β	*p* value
	R^2=0.33	
Intercept		**0.0001**
Age	-0.53	**0.0001**
Gender(male)	-0.12	0.2
Education	0.12	0.5
Myopic group	0.19	**0.04**

*β is the standardized regression coefficient

4. Discussion

According to literature, there have been many inherited and

acquired factors affecting intelligence scores (21). Some studies relieved the relationship between reading and IQ scores; anymore reading books may cause pathological eye growth and myopic refractive error. There was a positive correlation between academic success and myopia as independent of relation with reading book and IQ test scores in another study by Saw et al (12). Like this, there are some finding about the association higher educational achievements more often were myopic than individuals with less education (22). Saw et all postulated that myopic children, with their cumbersome glasses, may be less likely to play sports outdoors and more likely to spend time on their school studies, thus attaining their full 'potential' in educational and IQ tests (7). So, it is thought that for the development of myopia, near work, level of education, reading book has been considered environmental risk factors. In our study, there was no difference in educational levels between the two groups. A cohort study of the population-based Gutenberg Health Study revealed that cognitive performance is linked to myopia, too. However, this study suggests that education level is more directly and strongly related to myopia than to cognitive performance. Cognitive ability has been interpreted as being related to myopia with its effect on education level firstly (23). In this respect, the confusing effect of the educational level must be considered.

Performance component of IQ test, such as personality, intelligence, and discipline may be related with myopia as inherited. The children's IQ is important risk factors for incident myopia (11). Williams et al indicated that shared genetic factors contribute significantly to the covariance between myopia and intelligence (24). In a study in school population, myopic students had higher score on IQ tests than non-myopias (10). Another study which done with twins was published in 2015 and revealed that evidence for genetic pleiotropy between IQ and myopia. And they found that a higher IQ polygenic risk scores were associated with a lower spherical equivalent (25). As Verma at al. reviewed with analysed studies that there may be a positive association between myopia and high intelligence. While the mechanism of the link between these two phenomena is not clearly understood and is confounded by a number of factors, there is evidence to suggest that both environmental and genetic factors may contribute to this relationship (26). In a study which done with 3 to 7-year-old Strabismic and Nonstrabismic Children in an Iranian Population, did not found a significant negative interference of strabismus on IQ score of preschool children (27). Considering all these studies, there seems to be a common genetic basis of between intelligence and myopia, in this respect, further studies on genetic regions are needed.

Therefore, we have thought that the developmental genetic foundations of the relation between intelligence and myopia must be investigated and the effects of the environmental factors must be revealed.

Another study compared a group of 14-year-old myopic children with hypermetropia children of the same age and reported that myopic students were more intelligent than that their poors of the same age (13). And there are some studies suggesting that hypermetropia is probably disadvantageous when performing a intelligence test and that it has lower scores as it cannot adapt to the test (28). In our study, a significant difference was found between these two groups and myopic group's intelligence scores were higher than hypermetropic group; cognitive ability in children with myopia is higher than that of hypermetropic children. In this respect, it is one of the rare studies comparing myopic and hypermetropic groups in terms of intelligence scores.

The mechanisms of high cognitive ability in children with myopia should be studied in further studies both environmental and genetic factors. Because of our study is cross sectional, causality must be revealed by follow-up studies. By increasing the number of the participants, it will be possible to obtain more reliable results with repetitive studies. Thus, it is possible to understand the factors affecting the intelligence score and its relationship with visual function.

Conflict of interest

The authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript. This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue.

Funding

No funding was used for the study.

Acknowledgments

None to declare.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 637-640
doi: 10.52142/omujecm.39.3.8

Efficacy and safety of platform myocardial stabilizer for off-pump coronary artery bypass grafting

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Received: 01.11.2021 • **Accepted/Published Online:** 24.06.2022 • **Final Version:** 30.08.2022

Abstract

It is important to have methods and devices for stabilizing the beating heart that are capable of maintaining atraumatic interaction with the surface of the beating heart over a wider range of conditions and orientations. Therefore, the development of simple and safe surgical methods that provide a stable and bloodless coronary anastomotic field is important for the successful coronary artery bypass grafting with a beating heart. In this article, we have tried to show the safety of our stabilizer in experimental operations.

Keywords: off-pump coronary artery grafting, CABG, OPCAB, myocardial, stabilizer

1. Introduction

In the 50 years since this landmark publication, coronary artery bypass grafting (CABG) has been enthusiastically developed by cardiac surgeons around the world. Currently, most coronary artery bypass grafting (CABG) procedures are performed using cardiopulmonary bypass (CPB). The use of CPB for CABG operations allows performing anastomosis of the coronary artery in a stable bloodless surgical field with myocardial protection, providing excellent long-term results (1).

The development of the off-pump coronary artery bypass grafting technique became possible with the development of technical aspects proposed by Lima et al. In the 1990s, the technique using a series of pericardial retraction sutures allowed access to the arteries of the lateral surface of the heart (2). Later, methods for improved visualization of the lateral wall were described using a traction suture in the oblique sinus of the pericardium (3). Further improvement in the technique of beating heart surgery is associated with Grundeman and Grunenfelder (4-5), who improved the OPCAB operation by applying vacuum fixation technologies to expose and stabilize coronary vessels. They studied spatial motion and the biological effects of stabilized absorption in an experimental laboratory and proved the superior stabilization of this method.

The ability to stabilize or immobilize the surgical site can significantly improve surgical accuracy and reduce the time required to complete a specific procedure (6). Methods and device for performing CABG on a beating heart are first described in the publications of Benetti et al. (7). In some cases, devices that provide mechanical stabilization of the myocardium by compression encounter difficulties in providing mechanical pressure on the myocardial surface. Similarly, devices that use a vacuum (vacuum stabilizers) have great difficulty in creating and maintaining effective "suction" to the moving surface of the heart (8-9). Even when the beating heart has been effectively stabilized, the target coronary artery may be covered by layers of fat or other tissue, making it very difficult for the surgeon to visualize it. Moreover, the stabilizing devices can lead to the deformation of the tissue surrounding the coronary artery, or the coronary artery itself, so that the arteriotomy remains in an unfavorable position for anastomosis.

It is important to have methods and devices for stabilizing the beating heart that are capable of maintaining atraumatic interaction with the surface of the beating heart over a wider range of conditions and orientations. Therefore, the development of simple and safe surgical methods that provide a stable and bloodless coronary anastomotic field is important for the successful CABG with a beating heart. We aimed to evaluate the efficacy and safety of newly developed myocardial stabilizer in an experimental model in dogs..

2. Material and Methods

The cardiac surgery team has developed a platform-type myocardial stabilizer. The myocardial stabilizer contains a movable handle (1) with an attachment point (2) on the retractor, a rigid fastening frame (3) connected to the handle

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with an opening framing the operating field. On one side of the frame there is a hinge joint (4) for fastening the frame (3) with the handle (1) of the stabilizer. The mounting frame (3) consists of two parts, connected to each other by means of rods (5), in each of the frame parts there are two holes (6). Nylon threads are passed through these holes for a more rigid fixation of the frame parts. On the attachment point (2) there is a valve (7) for rigid fixation of the stabilizer on the retractor (Fig. 1.).

The mounting frame is square with a side length of 4 cm. This size fits easily into the incision in the patient's chest, providing good stability and visibility during surgery. The device is completely reusable, all parts are made of medical steel (ferrite-chromium), which allows them to be subjected to repeated sterilization. The device provides the required local stabilization and, due to its simplicity and the collapsible design of the mounting frame, allows the surgeon to quickly adjust and remove the stabilizing device.

This experimental study was performed in Experimental Department of Republican Research Center of Emergency Medicine (Tashkent. Uzbekistan), under the permission of the Republican Committee of Ethics (protocol №2/1-1496) during 2021. All animals were provided with humane care in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC), UK National Medical Research Society's Guidelines for the Care and Use of Laboratory Animals and the Canadian Council on Animal Care (Association of Universities and Colleges of Canada) (10-11). Four mongrel dogs (12 to 20 kg) were included in this pilot study. Anesthetic management was provided with ketamine (20 mg / kg). (Fig. 2).

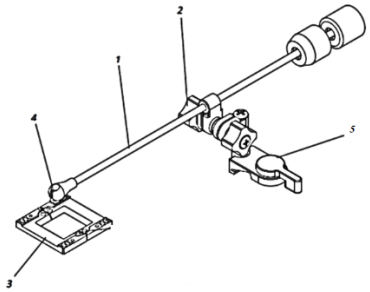

Fig. 1. Detailed view of the mechanical stabilizer: 1 - handle; 2- attachment point; 3- stabilizing frame; 4 - articulated joint; 5 - locking handle

The animals were ventilated with 100% oxygen at a rate of 0.5 l / s. Neuromuscular blockers were not used in this study. Then the animals were prepared for hemodynamic monitoring. Heart rate was measured using a continuous electrocardiographic monitor (model 90903A; SpaceLabs Inc, Redmond, Washington). Arterial oxygen saturation was measured with a sensor (Pulse-Oxymeter; Criticare Systems Inc, Redmond, WA) attached to the tongue.

Then a small anterior thoracotomy on the left side (8 cm) was performed in the fifth intercostal space, followed by complete mobilization of LIMA under direct visual control. The pericardium opposite to the LAD was fixed with silk sutures to the chest wall. At all stages of experimental interventions, hemodynamic parameters were continuously recorded. The LIMA - LAD anastomosis was performed according to the standard technique using the newly developed stabilizer using 8-0 Prolene sutures (Ethicon, Somerville, NJ). After making sure of adequate hemostasis and hemodynamic stability, the experimental intervention was completed in a standard manner, the pericardium was left open, the pleural cavity was drained with silicone drainage, followed by connection to a vacuum aspiration system.

Fig. 2. Intraoperative photos describing the experimental technique and monitoring of experimental animals

3. Results

All animals survived the procedure and maintained stable hemodynamic parameters both during and after manipulation (Table 1).

The use of the stabilizer did not adversely affect heart rate, cardiac output, or arterial oxygen saturation (Fig. 3).

As can be seen from the above graph, hemodynamic parameters and arterial blood saturation did not undergo any changes in dynamics, which allows us to conclude about the safety and effectiveness of the used myocardial stabilizer. Complications and deaths after the performed operations were not observed in experimental animals.

Table 1. Intraoperative parameters of experimental animals

Parameters	Before installing the stabilizer	After installing the stabilizer	p
Blood pressure	81.2±3.5	79.8±4.1	0.1
HR	178.9±12.8	182.6±11.2	0.1
Saturation	96.5±3.2	95.5±4.1	0.1
ST segment	N	N	0.1

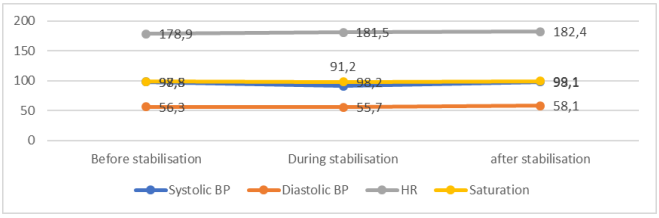

Fig. 3. Changes in key hemodynamic parameters before, during and after coronary artery bypass grafting using a myocardial stabilizer (BP - blood pressure; HR - heart rate)

4. Discussion

The myocardial stabilizer developed by our team allows performing coronary artery bypass grafting on a beating heart. No undesirable effects on any of the measured hemodynamic parameters were observed when using this stabilizer. Despite the small diameter of the coronary arteries in dogs and a high heart rate, coronary artery bypass grafting was successful and was not accompanied by complications and mortality in the experimental part of the work.

Several difculties, however, have limited the use of the dog for such a purpose—mainly the high infection rate resulting from incision and uncontrolled tachycardia with the slightest manipulation of the heart, and even the risk of short periods of myocardial ischaemia.

Recent studies confirm that OPCAB should be considered technically more challenging than conventional CABG. To ensure optimal conditions for surgical intervention, three fundamental rules must be followed: the surgical field must be well open and accessible, stable and bloodless. During OPCAB, cardiac surgeons voluntarily violate these rules. However, to date, none of the available mechanical stabilization devices has been able to achieve a stable bloodless field comparable to the surgical field achieved with cardioplegic cardiac arrest and cardiopulmonary bypass. Moreover, a number of surgeons express doubts about the quality of coronary anastomoses in interventions on a beating heart. There is still no completely clear evidence. In 2001, Puskas et al. (12) reported an impressive 98% OPCAB patency rate at hospital discharge, while more recently Khan et al. (13) reported the results of a prospective randomized study with a graft patency rate at three months of 98% for patients operated on -pump, compared with 88% for patients operated off-pump (P = 0.002). The learning curve appears to play an important role in OPCAB, and many series have been published showing higher patency rates and lower incidence of unsatisfactory anastomoses as the surgeon grows in experience (14-15).

The movement of the heart occurs in three dimensions of space and can be described as a smoothly varying combination of sinusoidal waves, we found only a few publications in which the movement of the surface of the heart is quantified (16). In a pig model, Borst et al. (17) calculated the two-dimensional area (x and y axes) covered by the control point on the epicardium during free heartbeat and after the installation of a mechanical vacuum stabilizer, showing a significant reduction in movement (from 73 ± 43 mm2 to 1.3 ± 0.5 mm2) at stabilization of the heart. Koransky et al., (18) analyzed the three-dimensional movement of the coronary artery LAD in pigs using sonomicrometry techniques. Movement and speed were analyzed alternately in the x, y and z planes using triangulation theory before and after placement of the vacuum stabilizer. Stabilization led to a significant decrease in travel (11.36 ± 1.74 versus 5.99 ± 1.30 mm; $p < 0.05$), maximum Cartesian speed (141.80 ± 29.73 versus 86.55 ± 29.45 mm / s; $p < 0.05$) and the average Cartesian LAD velocity (44.30 ± 7.02 versus 21.46 ± 4.54 mm / s; $p < 0.05$).

In conclusion, it can be noted that the myocardial stabilizer developed by heart team of Republican Research Center of Emergency Medicine provides excellent stabilization of the target segments of the coronary artery and facilitates vascular anastomosis on the beating heart.

Limitations of this study are that this is an experimental study, which was conducted on a small amount of experimental animals.

Conflict of interest

Authors declared no conflict of interest.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Acknowledgments

This experimental study was performed in Experimental Department of Republican Research Center of Emergency Medicine (Tashkent. Uzbekistan), under the permission of the Republican Committee of Ethics (protocol no: 2/1-1496) during 2021.

Authors' contributions

Concept: A.A.A., Design:A.A.A., Data Collection or Processing: O.M.A., Analysis or Interpretation: A.A.A., Literature Search: O.M.A., Writing: A.A.A.

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Journal of Experimental and Clinical Medicine
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Research Article

J Exp Clin Med
2022; 39(3): 641-644
doi: 10.52142/omujecm.39.3.9

Effects of repeated use of disposable FURS on efficiency, safety and cost analysis

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Received: 03.11.2021 • **Accepted/Published Online:** 17.07.2022 • **Final Version:** 30.08.2022

Abstract

In this study, we aimed to examine cost analysis and evaluate the results of retrograde intrarenal surgery (RIRS) performed with reusable and disposable flexible ureteroscope (FURS) devices in our clinic. In total, we included 96 patients who underwent RIRS surgery for kidney stones with reusable FURS (n = 49) and disposable FURS (n = 47). There were noted preoperative demographic data and stone characteristics of the patients. Also, operation time, fluoroscopy time, hospitalization time, urethral catheter removal time, return to work time, complication rates and cost per procedure were collected and analyzed. There were no significant differences between each groups in terms of demographic characteristics of retrospectively analyzed the data of 96 patients. In addition, the comparison made in terms of operative and postoperative results in both groups was similar. We determined significant difference for mean cost per case compared two groups. There were cost per case $ 293,87 in group 1 and $ 191,48 in group 2. We determined that the repeated use of disposable FURS compared in terms of cost analysis is a safe, effective and low cost method without increasing the infection frequency.

Keywords: Disposable FURS, reusable FURS, cost analysis, repeated use

1. Introduction

Flexible Ureteroscopy (F-URS) is minimal invasive surgical treatment of kidney stone which provides minimal complications and early return to daily life. But it has seem like a disadvantage with its high initial purchase cost. In the recent years, treatment success with the development of disposable F-URSs has been comparable to reusable F-URS results (1, 2, 3).

Some studies have shown that disposable F-URS performs stone free rate (SFR), operative time, and complication rate by high maneuverability and image quality comparable to existing reusable F-URS. The cost of surgery began to raise day by day depend on using high technological materials (4, 5).

We planned to make a comparison between disposable F-URS and reusable F-URS in terms of cost analysis, efficacy and safety in the treatment of kidney stone. In addition, we aimed to investigate the effect of re-use of single-use F-URS with proper cleaning and sterilization after use on treatment costs. To the best of our knowledge, the study presented here is the first attempt to compare reusable F-URS by repeated use of disposable F-URS.

2. Material and Methods

The data of 96 patients who underwent retrograde intrarenal surgery (RIRS) using reusable and disposable F-URS for kidney stones were retrospectively evaluated. Patients between the ages of 19-71 and without comorbidities were included in the study. Exclusion criteria were patients in whom concurrent ureteral stones, kidney anomalies and residual stone after procedure. We formed two groups. Group 1 consisted of 49 patients were operated with reusable F-URS, group 2 consisted of 47 patients were operated with disposable F-URS. The study was approved by the research ethics committee at the institution where the study was conducted.

All operations were performed under general anesthesia and in the dorsal lithotomy position. The ureter was examined endoscopically by entering through the ureteral orifice with a guidewire with a semi-rigid URS (Ultrathin Ureterorenoscope 4.5/6.5 Fr, Richard Wolf GmBH, Knittlingen, Germany) and possible ureteral pathologies were ruled out. There were used 7,95 F superslim reusable F-URS (URF-P7, Olympus, USA) in group 1 and 8.7 F disposable AnQing EU-scope (Anqing medical, China) in group 2. Also, a total of 5 disposable F-URS devices were used to complete the operations of 49 patients in group 2. Stone fragmentation was performed using a 272 micron holmium yag laser probe in the energy range of 1.0–1.5 J and 5-10 Hz by dusting method. A 10.7 F ureteral access sheath (Cook®, Bloomington, IN, USA) was placed in patients with stones of 15 mm and above and in cases where we predicted that the procedure time could be prolonged. The access sheath was successfully attached to all patients we

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planned. Routine antibiotic (cefuroxime) was used for 3 days after discharge for each patient. There were noted preoperative demographic data of the patients and standard preoperative investigation protocol that included stone side, stone diameter and Hounsfield unit (HU). Also, operation time, fluoroscopy time, hospitalization time, urethral catheter removal time, return to work time, complication rates and cost analyzes were collected and analyzed.

2.1. Statistical analysis

Statistical analyses were performed with SPSS version 18. 0 and data were displayed as mean±standard deviation (SD) (range). Wilcoxon Rank Test and independent sample t-test were used for statistical comparisons. A 5% level of significance was used for all statistical testing. A P-value<0.05 was considered significant.

3. Results

There were retrospectively analyzed the data of 96 patients. Mean age of the patients was 45.4±13.1(21-70) years in group 1and 44.6±12.8(19-71) in group 2. There were no significant differences between the two groups in terms of demographic characteristics, age, gender, height, weight, stone characteristics and Hounsfield Unit of the patients (Table 1).

Table 1. Demographic and preoperative parameters of the patients

	Reusable f-URS (n=49) (Group 1)	Disposable f-URS (n=47) (Group 2)
Age(years)	45.4 ±13.1 (21-70)	44.6 ±12.8 (19-71)
Sex (M/F)	26/23	23/24
Height (cm)	165.3±9.2 (152-182)	166.2 ±9.4(151-186)
Weight (kg)	73.7±6.4 (58-96)	74.6±7.8 (54-94)
R/L kidney	27/22	24/23
Stone diameter mm	15.6±4.9 (10-20)	15.4±4.9 (10-20)
Hounsfield unit	884.3±195.7	Hounsfield unit

Our mean operation time were 43±14.3(20-68) minutes in group 1 and 43.5±13.9(20-65) in group 2 respectively. Mean flouroscopy time were 2.9±1.7(0-9) seconds n group 1 and 2.8±1.7(0-8) in group 2. Ureteral access sheath were used in 15 (30%) patients in group 1 and in 16 (34%) patients in group 2. Mean hospitalization period of patients were 16.3±6 (6-24) hours in group 1 and 16.9±6.6(6-24) hours in group 2. Mean return to work time were 71.5±17.9(48-96) hours in group 1 and 72.5±13.6 (48-96) hours in group 2. According the data of the patients, it was observed that there was no statistically significant difference between the two groups in terms of operation time, fluoroscopy time, using ureteral access sheath, hospitalization time and return to work time. (Table 2) The urethral catheter was removed before all of patients were discharged. None of patients were required re-hospitalization. In both groups, urinary tract infection was detected in 3 (6%) patients in the postoperative period and using non-routine antibiotic was required. There were not detected complications such as acute renal injury, subcapsular hematoma, and stent migration any patient in both groups. We determined significant difference for mean cost per case compared two groups. There were cost per case $ 293,87 in group 1 and $ 191,48 in group 2.

Table 2. Comparison of operative, postoperative parameters and cost analysis of RIRS (reusable F-URS and disposable F-URS)

	Reusable f-URS (n=49)	Disposable f-URS (n=47) (Group 2)
Operation time (min)	43±14.3 (20-68)	43.5±13.9 (20-65)
Fluoroscopy time (sec)	2.9±1.7 (0-9)	2.8±1.7 (0-8)
Using ureteral access sheath	15 (30%)	16 (34%)
Hospitalization (h)	16.3±6 (6-24)	16.9±6.6 (6-24)
Return to work (h)	71.5±17.9 (48-96)	72.5±13.6 (48-96)
UTI n (%)	3 (%6)	3 (%6)
Subcapsular hematoma	0	0
Acute renal injury	0	0
Stent migration	0	0
Using non-routine antibiotic	%6 (3)	%6 (3)
Using non-routine analgesic	%12 (6)	%10 (5)
Initial purchase cost	$ 14.400	$ 1.800
Mean cost per case	$ 293,87	$191,48

4. Discussion

F-URS provides lots of advantages to treatment kidney stone. it has been possible to achieve higher success with the advancing technology in kidney stone treatment, and lower complication rates. In addition, operation times have been shortened thanks to the improvement in image quality (6, 7,8). In recent studies, F-URS procedure has begun to be recommended even for kidney stones larger than 2 cm, and higher success rates have been shown (2, 9). However, initial purchase cost, maintenance cost, performance degradation, and poor durability have been limitations of reusable ureteroscopes and led to the development of disposable ureteroscopes (10).

Initial purchase price and costly repairs are limitations on reusable F-URS and these costs show both local and international variations (1,11). In a recent review, purchasing costs were reported to range from $ 13.611 to $ 85.000 for reusable F-URS and $ 800 to $ 3.180 for disposable F-URS, depending on the country and device brand (1). The purchasing costs of the devices we used in our study were $ 14.400 for reusable F-URS and $ 1.800 for disposable F-URS.

The factors determining the durability of the instruments were shown as surgeon experience, repeated instrument passage through the working channel, laser activation in the canal, and increased operation time (12, 13). In a study conducted on the average number of uses until the need for repair, it was shown that a resuable digital ureteroscope was

used 21 times on average, while a resuable fiberoptic ureteroscope was used 6-15 times (14). However, we think that the current numbers are higher due to the increasing experience and the effect of developing devices during the time passed over this study. The study, reusable F-URS using in group 1 which was completed all cases without the need for repair. While a total of 5 disposable F-URS was used in Group 2, 10 cases were made with an average of 1 device. There was no deterioration in the disposable devices. Counter allowing 10 hours of use which set by the manufacturer caused us to change the device. When disposable F-URSs were first released, there was no counter program limiting their hourly use. Later, we think that these counters were added due to financial concerns. It is known that different brands have different durations were known. The disposable F-URS we used had a 10 hours' limit.

The average repair cost per case has been shown to range between $ 355 and $ 511 (15). In another study, the average total cost per case was calculated to be $ 2.799 for reusable F-URS and $ 2.852 for disposable F-URS. Also, the total operating room time was found to be shorter in the disposable F-URS group and it was stated that the time was effective on the cost (16). In our study, no damage was found for the devices used in both groups, and there was no repair cost. Our average total costs per case were calculated $ 293,87 for group 1 and $ 191,48 for group 2. We also found that the total operation times were similar in both groups, so the cost was not affected in this respect in our study.

Legemate, et al. stated in their study that the use of cumulative ureteroscope was not associated with higher microbial contamination and positive urine culture (17). The study, urinary tract infection was detected in 3 (6%) patients in both groups and the patients were treated on an outpatient basis. When considering possible device-related infection, there was no difference between two groups. We think that disposable devices can be reused with appropriate sterilization as indicated by the companies. Considering postoperative complications such as the need for reoperation or re-hospitalization that may affect the cost, this situation was not detected in both groups.

Our study has several limitations. We evaluated our results retrospectively. Other equipment such as laser, working equipment, light sources, video tower and supplies as ureteral access catheter and jj stent were not calculated. But, we primarily aimed to make a comparison between disposable F-URS and reusable F-URS in terms of cost analysis.

In developing countries like ours, surgery cost analysis is an important element, and it is important to reuse instruments with appropriate sterilization without harming patients as much as possible.

In terms of efficiency and safety, reusable F-URS and re-use of disposable F-URS with proper cleaning and sterilization show similar success results. When we compare it in terms of cost analysis, it has been seen that repeated use of disposable F-URS significantly reduces the cost without increasing the frequency of complications.

Conflict of interest

The authors declared no conflict of interest.

Funding

No funding was used for the study.

Acknowledgments

None to declare.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 645-648
doi: 10.52142/omujecm.39.3.10

The location of the mandibular foramen as a guide in mandibular block anesthesia in children by age: A radiographic analysis

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Received: 10.11.2021 • **Accepted/Published Online:** 05.05.2021 • **Final Version:** 30.08.2022

Abstract

Knowing the correct location of the mandibular foramen (MF) is a significant factor in the success of inferior alveolar nerve block (IANB). The primary clinical guide for this anesthesia in children with continued growth and development may be the location of MF. The present study was carried out retrospectively on panoramic radiographs of children aged 5-14 years. Three linear measurements were performed on panoramic radiographs to evaluate the distance of the MF to the occlusal plane (OP), anterior border of the ramus (ABR), and mandibular base (MB). The participants' ages were divided into five groups: (G1) 5-6 years, (G2) 7-8 years, (G3) 9-10 years, (G4) 11-12 years, and (G5) 13-14 years. The results revealed a statistically significant increase in the mean values from G2 to G4 in all measurements. However, it was determined that there were no statistically significant differences between G1 and G2 and between G4 and G5 by the mean measurement values, respectively. Besides, the results of the measurements did not significantly differ by sex. Overall, it was concluded that MF was slightly below OP under 8 years of age and moves in the poster superior direction until the permanent dentition period. This result may guide clinicians in IANB in children.

Keywords: mandibular foramen, panoramic radiography, children, inferior alveolar nerve, local anesthesia

1. Introduction

Pain management is a far-reaching consideration in behavioral management in pediatric dental care. The first method in pain management is still local anesthesia practice. The inferior alveolar nerve must be blocked when deep restoration or surgery is required on mandibular permanent or primary teeth (1-3).

Preventing pain seems essential for a child to have a positive experience during a dental visit, build trust and cooperation, and enjoy future visits (4). An inferior alveolar nerve block (IANB) is the prevalently adopted technique in children, but it is famous for its high failure rates (5). Yet, the exact location of the mandibular foramen (MF) must be detected to achieve success in the IANB (6). The MF, formed at the beginning of the mandibular canal and covers the mandibular nerve and vessel, is an anatomical structure located slightly above the center of the inner surface of the mandibular ramus (7, 8).

The most common causes of failure of the IANB are lack of anatomical knowledge, lack of a specific anatomical landmark, variations in mandibular ramus sizes, and changes in the location of the MF (9). Panoramic radiography is an advantageous imaging method in children and the disabled and patients with sensitivity to the gag reflex, as well as offering less radiation exposure compared to intraoral radiography (10). On panoramic radiography, the mandibular canal appears radiolucent as a curved plane extending from the MF to the mental foramen. On the other hand, it is reported that panoramic radiography, which is more cost-effective than other imaging methods, helps clinicians identify anatomical landmarks, particularly in developed countries (e.g., MF) (11).

Considering the common failure rates of IANB in children, knowing the exact anatomical location of the MF becomes important to ensure successful anesthesia of IANB during dental procedures in these patients. On the other hand, relevant studies in the literature suggest the location of the MF differs by race and age (12, 13). Although the MF in children is expected to be located lower than in adults during the primary dentition, there are some contra versions about its location in children (14).

Although the literature hosts many studies on the location of the MF in children and adolescents, it is evident that the previous findings highly differ; therefore, further research is needed (12, 13, 15-17).

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Ultimately, this study aimed to reveal the location of the MF in a Turkish pediatric sample from the Eastern Mediterranean region by the occlusal plane (OP), the anterior border of the ramus (ABR), and the mandibular base (MB) using panoramic radiographs. Thus, it was intended to guide clinicians about the correct location of the MF while applying anesthesia to pediatric patients.

2. Materials and Methods

The Clinical Research Ethics Committee of Sutcu Imam University granted ethical approval to the study (No: 2021-17). The present research retrospectively employed panoramic radiographs taken during dental examinations of children who applied to Sutcu Imam University, School of Dentistry, Pedodontics Clinic between 2019-2020.

No additional radiographs of the children were needed in this study. The imaging was taken by the same staff following the proper shooting techniques of the device. The analyses were performed on the panoramic radiographs of the determined sample with complete posterior teeth and those with high image quality without exposure and positioning errors. Nevertheless, the following cases were excluded from the study: patients with low-image quality radiographs, craniofacial syndrome, temporomandibular joint disease, missing posterior teeth, supernumerary teeth, dental agenesis, orthodontic treatment, surgical treatment, trauma, and pathology in the maxillofacial region.

The radiographs were taken by the same staff on the GENDEX GDP -700 (Magnification 1.3) device at 66 Kv, 6.3 mA 14 sec. in the child module following the manufacturer's recommendations. The relevant measurements were made in Adobe Photoshop CS6.

The pediatric patients were divided into five groups by age: Group 1 (G1): 5-6-year-olds, Group 2 (G2): 7-8-year-olds, Group 3 (G3): 9-10-year-olds, Group 4 (G4) 11-12-year-olds, and Group 5 (G5) 13-14-year-olds.

The points and planes in the studies by Apaydın and Shukla were taken as reference in this study (Fig 1.) (15, 18).

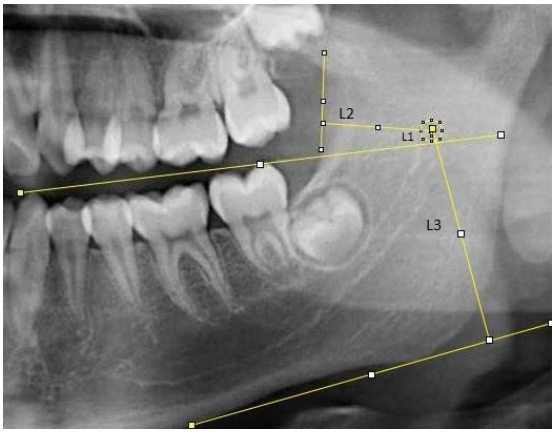

Fig 1. The locations of L1, L2, and L3 on a panoramic radiograph

An experienced oral and maxillofacial radiologist performed all measurements on the radiographs in mm. Since there were no differences between the measurements by right and left areas in the previous studies, the left area was considered while taking the measurements from the patients (18-20).

Meanwhile, to test the reliability of the measurements, the distances were remeasured among 1/10 of the participants two weeks after the main measurements.

L1: MF-OP distance

L2: MF-ABR distance

L3: MF-MB distance

Considering the reference article, a Cohen's d value of 1.13 was settled to be sufficient for deciding a finding to be significant (15). For the total sample size, it was calculated that 85% power would be achieved when 15 participants were recruited for each group. In this study, 30 children (15 girls, 15 boys) were included in each age group. The analyses were performed on the Jamovi (Version 1.0.4) software. The normality of distribution was checked through the Shapiro-Wilk test. The normally distributed data were analyzed using a one-way analysis of variance (ANOVA) with Tukey's multiple comparison tests to compare the locations of the MF by sex and age. Finally, Pearson's correlation test was performed to reveal the associations between the continuous variables. Correlation analysis was performed on repeated within-observation measurements. In all the analyses, a *p*-value <0.05 was considered statistically significant.

3. Results

Intra-observer correlation coefficients ranged from 0.90 to 0.92 for all the measurements (robust positive correlation).

We evaluated panoramic radiographs of 30 patients (15 boys, 15 girls) in each age group, 150 children in total. The results revealed that the measurements did not differ by sex (Table 1).

Table 1. Comparison of the location of the mandibular foramen by gender, one-way anova test (p<0.05)

Locations	Male	Female	P value
L1	1.54±1.26	1.43±1.57	0.611
L2	15.7±2.36	15.08±2.49	0.087
L3	23.59±2.48	23.1±2.78	0.213

The same superscript letters indicate no significant difference within row lines (p>0.05)

There were no significant differences between G1 and G2 and between G4 and G5 by the mean L1 value, respectively (p>0.05). However, the results revealed a significant increase in the mean L1 value from G2 to G4 (p < 0.05).

There were no significant differences between G1 and G2 and between G4 and G5 by the mean L2 value, respectively (p > 0.05) Nevertheless, it was found that the mean L2 value significantly increased from G2 to G4 (p < 0.05).

There were no significant differences between G1 and G2 and between G4 and G5 by the mean L3 value, respectively ($p > 0.05$). Nevertheless, it was found that the mean L3 value significantly increased from G2 to G4 ($p < 0.05$).

The mean values and standard deviations by age group are shown in Table 2.

Table 2. Comparison of the locations of the MF by age, one-way ANOVA and Tukey post-hoc tests ($p < 0.05$)

Locations	5-6 years (G1)	7-8 years (G2)	9-10 years (G3)	11-12 years (G4)	13-14 years (G5)
L1	-0.15±1,43[a]	-0.36±0.98[a]	1.07±0.51[b]	1.99±0.56[c]	2.59±0.59[c]
L2	13.11±1.12[a]	13.14±1.88[a]	14.69±1.48[b]	16.81±1.82[c]	15.18±2.17[c]
L3	19.22±2.34[a]	20.48±1.34[a]	22.56±2.30[b]	23.99±1.32[c]	25.26±1.29[c]

The same superscript letters indicate no significant difference within row lines ($p > 0.05$)

4. Discussion

It is estimated that the locations of the mandibular lingula and foramen may change on the ramus of growing children. Therefore, considering variability in the location of the MF may reduce the incidence of injection failure in pediatric patients (13).

In this study, the MF was slightly located below the OP among those under 8 years of age while being above the OP in the patients 9 years and older. Similarly, the previous research reported that the MF is below the OP in children under 8 years of age (3, 12, 17). Yet, some other studies demonstrated it to be at or above the OP at the beginning of mixed dentition (1, 2, 15, 18, 20). Akbari et al. recommended needle insertion in the same plane as the OP among 7-8-year-olds, while it was recommended to enter from a distance of approximately 2 mm in the 9-10 and 11-12 age groups. They attributed this situation to the incompleteness of teeth among those under eight years of age (1). Accordingly, it can be recommended the injection be made slightly below the OP in children under eight years of age in the INAB application.

The previous research also determined a gradual increase in the distance from the MF to the OP from the early primary dentition to the permanent dentition (2, 13, 15, 18, 20). In this study, it was determined that L1 (MF-OP distance) increased gradually from 7-8 years to 11-12 years, but there was no statistically significant increase at the beginning of early mixed dentition (G1-G2) and permanent dentition (G4-G5). Poonacha et al., on the other hand, reported that the location of the MF shows a slight change with age. In their study, they also found both an increase and a decrease in MF-OP distance (21). Altunsoy et al. evaluated the location of MF in children and adolescents aged 8-18 years through CBCT and, unlike this study, concluded insignificant differences between different age groups by MF-OP distance (16). Contrary to the findings of this study, Afsar et al. concluded that the linear distance between the MF and the OP does not differ significantly by age (9). Similarly, a recent study by Feuerstein et al. with 4-23-year-olds concluded that the location of the MF does not differ significantly by age (22).

On the other hand, although there were no significant differences between 5-6 years of age and 7-8 years of age and between 11-12 years of age and 13-14 years of age by L2 (MF-ABR distance) and L3 (MF-MB distance), it was discovered that the MF increased gradually from 7-8 years to 11-12 years, In the literature, some studies reported an age-associated increase in MF-MB distance (18, 20, 23). It was previously asserted that vertical position changes in the MF may be related to child maturation resulting in enlargement of the ramus and bone formation at the lower border of the mandible (19). It was also suggested that the horizontal location of the MF in children is more inferior-anterior in the ramus of the mandible than in adults and that the MF approaches posterosuperior more with age (15, 20, 21, 23). However, Altunsoy et al. discovered that the distance of the MF from the ramus anterior does not change by age (16).

Nevertheless, no significant differences were found between the measurements by sex. Similarly, the literature previously reported that sex does not have a substantial impact on these distances (15, 19, 22, 23). On the other hand, except for the measurements between the OP and the MF, Altunsoy et al. determined that MF-mandibular lower border and MF-ABR distances are significantly greater in girls than in boys (16). Movahhed also found that the location of MF differed by sex and that it was below the OP in boys and above the OP in girls at the age of 9 years. This situation was attributed to different growth patterns between boys and girls (3).

This study recruited the patients' panoramic radiographs. Although there are studies showing that linear measurements with cone beam computed tomography (CBCT) give precise results for measurements in the region oral and maxillofacial, panoramic radiographs with less radiation are advantageous in pediatric patients (24).

In conclusion, it was discovered that the MF is located below the OP under 8 years of age, and moves in the poster superior direction until the permanent dentition period, which had better be considered by clinicians in IANB.

Conflict of interest

The authors declare no conflict of interest

Funding

No funding was used for the study.

Acknowledgments

None to declare.

Authors' contributions

Concept: K.T.T., A.S.O., Design: K.T.T., A.S.O., Data Collection or Processing: K.T.T., A.S.O., Analysis or

Interpretation: K.T.T., Literature Search: K.T.T., A.S.O., Writing: K.T.T., A.S.O.

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Journal of Experimental and Clinical Medicine
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Research Article

J Exp Clin Med
2022; 39(3): 649-653
doi: 10.52142/omujecm.39.3.11

Causes of parity-related pelvic pain found incidentally on sacroiliac joint MRI: Osteitis condensans ilii and pelvic congestion syndrome

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Received: 13.11.2021 • **Accepted/Published Online:** 05.01.2022 • **Final Version:** 30.08.2022

Abstract

Chronic Pelvic Pain (CPP) is defined as severe pain that persists continuously or intermittently for six months or longer in women, is unrelated to sexual intercourse or menstruation, is likely to cause functional limitation, and requires medical treatment. Osteitis Condensans Ilii (OCI) and Pelvic Congestion Syndrome (PCS) are known causes of CPP. Sacroiliac joint Magnetic Resonance Imaging (MRI) can show different clinical mimickers of sacroiliitis and pelvic pain causes such as OCI and PCS. In this study, our aim was to investigate the frequency of OCI and PCS in patients referred to our clinic for Sacroiliac joint MRI due to low back-pelvic pain and to analyze their association with age, presence of sacroiliitis and multiparity. The the data of 700 female patients who attended the hospital with complaints of low back pain were investigated and it was found that the number of births experienced by the patient significantly correlated with OCI (OR:3.3) and PCS (OR:5.1). Even when MRI shows no evidence of sacroiliitis in a multiparous woman admitted with low back pain, these two differential diagnoses should still be considered by clinicians and radiologists.

Keywords: Sacroiliac joint, chronic pelvic pain, pelvic congestion syndrome, osteitis condensans ilii

1. Introduction

Chronic pelvic pain (CPP) is defined as severe pain that persists continuously or intermittently for six months or longer in women, is unrelated to sexual intercourse or menstruation, is likely to cause functional limitation, and requires medical treatment (1). The prevalence of CPP worldwide is reported to be 16-25% of multiparous women (2). Although there are a variety of causes of CPP, including gynecological, urological, gastrointestinal, musculoskeletal, and/or nervous system disorders, one study found no pathology in 35% of women with CPP who had undergone diagnostic laparoscopy (3). However, Osteitis Condensans Ilii (OCI) and Pelvic Congestion Syndrome (PCS) are known causes of CPP.

OCI is sclerosis secondary to mechanical stress on the auricular surface of the ilium, occurring often during or after latency. It is commonly bilateral and symmetrical (4). The mechanical stress caused by pregnancy is the most widely accepted theory for the cause of OCI (5). On physical examination, sacroiliac tests are negative in these patients, with minor sensitivity found only on the joint (6). OCI should be considered in the differential diagnosis of postpartum CPP, particularly because the articular surfaces of the sacroiliac and hip joints, as well as the soft tissue structures that joints, reflect pain to the lower back and pelvis (7).

PCS, as a known cause of CPP, is defined as the presence of enlarged pelvic veins as a result of ovarian vein failure (8). Some studies describe the presence of reflux as an ovarian vein diameter greater than 8 mm and a parauterine vein diameter greater than 5 mm in color Doppler ultrasonography, dynamic computed tomography, magnetic resonance imaging (MRI), conventional catheter angiography, or venography diagnostic imaging criteria (9-12). The diagnosis of PCS, on the other hand, necessitates a multidisciplinary approach, and diagnosis is usually achieved after ruling out secondary causes.

Pains arising from the hip, pelvis, and sacroiliac joint are entangled due to the complicated anatomy of the lower abdomen and pelvis. PCS-induced pain, particularly in multiparous women, can mimic sacroiliac joint pain (13). To investigate musculoskeletal problems in women presenting with CPP, MRI should be used to thoroughly examine the sacroiliac joint (14). Sacroiliac joint MRI can show conditions such as OCI and PCS clinically mimicking low back pain and sacroiliitis-related pain. Evaluation of pathologies in pelvic varicose veins using sacroiliac joint MRI helps the diagnosis of PCS (11). However, the number of studies examining PCS findings using sacroiliac joint MRI and other causes of CPP is insufficient to draw conclusions.

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The purpose of this study was to assess the prevalence of PCS and OCI findings in sacroiliac joint MRI in patients with low back pain, as well as their association with age, multiparity, and the presence of sacroiliitis.

2. Methods and Materials

Sacroiliac joint MRIs performed on a total of 1003 patients aged 15–56 years who were attended to at the Ondokuz Mayıs University Faculty of Medicine, Department of Radiology, between January 2017 and December 2018, were evaluated retrospectively. Because of the inability to clearly measure the diameter of the parauterine vein due to the imaging technique, motion artifacts, poor resolution, or the presence of an incidentally detected malignant mass or metastasis in the pelvic region, the data of 303 out of 1003 patients were excluded from the data group. Research analyses were performed on the data of the remaining 700 patients.

All sacroiliac joint MRIs included in the study were examined for the presence of active and chronic sacroiliitis using the Ankylosing Spondyloarthritis International Society's criteria. Diagnostic criteria are summarized in Table 1.

Table 1. Diagnostic criteria for sacroiliitis according to ASAS Classification

SpA findings	Sacroiliitis in Screening
Inflammatory low back pain	Active (acute) inflammation on MRI is highly significant for SpA-associated sacroiliitis.
Arthritis	
Enthesitis (heel)	
Uveitis	Definitive radiographic sacroiliitis according to modified New York criteria
Dactylitis	
Psoriasis	
Crohn's disease/Colitis	
Good response to NSAIDs	
Family history of SpA	
HLA-B27	
High level of C-reactive protein (CRP)	

The presence of a dilated vein of ≥ 6 mm in overall dimension surrounding the ovaries and uterus was acknowledged as the diagnostic criterion for PCS (12). The presence of a triangular sclerosis area on the iliac wing inferior to the sacroiliac joints was acknowledged as the diagnostic criterion for OCI (6). The number of deliveries for each patient were obtained from the hospital's informatics system. The patients in the study were assessed based on their age, parity number (patients with two or more deliveries were considered multiparous), active and chronic sacroiliitis, bone marrow edema (BME), and OCI and PCS results.

2.1. MRI Protocol

A 1.5-Tesla (Magnetom Symphony, Siemens Healthcare, Erlangen, Germany) MRI system and a 3-Tesla (Ingenia, Philips Healthcare, Netherlands) MRI system were employed. Before and after axial contrast, fat-suppressed T1-weighted Turbo Spin Echo (TSE), coronal fat-suppressed T2, and/or STIR images were assessed.

2.2. Statistical analysis

The study data were entered into SPSS v21 (Chicago, IL). Descriptive data were presented as mean, standard deviation, and percentage. For group comparisons, T-tests and chi-square tests were employed. For modeling, logistic regression analysis was performed using the enter method. The statistical significance level was accepted at $p<0.05$.

3. Results

In this study, the data of 700 female patients who attended the hospital with complaints of low back pain were investigated. The patients' mean age was 38.1±10.7, of which 55% (n:388) were multiparous and 26.0% had active sacroiliitis. Having acknowledged vein diameter as 6 mm and beyond as an indication of PCS, this finding was found to be positive in 9.3% (n:65) of the patients (Fig. 1). Of these, 24 (3.4%) were found to have OCI, 9 of which (1.3%) had BME. All patients (100%) with BME were found to have OCI; however, none showed any active sacroiliitis (Fig. 2). The measured PCS diameters ranged from 2 mm (n:141) to 11 mm (n:1), with 3 mm being the median (Fig. 3). Table 2 shows the descriptive data of the patient group.

The mean age of patients (n:65) with PCS (vein diameter of 6 mm and above) was found to be 43.2±8.4, and the mean age of patients with OCI (n:24) was 46.0±9.1. PCS (15.7%) and OCI (5.2%) were found to be statistically significantly higher in multiparous (n:388) women compared to others. Examination of the PCS diameters revealed that the PCS diameters of the multiparous group were significantly higher. Table 3 shows the comparison of the data of patients with and without multiparity.

Patients with and without PCS were grouped in the logistic regression analysis for PCS, and the number of deliveries, active and chronic sacroiliitis, BME, OCI, and age were included in the model. The analysis revealed that the model was significant (Nagelkerke R2:0.350, p:0.001), and a one-unit increase in the number of deliveries increased the likelihood of PCS 5.1 times (p:0.001, OR:5.128). Table 4 shows the logistic regression analysis for PCS.

In the logistic regression analysis for OCI, the number of deliveries, active and chronic sacroiliitis, bone marrow edema, and age of patients with and without OCI were included in the model. The analysis revealed that the model was significant (Nagelkerke R2:0.559, p:0.001), and a one-unit increase in the number of deliveries increased the likelihood of OCI 3.3 times (p:0.001, OR:3.287). Table 5 shows the logistic regression analysis for OCI.

4. Discussion

Chronic pelvic pain accounts for 10–15% of gynecological outpatient clinic visits and is a clinical entity with an unclear etiology (12). In patients being examined for CPP, the frequency of PCS and OCI should be taken into account and used in the differential diagnosis.

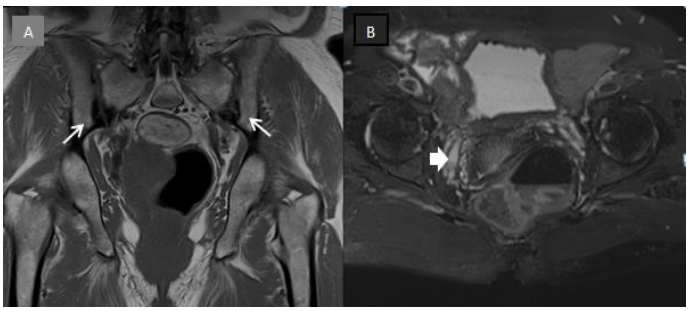

Fig. 1. A 38-year-old grand multiparous patient with OCI and PCS findings together and who has intermittent lower back pain for 3 years (A) Coronal oblique T1 weighted magnetic resonance image show symmetric sclerosis of iliac subchondral bone as very dark signal. Note bilateral SIJ spaces and surfaces are normal (white arrows). (B) Oblique axial T1-weighted postcontrast image demonstrates enlarged parauterine vein of the largest measuring 8.5 mm in diameter on the right side (thick arrow)

Table 2. Descriptive data of the patient group

Study parameters	Mean±SD (%)	n
Age	38.1±10.7	700
Active sacroiliitis	26.0%	182
Chronic sacroiliitis	21.1%	148
PCS diameter (mm)	3.9±1.6	700
PCS ≥ 6 mm	9.3%	65
Osteitis condensans ilii	3.4%	24
Bone marrow edema	1.3%	9
Multiparity	55.4%	388

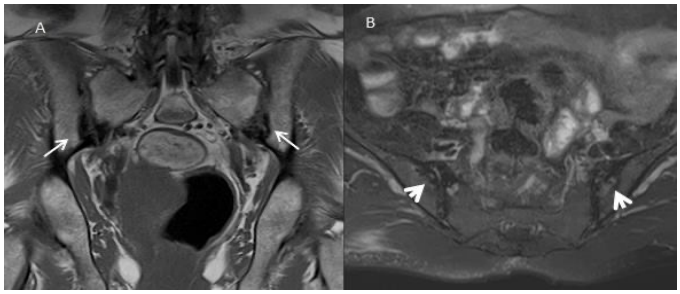

Fig. 2. Bilateral bone marrow edema (BME) of the sacroiliac joints in a 35-year-old OCI patient with an intermittent lower back pain duration of 4 months. (A) Coronal oblique T1 weighted magnetic resonance image show typical bilateral triangle bone sclerosis beneath auricular surface of iliac bone (white arrows). (B) Short tau inversion recovery (STIR) MRI reveals bilateral BME of iliac bone beneath the iliac sclerosis without joint space changes and joint surface erosions (short arrows)

Table 3. Comparison of the data of patients with and without multiparity

Study parameters	Multiparity – (n:312)	Multiparity + (n:388)	Statistics	p
Age	31.6±10	43.3±7.8	T:16.517	0.001*
Active sacroiliitis	20.3% (n:63)	30.7% (n:119)	χ^2:9.868	0.002*
Chronic sacroiliitis	10.9% (n:34)	29.4% (n:114)	χ^2:35.439	0.001*
PCS diameter (mm)	3.1±1.0	4.5±1.7	T:12.731	0.001*
PCS ≥ 6 mm	1.3% (n:4)	15.7% (n:61)	χ^2:42.806	0.001*
Osteitis condensans ilii	1.3% (n:4)	5.2% (n:20)	χ^2:7.833	0.005*
Bone marrow edema	1.0% (n:3)	1.5% (n:6)	χ^2:0.466	0.495

In the comparisons, Chi-square and T-test were employed. *p<0.050

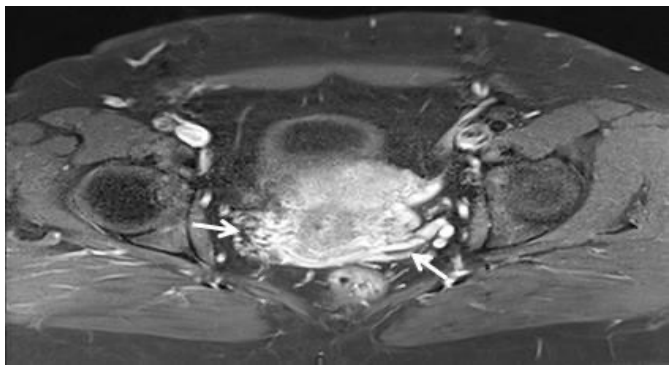

Fig. 3. A 42-year-old multiparous woman with hip and low back pain radiating to the groin and medial buttocks. Normal sacroiliac joint MRI shows axial FS T1-weighted postcontrast image reveal bilateral enlarged and tortuous parauterine veins of varying caliber, the largest measuring 9.4 mm in diameter on the left side (white arrows)

In recent years, some researchers have suggested that sacroiliac joint MRI may clinically demonstrate conditions that mimic low back pain and sacroiliitis and that PCS and OCI may be among these conditions (15). The aim of this study was to identify the frequency of findings in favor of PCS and OCI in sacroiliac joint MRI using a greater number of patients, as well as to show their confusion with SpA clinical criteria and their association with parity.

The sacroiliac joint MRIs taken prior to diagnosis of sacroiliitis in 700 female patients who presented at our clinic with complaints of low back pain were retrospectively evaluated, and the presence of OCI and PCS, which are the sources of parity-related pain, was investigated. In our study, only 26.0% of the patients showed active sacroiliitis symptoms; 9.3% of the patients had positive MRI results that met the diagnostic criteria for PCS. This rate was found to be 15.7% in multiparous patients, and the number of deliveries raised the probability of PCS incidence by 5.1 times. According to the current literature, 6% of women have ovarian varices, 10% have pelvic varices, and PCS develops in 50–60% of these women (15-18). Similarly, in a study by Cimşit et al. (19) sacroiliac joint MRIs taken with the pre-diagnosis of sacroiliitis were analyzed, and it was proposed that venous engorgement could induce low back pain mimicking sacroiliitis; it was subsequently reported that 5.3% of the patients exhibited PCS findings on MRI. The results of our study are consistent with the existing literature and clearly show that the frequency of PCS is higher in multiparous women. The most commonly stressed hypothesis regarding the prevalence of PCS in multiparous women during the premenopausal period is that the vascular capacity in the ovarian-uterine veins increases to 60 times the normal values during pregnancy. Excessively increased venous load is thought to cause deficiency and varicosity 12). Our results support this hypothesis and make an important contribution to the literature. Given these results, it would be prudent to include PCS in the differential diagnosis of CPP, particularly in multiparous women, and to examine PCS using more specific diagnostic methods (20).

Table 4. Logistic regression analysis for PCS

	B	Standard failure	Wald	p	Exp(B)
Number of deliveries	1.652	0.198	69.397	0.001*	5.218
Osteitis condensans ilii	-1.932	1.002	3.717	0.054	0.145
Age	-0.033	0.019	2.923	0.087	0.968
Active sacroiliitis	-0.716	0.528	1.836	0.175	0.489
Bone marrow edema	1.377	1.418	0.943	0.331	3.961
Chronic sacroiliitis	0.018	0.513	0.001	0.972	1.018
Constant	-4.401	0.759	33.658	0.000	0.012

Table 5. Logistic regression analysis for OCI

	B	Standard failure	Wald	p	Exp(B)
Constant	-6.979	2.152	10.516	0.001	0.001
Number of deliveries	1.050	0.302	12.062	0.001*	3.287
Age	0.045	0.045	0.978	0.323	1.046
Pelvic congestion syndrome	-1.015	0.850	1.427	0.232	0.362
Active sacroiliitis	-15.677	2541.368	0.001	0.995	0.001
Bone marrow edema	40.459	10921.958	0.001	0.997	0.001
Chronic sacroiliitis	-16.118	2674.186	0.001	0.995	0.001

Additionally, 3.4% of the patients in this study were diagnosed with OCI; OCI was found in all patients with BME, and none of these patients had signs of active sacroiliitis. In 2009, BME was shown to be a major criterion in the diagnosis of active sacroiliitis using sacroiliac MRI and according to the ASAS diagnostic criteria (21-22). Hence, clinicians are increasingly relying on sacroiliac joint MRI data to identify or rule out early sacroiliitis. Some studies have reported on cases in which BME was found in the MRI of individuals presenting with low back pain; these cases were initially followed as active sacroiliitis, but the diagnosis was changed during the follow-up to OCI (23). The reason for this confusion may be that in clinical practice, the diagnosis of OCI is made after radiological examinations and the exclusion of all other diagnoses that may cause low back pain, particularly sacroiliitis. The results of our study support OCI diagnosis following BME detection. However, thorough examination of the location and distribution pattern of the BME observed on MRI may aid in making the differential diagnosis between OCI and active sacroiliitis.

The rate of OCI detection in multiparous women was found to be 5.2%, which is higher than in nulliparous women and those who have had only one delivery. It was observed that each increase in the number of deliveries increased the incidence of OCI by 2.9 times. The incidence of OCI in the general population has been reported as 1–2.5% (6, 14, 24), and this rate may increase up to 8.9% in patients evaluated for inflammatory arthritis (25). The main reason why the incidence of OCI is unclear is that most patients are asymptomatic and have normal physical examination findings. OCI is typically detected incidentally through radiological examination (26,27). OCI is known to be more common in women in their third trimester of pregnancy and in the early postpartum period, and it often recurs in subsequent pregnancies. Based on the current literature, pregnancy can be viewed as a possible triggering factor leading to sclerosis (28,29). The results of this study pertaining to multiparity, unlike previous studies, underline the importance of investigating OCI in addition to PCS in multiparous women presenting with low back pain.

This study had both strengths and limitations. The most significant strength of this study is the sample size. The inclusion of a large sample group of 700 patients improved the reliability of our results and makes this study a valuable reference for other researchers. Another strength is the determination of varicose diameter width reported for diagnosis as 6 mm rather than 5 mm, which excluded asymptomatic enlargement that may occur in women with PCS. The first limitation of this study is its retrospective design. Thus, the level of evidence is not as high as that obtained from a prospective study. Second, the study used MRI taken for another diagnosis, and thus the MRI only contributes as a recommendation in differential diagnosis because of the lack of confirmation through physical examination specific to both PCS and OCI and the lack of data such as the duration of the disease, type of complaints, and the treatment approaches taken. Lastly, the imaging of symptomatic patients was assessed, implying that asymptomatic OCI or OCI patients with a final diagnosis were excluded from the study. Nevertheless, the similarity of our results with the literature and the identified correlation with multiparity are noteworthy.

This study revealed incidentally detected PCS and OCI findings in patients with a pre-diagnosis of sacroiliitis who underwent MRI. In conclusion, even though MRI shows no evidence of sacroiliitis in multiparous women presenting with low back pain, these two differential diagnoses should still be considered by clinicians and radiologists.

Ethical approval

This study was conducted in compliance with the ethical principles according to the Declaration of Helsinki, and it was approved by the Ondokuz Mayıs University Ethics Committee (approval number 2019/782). As this study was retrospective, the patients' consent was waived.

Conflict of interest
The authors declared no conflict of interest.

Funding
No funding was used for the study.

Acknowledgments
None to declare.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 654-659
doi: 10.52142/omujecm.39.3.12

Investigation of long-term fall prevalence after total knee arthroplasty in Hatay: A cross-sectional study

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Received: 26.10.2021 • **Accepted/Published Online:** 04.01.2022 • **Final Version:** 30.08.2022

Abstract

Falls and fractures caused by falls in the elderly affect their daily activities, creating fear of movement and alienating the individual from social participation. Fall related-injuries are some of the main afraid complications after total knee arthroplasty surgery. The aim of this study is to determine the incidence of falling and fear of movement in the long period (one year and more after surgery) in patients who had total knee arthroplasty surgery. Patients who had total knee arthroplasty surgery in Hatay Mustafa Kemal University Department of Orthopedics and Traumatology between 01.01.2016 and 01.10.2020 enrolled in the study. Demographic information was recorded from the patient file or asked to the patients/caregivers via telemedicine. The falls before the surgery, falls after the surgery within 3 months, within 3 to 6 months, within 6 to 12 months which caregiver/relative's remember recorded via telemedicine. Fear of movement assessed with the Tampa Kinesiophobia Scale was asked. 149 Patients with, mean age 67.06±8.72 years, female (85.9%), mostly house wives (80.5%) was the cohort. Their education time (89.8 %) were less than 5 years, and the huge majority of the patients did not have exercise habits (83.2%). The fall rate before the surgery was (35.6%), after the surgery was (36.9%), within the first 3 months (6.7%), 3 to 6 months (6%) and 6 to 12 months (11.7%). 7. 4 % of our patients had a fall related-fractures including: 2 radius distal fractures (1.3%), 3 hip fractures (2%), 3 periprosthetic fractures (2%), 1 patella fracture (0. 7%) and 2 vertebral compression fractures (1.3%). Tampa Kinesiophobia Scale score was 41(38-44). There was a positive correlation between kinesiofobia and those who did not have exercise habits and those who fell preoperatively (r: 0.31, p:0.01). Some of TKA patients were falling and had fear of movement. New researches should be conducted about what is the reason that makes the patients posture and movement more stable or which mechanism alters the balance. In order to prevent complications that may occur due to falls, new studies, treatment modalities and rehabilitation programs focusing on the etiology of falling in the elderly individuals should be organized.

Keywords: knee replacement, kinesiophobia, fall risk, osteoarthritis

1. Introduction

Falls and fractures caused by falls in the elderly affect their daily activities, creating fear of movement and alienating the individuals from social participation. Thus, prevention of falls is extremely important for elderly individuals to be independent in their daily living activities and to increase their mobility. Deformed and painful joints are among the risk factors for falls. The knee, foot or spine's age-related deformities impair the stability and balance of the spine, increasing the frequency of falls. Especially, it has been reported that osteoarthritis causes deformation of the knee joints and pain during walking, thus increasing the risk of falling and fracture and triggering the fear of movement (1, 2). Knee arthritis is an established risk factor for falls due to pain, stiffness and functional limitation that are more associated with fall risk. It has been reported significant improvements in pain, function and proprioception after total knee arthroplasty (TKA) in patients with knee osteoarthritis. Although these factors are expected to reduce the frequency of falls in elderly people after TKA, Some studies have shown that balance deficits may develop due to proprioceptive reasons following TKA. Also, it has been shown that this situation can increase the risk of falling and lead to fear of movement (3). In another study, it was reported that the functions of many mechanoreceptors were affected as a result of loosening or removing some ligaments in order to correct the intra-articular geometry during knee arthroplasty surgery, and as a result, motion control and balance were affected (4). Therefore, it is seen that the probability of falling is affected, although not as much as before the surgery and that those who did not fall before the surgery also fell after the surgery. It has also been reported that the incidence of falls after TKA is higher compared to asymptomatic healthy elderly, which ranges from 17-48% (5, 6). Swinkels et al., 2009 reported a 10% fall after TKA in the patients with no previous fall history (3). 54.2% of the group reporting preoperative fall did not fall in the first year after TKA; in 45.8%, it was stated that the falling action continued. They reported that 17.3% (13/75) of those who did not fall before

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the operation fell after the operation. 24.2% of the people who had TKA surgery were those who fell within the 3 months before the surgery. The rate of falling in the postoperative follow-up of this group was 11.7–11.8%for every 3 months in the first year. Functional scales and balance confidence improved after TKA. However, it is known that the falling action continues in 45.8% of patients with a history of falling preoperatively (7). Therefore, rehabilitation programs should be developed to prevent the fear of movement (kinesiophobia) and falls that may develop in these patients and to increase independence in daily living activities. In order to contribute to these rehabilitation programs, it is important to determine the prevalence of long term fall and kinesiophobia after TKA applied. The aim of this study is to determine the incidence of falling and fear of movement in the long period (one year and more after surgery) in patients who had TKA surgery in Hatay Mustafa Kemal University Health Practice and Research Hospital.

2. Materials and Methods

2.1. Participants

All patients who had surgery in Hatay Mustafa Kemal University Department of Orthopedics and Traumatology between 01.01.2016 and 01.10.2020 and completed the first year after surgery were scanned from the archives. Patients were called via telemedicine method. The information of 268 TKA patients was obtained. 115 patients refused to participate. Four patients with TKA could not be interview because of the unreached (Fig. 1). One hundred fourty nine patients with TKA agreed to participate in the study. The inclusion criteria were as follows: patients operated as primary TKA, completed the first year after surgery. The exclusion criteria were as follows: history of lower extremity congenital deformation or musculoskeletal injury, neurologic or orthopedic diseases leading to gait disturbance, balance and postural problems, postoperative infection, deep venous trombosis and revision TKA surgeries.

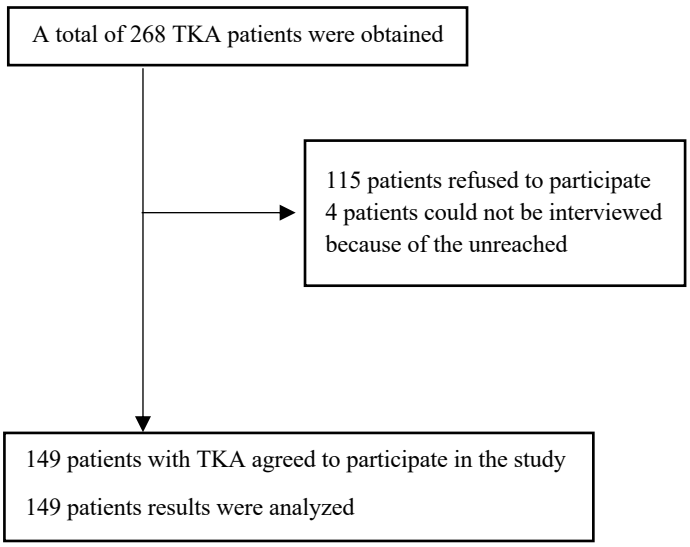

Fig. 1. Flow chart of the study

2.2. Study design

The study was approved by the Ethics Committee of the Hatay Mustafa Kemal University (Decision No. 6, dated 4.10.2021) and performed in accordance with the Declaration of Helsinki. Verbal informed consent was obtained from all patients to participate in the study. Demographic information was recorded from the patient file or asked to the patients. The preoperative fall status of the patients who accepted to participate in the study was recorded from the patient file and by asking the patient and the caregiver/relatives, and the number of falls in the last one year after surgery was recorded with questions prepared by the researchers. Falls before the surgery from patients' records, falls before the surgery which patients remember, falls before the surgery which caregiver/relatives remember, falls after the surgery, falls after the surgery within 3 months, falls after the surgery within 3 to 6 months, falls after the surgery within 6 to 9 months, falls after the surgery within 6 to 12 months, falls after the surgery which caregiver/relative's remember recorded via telemedicine. Patients' relatives/caregivers were called at after three days and falls status of the patients before and after the surgery were asked. Fall related-fracture rate calculated by questioning the patients and searching the medical records. All the replies were recorded.

Fear of movement assessed with the Tampa Kinesiophobia Scale (TKS) was asked via telemedicine. This 16-item questionnaire, which includes the subjective evaluation of kinesiophobia, has validity and reliability in Turkish. Each statement of the 16 items is scored between "strongly disagree" (score 1) and "strongly agree" (score 4) on a four-point Likert scale. The 4th, 8th, 12th and 16th items of the questionnaire are reversed and scored. Total score is between 17-68 points. A high score on the scale indicates that kinesiophobia perception is also high (8).

2.3. Statistical analysis

Statistical analysis was performed via the Windows based SPSS 20 (IBM Corp. Armonk, New York, USA.) The suitability of the variables to normal distribution was examined by visual (histogram and probability graphs) and Kolmogorov-Smirnov, an analytical method. Mean and Standard deviation values were given fornormally distributed variables and percentages (%) for variables uncountable variables. Non-normally distributed data were expressed as median (quartiles) and were compared using the Mann–Whitney U test. Correlations were analysed using Pearson's and Spearman's rank correlation coefficients, as appropriate.

3. Results

Clinico-Demographic data of the patients with TKA were presented in table 1. Mean age was 67.06±8.72 years and most of the patients (85.9%) were female. Most of the participants (80.5%) were housewives, the education time of the participants was mostly (89,8 %) less than 5 years and the highest education level was 'literate' (61.1%). The huge

majority of TKA patients did not have exercise habits (83.2%). The ratio of the operated side was left (34,2), right (33.6) and both knee (32.2), respectively.

Fall incidance and kinesiophobia in patients with TKA were shown in table 2. According to the archive, the highest rate of preoperative fall was "none". Most of the patients stated that they did not fall before the operation (64,4%), similar to this their relatives/caregivers (73.8) mostly stated that there was no fall in TKA patients before surgery. In the period of after the surgery till the questionaire the highest falling status was 'none fall'' with the rate of (63.1%), within the first 3 months (93,3%), 3 to 6 months (94%) and 6 to 12 months (89.3%). After the surgery falls rate was in "1 fall" 34,9%, in "2 fall" 1.3%. Most patients were asked, "Have you fallen in the last 12 months from today?" and mostly of the patients (66.4%) answered the question "none fall". Falls after the surgery which caregiver/relatives's remember was mostly (65.1) in 'none fall'. According to caregiver/relatives's the rate of falling after surgery was 30.3 %, while it was 35,6% according to patients (Table 2, Fig. 2). Falls rate before the surgery was 34.2 % according to patients, while it was 22,7% according to caregivers/relatives (Fig. 2, Table 2).

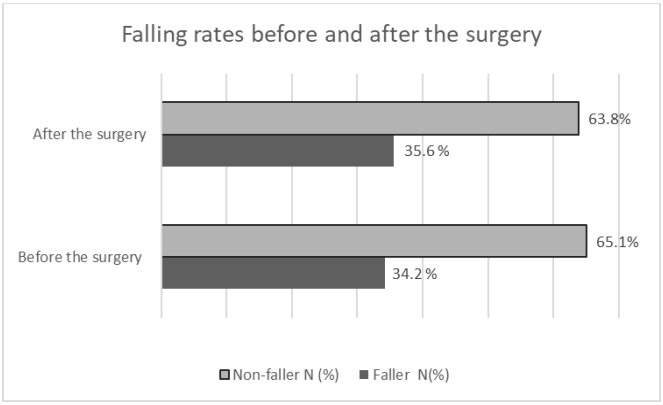

Fig. 2. Falling rates before and after the surgery in patients with TKA 7.4% of our patients had a fall related-fracture similar with the literature including: 2 radius distal fractures (1.3%), 3 hip fractures (2%), 3 periprosthetic fractures (2%), 1 patella fracture (0.7%) and 2 vertebral compression fractures (1.3%)

The fear of movement (kinesiophobia) was found with TKS score which was 41(38-44) (Table 2). The relationship between kinesiophobia and age, fall-related fracture, BMI, exercise habits, preoperative and postoperative fall frequency was evaluated. There were no statistically significant relation between kinesiofobia and age (p:0.67 r:-0.03), fall related-fracture (p: 0.49 r:-0.05), BMI (p: 0.54 r: 0.05). However, there was a positive correlation between kinesiofobia and those who did not have exercise habits (p.0.03 r:0.17) and those who fell preoperatively (p: 0.01 r: 0.31). Total kinesiophobia scores of falling and non-falling patients were similar (p>0.05, Table 4). Also, the TKS scores between the patients who have and don't have fall related-fracture were similar. Also, when the answers of the patients and their caregivers to the questions asked about fall data were examined, there were differences before surgery (35.4 & 27.2%), but when they were asked about their recalls about fall rates after surgery, the comparison was similar.

There was no relationship between falling in the perioperative or postoperative period and knee surgery side or unilateral or bilateral TKA surgery.

Table 1. General informations about participants

	N (%) / Mean±SD / Median (IQR)
Age (years)	67.06±8.72
BMI (kg/m^2)	32.85(29.06-36.82)
Gender n (%)	
Male / Female	21 (14.1) / 128 (85.9)
Profession n (%)	
Housewife Farmer Old age pensioner Driver Self-employment Other	120 (80.5) 7 (4.7 %) 10 (6.7 %) 3(2 %) 4(2.7 %) 4(2.7%)
Education Time n (%)	
<5 years 6-8 years 9-11 years >11 years	134(89.8%) 8(5.4%) 2(1.4 %) 5 (3.4%)
Eduacation Level n (%)	
Literate Primary school Middle school High school University	91 (61.1) 46(30.9) 5 (3.4) 3(2) 4 (2.7)
Exercise Habit n (%)	
Yes/No/No stated	24(16.1%)/124 (83.2%) / 1(0.7)
Surgical Side n (%)	
Right knee /Left knee/both of knee	50 33.6%) /51(34.2%)/48(32.2)

Table 2. Fall risk and kinesiophobia in patients with knee artroplasty

	n (%) / Median (IQR)
Falls before the surgery	
None	98 (65.8)
1 fall	49 (32.9)
2 fall	1 (0.7)
Not stated	1(0.7)
Falls before the surgery which patients remember	
None	96(64.4)
1 fall	23(15.4)
2 fall	11(7.4)
3-4 fall	7(4.7)
4-5 fall	3(2.0)
5-6 fall	3(2.0)
6-7 fall	1 (0.7)
Not state	5 (3.4
Falls before the surgery which caregiver/relatives remember	
None	110 (73.8)
1 fall	16 (10.7)
1-2 fall	10 (6.7)
3-4 fall	3 (2.0)
4-5 fall	2 (1.3)
5-6 fall	3 (2.0)
Not stated	5 (3.4)
Falls after the surgery	
None	94(63.1)

1 fall	52 (34.9)
2 fall	2 (1.3)
Not stated	1 80.7)
Falls after the surgery within 3 month	
None	139 (93.3)
1 Fall	1 (0.7)
2 Fall	1 (0.7)
3 Fall	1 (0.7)
Not stated	7 (4.7)
Falls after the surgery within 6 month	
None	136 (91.3)
1 fall	5 (3.4)
2 fall	0 (0)
3 fall	1 (0.7)
Not stated	7 (4.7)
Falls after the surgery within 9 month	
None	140(94)
1 fall	0(0)
2 fall	0(0)
3 fall	2 (1.3)
Not stated	7 (4.7)
Falls after the surgery within 12 month	
None	133 (89.3)
1 fall	6 (4.0)
2 fall	0 (0)
3 fall	2 (1.3)
4 fall	1 (0.7)
Not stated	7 (4.7)
Have you fallen in the last 12 months from today?	
None	99 (66.4)
1 fall	29 (19.5)
2 fall	8 (5.4)
3 fall	4 (2.7)
4 fall	0 (0)
5 fall	1 (0.7)
Not stated	8 (5.4)
Falls after the surgery which caregiver/parent's remember	
None	97 (65.1)
1 fall	28 (18.8)
2 fall	12 (8.1)
3 fall	4 (2.7)
10 fall	1 (0.7)
Not stated	7 (4.7)
Tampa Kinesiophobia Scale median (IQR)	
Total score	41(38-44)

4. Discussion

The most important findings in the present study were as follows: (1) Fall rate before the surgery was 35.6 % (one or more time fall event) (Table 2) (2) Fall rate after the surgery was 36.9% (Table 2) (3) The proportion of those who did not fall both before and after surgery was 41.9 %, those who did not fall before the operation but fell after the operation was 24.3 %, those who fell before surgery but did not fall after surgery 23 % and those who fell both before and after the operation was 10.8 %.

According to Centers for Disease Control and Prevention Center's (United States) Morbidity and Mortality Weekly Report in 2018, 27.5% of adults aged ≥65 years had at least one fall in the past one-year period (9). Knee osteoarthritis (10) and TKA (11) has been implicated as a risk factor for falls. In previous studies, preoperative fall status was determined as a factor predicting postoperative fall events. According to the study of Hill; of the 283 patients who completed the questionnaire, 41% reported one or more falls in the previous year of TKA surgery (12). Swinkels et al reported a 24.2 % of preoperatively fall rate in the 3 months prior to TKA surgery (7). We found a higher fall rate before surgery which is stated at the CDC MMWR (9). Although preoperative fall rate was less but correlated with the literature; 50 (33.6%) of our patients fell 1 or more times before surgery.

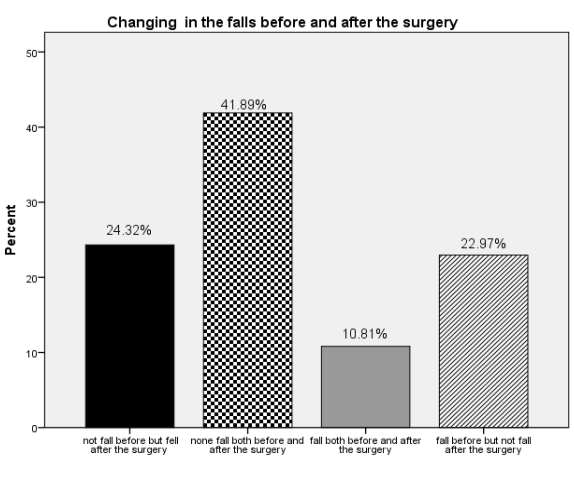

Fig. 3. Changing in the falls before and after the surgery

Table 3. Changes in the falls before and after the surgery

	n (%)
Preop none fall but fall after the surgery	36 (24.3)
Both pre and post-surgical fall	62 (41.9)
Both pre and post-surgical fall	16(10.8)
Fall before but not fall after the surgery	34(23)

Table 4. Kinesiophobia results between the faller and non faller patients

	Faller	Non- faller	
	Median (IQR)	Median (IQR)	p
Preop Tampa Total score	42(39-44.5)	41(38-43.75)	0.18
Postop Tampa total score	42 (39-45)	41(38-43)	0.14

Mann-Whitney Test, $p<0.05$

According to the literature there are some studies reporting the prevalence of falls between 17% and 48% in patients 12 months following TKA (5, 6, 7, 13). Matsumoto et al. designed a study with monthly pre-stamped postcards sent for assessing the incidence of falls. They reported that 23 of 70 patients (32.9 %) fell during the observation period (13). Also opposite the literature Riddle and Golladay stated that the fall rates of the persons with and without kneearthroplasty were similar over an 8-year period among (14). However we found a similar fall rate after surgery as 36.9% (Table 3).

In the report of a retrospective review to identify falls in patients admitted for elective orthopaedic procedures conduted By Mandl, There were 868 falls among orthopaedic

patients (Of 38,2% TKA surgery). The fall rate was 0,9% of admissions, or 2,0 per 1000 inpatient days (15). Johnson et al reported that the fall rate during hospital stay after surgery was 15, 3 per 1000 patients (16). No fall accident occurred during the hospital stay in our patients. In our studynone of our patient had a fall history during the postoperative hospital stay. This may be explained because of the small sample size.

In a prospective study, Levinger et al questioned the patients about physical activity, fear of falls and history of falling in the 12 months post-surgery. 60 of the 243 participants (34,1%) reported1 or more fall in the 12 months post-operatively following TKA. Also they stated that increased planned physical activity and previous falls in the preceding year were predictors of falls. According to the results patients who fell 1 or more times pre-operatively were three times more at risk of fall post-operatively (17). Our patients were predominantly sedentary (83,2%) and TKS scores were signaficantly correlated.

In many previous studies it was shown that there is a relation between mobility status, falls and developing fear of falling (18, 19). Although there was a positive correlation between kinesiophobia and exercise habit of our patients who did not have exercise habits and those who fell preoperatively, we could not explain this activity level-fall-fear of falling relation because of the similar TKS scores between fallers and nonfallers.

Fall related-injuries are some of the main afraid complications after TKA surgery. In the CDC MMWR report it's been stated that 10.2% of adults aged ≥65 years had a fall-related injury (9). Thus, the results of the fall events must be considered in a separate view. According to the results reported by Jorgensen, they classified the fall injuries as "none" or minor in 39.8%, moderate in 9.6%, and major in 50.6%. In addition, they reported that of 1.6% fall-related hospital admissions (20). Matsumoto et al stated the fracture rate due to falling as 6.2% (13). In addition, fall-related fractures are seen at a substantial rate after TKA. According to the results of a cohort study by Tromp et al, the total number of fractures was 85 of 5145 patients, including 23 wrist fractures, 12 hip fractures, and nine humerus fractures (5.8%) (21) In our cohort 7,4 % of our patients had a fall related-fracture similar with the literature including: 2 radius distal fractures (1,3%), 3 hip fractures (2%), 3 periprosthetic fractures (2%), 1 patella fracture (0,7%) and 2 vertebral compression fractures (1.3 %).

Swinkels and Allain determined the fall rate timing period. In their study seven of 30 (23.3%) patients fell in the 3 months before surgery and 5 (22.7%) in the 6 months after surgery. Also, per quarter fall rate was 11.7-11.8% and overal fall rate was 24,2% in the first year after surgery (22). In comparison with these rates after surgery our results were lesser as: within the first 3 months (6,7%), 3 to 6 months (6%) and 6 to 12 months (11,7%). On the other hand after surgery a overall fall rate was higher as 35.6%. Other results reported by Swinkels and Allain should be noted: 45.8% of preoperatively falled patients continued to fall, 17.3% of patients who did not fall preoperatively fell postoperatively. When our data is analyzed in this perspective, patients who did not fall before the surgery but fell after the surgery was 24.3 %, patients who fell before surgery but did not fall after surgery 23 % and patients who fell both before and after the operation was 10.8 %. An important question has been raised as a result of our findings: 'What is the mechanism or reason that make the patients' fall or doesn't fall?' or 'Which parameters change after the TKA surgery?'

Our recommendation is not to ignore the high risk of falling before and after TKA in the elderly. To the best of our knowledge, this is a rare study that assesses the fall rate and fear of falls in patients with total knee replacements in terms of both preoperatively and postoperatively.

New researches should be conducted about what is the reason that makes the patients posture and movement more stable or which mechanism alters the balance. In order to prevent complications that may occur due to falls, new studies, treatment modalities and rehabilitation programs focusing on the etiology of falling in the elderly individuals should be organized.

There are some limitations in our study. Although many factors such as health status, posture, static and dynamic balance problems are shown to be responsible among the causes of falls in the elderly, we did not evaluate our patients in terms of the etiology of falls. Although the fear of falling and the events of falling were questioned in our patients, the injuries accept fractures that developed around the knees or other body parts due to these falls were not questioned. İn addition, it was not questioned whether TKA complications due to falling occurred or not. Therefore, the potential effects of falling in TKA patients are unknown. No research has been conducted on the etiology of falls, such as prosthetic designs and surgical approaches the contribution of the other knee with osteoarthritis to the fall has not been examined.

Conflict of interest

The authors declare that there is no conflict of interest.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Acknowledgements

All the authors would like to thank to Yurdagül Yılmaz and Ramazan Görkem Çay for their assistance in collecting data.

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Journal of Experimental and Clinical Medicine
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Research Article

J Exp Clin Med
2022; 39(3): 660-663
doi: 10.52142/omujecm.39.3.13

The relationship between the degree of prolapse and bone mineral density in postmenopausal women with pelvic organ prolapse

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Received: 13.12.2021 • **Accepted/Published Online:** 07.06.2022 • **Final Version:** 30.08.2022

Abstract

This study aimed to investigate the relationship between the degree of prolapse and bone mineral density (BMD) in postmenopausal women with pelvic organ prolapse (POP). Materials and Methods: This retrospective study includes 40 postmenopausal women who were operated for POP. While patients who had grade <3 vaginal cuff prolapse were accepted as low grade prolapse, patients with grade ≥ 3 vaginal cuff prolapse were accepted as high grade prolapse. Patients' demographic characteristics, obstetric and gynecological history, laboratory findings and dual-energy X-ray absorptiometry results (lumbar spine BMD, lumbar spine T and Z scores, femur neck BMD, femur neck T and Z scores) were recorded as study parameters. Results: There were no significant differences between the groups in terms of age, body mass index, gravida, parity, follicle-stimulating hormone levels. BMD of the femur neck and lumbar spine were significantly higher in the low grade prolapse group than in the high grade prolapse group. T score of the femur neck were significantly higher in the low grade prolapse group than in the high grade prolapse group. In addition, estradiol levels of the high grade prolapse group was lower than the low grade prolapse group. BMD and T score of the femur neck and BMD of the lumbar spine were found to be significantly lower in the high grade prolapse group, suggesting that, there may be an association between the severity of POP and the risk of osteoporosis in postmenopausal women.

Keywords: Proplapse, bone mineral density, osteoporosis, menopause

1. Introduction

Pelvic organ prolapse (POP), is downward descent of female pelvic organs, including the bladder, bowels, uterus or post-hysterectomy vaginal cuff, resulting in protrusion through the genital hiatus (1). Osteoporosis is a disease that is characterized by low bone mass, deterioration of bone tissue, and disruption of bone microarchitecture (2). The predisposing factors for POP are parity, advancing age, genetic predisposition leading to reduced connective tissue and muscle strength and increased body mass index (BMI) as the most consistent risk factors (3, 4). Predisposing factors for osteoporosis are almost the same, it is more common in older people (5). Estrogen receptors play an important role in controlling musculoskeletal growth and maintenance of bone mass and also in the etiology of POP (6, 7).

When the literature is analyzed, it is seen that, a few studies have been conducted on the relationship between POP and osteoporosis (8-11). It has been shown that moderate-to-severe POP in postmenopausal women as a risk factor for hip fracture (10). After adjusting for age, advanced POP was correlated with osteoporosis (11).

With an aging population and longer life expectancy, POP and osteoporosis is of increasing importance. In this study, given the association between menopause, POP and osteoporosis we wanted to investigate the relationship between the degree of prolapse and bone mineral density (BMD) in postmenopausal women with POP.

2. Materials and Methods

After receiving approval from the institutional review board, the medical records of consecutive postmenopausal patients who were operated for POP and have undergone DXA in a tertiary center were retrospectively reviewed. The study was performed in accordance with the 1964 Helsinki declaration.

Patients who have undergone dual-energy X-ray absorptiometry (DXA) in the last 3 months before surgery were included in the study. The patients had at least 12 months of amenorrhea. This was accepted as menopause (12). Patients' demographic characteristics, obstetric and gynecological history, and laboratory findings, DXA results were recorded as study parameters.

Baden Walker halfway classification system which consists of four grades was used for the assessment and

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documentation of POP (grade 0 – no prolapse, grade 1– halfway to hymen, grade 2 – to hymen, grade 3 – halfway past hymen, grade 4 –maximum descent) (13). Patients who had grade < 3 prolapse were accepted as low grade prolapse, patients with grade ≥ 3 vaginal cuff prolapses were accepted as high grade prolapse.

The difference between the patient's BMD and mean BMD of young females aged in the range of 20–29 years (divided by the standard deviation (SD) of the reference population) yields the T-score. The expected BMD for the patient's age and gender yields the Z-score. A T-score less than -2.5 SD was defined as osteoporosis (5, 14).

Patients with a systemic disease, previous pelvic surgery, patients on hormone replacement therapy were excluded. Additionally, patients using drugs that can affect BMD, smokers were excluded. Patients who did not have sufficient records were excluded.

Data were analyzed via SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). Kolmogorov- Smirnov analysis was used to evaluate the normal distribution of continuous variables. Continuous variables were compared via the independent simple t test. The comparison of categorical variables was tested via $\chi 2$ test and Fisher exact test. Nominal data with normal distribution is shown as mean ± standard deviation in the tables. Categorical data are shown in numbers (n) and percentages (%). A p value of less than 0.05 was taken to be significant.

3. Results

The present study was performed on a total of 40 postmenopausal patients who were operated for POP. While there were 16 patients who had grade < 3 prolapse and formed the low grade prolapse group, patients with grade ≥ 3 prolapse formed high grade prolapse group.

Clinical, demographic and laboratory data of patients are shown in Table 1. The mean age of the low grade and high grade patients was 55.2 ± 6.5 years vs 57.8 ± 5.7 years respectively (p = 0.222). Body mass index (BMI) was similar between the groups. Also, there were no significant differences between the groups in terms of gravida, parity, follicle stimulating hormone (FSH) levels, prolapsed compartment. However, whereas the mean estradiol (E2) level of the low grade patients was 26 (4-56), it was 10 (5-35) in high grade prolapse patients (p=0.019) (Table 1).

Femur neck and lumbar spine DXA results of the patients are shown in Table 2. BMD and T score of the femur neck were significantly higher in the low grade prolapse group than those in the high grade prolapse group (p = 0.043 and p = 0.028, respectively). In addition, lumbar spine BMD was higher in the low grade prolapse group than in the high grade prolapse group (p = 0.002). Although, lumbar spine T and Z score were higher in the low grade prolapse group, no statistically significant difference was found between the groups (Table 2).

Table 1. Clinical, demographic and laboratory characteristics of the patients

	Grade < 3 prolapse cases (n=16)	Grade ≥ 3 prolapse cases (n=24)	p
Age (years)	55.2 ± 6.5	57.8 ± 5.7	0.222
Gravida	4 (1-7)	4 (2-8)	0.987
Parity	3 (1-5)	3 (2-6)	0.765
BMI	26.8 ± 2.3	27.5 ± 1.8	0.876
FSH	53.4 ± 4.8	54.5 ± 4.7	0.965
E2	26 (4-56)	10 (5-35)	0.019
Compartment prolapse*			0.784
Anterior	13 (81.3)	19 (79.2)	
Posterior	8 (50)	16 (66.7)	
Apical	6 (37.5)	10 (41.7)	

Values were presented as mean±standard deviation, median (min-max), number (%).
*Some patients had more than one compartment prolapse
BMI: Body Mass Index, FSH: Follicle Stimulating Hormone, E2: Estradiol
p<0.05 was considered statistically significant

Table 2. Femur neck and lumbar spine dual-energy X-ray absorptiometry results of the patients

	Grade < 3 prolapse cases (n=16)	Grade ≥ 3 prolapse cases (n=24)	p
Lumbar spine BMD (g/cm^2)	0.889 ± 0.127	0.799 ± 0.382	0.002
Lumbar spine T score		- 0.72 ± 0.20	- 1.09 ± 0.88
Lumbar spine Z score	0.64 ± 0.74	0.58 ± 0.76	0.719
Femur neck BMD (g/cm2)	0.661± 0.332	0.622 ± 0.231	0.043
Femur neck T score	- 0.75 ± 0.65	- 1.22 ± 0.63	0.028
Femur neck Z score	0.59 ± 1.04	0.49 ± 1.10	0.786

Values were presented as mean±standard deviation.
BMD: Bone Mineral Density
p<0.05 was considered statistically significant

4. Discussion

POP and osteoporosis affects quality of life to an important extent and nowadays, due to ageing population are of increasing importance. In this study, we aimed to investigate the relationship between the degree of prolapse and BMD in postmenopausal women with POP. In this study, we found statistically significant difference between low grade and high grade prolapse group in terms of BMD of the femur neck and lumbar spine. Additionally, T score of the femur neck were found to be significantly higher in the low grade prolapse group.

When the literature is analyzed, there are few studies available on the association between POP and BMD. Pal et al, demonstrated a relationship between moderate to severe POP and low bone mineral density in postmenopausal women enrolled in the Women's Health Initiative Estrogen Plus Progestin trial (15). In another study, lumbar spine BMD was found to be inversely correlated with POP severity. The BMD

and T score of the femur neck were found to be significantly higher in the absent to mild POP group (8). Additionally, in a recent study, advanced POP was shown to be correlated with osteoporosis in Korean women aged 50 years and above (11). These results address a possible relationship between POP and BMD. In our study, in line with the results of these studies, BMD was found to be significantly lower in the high grade prolapse group in both the femur neck and lumbar spine. There we think, may be due to the same associated risk factors of BMD and osteoporosis, such as collogen and connective tissue disorders, advancing age, BMI (3-5, 16).

In contrast, Yoldemir et al. investigated whether there is a possible association between the presence of POP and osteoporosis. In this study, eighty-seven early postmenopausal women between the ages of 55 and 60 years were enrolled. And the results of this study, suggested that presence of pelvic organ prolapse in early postmenopausal women was not helpful in predicting osteoporosis (17).

Estrogen receptors were shown to play an important role in controlling skeletal growth and maintenance of bone mass and also in the etiology of POP (6). In a study held by Lang et al., serum estrogen levels and estrogen reseptor values were found to be significantly lower in the uterine ligaments of premenopausal women with pelvic organ prolapse, and there found a positive correlation between estrogen receptor values in the uterine ligaments and the duration of postmenopausal years (7). Also, it is known that, the decrease in the estrogen levels results in rapid bone loss and osteoporotic patients should be counseled regularly about cigarette cessation, alcohol intake, and estrogen status (18, 19). Similar to these studies, in our study, we found statistically significant difference between E2 levels of the groups showing that E2 may play a role in both diseases. While the median E2 levels of the high grade prolapse group was 10 (5-35), it was 26 (4-56) in the low grade prolapse group (p=0.019).

The strength of this study is that there we found no significant difference between the groups in terms of age and BMI. This can eliminate some factors that can affect the BMD. Moreover, this study differs from other studies by evaluating patients' FSH and E2 levels.

However, there are several limitations to this study. Once, being a retrospective study may limit the reliability of data. And also, in this study, there were 40 postmenopausal patients who were operated for POP. These numbers may not be sufficient to reach a reliable result. Another weakness is that lifestyle characteristics (eating habits, alcohol use, and exercise status of patients) and the duration of menopause were not determined.

In conclusion, in the current study, BMD and T score of the femur neck and BMD of the lumbar spine were found to be significantly higher in the low grade prolapse group than in the high grade prolapse group. So that, we think, there may be an association between the severity of POP and the risk of osteoporosis in postmenopausal women. Early recognition of patients under the risk of osteoporosis and fractures is very valuable since fractures increase morbidity and mortality. However, in order to apply the validity of these findings in the clinics, further studies are needed.

Conflict of interest

The authors declared no conflict of interest.

Funding

No funding was used for the study.

Acknowledgments

All procedures performed were in accordance with the ethical standards of the institutional committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 664-669
doi: 10.52142/omujecm.39.3.14

Objective and subjective voice evaluation in Covid 19 patients and prognostic factors affecting the voice

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Received: 15.12.2022 • **Accepted/Published Online:** 24.06.2022 • **Final Version:** 30.08.2022

Abstract

Coronavirus disease 2019 (COVID-19), a respiratory and systemic zoonosis form caused by a virus belonging to the Coronaviridae family. Although several studies have shown the otolaryngology symptoms are affected in COVID-19 patients, the number of studies regarding the COVID-19 effects on voice is limited. Our study aims to evaluate the effect of COVID-19 on voice objectively - subjectively and compare it with the control group. 50 hospitalized patients with laboratory-confirmed COVID-19 and 50 healthy individuals were included in the study as study and control group, respectively. All subjects were trained to vocalize a continuous/a/ vocal pattern at speech sound intensity for Maximum Phonation Time. Voice samples were recorded using a Sony (ICD-PX470) audio recorder and analyzed by the Praat program. Dysphonia grades were ranked on 4-point scales (grade: 0=none; 1= mild; 2= moderate; 3=severe). It is seen from the results that, there were significant differences between the male and female participants in acoustic parameters of fundamental frequency (F0) ($p<0.001$), shimmer and mean harmonic to noise ratio (HNR) ($p=0.011$). There was also a significant difference in F0 values of infected and healthy participants ($p=0.008$). However, there was no significant interaction between gender and health status in any acoustic parameters ($p>0.05$). The degree of thoracic computed tomography (CT) involvement had no significant effect on parameters ($p>0.05$), while there was a weak positive relationship between the duration of hospitalization and F0 ($rs=0.397$, $p=0.004$). Dysphonia was positively associated with health status ($rs=0.682$, $p<0.001$), and female infected participants reported more frequent dysphonia than males. In our study, we examined the effect of COVID-19 on voice both objectively and subjectively and evaluated the relationship between CT involvement and duration of hospitalization, which made our study more reliable. Future studies with larger and more specific patient groups to investigate the relationship between COVID-19 and dysphonia will shed a light on the subject.

Keywords: COVID-19, praat, acoustic analysis, dysphonia

1. Introduction

Coronaviridae can cause respiratory and gastrointestinal infections in animals and humans, settle in the lower respiratory tract, and cause pneumonia and death due to respiratory failure (1).

Phonation can be defined as the process of producing a human voice. The human voice is controlled by three systems that create energy, vibrate, and resonate. The lungs produce the energy to form a voice through high-pressure airflow during expiration, while vibration is produced by the vocal folds. Finally, resonance is provided by nearly all the structures above the glottis, which shape the vibrations produced by the vocal folds. Knowing the anatomophysiology of these systems helps us better understand the causes of voice disorders and the associated diagnosis and treatment processes (2, 3). We argue that some COVID-19 patients experience voice problems because the required airflow for phonation is restricted by COVID-related exhalation issues; other symptoms, such as a recurring dry cough and sputum, inhibit the systems creating vibration and resonance (3).

New clinical signs developed rapidly during the COVID-19 pandemic. Voice dysphonia or distortion has recently been identified as a COVID-19 symptom (4, 5). A quarter of patients with mild-to-moderate COVID-19 may experience dysphonia, which should therefore be added to the list of infection symptoms (4). Numerous etiological factors, such as postviral vagal neuropathy (the inflammatory factor causing vocal cord edema or inflammation), vocal cord injury due to strong coughing or vomiting, intubation injury involving vocal cord granulomas, vocal cord paralysis, cricoarytenoid joint dislocation, poor lung function, and psychogenic dysphonia, may be associated with dysphonia in COVID-19 patients (1, 4, 5). Dysphonic COVID-19 patients have been reported to be more symptomatic than non-dysphonic individuals (4).

Voice analysis was performed subjectively and objectively by an otolaryngologist (6). In subjective voice

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evaluation, the degree of dysphonia was evaluated using a 4-point scale (1, 4). Acoustic voice analysis is a valuable technique for diagnosing and monitoring voice disorders. The parameters obtained by acoustic analysis have the advantage of objectively defining voice rather than subjective perceptual analysis (3, 7, 8). Various types of software have been developed for acoustic analysis, including Praat, objective voice analysis software that is recommended by scientists and clinicians worldwide (9). Praat is a free, easy-to-use program that allows clinicians to obtain objective data at a low cost (6, 8, 10). Studies of vocal cord pathology have found that Praat is reproducible and reliable in distinguishing between normal and pathological voices. Because of these features, we used Praat to objectively evaluate the voices in our study.

This study aimed to evaluate the effect of COVID-19 on the voice objectively, using Praat, and subjectively, with a 4-point questionnaire, and compare it with the control group.

2. Materials and Methods

This study was performed between March and April 2021 in the Department of Ear, Nose and Throat Diseases at the Samsun Training and Research Hospital after obtaining approval from the Samsun Training and Research Hospital's Human Ethics Committee (Decision number: 2021/5/1). This study adhered to the rules of the Helsinki Declaration.

All participants were informed about the study and provided written informed consent for us to use their speech samples for research purposes.

Patient COVID-19 diagnoses were based on serological tests with COVID 19-specific IgM or IgG and/or reverse transcriptase-polymerase chain reaction (RT-PCR). Researchers applied current tests to patients with appropriate protective equipment to avoid viral transmission. All patients' age, gender, comorbid diseases, smoking status, and nasal surgery history were recorded. Additionally, the grade of dysphonia was assessed using a 4-point scale (grade: 0 = none, 1 = mild, 2 = moderate, 3 = severe).

The inclusion criteria were dysphonia according to a 4-point scale, an age of between 18 and 70 years, and providing informed consent. The exclusion criteria were a history of head and neck trauma or head and neck cancer surgery, previous head and neck chemoradiotherapy treatments, benign, or malignant laryngeal lesions, dysphonia in anamnesis, abnormalities of the vocal tract and/or auditory problems, laryngitis, history of asthma, being than 18 years old or older than 70 years old, no informed consent, and any previous formal voice training or voice therapy.

Table 1. Descriptive statistics for acoustic parameters

Control group			COVID Group		
	Mean+SD	Range		Mean+SD	Range
F0 (Hz)	247.491+30.3521	191.654-315.4210	F0 (Hz)	253.5240+33.0266	193.9410-311.0370
Jitter (%)	0.2599+0.1283	0.1200-0.6800	Jitter (%)	0.3367+0.1783	0.1040-0.7700
Shimmer (%)	2.9014+1.0623	1.4650-6.4980	Shimmer (%)	2.8695+1.2896	1.1780-6.8320
HNR (dB)	22.7119+3.2479	16.7640-28.7970	HNR (dB)	23.4114+3.4939	14.9160-30.4690
MALE					
Control group			COVID Group		
	Mean+SD	Range		Mean+SD	Range
F0 (Hz)	140.619+24.90	105.9930-213.2650	F0 (Hz)	161.6380+29.1236	117.4220-231.2720
Jitter (%)	0.2885+0.1178	0.1400-0.6190	Jitter (%)	0.3092+0.1257	0.1030-0.6360
Shimmer (%)	3.4294+1.6894	1.5740-9.4390	Shimmer (%)	3.7389+2.0823	1.1970-11.0730
HNR (dB)	21.3531+2.8389	17.370-27.309	HNR (dB)	21.2637+3.8514	14.2370-28.6570

There were 50 hospitalized patients (25 females, 25 males) with laboratory-confirmed COVID-19 (COVID group) and 50 healthy individuals (25 females, 25 males; control group) in the study as the study and control groups, respectively. Healthy participants were chosen using a simple random sampling method. The groups were similar in terms of age and gender.

Before starting the recording sessions, an otolaryngologist explained the recording process to each participant. The same otolaryngologist supervised all the recording sessions with the following safety precautions: a face mask, face shield, disposable gloves, and suit were worn. The recorder was also sterilized before and after each recording session using alcohol pads. All recordings were taken with a Sony (ICD-PX470) digital audio recorder with a sampling rate of 44,100 Hz and 16-bit quantization. The recorder was held 20 cm from the participants' mouths at a 45° angle. All participants were asked to say a vowel, /a/, as long as possible.

The acoustic parameters related to voice quality were extracted using Praat (version 6.1.40, Institute of Phonetic Sciences, University of Amsterdam, Amsterdam, Netherlands) (9) with default settings. The stable middle part of the vowel /a/ (3 seconds) was selected, and the fundamental frequency (F0), jitter (local, %), shimmer (local, %), and mean harmonic to noise ratio (HNR) in dB were obtained and recorded for further evaluation. Routine thoracic computed tomography (CT) of the COVID-19 patients was grouped according to normal, focal, and diffuse involvement,

and the relationship between the groups and acoustic parameters was evaluated. Patients without pulmonary involvement were accepted as normal, those limited to one lung lobe as focal, and those with widespread involvement in both lungs as diffuse. The correlation between the duration of hospitalization and acoustic parameters, dysphonia, and health status were also investigated. Table 1 presents the mean, standard deviation, and range of the parameters in the healthy and infected female and male participants.

2.1. Statistical analysis

Extracted data were analyzed using IBM SPSS Statistics Software (version 26; IBM, New York, USA). In all analyses, acoustic parameters were transformed using a natural logarithm to provide normal distribution. All estimates were back-transformed to the original scale and represented multiplicative effects on the geometric mean of acoustic parameters. A two-way MANOVA (Multivariate Analysis of Variance) was conducted to compare the main effect of gender, health status, and their interaction effects on F0, jitter, shimmer, and HNR.

3. Results

Gender was statistically significant ($p<0.001$), and the effect of gender yielded an effect size of 0.75, indicating that 75% of the variance in F0 was due to gender ($F(1,96)=287.281$, $p<0.001$). Additionally, gender affected shimmer and HNR ($p<0.05$), 4.7% and 6.6% of the variance in shimmer and HNR was attributable to gender ($F(1,96)=4.685$, $p=0.033$ and $F(1,96)=6.742$, $p=0.011$). The analysis showed a significant difference in F0 values between the healthy and infected participants ($p=0.008$). The interaction effect was not significant, indicating no combined effect for gender and health status on parameters F0, jitter, shimmer, and HNR with p-values of 0.064, 0.375, 0.550, and 0.511, respectively (Table 2). In order to determine whether CT involvement type had a statistically significant effect on the parameters, a one-way MANOVA was performed, and the test of the between-subject effect was determined. Based on the results, there was no significant effect on parameters ($p=0.119$, $p=0.277$, $p=0.377$, $p=0.683$), (Table 3). Spearman's Rho test results revealed a weak positive relationship between the duration of hospitalization and F0 ($rs=0.0397$, $p=0.004$), but there was no significant correlation for the rest. Additionally, there was a weak positive linear relationship between F0 and HNR ($rs=0.243$, $p=0.015$), a strong negative relationship between jitter and HNR ($rs=-0.676$, $p<0.001$), and a very strong negative correlation between shimmer and HNR ($rs=-0.813$, $p<0.001$), (Table 4).

Table 2. MANOVA results for gender and health status

	Parameters	Type III Sum of Squares	F Value	p	Partial η^2
Gender	F0	6.593	287.281	0	0.750
	Jitter	0.051	0.253	0.616	0.003
	Shimmer	0.837	4.685	0.033	0.047
	HNR	0.164	6.742	0.011	0.066
Health Status	F0	0.166	7.248	0.008	0.070
	Jitter	0.497	2.469	0.119	0.025
	Shimmer	0.002	0.012	0.913	0
	HNR	0.002	0.069	0.793	0.001
Gender * Health Status	F0ln	0.080	3.506	0.064	0.035
	Jitln	0.160	0.794	0.375	0.008
	Shimln	0.064	0.359	0.550	0.004
	HNRln	0.011	0.434	0.511	0.005

Table 3. MANOVA results for CT involment type

	Parameters	Type III Sum of Squares	F Value	p	Partial η^2
CT involment type	F0	0.532	2	0.119	0.059
	Jitter	0.785	1.305	0.277	0.039
	Shimmer	0.570	1.043	0.377	0.032
	HNR	0.039	0.5	0.683	0.015

Table 4. Correlation table between duration of hospitalization and acoustic parameters

		Days	F0	Jitter	Shimmer	HNRl
Days	r_s	1				
	p	.				
F0	r_s	**0.397**	1			
	p	0.004	.			
Jitter	r_s	-0.034	-0.086	1		
	p	0.815	0.394	.		
Shimmer	r_s	0.028	-0.144	**0.520**	1	
	p	0.848	0.153	0	.	
HNR	r_s	0.181	**0.243**	**-.676**	**-.813**	1
	p	0.207	0.015	0	0	.

rs : Spearman's Rho correlation coefficient

Table 5. Correlation table between dysphonia and health status

		Dysphonia	Health Status
Dysphonia	r_s	1	
	p	.	
Health Status	r_s	**0.682**	1
	p	0	.

r_s : Spearman's Rho correlation coefficient

Dysphonia was reported by a total of 34 patients. It was mild and/or moderate in 32 while severe for 2. 13 male and 21 female infected participants were dysphonic, and the female ratio was higher. Dysphonia was positively correlated with health status, and the correlation was strong between dysphonia and health status ($rs=0.682$, $p<0.001$), (Table 5).

4. Discussion

COVID-19 is an infectious disease caused by the coronavirus, starting by infecting the mucous membranes in the throat and descending the respiratory tract to the lungs, where cough is a common symptom (11).

Fever, fatigue, and dry cough are considered the most common manifestations of COVID-19. Anorexia, shortness of breath, sputum production, and myalgia has been reported in more than 25% of cases. Sore throat, rhinorrhea, headache, nausea and diarrhea common or frequent in mild or moderate disease. Cough, dyspnea, sore throat, rhinorrhea, nasal congestion, throat congestion, tonsil edema, enlarged cervical lymph nodes, or dizziness are symptoms that an otolaryngologist may encounter when examining COVID-19 patients (11, 12, 13).

Recent studies reported that mild to moderate COVID-19 patients exhibit a different clinical picture, and dysphonia has been observed in some COVID-19 patients (1, 3, 4, 5, 14).

Since the SARS-CoV-2 affects both the upper and lower airways, there are likely multiple possible causes for dysphonia with this viral infection. A study conducted in the Department of Anatomy at Mons University observed that vocal cords are associated with high expression of angiotensin-converting enzyme 2 (ACE 2), the COVID-19 receptor (4).

Therefore, dysphonia may result from the direct entry of SARS-CoV-2 into the glottic epithelium, resulting in infection and damage (15). ACE 2 receptors are also known to be present in the nasal and lung epithelium and abdominal and chest muscles. Thus, the larynx may also be indirectly affected by the inflammatory process of the nasal airway. Also, the effectiveness of voice production can be blocked by respiratory failure due to lung infection and muscle fatigue (4).

Acoustic voice analysis is considered a beneficial technique for detecting voice disorders. Subjective evaluation methods depend heavily on the experience of professionals and can lead to different results. This requirement encourages objective measurement of voice (6). Processing a speech signal is used to obtain a set of voice parameters. It allows detecting vocal cord pathologies or other related pathologies by comparing patients' data with other individuals with normal healthy voices (8). The objective evaluation of voice, especially acoustic analysis, has attracted our attention due to its relatively low cost, ease of application, and quantitative output.

Various types of software have been developed for acoustic analysis. Praat software (version 6.1.40) was used in our study. This software was first designed in 1992 by Paul Boersma and David Weenick of the University Of Amsterdam Institute Of Phonetic Sciences. Praat uses the best algorithms available in various operating systems, including the most accurate step analysis algorithm for free variation, articulation synthesis, and progressive learning algorithm (6, 10).

The prevalence of dysphonia in patients with COVID-19 in the literature was reported to be between 5.1% and 43.7% (1, 3, 4, 11). In our study, the prevalence of dysphonia was determined as 68%. This difference is thought to be due to the severity of the disease, epidemiological differences according to race, gender and age, the tests being objective and subjective, the test being performed by one or more researchers, the day the test performed, and the duration of the disease (7, 8, 10).

Based on this information in the literature, we evaluated the effect of COVID-19 on voice with parameters F0, jitter, shimmer, and HNR. We evaluated the relationship between the duration of hospitalization and the severity of lung involvement and voice parameters by a single researcher using the Praat program, an objective voice analysis, and subjectively evaluated it with a 4-point questionnaire and compared them with the control group.

In the study that Cantarella et al. (1) evaluated the degree of dysphonia with 4-point scales in outpatients diagnosed with COVID-19 by PCR test and by phone calls by physicians, they reported that the prevalence of dysphonia was 43.7% and that dysphonia was positively correlated with vocal fatigue, cough, rhinitis, and shortness of breath. They reported that one of the study's shortcomings was that the study was based on information gathered through telephone interviews and that patients reporting dysphonia were not subjected to an objective evaluation. They reported that dysphonia is a very common and long-lasting symptom in this series and has been underestimated to date.

In the study in which Lechien et al. (4) investigated the prevalence of dysphonia in patients with mild to moderate COVID-19 and clinical characteristics of dysphonic patients, they reported that the prevalence of dysphonia was 26.8% and that dysphonia developed more frequently in women than in men. They reported that dysphonic patients were more symptomatic than non- dysphonic individuals, and dysphonia should be considered as the symptom list of COVID-19. In this study, dysphonia was evaluated by the patients on a 4-point scale and an objective evaluation was not made.

In the study of Asiaee et al. (3), in which the acoustic parameters of voice were objectively evaluated between healthy individuals and COVID-19 patients, they reported significant differences between CPP, HNR, H1H2, F0SD, jitter, shimmer, and MPT parameters in individuals affected by COVID-19.

In our study, we increased the reliability of our study by using both objective and subjective methods to evaluate the voice. No studies have evaluated the effect of COVID-19 disease on voice both objectively and subjectively in the

literature. This study is critical because it is the first study to evaluate the effect of COVID-19 disease on voice with an objective and subjective method.

Due to high virus concentrations in the nasal cavity, nasopharynx and oropharynx and close contact of otolaryngologists with the upper respiratory mucosa of the patients, the highest nosocomial transmission rates were reported among otolaryngologists (8, 16, 17). The procedures that are especially at high risk of contamination are clinical and flexible endoscopic larynx examinations. We think that developing methods that automatically examine these voice analyses and help diagnosis and treatment will reduce the risk of disease transmission. More research is needed to develop these methods (8, 18).

One of the critical limitations of our study is the relatively small number of patients. We think that studies with a larger sample size may contribute to further knowledge.

Although the flexible endoscopic examination is a routine procedure in examining patients with complaints about voice in otorhinolaryngology practice, the fact that it could not be performed on every patient due to the risk of transmission in the COVID-19 pandemic is one of the deficiencies of our study.

We applied the voice analysis in COVID-19 patients who were followed up in the inpatient clinic and had mild and moderate symptoms. At the beginning of the pandemic, every patient who was positive for COVID-19 was hospitalized and the prevalence and characteristics of symptoms were more reliable. Since the pandemic progresses rapidly and the patient density increases, patients whose complaints do not regress with home treatment are now hospitalized, which causes us to clearly identify the prevalence and characteristics of the complaints in the early stages of the disease. Besides, since the test we used requires patient adherence, we believe that a testing method applicable to patients with severe complaints and patients hospitalized in the intensive care unit may change our study results. The rates of hoarseness among those with more severe illness and those in critical care are not known, but we think it will be higher. Performing voice analysis and repeating periodically for each patient with a positive diagnosis will ensure that our results are more reliable.

Analyzing the characteristic of voice has a substantial prognosis value as it can show the progression of a disease or the effectiveness of treatment. Dysphonia can be a silent symptom of COVID-19. Since COVID-19 is a disease affecting the upper and lower respiratory tract, we think that evaluating the characteristics of the voices obtained from individuals with a positive diagnosis will be essential in diagnosis, treatment, and prognosis. More research is needed to shed light on the pathophysiology of vocal disorders in COVID-19 patients.

Conflict of interest

None to declare.

Acknowledgments

None to declare.

Authors' contributions

Concept: A.Ç., B.K.E., Design:A.Ç., Data Collection or Processing: B.K.E., Analysis or Interpretation: A.Ç., Literature Search: A.Ç., B.K.E., Writing: A.Ç., B.K.E.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 670-674
doi: 10.52142/omujecm.39.3.15

Liver trauma in children with Syrian Civil War: How should treatment management?

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Received: 17.12.2021 • **Accepted/Published Online:** 06.06.2022 • **Final Version:** 30.08.2022

Abstract

Patients with liver injuries should be considered multidisciplinary cases, and the decision to operate should be made according to each patient's clinical and hemodynamic stability. In this study, we aimed to describe treatment management approaches and appropriate operation times for children with liver injuries resulting from the Syrian Civil War. A total 32 patients who were admitted to a pediatric surgery clinic between 2010 and 2020 with liver injuries resulting from Syrian Civil War were examined retrospectively. Patients were evaluated according to age, gender, type of injury, accompanying trauma, treatment modality, and mortality. A total of 21 patients were injured with shrapnel, while 11 patients suffered from blast effect injury. The mean pediatric trauma score of the patients was 5.2, while the mean pediatric trauma score of the six patients who died was 3.11. Liver suturing was performed in four patients due to bleeding. A segmentectomy was performed in one patient with active bleeding in segment 7. This bleeding was stopped by ligating the branches of the hepatic artery. Two patients who were operated on in Syria and to whom packing was applied due to uncontrolled bleeding were referred to Turkey. One patient with inferior vena cava injury died due to excessive blood loss and instability at the time of admission, and six patients died due to accompanying head trauma and/or multiple body trauma. The main purpose in emergency operations is to stop bleeding. Rarely, however, suturing or even segment resection in the bleeding area may be required.

Keywords: blast effect, liver, laceration, shrapnel, Syrian Civil War

1. Introduction

The liver is the most commonly injured intra-abdominal solid organ (1). Whether the trauma is penetrating or blunt, the most common cause of morbidity and mortality is bleeding (2). Most liver damage occurs in the posterior segment of the right lobe (3). The management of liver injuries is a clinical challenge for pediatric surgeons, especially in children with multiple traumas. Although penetrating traumas are uncommon, it remains a challenge to determine optimal surgical timing (4). Fortunately, isolated injuries of the extra hepatic bile ducts are rare. Moreover, long-term sequelae are infrequently seen due to the highly regenerative capacity of the liver (5).

While aggressive operative approaches were more popular at the beginning of the twentieth century, non-operative treatment was replaced by the surgical approach with the development of imaging modalities after World War II (6, 7). Thus, patients with liver injuries should be considered multidisciplinary, and the decision to operate should be made according to each patient's clinical and hemodynamic stability. Computed tomography (CT) the preferred imaging method for evaluating post-traumatic abdominal and pelvic injuries in children with stable hemodynamics.

Types of liver damage include laceration, hematoma (subcapsular or intraparenchymal), active hemorrhage, hepatic vascular injury, A-V fistula, and bile duct injury. Lacerations are the most common type of liver parenchymal injury and they are observed as hypodense areas that extend linearly or show branching on contrast-enhanced CT (8).

Since most bleeding that develops as a result of liver injury in children stop spontaneously without the need for operation (9), it is necessary to evaluate the patient's laboratory findings and CT findings together, and then the decision for the operation should be taken. While rapid physical examinations and further investigations are performed in stable patients, unstable patients, despite resuscitative efforts, should be taken for emergency laparotomy (10).

In this study, we aimed to describe treatment management and appropriate operation timing in children with liver injuries resulting from the Syrian Civil War.

2. Materials and Methods

2.1. Study design and patients

All patients with liver injuries due to the Syrian Civil War who were admitted to the Department of Pediatric Surgery between 2010 and 2020 were included the study. Patients who had already been operated on in Syria and who did not require

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further re-operation were excluded from the study. The permission of the ethics committee was granted with decision number 10 (dated May 02, 2019).

Patients were evaluated according to age, gender, type of injury, pediatric trauma score (11), accompanying trauma, treatment modality, and mortality. All patients who had firearms injuries and were brought to the emergency department received a rapid physical examination and appropriate fluid resuscitation.

The presence and degree of laceration were evaluated with CT imaging. The most important determinant of the decision to whether operative management or non-operative management was the assessment of hemodynamic stabilization. The most important determinant of the decision to operate was the lack of hemodynamic stabilization (12). The oral intake of the patients who were decided to be followed by non-operative treatment was discontinued, and appropriate fluid treatment and antibiotherapy were initiated. These patients were also immobilized.

2.2. Statistical method

The Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) version 18.0 for Windows was used for statistical analysis. Categorical variables were arranged by frequency (n, %), and scaled measurements were arranged with mean ± standard deviation. Since our data showed a normal distribution, the mean was used as a measure of central tendency, and the standard deviation was used to show the spread.

3. Results

The mean age of the patients was 8.1 years. A total of 25 (78%) patients were male, and 7 (22%) were female. While 21 patients had been injured with shrapnel, 11 patients had blast effect injury. The mean pediatric trauma score of the all patients was 5.2±2.94, while the mean pediatric trauma score of six patients who died was 3.11±1.86.

In terms of injury grading according to the Organ Injury Scale of the American Association for the Surgery of Trauma (13), four patients had grade 1 injuries, seven patients had grade 2, nine patients had grade 3, seven patients had grade 4, and five patients had grade 5 injuries. Further, eleven patients had contusions in the lung, eight patients had intestinal perforations, three patients had head trauma, two patients had right kidney lacerations, two patients had colon perforations, two patients had fractures in the ribs and extremities, two patients had splenic injuries, one patient had a pancreatic injury, one patient had diaphragm perforation (Fig. 1), and one patient had an inferior vena cava injury (Table 1 and Table 2).

All operated patients had grade 4 or 5 liver laceration. It was noted that liver segments 7 and 8 were damaged in all operated patients. Liver suturing was performed in four patients due to bleeding. A segmentectomy was performed in a patient with active bleeding in segment 7. Bleeding was stopped by binding the branches of the hepatic artery. Four patients who had been operated on in Syria and had packing applied due to unstoppable bleeding were referred to Turkey (Fig. 2).

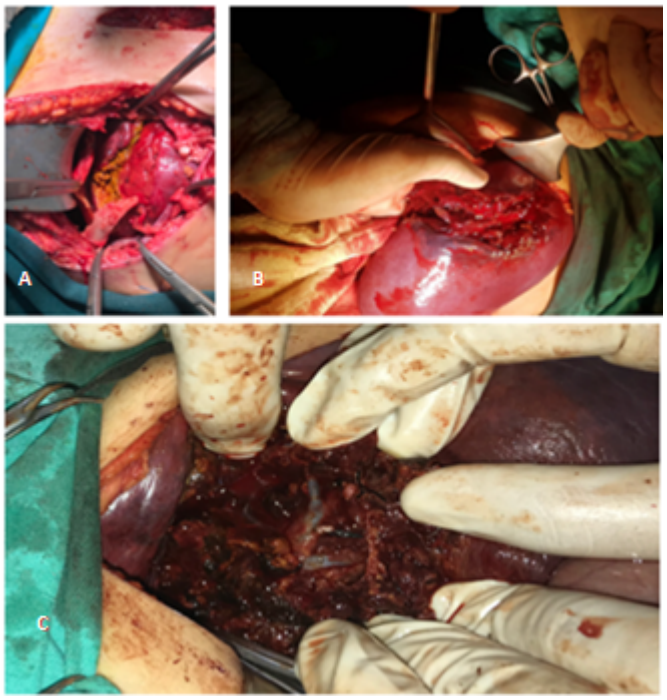

Fig. 1. a: Liver injury with diaphragm rupture and rib fracture places held by clamp are diaphragm edges **b:** Liver parenchymal laceration **c:** Intraoperative view after resection of the lacerated segment which active bleeding in the liver

Table 1. Clinical features of patients

	n	%
Number of Patients	32	
Male	25	78
Female	7	22
Mean age all of the patients	8.1	
Male	7.42	
Female	9.25	
Type of injury		
Penetrating	21	65
Blast	11	35
Grade of injury		
Grade 1	4	12
Grade 2	7	22
Grade 3	9	28
Grade 4	7	22
Grade 5	5	15
Accompanying injuries		
Pulmonary laceration	11	34
Intestinal perforation	8	25
Head trauma	3	9
Right kidney laceration	2	6
Colon perforation	2	6
Fracture of the ribs and/or extremities	2	6
Spleen injury	2	6
Pancreatic injury	1	3
Diaphragm perforation	1	3
Vena cava inferior injury	1	3

Table 2. Features of patients undergoing surgery

	Age/Sex	Degree of liver injury	Treatment	PTS*	Type of injury	Hemoglobin** (g/dL)	Hematocrit** (%)	Survey
Patient 1	4 years/male	5	Primer suturation	5	Shrapnel	7.4	21.5	Healing
Patient 2	13 years/male	4	Primer suturation	2	Shrapnel	13.6	39.7	Healing
Patient 3	12 years/male	4	Primer suturation	1	Blast	10.1	30.2	Healing
Patient 4	11 years/male	4	Primer suturation	4	Blast+shrapnel	8.8	25.1	Healing
Patient 5	7 years/male	5	Segmentectomy	6	Blast	6	18.9	Healing
Patient 6	15 years/male	4	Packing removed	11	Shrapnel	12	35	Healing
Patient 7	12 year/male	4	Relaparotomy	10	Blast+shrapnel	10.2	32.7	Healing
Patient 8	13 years/female	4	Relaparotomy	4	Blast	6	18.9	Healing
Patient 9	15 years/female	4	Laparotomy	7	Shrapnel	8.1	24.6	Healing
Patient 10	8 years	5	Inferior vena cava repair+primer suturation	-1	Shrapnel	4	12.1	Exitus

*Pediatric Trauma Score

** Values of the patients on arrival who were taken into surgery

When the compressions were removed in relaparotomy, it was observed that active bleeding had stopped. Only one of the operated patients died. The patient with the inferior vena cava injury died due to excessive blood loss and instability at the time of admission. Six patients who followed by non-operative management died due to accompanying head trauma and/or multiple body trauma.

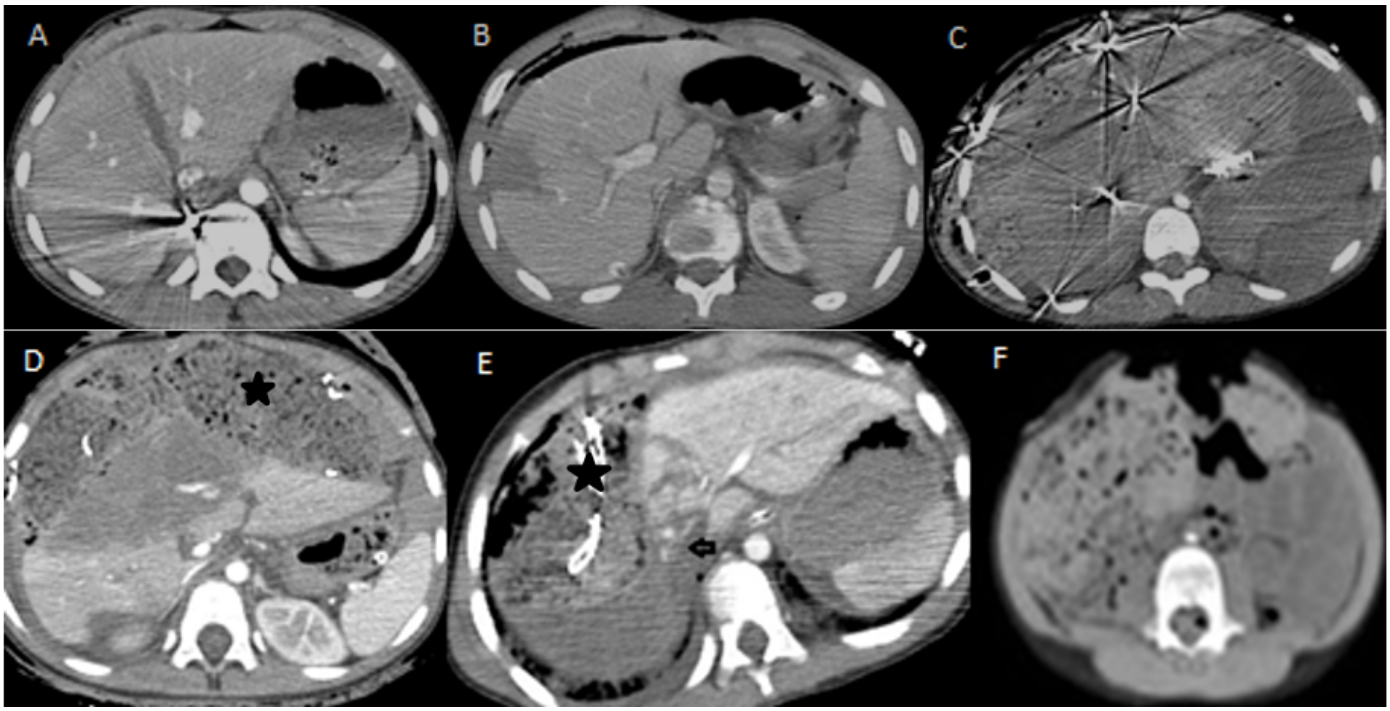

Fig. 2. Lesion patterns on CT images. **A.** A laceration with parenchymal extension of more than 3 cm, and shrapnel particle, **B.** Intraparenchymal hematoma with laceration, **C.** Multıple shrapnel particles in liver parenchyma, **D.** Oversized devascularized area and contrast extravasation, buffer placed in Syria for bleeding control (blackstar), **E.** Large devascularized area in the right lobe of the liver, hepatic vein injury (blackarrow) and buffer placed in Syria for bleeding control (black star), **F.** Packing with open abdomen

It was observed that bile drainage from the abdomen of the patient who underwent liver segment resection began on the fourth postoperative day. Bile leakage stopped spontaneously on the postoperatively 26th day. None of the patients developed late complications such as hemobilia, biliary peritonitis and ileus.

All ex-patients had comorbid organ damage. All these patients had thoracic injuries (6/6, %100), 3 patients (3/6, %50) had cranial injuries and 3 patients (3/6, %50) had abdominal trauma. One patient had major vascular injury. Interestingly, all of the ex-children had injured with blast effect (6/6, %100). The majority of patients (n=16, 80%) were brought to the hospital at night. Twelve patients (60%) did not have any family members at the time of admission. Two patients (40%) were brought from battlefield with injured other family members at the same time. Therefore, clear information and medical history about the injured children could not be obtained.

4. Discussion

According to the UN High Commissioner for Human Rights, more than 1,900,000 civilians were killed in the war in Syria between 2011 and 2014, and more civilian were injured due to

weapons and bombs. 85.1% of the dead civilians were males and 9.3% were females (14). Similar to the literature, in our study; 78% of the patients were male and 22% were female. The majority of patients are male, and extremity injuries in adolescent age group suggests that children are actively used in war.

Trauma surgery in war condition has many different features according to civil trauma surgery. Weapons have more energy, so more destructive (15). In a study, it was observed that 67% of ex children in battlefield had injured by blast effect (16).

Despite its not seen an external injury, blast trauma may be more mortal than penetrant trauma due to affects the whole body. Explosions, especially in closed areas, may be more lethal than in open areas. The determination of trauma related blast injuries is more difficult than the detection of penetrating trauma. Radiological examination is valuable in these patients (17). Invasive procedures should be avoided to save time, especially in injured children. CT scans should be the most preferred imaging method for patients with multisystemic injuries (18). CT shows intraabdominal extraperitoneal and intraperitoneal hemorrhages as well as solid organ injuries. In addition, intestinal perforation, bone damages, subcutaneous soft tissue damage, pulmonary injuries and central nervous system injuries within the image can be detected. In our study, 13 of our patients had shrapnel injury while 7 patients had blast effect injury. 6 patients (6/7, 86%) who had blast injuries were observed to be ex because of the accompanying multiple traumas.

Liver damage mostly occurs in the posterior part of the right lobe. Because the posterior right lobe is fixed by the coronary ligaments so when the liver is exposed to trauma, the movement of this segment is restricted while the rest of the liver is free to move (10). In our study, similar to literature, liver segments 7 and 8 were damaged in all operated patients.

Accompanying injuries are one of the most important obstacles to the success of the treatment. It has been reported that cranial injury, thoracic injuries and major vascular injuries increase morbidity and mortality significantly. In addition, retroperitoneal organ injuries, such as duodenum, cause high mortality (6). According to Iraq and Afghanistan war, it was observed that there was relationship between thoracic trauma, head trauma, severity of trauma, large vessels trauma and mortality (19, 20). In our study, among dead patients, all of these had lung contusion, 3 patients had cranial injury, 1 patient had major vascular injury, 1 patient had non-functioning spleen and 1 patient had pancreatic laceration. And also, severe trauma (pediatric trauma score<6) was seen in all these patients. These results were similar to findings of Iraq and Afghanistan war.

Transfer time of the injured from the battlefield to the hospital is closely related to mortality. The first sixty minutes are known as “golden hours” (19). Almost all of our patients were not brought in this first hour. These patients were brought to the emergency service at night.

Approximately 80-90% of hepatic injuries can be managed safely without operation (21, 22). Hemodynamic stability is the main factor in determining whether or not conservative treatment can be applied (23). In a study reported that in children, organ injury scales are not determinative factor for operative management. Because regardless of the severity of trauma bleeding generally stops spontaneously so most frequently liver injuries can be successfully managed nonoperatively (10). Conservative treatment is possible in patients with blood pressure greater than 80 mmHg, heart rate less than 120/min, blood transfusion requirement less than half the blood volume. Bleeding stops spontaneously in more than half of liver traumas. The liver has an incredible post-traumatic self-healing capacity. The most important among the non-surgical management criteria are; hemodynamic stability is the absence of other abdominal organ injuries. Grade I and grade II liver injuries should be observed with hematocrit follow-up and bed rest. More severe injuries should be followed up in the intensive care unit if hemodynamics are stable. In some studies, it has been reported that 92% of patients with grade IV and grade V liver injury will develop complications that will require intervention. Interventional radiology may be required to embolization with angiogram for stop bleeding or to percutaneously drainage for biloma or abscess (6, 24). Many surgical approaches such as simple hemostasis, liver suturing, segment resection, lobe resection and abdominal packing have been described in liver injuries. It has also been reported that hepatic resection whether anatomic or nonanatomic has a high mortality rate when performed under emergency conditions (25, 26). In a study, the operative mortality rate of trauma patients who operated for grade 4 and grade 5 liver injury was reported as 66% (21). In the majority of recently studies in the literature, the mortality rate in grade 4 and grade 5 liver injury has been reported to 15% (6). In our study, nearly 70% of the patients were followed conservatively. Since all our patients were brought under war conditions and at night, open surgery was performed for all operated patients. Segmentectomy was performed in a patient who already had avulsion injury. In spite of being an old method, packing can be applied in unstoppable hemorrhages in such desperate situations as war environment. Compressions filled in the bleeding area should be removed with a second laparotomy after 48-72 hours. In our study, packing had performed on two patients and it was observed that hemorrhages stopped spontaneously in the laparotomy.

As a result; the widespread use of high-quality radiological imaging systems has been an important support in non-operative management. Noninvasive imaging methods provide an important and noninvasive assessment of the severity of the injury, as well as valuable information in the patient's follow-up. Patients should be hemodynamically stable and have no

signs of peritonitis in order to perform nonoperative management (27).

Careful physical examination and vital signs should be followed in patients who are decided to undergo nonoperative management (26). Patients who have firearm injury of the liver to be treated nonoperatively should be selected very well. The main purpose in emergency operations is stopping the bleeding. Rarely though, suturing or even segment resection in the bleeding area may require. Reducing the transfer time from the battlefield to the hospital and knowing the applied first medical interventions especially in the medical center close to the battlefield will reduce the mortality, thus it can be avoided from unnecessary operation and cost.

Conflict of interest

The authors declare that they have no conflict of interest to the publication of this article.

Funding

The authors declare that there is no funding.

Acknowledgments

None to declare.

Authors' contributions

Concept: M.E.Ç, A.A, Design: M.E.Ç, Ç.E, Data Collection or Processing: M.E.Ç, İ.K., B.A, Analysis or Interpretation: M.E.Ç, B.A., A.A, Literature Search: M.E.Ç, İ.K., Writing: M.E.Ç, Ç.E.

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Research Article

J Exp Clin Med
2022; 39(3): 675-680
doi: 10.52142/omujecm.39.3.16

Effectiveness of robotic assisted laparoscopic nissen fundoplication in neurologically impaired children with severe gastroesophageal reflux disease

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Received: 06.01.2022 • **Accepted/Published Online:** 18.04.2022 • **Final Version:** 30.08.2022

Abstract

Laparoscopic Nissen Fundoplication is still the most widely used surgical technique in the treatment of gastroesophageal reflux disease (GERD) in children. However, there are some technical and anatomical difficulties in the treatment of reflux in NIC with GERD. All these difficulties lead to the high rate of surgical failure observed in neurological impaired children (NIC). Robotic surgery provides several advantages in overcoming these difficulties. This study is the first study to evaluate the effectiveness of robotic surgery in the treatment of GERD in children with severe or moderate neurological impairments reported in Turkey. This study took place between January 2018 and February 2020. We retrospectively evaluated the records of eleven children with severe or moderate neurological problems who were treated using the robotically assisted laparoscopic Nissen fundoplication (RALNF) technique for demographic data, anesthesia time, pre-console time, console time, and postoperative complications. Nine of the patients (81.8%) had serious and two had moderate neurological problems. All of the patients applied to the emergency department at different times due to recurrent lung infections. While the first RALNF console time was 240 minutes, this study revealed that this time decreased to 45 minutes. None of the patients had complications related to the surgical procedure, but four patients required postoperative intensive care unit up to two months due to several problems related to NIC. RALNF can be safely applied to pediatric patients with GERD with severe and moderate NIC.

Keywords: fundoplication, gastroesophageal reflux, laparoscopy, neurologic disorders, robotic surgical procedures

1. Introduction

Gastroesophageal reflux disease (GERD) is a common and important gastrointestinal tract disease of childhood (1, 2). Neurologically impaired children (NIC) have an increased risk for GERD and GERD dependent comorbidities, such as recurrent upper and lower respiratory tract infections, severe feeding and growth (3). Serious neurological damages in these children result in frequent emergency service visits and persistent pulmonary problems, such as persistent coughing, asthma, apnea and apparent life-threatening situations (4). Thus, the development of progressive lung infections and chronic lung diseases is often inevitable (5). Symptomatic GERD incidence has been reported as high as 20-30% in NIC. These high rates are mostly due to several sub factors causing decreased esophageal acid clearance and increased reflux episodes. Medical treatment is the first choice in NIC with GERD. However, unsuccessful medical treatment brings surgical options to the fore. Open or laparoscopic Nissen fundoplications are the most acceptable surgical techniques in NIC with GERD. While recurrence rates after open or laparoscopic Nissen Fundoplication in non-NIC have been reported as 2-5%, they were reported as high as 45% in NIC. Therefore, surgical treatment of GERD in NIC is still a significant challenge for surgeons and patients (3, 6, 7). Laparoscopic anti-reflux surgery is still the first preferred technique in children (5). However, Laparoscopic Assisted Nissen Fundoplication (LANF) has some disadvantages, especially for surgeons, such as the need for advanced laparoscopic skills, difficulties in the exposure of the esophageal hiatus, knot tying in a very small space and dissection of the gastro esophageal junction, etc. (8).

The first cases for robotic assisted laparoscopic surgery of GERD were reported in 2001 (9). Afterwards, robotic assisted laparoscopic surgery for GERD has been widely accepted and performed. One of the reasons for the development of Robotic surgery (RS) is to overcome the difficulties present in traditional laparoscopic surgery (10). RS has some unique advantages for surgeons and patients while performing fine surgical techniques in small spaces (8, 11, 12).

Endowrist function which allows 180° articulation and 540° rotation and greater 3D optical magnification, delicate instrument motion, tremor filtration, operator controlled highly versatile camera movement, elimination of the fulcrum effect are the striking features of the Robotic Surgical system

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(Da Vinci® surgical system, Sunnyvale, CA, USA) (8, 10, 13).

There are only limited data about the effectiveness of RALNF in NIC with severe GERD.

2. Materials and Methods

This study was designed according to the Helsinki Declaration and approved by the Ethics committee of the hospital (10.03.2020/ 46418926). Between January 2018 and February 2020, we reviewed retrospectively eleven children with NIC who were operated for GERD. All of the patients were diagnosed with GERD according to Rome 4 criteria and followed by an experienced pediatric gastroenterologist. We performed RALNF on the patients who were resistant to medical treatment. We evaluated the patients' socio-demographic data, body weight, body mass index, associated disorders, intraoperative findings, operative protocols, complications and operation time and length of hospital stay.

2.1. Surgical techniques

The patients were accepted to the clinics a day before the operation. Fig. 1 shows the patient, robot and trocar positions. After fixing the patients to the table for the operation, we gave the table Trendelenburg position close to 30 degrees (10-30°). One of the main differences between LANF and RALNF is the positions of the trocars. Due to the length of the robotic arms and instruments and the thoracoabdominal deformities of the patients, trocar access sites are customized for each patient.

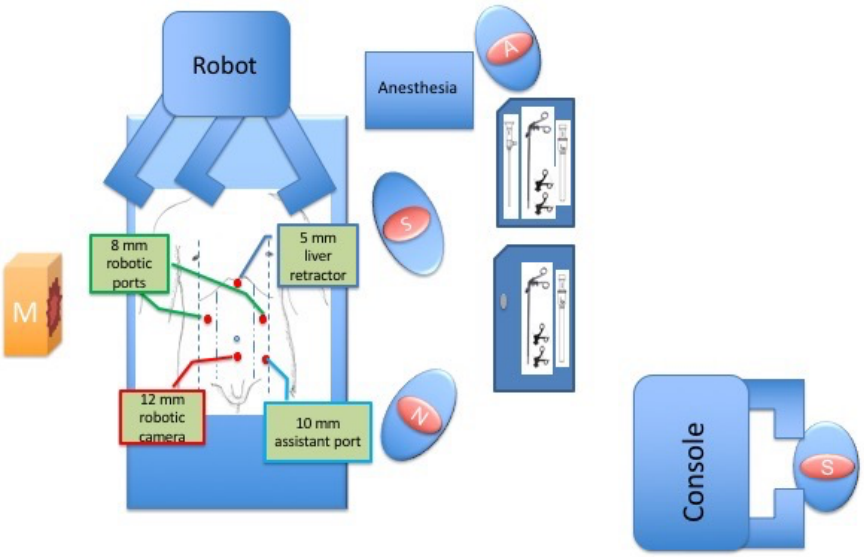

Fig.1. Operation room set up, patient, robot and trocar position during RALNF

We used the Da Vinci SI® surgical system (Intuitive Surgical, Sunnyvale, CA, USA) with a camera arm and three instrument arms. As standard, we used one camera port (12 mm), two working ports (8 mm) and 5 mm incision for liver retractor (Strong Arm™, Nathanson liver retractor system, USA), and 10 mm trocar for an assistant port (we used an additional assistant port for the use of steppes for a patient, who also underwent gastrojejunostomy). All surgical procedures were performed by two pediatric surgeons.

We selected the umbilical trocar position according to the patient's body length; the younger the child, the lower the trocar entry. We used Maryland® bipolar forceps, needle driver and the harmonic scalpel (Ultracision, Ethicon, Cincinnati, OH, USA) for robotic arms in all cases and selected hook cautery or scissors according to availability. We found hook cautery more useful due to articulation.

We drove a laparoscopic grasper through the left robotic trocar to grasp the stomach of children who required gastrostomy and performed gastrostomy under camera view on two layers using 2-3 / 0 polyglactin (YUCE, Turkey).

2.2. Definition of terms

Severe neurological impairment: Bed dependent neurological problems which result in motor and cognitive impairments.

Moderate neurological impairment: Non-bed dependent children with a lesser degree of motor and cognitive impairments (13).

Pre-console time: Starts from placing the patient on the operating table and ends with the surgeon sitting on the console

Console time: Time between the end of the pre-console time and leaving the robot

Docking: Attachment of the robotic system to the patient and trocar placement

Anesthesia time: Includes the total time that the patient was taken to and removed from the table.

3. Results

We included in the study eleven children who were followed up for GERD and underwent RALNF. Two of the patients were girls, and nine were boys. The median age of the

patients was 10 (range: 25 months- 17 years). The median body weight was 19 kg (range: 8-44 kg), while the median body mass index was 16.7 kg/m^2 (range: 7.7-36.4 kg/m2). Nine of the children had cerebral palsy (CP) (81.8%), while seven had coexistent epilepsy (63.6%). One patient had pons hypoplasia with corpus callosum agenesis, while the last one had Klinefelter's syndrome with CIT gene mutation (citron rho-interacting serine/threonine kinase) and severe growth retardation (less than 1% percentile). One patient without CP had HERC2 and BCKDHA genes associated with maple syrup urine disease (MSUD) and severe respiratory insufficiency (Table 1). Two of the patients were moderate, while nine were severe NIC patients.

Table 1. Demographic and clinical features of eleven children treated using the RALNF technique

#	Gender	Age (year)	Height (cm)	Weight (kg)	BMI	ASA score	Surgery procedure	Notes	TLHS (day)	Additional disease
1	W	11	150	33	14,70	II	HHR+ RALNF + G	ICU	10	CP + Epilepsy + PEG(+)
2	M	16	150	42	18,70	III	HHR+ RALNF + G	ICU	1	CP + Epilepsy
3	M	2(+1 month)	70	8	16,30	II	HHR+ RALNF + G	ICU	2	CP + Epilepsy+ Pons hypoplasia + DCCH
4	M	11	110	44	36,40	II	HHR+ RALNF		7	CP
5	M	8	100	30	30,00	III	HHR+ RALNF + G		6	CP + Epilepsy + Tracheostomy
6	M	17	130	31	18,30	II	HHR+ RALNF + G+GJ		7	CP + MMR
7	M	17	100	17	17,00	II	HHR+ RALNF + G	ICU	2	CP + Epilepsy+ MMR
8	W	6	150	19	8,40	II	HHR+ RALNF + G		6	CP + Epilepsy
9	M	4	100	12	12,00	II	HHR+ RALNF + G		4	KS+ Epilepsy + CIT gene mut + MMR
10	M	10	140	15	7,70	III	HHR+ RALNF		6	CP
11	M	2(+3 month)	98	16	16,70	III	HHR+ RALNF+ G		6	MMR + HERC2 and BCKDHA+ PEG(+)

DCCH: Diffuse corpus callosum hypoplasia; PEG: percutan endoscopic gastrostomy; G: Gastrostomy; TLHS: Total length of hospital stay; CP: cerebral palsy; ASA: American Society of Anesthesiologists risk score; RALNF: Robotic assisted laparoscopic Nissen fundoplication; KS: Klinefelter syndrome; MMR: mental motor retardation; HHR: Hiatal hernia repair; GJ: Gastrojejenostomy; ICU: intensive care unit

Two children had big hiatal hernia detected by an upper gastrointestinal contrast study.

The surgical protocols of patients were as the following: Eight children (72.7%) RALNF and gastrostomy, two children (18.1%) RALNF and one child (9%) RALNF, gastrojejunostomy and gastrostomy (Table 1).

Two patients had a previously opened percutaneous endoscopic gastrostomy tube. Gastrostomy was taken down by RS, and gastrostomy was performed again after fundoplication.

The mean total anesthesia time was 4.5±1.39 hours, while the mean pre-console time was 81.2±13.8. The mean docking time was 26.7±4.5 min, while the mean console time was 145±84.7 min (Table 2). We completed all of the RALNF procedures successfully without any need for conversion to open or laparoscopic surgery. No intraoperative complications were observed, and no need arouse to give blood for any reason. Three children were followed up in the intensive care unit due to their associated diseases. We used pethidine HCl (1 mg/kg/dose) and paracetamol (10 mg/kg/dose) for the first postoperative day and two to four days, respectively. We removed the nasogastric tube right after the operation. We started oral feeding/gastrostomy on postoperative day two and increased it gradually according to the patients' tolerance. The mean length of hospital stay was six days (range: 4-10 days). Two children, who were discharged before without any complications, were readmitted with fever to the clinic, caused by non-operative problems. The mean follow-up period was 17.5 months (range: 7-34 months). There were no major complications after RALNF. However, considering our experiences from Laparoscopic Nissen fundoplication, trocar side wound healing was better than RALNF. A patient died from an oncological problem on postoperative month eight.

Superficial and limited liver injury occurred in two patients due to liver retraction. We observed in all patients liver enzyme elevation which decreased to normal values in a short time. None of the patients had vagal nerve, spleen or esophageal injury. We observed no recurrence during the follow-up period.

Table 2. Surgical time subdivisions of the eleven children

Case no	Anesthesia time (h)	Pre-console time(min)	Docking time (min)	Console time (min)	Follow-up time (months)
1	6,50	78,00	35,00	240	34
2	7,00	88,00	30,00	300	24
3	3,00	84,00	20,00	60	22
4	3,50	75,00	22,00	90	21
5	4,50	75,00	25,00	180	17
6	6,00	90,00	30,00	240	17
7	4,50	116,00	24,00	100	15
8	4,00	80,00	25,00	90	15
9	3,50	72,00	27,00	90	12
10	3,00	65,00	24,00	45	8
11	4,50	70,00	32,00	160	8

4. Discussion

There is limited data about RALNF in NIC with GERD. The surgical treatment of NIC with GERD continues to be a challenging problem due to some difficulties unique to such patients. Severe musculoskeletal deformities (severe scoliosis, kyphosis, etc.), reduced lung reserve caused by recurrent lung infections (fig. 2), epilepsy which causes wrap dehiscence, and migration are only some of such difficulties (15, 16).

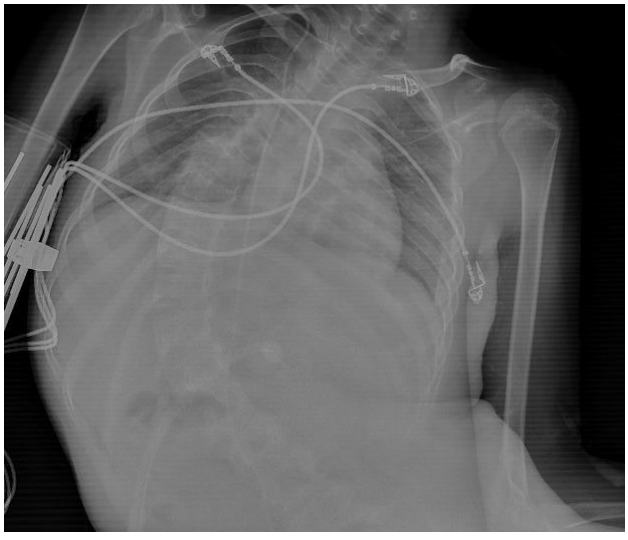

Fig. 2. Thoraco abdominal X-ray of patient #7, the patient has severe scoliosis and upper and lower extremity contractures

During open or laparoscopic Nissen fundoplication in NIC with GERD, extreme abdominal wall deformities and limb contractures are usual challenges limiting the exposure of the esophageal hiatus and the dissection of the intrathoracic esophagus essential for a successful surgical procedure. These difficulties are also responsible for the high surgical failure rates in NIC with GERD (15, 16). Robotic surgery makes significant contributions to successfully coping with these common difficulties in the surgical treatment of children with NIC with GERD.

Today, robotic surgery (RS) is frequently used by some surgical disciplines such as urology, general surgery, gynecology, cardiovascular surgery and is accepted as the gold standard for the treatment of some diseases such as prostatic cancer. However, RS has some disadvantages for routine clinical use in pediatric surgery. Some of these disadvantages are the instrument size, high cost, difficulties in reaching the robotic system due to their limited number and resistance to the new surgical technique to a certain extent. After reporting the first pediatric RALNF case in 2001 (10, 13), an increasing number of pediatric cases have been operated using robotic techniques. This study is the first pediatric RALNF case series from Turkey.

Unlike adult patients, a significant proportion of children undergoing anti-reflux procedures have neurological sequelae and serious additional pathologies (17, 18). In our series, all patients had severe or moderate neurological impairments. Serious advantages of the RS are a short learning curve thanks to articulating instruments, 3D high definition imaging, and fine surgical simulation capacity (8, 19). While the total surgical time (including pre-console and console times) was 6.5 hours in the first RALNF and gastrostomy case, it was 7.5 hours in the last two (case 1: RALNF + gastrostomy, case 2: RALNF) operated the same day. Comparison of the console time between the first and the cases revealed that the time reduced from 240 minutes to 45 minutes (Table 2). These rates are approximately similar to the learning curves specified in the literature (8, 17, 20).

Unlike LANF, re-positioning the patient after performing "docking" in RS significantly prolongs the operation time. In our cases, the most important factor causing the pre-console time to be longer was the positioning difficulties of patients with severe limb contracture. All of the patients except one had moderate to severe upper, lower or both extremity contractures, significantly extending the docking time despite increasing experience. Unfortunately, this problem persists as a challenge in either RALNF or LANF. Anderberg et al. (1) showed that robotic fundoplication in children was comparable to conventional laparoscopic surgery in terms of operative time, postoperative pain, and postoperative hospital stay. A study showed that long pre-console time in RS was compensated with faster dissection and knot tying (21).

The most common acute surgical complications of Nissen fundoplication are *N.vagus* damage, esophageal perforation, and spleen and liver injuries, which are also similar in LANF

and RALNF except with different rates (22).

In our series, the length of hospital stay time was too long, especially for four patients due to severe neurological and pulmonary problems which needed intensive care unit treatment up to two months. The mean length of hospital stay was six days (range: 4-10 days) for the remaining seven patients (Table 1).

Probably the most commonly accepted disadvantages of RS are the high price of robot and robotic instruments, in addition to high annual maintenance expenses, which seriously limit the usage of robotic surgical systems (1). The average cost of a surgical procedure using two robotic arms (excluding annual maintenance costs) is about US$ 2000. Another problem in Turkey is that pediatric robotic cases can still not be billed due to the lack of appropriate procedure codes.

This study had several limitations such as small case numbers, short follow-up periods and the patient groups including only those with severe neurological and pulmonary disabilities.

The usage of the robotic surgical system in pediatric cases has some difficulties caused by their design for adults. We hope the problem will be solved with an increasing in the number of pediatric cases. RALNF could be performed safely in children with severe or moderate neurological impairments. However, low cost-effectiveness and adult size instruments are the most important problems of RS in pediatric cases. Solving such problems will lead to the RS being used more commonly in the treatment of GERD in NIC.

Conflict of interest

The authors declared no conflict of interest.

Funding

No funding was used for the study.

Acknowledgments

The study was designed according to Helsinki Declaration and approved by Ethics committee of hospital (10.03.2020/ 46418926). All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version. Additionally, there are no conflicts of interest in connection with this paper, no financial support in connection with this article, and the material described is not under publication or consideration for publication elsewhere.

Authors' contributions

Concept: M.U., T.A., Design: M.U., T.A., Data Collection or Processing: M.U., T.A., Analysis or Interpretation: M.U., T.A., Literature Search: M.U., T.A., Writing: M.U., T.A.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 681-685
doi: 10.52142/omujecm.39.3.17

Peculiarities of the physical growth of school children and teenagers with chronic diseases of the gastroduodenal area

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Received: 13.01.2022 • **Accepted/Published Online:** 29.11.2022 • **Final Version:** 30.08.2022

Abstract

The objective of this research was to study the physical development of schoolchildren and teenagers with chronic gastroduodenal pathology (CGDP). The basis of the work was a multifactor analysis of 1547 schoolchildren from 6 to 15 years old. We carried out a study of physical growth indicators, including body height for age and weight for age and body mass index (BMI). To maintain the reliability of the study, we selected only children with CGDP, excluding children with functional impairments not associated with damage to these organs. CGDP included chronic gastritis, gastroduodenitis, duodenitis, gastric ulcers, and duodenal ulcers. The results indicated weight loss in children with CGDP against a background of increased growth compared to the control group (63.64%). These were mainly children with various forms of chronic gastritis and gastroduodenitis. Analysis of BMI evinced that 35.66% of children showed a decrease in the index (BMI<18.5), while 55.24% had BMI=20-25, and 7.69% BMI=25-29.9. We observed in children and teenagers with CGDP physical growth disorders, such as a reduction or increase in body weight compared with healthy coevals. The differences observed were dependent on the gastroduodenal area pathology type. Registered with ClinicalTrials.gov PRS ID: NCT04702542 (https://clinicaltrials.gov/ct2/show/NCT04702542).

Keywords: chronic diseases, schoolchildren, teenagers, gastroduodenal area

1. Introduction

The health state of the population is one of the important indices of the welfare of society, reflecting the characteristics of the economic and social tendencies in the country (1, 2). Thus, the problem of maintaining the normal physical growth of schoolchildren and teenagers has great significance in modern paediatrics (3). In the practice of paediatrics, gastrointestinal tract (GIT) organ pathology is often observed in association with predominantly chronic inflammatory diseases, including gastroduodenitis and ulcers with a long relapsing course, which lead to a considerable reduction in the quality of life for children and to the development of different complications (anaemia, obesity, insufficient body weight, bleeding, perforation and so on) (4,5).

According to some authors, studying the physical development of these patients remains controversial due to the small number of studies (6, 7). However, relatively little attention is given to the factors related to the daily activities of schoolchildren and their feeding (1, 8). In recent years, the study factors leading to disorders in the physical development of growing children has been an actual direction in medicine.

The impact of environmental and social factors on the development of chronic gastroduodenal pathology (CGDP) in children and teenagers remains a question.

2. Patients and methods

2.1. Study design

The clinical observation of patients was based on a randomized controlled scientific study. The test reports are in accordance with the CONSORT 2010 recommendations and presented with their protocols on the website: https://clinicaltrials.gov/ct2/show/NCT04702542. We conducted this study in three stages: Stage 1 – We conducted a screening survey based on a comprehensive questionnaire. The children in middle and high schools completed their own questionnaires, while the children in primary schools did so with the help of medical professionals through a "question-and-answer" method. Medical workers completed the questionnaires for children of younger age groups. Teens self-administered the questionnaires.

Stage 2 – We performed a common outpatient examination of children, including a comprehensive survey with other specialists (neurologists, endocrinologists, ophthalmologists, otolaryngologists, paediatric surgeons, etc.). We also evaluated the physical development of children (body length/height, weight, body mass index, BMI).

Stage 3 – We conducted clinical laboratory and instrumental studies, and performed EF om 6 to 15 years old

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living in Tashkent City and the Tashkent area (Uzbekistan). The median GDS.

2.2. Patients

The basis of this work was a multifactor analysis of 1547 schoolchildren fr age was 11.8 ±2.5 years. We examined the children after taking permission from their parents. We carried out examinations based on the regular medical examination schedule of schoolchildren. We divided the examined children into age groups according to WHO recommendations.

The first group included children from 6 to 8 years old, who made up 39.49% (n=611) of the total number of examined children, while the second group included children from 9 to 11 years old with 32.84% (n=508), and teenagers (12-15 years old) composed the third group with 27.67% (n=428). The ratio of girls to boys was 1.2:1.

2.3. Research methods

Research methods included the following:

• General clinical research: complete blood count, complete stool analysis.

• Instrumental research: fibrogastroduodenoscopy (FGDS) of the upper digestive system.

• Study of the physical development of patients.

We evaluated the physical growth of schoolchildren (body height for age, weight for age, index of body weight - BMI), performed GDS (gastroduodenoscopy), and determined the level of somatotrophin (STH). Subsequently, we treated all patients.

2.4. Ethical review

We conducted the described study in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. To conduct clinical and laboratory studies on children in an outpatient setting, we obtained a certificate from the Ethics Committee under the Ministry of Health of the Republic of Uzbekistan (protocol no. 3 of 20.04.2017). We registered the study on the ClinicalTrials.gov PRS site (https://clinicaltrials.gov/ct2/show/NCT04702542). Before the clinical trials, we also obtained written permission from the parents or guardians to examine their children and adolescents. Furthermore, we recorded all the study results in the patients' outpatient documentation.

2.5. Statistical analysis

In the course of the study, we entered all the mathematical data obtained into the primary patient database using MS Excel 2013 spreadsheets (based on Microsoft Office 2013), followed by data processing using Statistical 10 application packages. We determined the statistical significance of differences in trait values in the study groups using the nonparametric Mann–Whitney and Kruskal–Wallis tests. We used the Spearman correlation coefficient to identify the relationships between the variables, and the nonparametric Wilcoxon test to compare the changes in indicators. Data are presented as the mean (M) and standard deviation (M±S). The obtained statistical results were considered significant at $P \leq 0.05$.

3. Results

The results showed that as age increased, the number of schoolchildren with functional disorders of the gastrointestinal tract increased. Intending to obtain “clear” results, we chose only children with chronic diseases of the gastroduodenal area (CGDP), excluding children with functional disorders that were not associated with damage to these organs. We diagnosed the following CGDP: chronic gastritis, gastroduodenitis, enterocolitis, and gastroduodenal ulcers. After all stages of the study, we diagnosed 437 (28.25%) schoolchildren from a total of 1547 with different clinical variants of functional disorder of organs in the gastroduodenal area. Among them, 12.81% (n=56) were 6 to 9 years old, 32.72% (n=143) were 10 to 13 years old and 54.46% (n=238) were teenagers (14-15 years old). For comparison, we chose 225 children and teenagers without chronic somatic diseases as the control group.

The study evinced that the physical development of schoolchildren from the first group with CDGDA did not practically differ from that of their healthy coevals. The differences in the weight and height indices in comparison to the control group were not significant.

We observed a trend of a reduced weight index for age on the background of an increased height index among the schoolchildren with CGDP from the second age group compared to the control group. We identified obesity only in 2 (1.40%) children (BMI>30) and diagnosed them with duodenal ulcers. Moreover, we observed an increase in the height index in 63.64% (n=91) of the children in this group in comparison to that in the control group. The difference was approximately 18.6%. These were mainly children with different forms of chronic gastritis and gastroduodenitis. The others accounted for 36.36% (n=48), and the height index was in accordance with that of the children from the control group, except for the retardation of height compared to the control group. During the analysis of the body weight index, we observed a reduction in the index (BMI < 18.5) in 51 (35.66%) children. The BMI was 20-25 in 79 (55.24%) schoolchildren with CGDP and 25-29.9 in 11 children (7.69%).

Among the teenagers, we marked the obvious trend towards differences in physical growth. An analysis of the weight index in relation to the height showed a marked reduction in comparison with the control group, at approximately 38%. The height index for age in 126 (52.94%) teenagers was also in accordance with that of the control group. The other 32 (13.45%) teenagers showed marked growth retardation, and 80 (33.61%) had increased

height compared to the control group. The analysis of the index body weight in 109 (45.8%) patients evinced a reduction in the index (BMI<18.5). We marked obesity in 6 (2.52%) teenagers (BMI>30), and in 19 children and teenagers (7.98%), the BMI was 25-29.9.

We found the BMI as 20-25 in only 104 (43.69%) teenagers with CDGDA.

4. Discussion

Early diagnosis and treatment of chronic diseases in children and adolescents are one of the most urgent problems in modern paediatrics. Digestive diseases occupy a leading position in the overall incidence of childhood diseases and thus represent a serious medical and social problem. Over the past 10 years, the frequency of diseases of the gastrointestinal tract in children under 14 years of age increased by 30%, and 15-17 years of age by 44% (9,10).

There is a large amount of information on the mechanisms underlying the development of digestive system pathology, and the resulting impact of pathological factors in various origins is a decrease in the barrier properties of the gastroduodenal mucosa, forming a cascade of adaptive reactions that affect the homeostasis of nearly the entire body (11,12).

Studies have shown that gastroduodenal area pathology influenced physical growth. In particular, obesity and elevated BMI were observed in children with ulcerous diseases of the duodenum.

According to Abuquteish D and Putra J. (2019), the prevalence of duodenitis in children with diseases of the upper digestive tract ranged from 33% to 48% (1). According to Styne DM et al. (2017), obesity or underweight in these diseases led to long-term complications; therefore, screening for concomitant obesity in patients with these diseases should be applied in a hierarchical, logical way for early identification before more serious complications arise (13). We noted in this study that chronic gastritis and ulcerous damage to the stomach in children resulted in a reduction in body weight and a corresponding reduction in the BMI, which was evidently displayed in the teenage period.

It should be noted that the teenage period sharply differed from all other stages of development owing to the pace and uniqueness of activities in this period of life.

As the endocrine system is in constant connection with the digestive system, disorders or deviations in one system are reflected in changes in the functional activity of the other system.

It should also be noted that the main factor in the pathogenesis of CGDP is nutrition disorders. The predominance or reduction of specific food ingredients promotes changes in specific fermentation products and hormones in organisms, which is especially observed in children and teenagers.

Furthermore, gastrointestinal hormones play a huge role in the humoral regulation of digestive functions (14). These substances are produced by the endocrine cells of the gastric mucosa, duodenum, and pancreas. Gastrointestinal hormones are involved in the regulation of secretions, motility, absorption, trophism, and the release of other regulatory peptides, and the disruption of their production affects metabolism. A representative gastrointestinal hormone is somatostatin (GHIF-growth hormone inhibiting factor, SRIF-somatotropin-release inhibiting factor).

It is worth mentioning that the concentration of somatostatin in the islets of Langerhans of the pancreas is higher than in the tissues of the hypothalamic zone of the brain. There are also high levels of somatostatin in the antral mucous membrane of the stomach and much less somatostatin in the intestinal mucosa. In general, 3/4 of all immunoreactive somatostatin is produced by D-cells located in the digestive organs, and the rest is produced in the brain (15,16).

In addition, the relationship between somatostatin and somatotropin (growth hormone, GH) is indisputable. GH increases the synthesis of cartilage tissue in the epiphyseal parts of the bones, stimulates the growth of the body in length and increases the thickness and width of bones in childhood (17).

Additionally, somatotropin has powerful anabolic and anticatabolic effects; that is, it accelerates protein synthesis and inhibits protein breakdown, providing nitrogen and phosphorus homeostasis and lowering urea levels, as well as helping to reduce the deposition of subcutaneous fat, increasing fat burning and the muscle mass-to-fat ratio (15).

In our previous study (4), we found a clear association between the hormonal background and the physical development of schoolchildren. Somatotropin is called the hormone of height because in children, teenagers, and young people, it causes an acceleration of linear (in length) height, mainly due to its effects on the long cylindrical bones of the extremities. Somatotrophin increases the synthesis of cartilaginous tissue in the epiphyses of bones, leading to an increase in the length of the epiphyses of bones, which then leads to changes in body height; and by increasing the periosteal height somatotropin also increases the width and thickness of bones. An increase in the mass of muscular and connective tissue occurs, and the mass of the inner organs also increases.

However, many physiological factors influence the balance of these hormones and the secretion of the growth hormone. The secretion of the growth hormone is stimulated by sleeping, physical exercise, increased consumption of high-protein foods, and hypoglycaemia. Under hypoglycaemic conditions, the level of GH sharply increases in the blood, which is one of the natural physiological

mechanisms for the fast correction of hypoglycaemia, and the secretion of GH is suppressed under hyperglycaemic conditions and with high blood plasma levels of free lipoic acids and glucocorticoids.

Therefore, there is a direct connection between the physical development of children and chronic gastroduodenal pathology.

We associated physical growth disorders in children and teenagers with CGDP with reduced (to 45% of the level of the observed patients) or increased body weight (to 8% of the lnevel of observed patients) compared to the healthy coevals. In a given category of schoolchildre, we observed an increase in the height index (to 64% of the level of the examined patients). Accordingly, we observed deviations in the body weight index in either direction. The differences observed were dependent on the gastroduodenal area pathology type. In children and teenagers with CGDP, the average increase in STH in relation to the control group was 24.35%, but in terms of normative indices. Given the controversial nature of the problem and the results of this research, clinical research in this area is ongoing, and the results will be communicated in the near future.

Conflict of interest

The authors declare that they have no competing or financial interests. Funding for clinical research was carried out at the expense of the authors' personal contributions and with the support of the administration of the medical institute.

Funding

None.

Acknowledgments

The author expresses gratitude to the chief physician of the Central District Polyclinic of the Yunus-Abad District of Tashkent, M.Kh. Umarova, for her assistance in carrying out and organizing this research.

Authors' contributions

We, Turdieva ShT and Ganieva DK, the authors of this scientific article, guarantee the provision of the Author Agreement after the acceptance of the article for publication. We are the only authors who claim that there is no one on the list of authors except us.

Authors jointly participated in conducting a clinical study and writing an article. Contribution of each author: Turdieva Sh.T. - contributed to study conception and design; contributed to data acquisition, analysis, or interpretation; drafted the manuscript; critically revised the manuscript, gave final approval; and agrees to be accountable for all aspects of the work, ensuring its integrity and accuracy. Ganieva DK - contributed to data acquisition and interpretation and drafted the manuscript and agrees to be accountable for all aspects of the work, ensuring its integrity and accuracy.

Both authors contributed to the writing of the manuscript and approved its final version.

Ethics approval

The manuscript does not contain the data of any person in any form (including any individual data, images, or videos); consent for publication obtained from parents or legal guardians - not applicable.

The work described has been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. To conduct clinical and laboratory studies of children on an outpatient basis, a certificate was issued by the Ethics Committee under the Ministry of Health of the Republic of Uzbekistan (protocol no. 3 of 20.04.2017).

We registered this study on the ClinicalTrials.gov PRS site (ID: NCT04702542). In addition, before clinical trial participation, we obtained written permission from the parents and guardians to examine their children and adolescents. We recorded all study results simultaneously in the patients' outpatient documentation.

Consent for publication

The manuscript does not contain information requiring consent for the publication of any individual person’s data in any form (including any individual details, images or videos). Consent for publication must be obtained from that person, or in the case of children, their parents or legal guardians.

Availability of data and materials

This published article includes all data generated or analysed during this study.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 686-692
doi: 10.52142/omujecm.39.3.18

Efficacy of combining clinical crown lengthening surgery and botulinum toxin A injection in gummy smile treatment

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Received: 18.01.2022 • **Accepted/Published Online:** 17.07.2022 • **Final Version:** 30.08.2022

Abstract

In many cases, the cause of a gummy smile is a combination of many simultaneously occurring factors. Therefore, a single method is certainly not effective in achieving the maximum aesthetic requirements. This study aims to evaluate the efficacy of combining clinical tooth crown lengthening surgery and subsequent BTX-A injection in gummy smile treatment using clinical and photographical assessment. 21 patients with an average length of excessive gingival display of at least 4 mm at maximum smiling point and with the height of keratinized gingiva ≥ 3 mm were recruited. Gingival exposure (GE) and clinical crown length (CCL) were measured at baseline (T0), two months after clinical crown lengthening surgery (T1), two weeks after BTX-A injection (T2), two months after BTX-A injection (T3). GE values at T1, T2 and T3 were significantly different ($p < 0.001$). CCL was significantly changed after gingivectomy surgery (T1) compared to preoperative value (T0) ($p < 0.001$). BTX-A could be considered a favorable adjunctive treatment for gummy smile patients after gingivectomy surgery.

Keywords: BTX-A, crown lengthening, smiling, injection

1. Introduction

A smile brings many benefits to oneself and makes everyone around feel better (1, 2). Because the mouth is the center of attention of one's face, the smile plays an essential role in facial aesthetics. Dentistry is a medical specialty most involved in smile improvement. However, the clinical assessment of dentists for a beautiful smile and smile cosmetic treatment planning remains challenging. Smile assessment criteria, which includes: the degree of gingival exposure, the proportion of anterior teeth, and the gum line, helps the dentist plan practical and effective treatment. According to recent studies, the maximum amount of gingival exposure allowed when smiling is 3 mm (3). Otherwise, it can be considered a gummy smile (GS), which is considered less aesthetic (4). According to a study in 2021 by Horn et al, the GS rate can be approximately 31% (5).

The demand for beauty is increasing in society as everyone is looking for cosmetic improvement, including gummy smile correction. Various methods have been proposed to correct gummy smiles, including orthodontic treatment, orthognathic surgery, bone resection, and gingivectomy.

While the orthodontic option is expensive and time-consuming, operations are also complex and invasive. Gingivectomy and smile line repositioning correct gummy smile cases when combined with passive erupted delay and when hyperactive lip muscles have been corrected (6, 7). However, both methods not only are invasive but also cause anxiety and pain after surgery. Botulinum Toxin A (BTX-A) has been adapted for other treatments and validated as a safe product in gummy smile treatment. In proper cases, excessive gum exposure can be adjusted by injecting BTX-A to reduce the contraction of the upper lip levator muscles, such as levator labii superioris (LLS), levator labii superioris alaeque nasi (LLSAN), zygomaticus major and minor (8, 9).

In many cases, the cause of a gummy smile is a combination of many factors acting simultaneously (10).

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Furthermore, the biomechanics of gummy smiles seem to involve the combined effects of teeth, gums, bones, and muscles. Therefore, no single method can reliably achieve the maximum aesthetic requirements for the patient. This study aims to evaluate the efficacy of a combination of clinical tooth crown lengthening surgery followed by BTX-A injection in gummy smile treatment based on clinical and photographic assessment.

2. Materials and Methods

2.1. Ethical statement

The study was approved by the UMPH ethical committee (approval number 20338-DHYD) in compliance with the Helsinki Declaration 2013. Informed consents were signed by each participant before enrolment in the study, after explanation of study objectives, intervention, risk, and potential benefits. Clinical trial registration: NCT05057286 at ClinicalTrials.gov.

2.2. Study design, setting, and participants

An uncontrolled longitudinal study was implemented at the Faculty of Odonto-Stomatology (FOS) of the University of Medicine and Pharmacy at Ho Chi Minh City (UMPH), Viet Nam, from August 2020 to May 2021.

Participants were recruited from patients with the chief complaint of excess gingival display at the Dental Clinic of FOS- UMPH. Study population included patients aged 18 and above with an average length of excessive gingival display ≥4 mm at maximum smiling (eight teeth counted, from the 14 to the 24 teeth) and height of keratinized gingiva ≥ 3 mm.

Inclusion criteria included: gummy smile due to combined etiologies: a short clinical crown of teeth due to the altered passive tooth eruption and hyperactivity of upper lip muscles (maxillary lip generally translates more than 8mm from the repose position to the position achieved at a full smile), with a normal lip length (20-22mm in females and 22-24mm in males).

Exclusion criteria were pregnant or breastfeeding patients, gummy smiles due to maxillary bone overgrowth (maxillary hypertrophy) or vertical maxillary excess, neuromuscular disorder, gingival hyperplasia due to medications, supplements, or neuromuscular transmission inhibiting agents, systematic diseases that can affect the result of surgical treatment (such as diabetes, anticoagulant use, hematologic disorders, immunodeficiency diseases etc.), allergy to BTX-A or albumin, a history of taking BTX-A injection in the head-neck area, or any contraindication of surgery.

Study procedure consisted of recruitment, pre & post-operative photography, gingivoplasty, BTX-A injection, visits, data collecting and analysis.

2.3. Photographic protocol

All participants were photographed before surgery at T0. The shooting procedure was standardized to ensure reproducibility for post-surgical photographs at two months after clinical crown lengthening surgery (T1), two weeks (T2), and two months (T3) follow-up visits after BTX-A injection. Frontal view photos of the patient's natural smile and maximum smile (Fig. 1A, Fig. 1B) were taken. Distance between the subjects and the camera was at least 1 meter and constant in every shooting. Patients sat on a chair without headrest in a relaxed position. Patients' Frankfort occlusal plane was parallel to the floor. The camera lens was placed on the same level as patients' occlusal plane and focused on the contact point of their upper central incisors (11). Before shooting, patients removed all their jewelry, tied their hair to show the face clearly. The patients were informed to dress up appropriately to see their necks in full. Face proportion was controlled in every photo using a grid board on the camera. An acceptable photo clearly showed the patient's eyes, both sides of the mandible's inferior border, the center of his/her nose, and two auricles. The photography protocol would be repeated in each subsequent visit.

Fig 1. A. Front smile photo
B. Maximum smiling photo
C. Initial stage at baseline time (T0)
D. Measurement of the width of the upper middle incisors
E. Measerement of the future length of the central incisors
F. Creation of the bleeding
G. Placement of internal bevel incision
H. Placement of sulcular incision for full-thickness flap reflection
İ. Full-thickness flap reflection, white border: CEJ, black border: alvoler crest
J. Alveolar bone adjustment according to biological width
K. Shaping the outer alveolar bone shape
L. Alveolar bone contour after shaping
M. Double-checking the biological width
N. Suturing the flaps
O. Follow-up 2 months after the surgery

2.4. Preoperative protocol

The same doctor performed clinical examination, history taking, evaluating, and recording the GS level of all patients. The keratinized gingiva height and initial clinical crown length (iCCL) were measured in millimeters using a digital caliper and periodontal probe.

Postoperative clinical crown length (pCCL) of teeth was calculated as below:

A surgical guided splint/ surgical template was fabricated from a plaster cast of the upper arch impressed by alginate. Patients were asked to submit a blood test result to make sure they were not contraindicated for for surgery. Preoperative

photos were taken by the same cameramen following the protocol mentioned above.

2.5. Clinical dental crown lengthening surgery protocol (Fig. 1C – Fig.1O)

Patients were asked to rinse their mouths with 10 ml chlorhexidine gluconate 0.12% (KIN) for 2 minutes. Next, nurses prepared the extra and intraoral cavity with antiseptic solution povidine 10%. Patients were locally anesthetized using Lidocaine 2% and 1:200,000 epinephrine. Distance from gingival margin to bone crest was measured by bone sounding with periodontal probe UNC-15. Usually, it is 3 mm at the buccal and lingual surface, 4.5-5.0 mm at the proximal sides.

The surgeon re-evaluated the height of keratinized gingiva (must be ≥ 3 mm) (12-14) before marking the bleeding points with a socket probe and surgical template.

The first incision was at the internal bevel gingivectomy 450 apically to the tooth axis. Then, the second incision was performed along the gingival sulcus. The gingiva was removed from the root surface, then the remaining tissue attaching to the root was cleaned with periodontal curettes. A full-thickness flap was performed in the gingivectomy border to the mucogingival junction to expose the alveolar at least 4-5mm. Alveolar was adjusted by a 2 mm diameter carbide bur to establish the prober biologic width, which was 2.5 mm at least from anterior teeth at every position (from CEJ to the crest of bone). Finally, 5-0 polyamide non-resorbable sutures were used.

2.6. Post-operation protocol

Patients were all prescribed antibiotics (Amoxicilline 500mg, three times a day in 7 days), anti-inflammatory drugs (Ibuprofen 400 mg, three times a day in 5 days), analgesics (paracetamol 500mg, three times a day in 5 days), and antiseptic mouthwash (15ml Chlorhexidine gluconate 0.12% (KIN), twice a day in two weeks). Patients were instructed to brush their teeth slightly with a soft toothbrush, to return to oral hygiene practice after two weeks, and to revisit every week within two months. The sutures were removed 14 days after surgery.

2.7. Botulinum Toxin A injection

One hundred units (U) of botulinum toxin were diluted with 2.5ml of sterile saline solution without shaking. 1 mm syringe with a 30-gauge needle was used to get 0.25ml BTX-A for four-point injection, or 0.6ml in case injection of 6 points. The anatomy points were located for BTX-A injection as in Fig. 2A. Cold compress was applied extraoral 5 minutes at injection site before injection. After local anesthesia, the doctor administered slowly 2,5 IU of diluted BTX-A at each point until white cellulite appeared. Patients were recalled two weeks (T2), and two months (T3) after injection.

2.8. Clinical outcomes

Another assessor determined, measured, and recorded clinical parameters at baseline T0, two months after performing clinical crown lengthening surgery (T1), two weeks after BTX-A injection (T2), two months after BTX-A injection (T3).

2.9. Measurements on photos

On frontal view photo, two types of parameters, including the highest point of the gingival margin (A) and the mesial-distal dimension (L0) (Fig. 2B), were measure for each of the eight maxillary anterior teeth. Photo scaling was performed using superimpose method with a given reference dimension (clinical L0). After synchronizing the clinical L0 with photo L0, the digital ruler on the software would be used to measure the study dimensions. All photos were processed using Keynote software version 9.2 (Fig. 2C – 2F).

Five selected photos were framed for each patient to focus on maximum smiles and named by numerical code in chronological order (For example, P1Tx) (Fig. 3). Photo scaling was performed using superimpose method with a given reference dimension (clinical L0). After synchronizing the clinical L0 with photo L0, the digital ruler on the software would be used to measure the study dimensions.

The tooth-axis parallel line (d line) was drawn from the A point vertically to meet the lower edge of the upper lip at B point. Thus, the clinical degree of gingival exposure (N0) was the length AB.

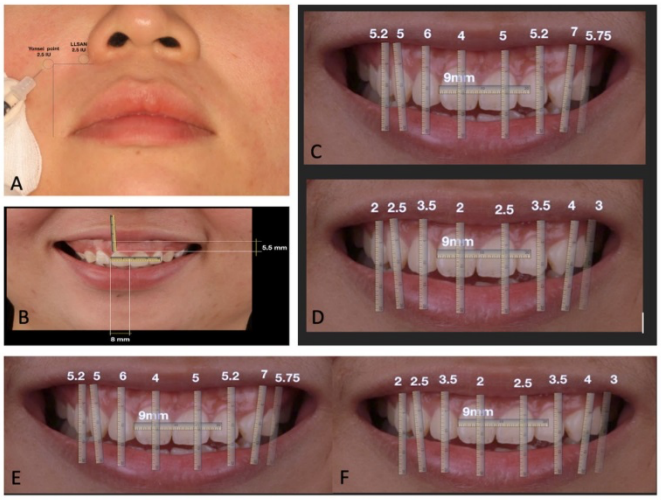

Fig 2.

A. The BTX-A injection site and dosage in gummy smile treatment
B. Mesial-distal tooth dimension and the clinical level of gingival exposure were measured with a digital caliper
C. Photos were processed using Keynote software version 9.2
D. Photos were processed using Keynote software version 9.2
E. Photos were processed using Keynote software version 9.2
F. All photos were processed using Keynote software version 9.2

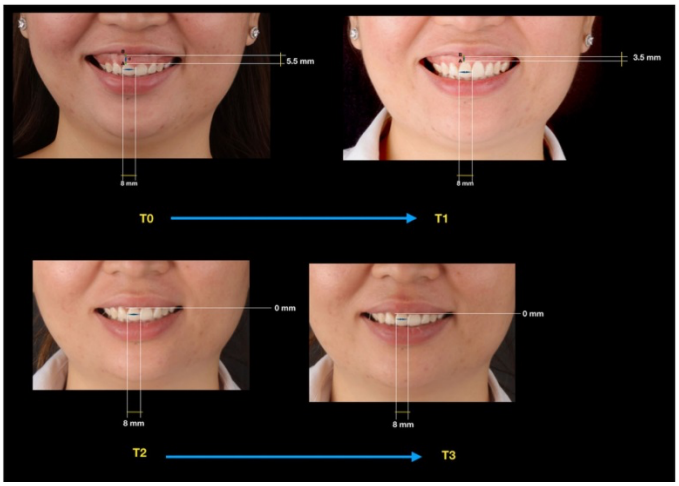

Fig 3. Processing smile photos with computer software.

2.10. Statistical analysis

Descriptive statistics were mean ± standard deviation values given for the numerical variables.

Analysis was performed to determine the difference between the clinical degree of gingival exposure before and after clinical crown lengthening surgery. Also, gingival exposure before and after the BTX-A injection was compared. Data were reviewed and analyzed using SPSS software (version 20.0; SPSS, Chicago, III). Student paired t-test was applied if variables were normally distributed. Otherwise, the Wilcoxon test was used for pair comparison.

It was considered statistically significant when p value < 0.05.

3. Results

Twenty-one patients (20 females and one male) aged 24.67 ± 3.44 visited the Dental Clinic of FOS-UMPH from August 2020 to May 2021 with the chief complaint of gummy smile. Among them, one patient dropped out of the study due to intermittent participation. After gingivectomy, the patient felt satisfied that she did not want to continue with the BTX-A injection. Thus, 20 patients (19 females and one male) at an average age of 24 years old completed all study requirements. Each patient had four photos taken at T0, T1, T2, and T3.

Average gingival exposure in each tooth and overall of one participant was shown in Table 1, Fig. 4A, B, and Table 2. The average gingival display at baseline was 5.35 ± 0.97 mm and 1.14 ± 0.79 mm at T3.

Gingival exposure in individual teeth after crown lengthening surgery (T1) and BTX-A injection after two weeks (T2) and two months (T3) were significantly different (p <0.001). Additionally, Mean gingival exposure change after crown lengthening surgery (T1) and BTX-A injection at two weeks (T2) and two months (T3) were statistically different (p <0.001) (Fig. 4C).

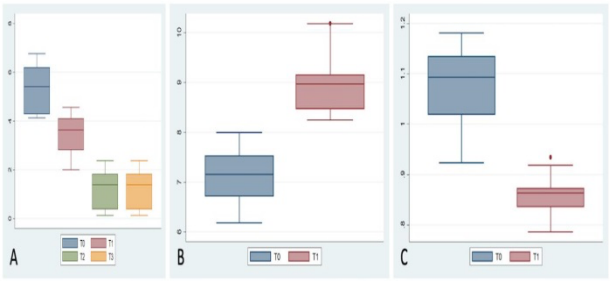

Fig 4.

A. Comparison of mean gingival exposure change after crown lengthening surgery (T1) and BTX-A injection at 2 weeks (T2) and 2 months (T3)
B. The mean clinical crown length change after crown lengthening surgery (T1)
C. Change of crown width/length ratio in individual teeth after crown lengthening

Clinical crown length was significantly changed after gingivectomy surgery (T1) compared to preoperative (T0), p <0.001 (Table 3). Differences of gingival exposure were 1.86 mm at T1, 4.20 mm at T2, and 4.21 mm at T3, compared to T0.

Width/length ratio of CCL also altered significantly, p < 0.001 (Table 4). No complication from BTX-A was noted.

4. Discussion

Our clinical trial suggested that a combination of gingivectomy surgery and botulinum toxin A injection effectively improved the esthetics of gummy smiles. The average age of our population was 24.67 ± 3.44. This age group has reached stabilization of craniofacial structures and has not been affected by physiological tooth wear. In

Table 1. Comparison of changes in gingival exposure in individual teeth after crown lengthening surgery (T1) and at two weeks (T2) and two months (T3) after BTX-A injection

Tooth	TO		T1		T2		T3		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	p value
14	5.58	1.51	3.88	1.04	1.33	1.24	1.28	1.19	
13	5.28	1.61	3.30	1.52	1.10	1.21	1.10	1.21	
12	6.30	0.99	4.15	1.08	1.80	1.01	1.80	1.01	
11	4.76	1.22	2.95	1.16	0.83	1.07	0.83	1.07	<0.001
21	4.85	1.51	3.03	1.21	0.83	1.07	0.83	1.07	
22	5.90	1.40	3.98	1.21	1.45	1.39	1.43	1.35	
23	5.43	1.29	3.58	1.31	0.93	0.89	0.93	0.89	
24	4.75	1.64	3.10	1.40	0.98	0.73	0.93	0.77	

Table 2. Comparison of mean gingival exposure change after crown lengthening surgery (T1) and at two weeks (T2) and two months (T3) after BTX-A injection

	Mean	SD	p value
T0	5.35	0.97	<0.001
T1	3.49	0.83	
T2	1.15	0.80	
T3	1.14	0.79	

Table 3. The clinical crown length change in individual teeth after crown lengthening surgery

Tooth	TO		T1		p value
	Mean	SD	Mean	SD	
14	6.25	1.01	7.65	0.84	< 0.001
13	7.50	0.71	9.28	0.83	
12	6.60	0.42	8.70	0.55	
11	7.88	0.79	9.93	0.52	
21	7.88	0.74	9.93	0.52	
22	6.95	0.54	8.80	0.44	
23	7.35	0.96	9.45	0.83	
24	6.48	0.88	7.70	0.73	

Table 4. Change of crown width/length ratio in individual teeth after crown lengthening surgery (T1)

Tooth	T0		T1		p value
	Mean	SD	Mean	SD	
14	1.15	0.16	0.93	0.08	< 0.001
13	1.06	0.09	0.85	0.07	
12	1.04	0.09	0.79	0.07	
11	1.06	0.14	0.84	0.06	
21	1.06	0.11	0.84	0.06	
22	1.01	0.10	0.80	0.09	
23	1.06	0.11	0.81	0.06	
24	1.16	0.15	0.98	0.07	

addition, the patients' smile lines and soft tissues have not changed significantly due to aging

Gender distribution in this study was 92, 86 % female and 7, 14% male, so the difference in the gender ratio was statistically significant (p <0.001). Hagai Miron et al. (15) studied subjects from 20 to 40 years old showed that the prevalence of gummy smiles in women was 2.5 times higher than in men. Thus there is a concordance in the gummy smile prevalence reported in literature (5). In addition, the percentage of women interested in smile esthetic is higher than that of men, which also contributes to the severe discrepancy in gender distribution. However, all comparisons were performed in the same group at different timelines so that sexual distribution did not affect the result.

The study sample was carefully selected at baseline to ensure that the average gummy smile calculated from teeth 14 to 24 was at least 4 mm (Table 1). Focused subjects were patients with gummy smiles caused by a combination of variable passive eruption and hyperactive upper lip muscles (the maxillary lip generally translates more than 8 mm from the repose position to the position achieved at a full smile). Other etiologies of gummy smiles, such as maxillary bone overgrowth or vertical maxillary excess or neuromuscular disorders, were not included in this study.

Literature has indicated the effectiveness of gingivectomy in gummy smile correction in mild and moderate cases (*). Consistently, our result reported that the gingival display level was statistically significantly reduced by about 1.8 mm ($p < 0.001$) after clinical crown lengthening surgery. Therefore, surgical treatment of gummy smiles is effective for patients with altered passive eruption. In addition, the decrease of gum exposure after the surgery from the upper incisors to the upper premolars was statistically significant. Therefore, clinical crown lengthening surgery should be indicated in aesthetics cases or before the restorations for subgingival decayed, broken teeth.

Furthermore, this surgical procedure helped reestablish the correct clinical crown and adjust gingival asymmetry (16) . However, it is still a challenge for clinicians to treat gummy smiles. A large amount of bone reduction can lead to gingival recession, while recurrence may occur with insufficient bone grinding.

To design an effective treatment plan for gummy smile cases, it is necessary to carefully analyze both the white aesthetic and the pink aesthetic. Gingivectomy surgery improves tooth height, bringing teeth to a favorable golden ratio. However, it is also essential to calculate the future width/length ratio of the teeth (17). A minimum of 2 to 5 mm of keratinized gingiva was required in clinical crown lengthening to maintain the periodontal tissue health (16).

Preserving interdental papillae is very important anatomically and esthetically. The alveolar bone crest at the gingival papillae should be carefully removed to ensure anatomical structure maintenance and establishment of reattachment after flap elevation, and the distance from the bone crest to the contact point must be sufficient (about 5 mm or less) (18). Bone should be removed carefully, respecting the minimum space for biological width. Reports showed that at least 3 mm of gingiva would remodel coronal after six months when the flap was sutured at bone crest (18). In the present study, the flap was repositioned at least 2.04 mm away from the bone crest, which is the minimum space for biologic width, to prevent recurrence.

On the other hand, clinical crown length after two months was significantly improved (about 1.82 mm), compared to the initial tooth position. Furthermore, gingival display level reduced significantly from T1 (2 months after the surgery) to T2 (2 weeks after BTX-A injection) with $p < 0.05$ (1.86 mm and 4.20 mm, respectively). Hence, BTX-A was effective in reducing gingival display level 3 days after injection. It is consistent with the study by Ahmet (2020) (19). The same dermatologist performed the BTX injection procedure with standardized doses and anatomical marks to eliminate confounding factors.

At two weeks post-injection (T2), BTX-A reached its peak effect (9, 20). Mean gingival display at T2 was 1.41 mm, while it reached 3.54 mm before the injection of BTX – A (T1). No complication from BTX-A was noted. This result was similar to a previous study by Polo (20), reporting that the average gingival exposure when smiling was 5.2 mm at the baseline and 0.09 mm after 15 days of BTX - A injection. Likewise, Suber et al. (21) showed that mean gingival display was 4.89 mm and only 0.75 mm at baseline and 15 days after BTX injection, respectively. There is a difference in the level of reduction of gingival display among studies, which may be due to the different sample sizes, inter-surgeon variation, and possible differences in metabolism among races, living in various geographical regions. However, we could safely conclude that BTX-A effectively reduced gingival exposure in mild gummy smile patients.

Although BTX-A injection is the least invasive treatment, results are temporary. BTX-A efficacy will fade away slowly after 3-6 months. If the patient wants to maintain that result, he/she has to re-inject BTX-A. Another common disadvantage of BTX injection is ptosis, Joker-like smiles, and difficulty functioning chewing function. This effect is common, caused by f BTX overdose or poor injection technique. Therefore, BTX-A injection should be performed by an experienced dermatologist. In 2010, Rosemarie M et al. (22) divided GS into four types dependent on excessive contraction of specific muscles, including gingival exposure more than 3mm at only six upper anterior teeth, gingival exposure more than 3mm at only upper posterior teeth, gingival exposure more than 3mm at both of upper anterior and posterior teeth with bilateral symmetry and gingival exposure more than 3mm at both of upper anterior and posterior teeth with bilateral asymmetry. Therefore, the position and dosage of BTX-A applied were different among GS types.

We recommended oral hygiene after clinical crown lengthening to achieve the best tissue healing outcome. The subjects were instructed to rinse their mouth with 15ml chlorhexidine gluconate 0.12% after surgery, twice a day (within two weeks), brush their teeth gently with a soft-bristle toothbrush. Oral instruction was repeated every visit. These may enhance the outcomes of gummy smiles (23).

Clinical crown lengthening surgery reduced gingival exposure significantly when smiling by increasing the crown height and adjusting the width: length ratio of the clinical crown to a proper aesthetical standard. The results were enhanced by BTX-A injection, proven by observations at three days, two weeks, and two months post-injection. There were no complications or side effects in the present study. Safe injection sites were levator labii superioris, levator labii superioris alaque nasi, zygomaticus minor and zygomaticus major muscle. From our findings, we suggest that BTX-A is a favorable adjunctive treatment for gummy smile patients after gingivectomy surgery.

Conflict of interest

The authors declared no conflict of interest.

Funding

No funding was used for the study.

Acknowledgments

We would like to thank Department of Periodontology, Faculty of Odonto-Stomatology, University of Medicine and Pharmacy at Ho Chi Minh City for supporting this study.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 693-698
doi: 10.52142/omujecm.39.3.19

Comparison of liver biomarkers with N/L ratio, CRP, d-dimer in Covid 19 pneumonia and its effect on mortality

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Received: 18.01.20212 • **Accepted/Published Online:** 14.08.2022 • **Final Version:** 30.08.2022

Abstract

Coronavirus Disease-2019 (Covid-19) is the cause of a pandemic that has high mortality and global effects. The liver damage in Covid-19 cases in mechanical ventilation was investigated in this study. A total of 60 patients (the Study Group) who were diagnosed with Covid-19 pneumonia, and 65 individuals (the Control Group) were included prospectively in the study. The cases were divided into 3 groups as those who were intubated (severe), those who were not intubated (mild), and the healthy Control Group. The lung tomography results of those who were diagnosed with Covid-19 were examined in the study. The cases with positive RT-PCR (Real Time Polymerized Chain Reaction) test results were recorded from the system. The liver tests of the patients were compared with those of the Control Group. The two groups with and without intubation were also compared. The results were evaluated and analyzed statistically. When all the data were evaluated, it was found that LDH, GGT, AST, and aPTT levels were significantly higher in the mild and severe patient group compared to the Control Group, and the T. Protein and albumin levels were low ($p<0.01$). The N/L Ratio, and the CRP levels, which are the other acute phase reactants, were significantly higher ($p<0.05$). No statistically significant differences were detected when all parameters of the groups with and without intubation were compared ($p>0.05$). Increased D-dimer, GGT, D. Bil, LDH, NLR, and AST levels in Covid-19 patients in invasive mechanical ventilation are associated with mortality.

Keywords: Covid-19, Liver Function, mortality, Invasive Mechanical Ventilation, d-dimer

1. Introduction

Coronavirus Disease 2019 (COVID-19) pandemic stems from a novel coronavirus infection called Severe Acute Respiratory Syndrome covid - 19 (1). The infection spread rapidly worldwide and was declared a pandemic by the World Health Organization. As of April 30, 2021, there were 150.110.310 documented cases worldwide and it was reported that 3.158.792 patients died (2). Since there is currently no special treatment or medication against the new virus, it is vital to determine the risk factors for mortality.

Lung involvement in severe infection is a serious complication that requires hospitalization in Intensive Care Units. The lungs are usually affected bilaterally. The severe respiratory failure caused may require mechanical ventilation. There are no specific drugs for the treatment of these patients, and supportive treatment is administered. The blood results of these patients may vary, and since there are comorbidities in COVID-19, examining them is guiding in severe pneumonia in terms of prognosis and treatment.

The total protein, albumin, AST, ALT, GGT, LDH, PT, PTT, T. Bilirubin, D. Bilirubin results, which show liver functions, may vary in patients in invasive mechanical ventilation. Furthermore, levels of acute-phase reactants, WBC (White Blood Cell), N/L (Neutrophil/Lymphocyte Ratio), and CRP (C-Reactive Protein), are guiding in demonstrating the severity of the disease.

The mechanism of the liver injury in COVID-19 patients is still not precise. It may stem from direct virus infection, immune damage, drug-related liver damage, systemic inflammatory response, ischemia, hypoxia, or relapse or exacerbation of the underlying liver disease.

This study investigated the relations between liver functions and other acute-phase reactants in COVID-19 patients in invasive mechanical ventilation and their effects on mortality.

2. Material and Methods

After obtaining the approval of the Ethics Committee of Ankara City Hospital (29/12/2021-2046), we took the blood samples from 60 COVID-19 patients and examined 65

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healthy volunteers on the computer in the laboratory.

We divided the cases into three groups: 1) the healthy Control Group, 2) the intubated, and 3) the non–intubated patients. We then examined the lung tomography results of the cases diagnosed with COVID-19. We recorded the PCR results from the system, entering the total protein, albumin, AST, ALT, GGT, LDH, PT, PTT, T. Bilirubin, and D. Bilirubin test results separately into the statistics program.

We excluded those under 18 years of age, trauma patients and pregnant women from the study.

2.1. Statistical analysis

We used the Statistical Package for Social Sciences for Windows, Version 22 (IBM, Armonk, NY, USA) for the statistical analyses; the Kolmogorov-Smirnov test for the normality of the variables; Mean±Standard Deviation (SD) for the parameters with normal distribution; median (interquartile range) (IQR) for the parameters not consistent with the normal distribution; and the One-Way ANOVA Test for the parameters normally distributed. We evaluated those without normal distribution with the Kruskal-Wallis Test and compared categorical variables using the Chi-Square Test or the Fisher's Exact Test. We used the Receiver Operation Characteristic (ROC) curve to analyze the efficiency of the COVID-19 severity and calculated the optimal cut-off values of the AST, GGT, procalcitonin, direct bilirubin, LDH, and NLR. A p-value <0.05 was considered to be statistically significant.

3. Results

Table 1 shows the demographic data of the COVID-19-related pneumonia cases and the control group. We found no significant differences between the cases regarding age and gender. The intubated 52 patients were under mechanical ventilation. The RT-PCR test results of all patients were positive. We examined the tomography results of all patients and entered the findings into the system.

We calculated the p-value for each parameter comparing the groups in the statistical analysis and divided the cases into three groups: 1) the healthy Control Group, 2) intubated (severe), and 3) not intubated patients (mild). As in Table 1, the data of the three groups evinced that the total protein and albumin levels were significantly lower in the serum ($p<0.01$). The LDH, GGT, AST, and ALT levels were higher at statistically significant levels in the mild and severe patient groups ($p<0.05$). The statistical analysis conducted between the severe and mild patient groups without including the Control Group indicated no significant changes in all these parameters. Fig. 1 shows the analysis in detail.

Table 2 includes the analysis of the cases who recovered from COVID-19 and who died. The serum T. protein and albumin levels were close to normal limits in fully recovering cases but lower in those who died ($p<0.01$). The LDH and GGT values were significantly higher in those who died ($p<0.01$). Comparing the two groups excluding the Control Group, we found significantly higher LDH levels ($p<0.01$).

Fig. 2 shows the event analysis of the blood routine parameters (i.e. the ROC curves) in estimating critical COVID-19 patients. We designated the COVID-19 Group with mortality as the positive and the Control Group as the negative group. We used the ROC curve to analyze the effectiveness of various blood routine parameters in diagnosing severe COVID-19 at admission and analyzed the optimal cut-off values calculated with the ROC Analysis. The D-dimer, NLR, AST, GGT, D. Bil, and LDH values had the highest AUC in the ROC analysis. Table 3 shows the laboratory parameters' EAA, optimal cut-off, and sensitivity and specificity values.

Table 1. Blood parameter characteristics of patient groups according to the intubated group

Characteristics	Control group (n=65)	Non-intubated group (n=68)	Intubated group (n=52)	p Value*	p Value**
Age, Median(IQR), range, years	58.2 ± 17.1, 18-75	66.3 ± 14.2, 23-80	69.8 ± 10.9, 23-80	0.272	0.683
Gender, male/female	34/31	36/32	29/23	0.861	0.848
Laboratory analysis					
PLT, (×109 / L)	259.9 ± 75.0	263.5 ± 136.9	241.1 ± 120.5	0.584	0.570
Albumin, (g/L)	46.5 ± 4.4	33.9 ± 6.2	33.1 ± 6.5	<0.001	0.789
T.protein, (g/L)	71.0 ± 4.1	58.9 ± 9.1	59.9 ± 77.4	<0.001	0.778
ALT, (U/L)	27.8 ± 17.0	51.3 ± 94.1	125.8 ± 61.1	0.034	0.102
AST, (U/L)	21.7 ± 12.9	58.1 ± 88.6	63.6 ± 106.1	0.008	0.927
LDH, U/L	206.8 ± 45.2	377.2 ± 212.7	469.0 ± 149.1	0.002	0.419
GGT, (U/L)	27.4 ± 18.0	63.2 ± 59.3	77.8 ± 117.4	0.001	0.514
D-dimer, mg/L	0.5 ± 0.6	2.3 ± 3.2	11.6 ± 12.6	<0.001	<0.001
T.bilirubin, (mg/dL)	0.8 ± 1.3	1.5 ± 5.1	0.8 ± 0.9	0.412	0.560
D.bilirubin, (mg/dL)	0.2 ± 0.2	0.4 ± 0.6	0.5 ± 0.7	0.022	0.753
PT, sec	13.3 ± 13.1	15.6 ± 13.8	13.9 ± 3.0	0.721	0.757
aPTT, sec	24.3 ± 3.5	29.9 ± 16.1	28.0 ± 8.0	0.019	0.691

All values are presented as Mean±SD. *Comparison of three groups, **Comparison of the intubated and non-intubated groups. p-values less than .05 were considered significant and highlighted in bold.

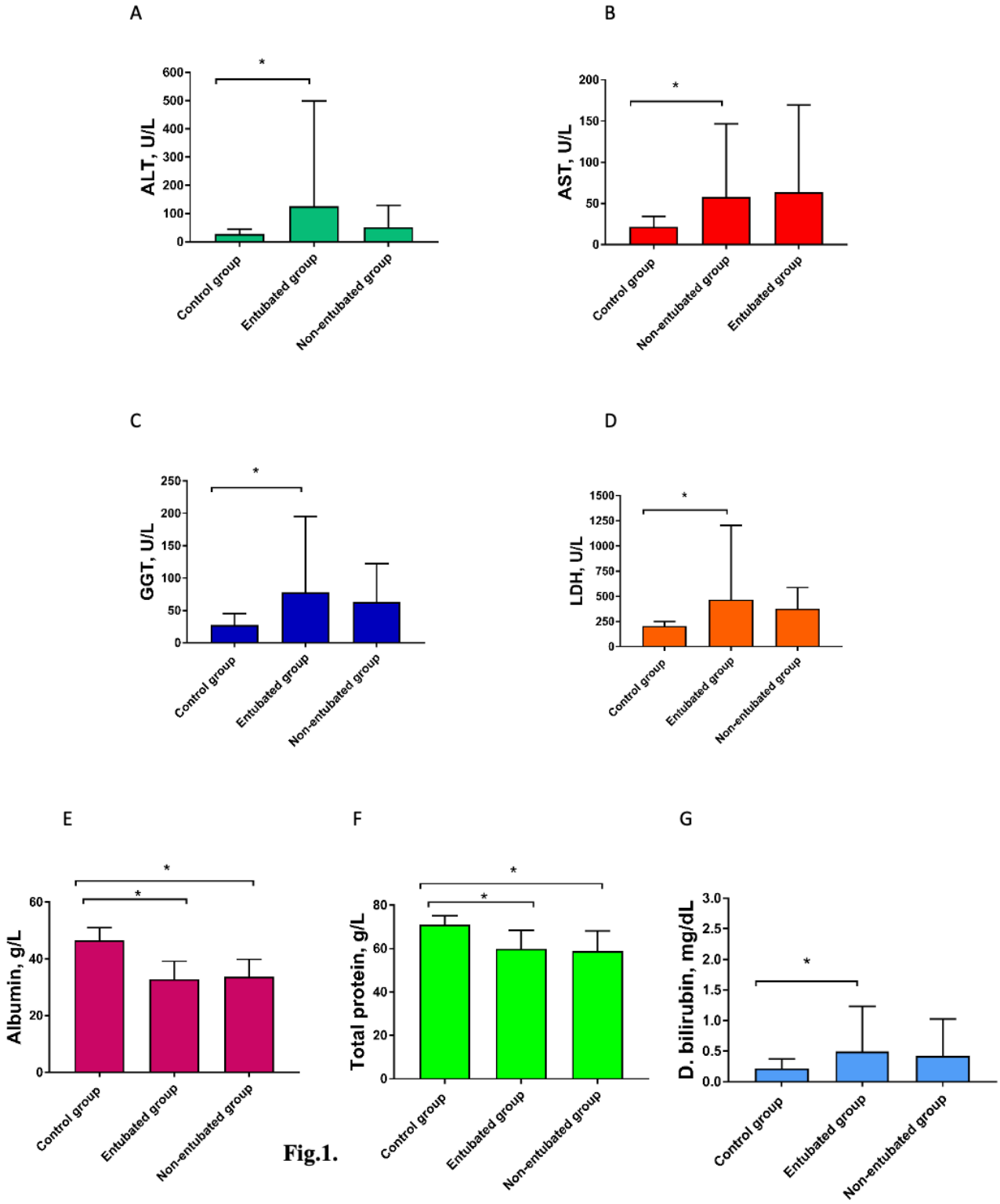

Fig.1.

Fig. 1. Comparison levels of ALT (A), AST (B), GGT (C), LDH (D), albumin €, total protein (F) and direct bilirubin (G) of control, entubated and non-entubated groups, p values less than. 001 were considered significant highlighted in asterisk

Table 2. Blood parameter characteristics of patient groups according to ex and recovery groups

Characteristics	Control group (n=65)	ex group (n=46)	recovery group (n=76)	p Value*	p Value**
Age, Median(IQR), range, years	63.2 ± 17.1, 18-75	67.4 ± 13.5, 42-90	72.7 ± 14.6, 43-88	0.324	0.149
Gender, male/female	34/31	30/16	44/32	0.637	0.474
Laboratory analysis					
PLT, (×109 / L)	259.9 ± 75.0	239.1 ± 137.7	265.9 ± 127.3	0.453	0.431
Albumin, (g/L)	46.5 ± 4.4	33.1 ± 6.4	33.6 ± 6.1	<0.001	0.906
T.protein, (g/L)	71.0 ± 4.1	56.2 ± 10.2	61.1 ± 7.6	<0.001	0.002
ALT, (U/L)	30.9 ± 22.0	62.9 ± 105.6	50.9 ± 68.2	0.058	0.631
AST, (U/L)	21.7 ± 12.9	64.0 ± 107.9	57.4 ± 85.7	0.008	0.893
LDH, U/L	206.8 ± 45.2	693.2 ± 96.4	299.1 ± 123.3	<0.001	<0.001
GGT, (U/L)	27.4 ± 18.0	77.4 ± 88.2	62.5 ± 80.6	0.001	0.483
T.bilirubin, (mg/dL)	0.8 ± 1.3	0.9 ± 1.0	0.8 ± 0.7	0.668	0.779
D.bilirubin, (mg/dL)	0.2 ± 0.2	0.5 ± 0.7	0.4 ± 0.6	0.020	0.685
PT, sec	13.3 ± 13.1	15.3 ± 3.6	14.8 ± 14.2	0.905	0.972
aPTT, sec	24.3 ± 3.5	35.1 ± 20.1	25.6 ± 5.3	<0.001	<0.001
D-dimer, mg/L	0.5 ± 0.6	10.7 ± 11.9	1.8 ± 2.1	<0.001	<0.001

All values are presented as Mean±SD. *Comparison of three groups, **Comparison of ex and recovery groups. p-values less than .05 were considered significant and highlighted in bold.

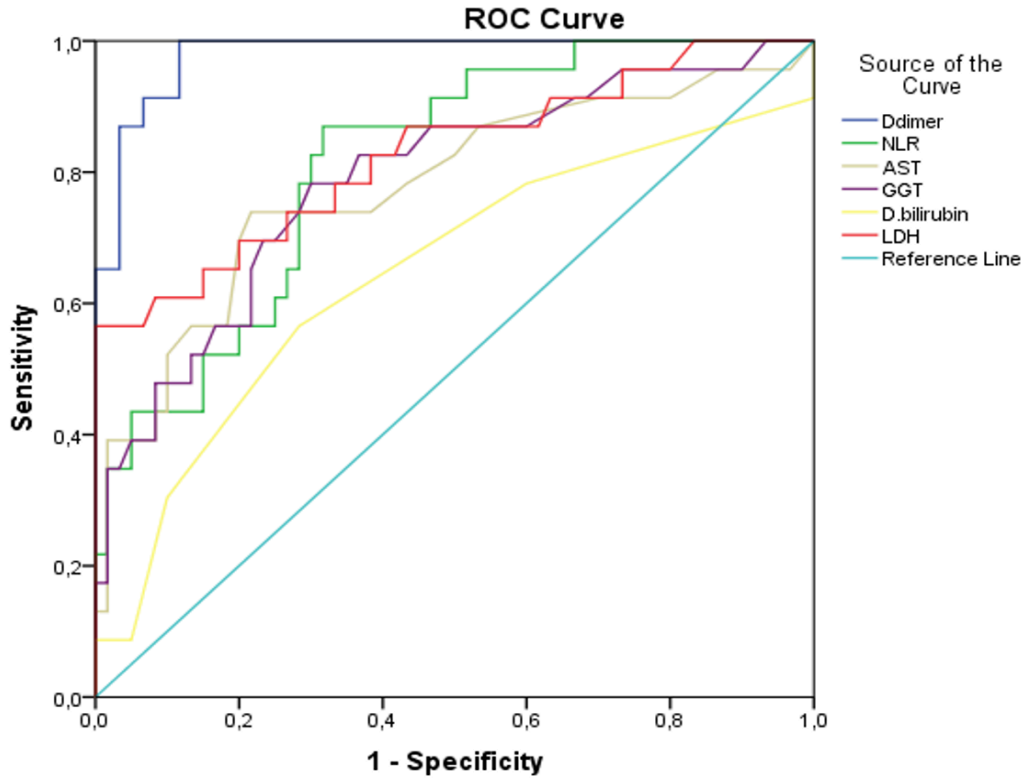

Fig. 2. The ROC curves of NLR, AST, GGT, D. bilirubin, LDH and D-dimer in predicting severity of COVID-19 infection on admission. NLR: Neutrophils-to-lymphocytes ratio

Table 3. Blood parameters in the diagnosis of ex patients with intubated COVID-19

Variables	Cut-off value	AUC (95% CI)	Sensitivity (%)	Specificity (%)	p-value
AST	>22.5	0.779 (0.686 - 0.871)	76.1	73.3	<0.001
GGT	>28.5	0.770 (0.679 - 0.862)	73.9	66.7	<0.001
D. bilirubin	>0.25	0.642 (0.531 - 0.754)	52.2	71.7	0.012
NLR	>3.875	0.799 (0.716 - 0.881)	76.1	70.0	<0.001
LDH	>204.5	0.749 (0.651 - 0.846)	80.4	55.5	<0.001
D-dimer	>0.985	0.980 (0.957 - 1.00)	95.7	88.3	<0.001

AUC: Area under the curve; NLR: Neutrophils-to-lymphocytes ratio. A p-value less than 0.05 were considered significant.

4. Discussion

SARS-COV-2 infection causes a wide clinical spectrum ranging from an asymptomatic state to severe pneumonia. Mechanical ventilation is inevitable in the treatment of cases with severe respiratory failure. The intensive care duration and improvement period increase in these cases (3). Evaluating the prognostic factors in this process is essential for prioritizing patients who need intensive care more. COVID-19 has no specific treatment except for infection control and support treatment. Multi-organ support treatment is the basis in the treatment of COVID-19 critical patients. Therefore, identifying the prognostic severity criteria is paramount in providing early intervention to patients who might require ICU support (3, 4).

Increasing numbers of liver damage have been reported since the Covid-19 pandemic began; however, the mechanism of the liver damage caused by COVID-19 is not yet precise. All genome sequencing results showed us that SARS-COV-2 is similar to SARS-COV with 82% genome sequence and the Middle East Respiratory Syndrome Coronavirus (MERS-COV), sharing 50% genome sequence homology (5). SARS-COV, MERS-COV, and SARS-COV- 2 are known coronaviruses causing severe respiratory symptoms. Previous studies reported liver damage in 60% of patients infected with SARS-COV and in some infected with MERS-COV (6, 7).

A study conducted in Wuhan with COVID-19 infected cases reported that abnormal Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) levels were observed in 43 of 99 patients (8). At least 12 clinical studies conducted with a single or multicenter design reported that approximately 14.8%-53% of COVID-19 patients had liver damage. These studies showed that liver damage is relatively common in COVID-19 patients (9).

Liver damage manifests with increased ALT, GGT, LDH, AST and TBIL levels, decreased albumin (ALB) levels, and as abnormal liver biochemical indicators in COVID-19 patients (9-12). We found in our study that the AST, ALT, T. Bil, and LDH have increased at significant levels in both groups of intubated and non-intubated patients. The T. protein and albumin levels were low. Our findings were in line with the literature data. Detecting that these biomarkers have increased abnormally suggests that they could be used as criteria in treating COVID-19. Clinicians should follow the changes in the liver biochemical indicators in COVID-19 treatment, detect the patients with liver damage in the early period, and initiate the transfer to Intensive Care Units.

COVID-19 infection may deteriorate blood coagulation functions. COVID-19 studies reporting liver damage also reported Prolonged PT, aPTT, elevated D-dimer, and thrombocytopenia (13-15). The clinical reflection of COVID-19-related coagulopathy is basically the dysfunction of organs, and hemorrhagic events are less frequent. The changes in hemostatic biomarkers represented by increased D-dimer and fibrin/fibrinogen destruction products show massive fibrin formation in the etiopathogenesis of coagulopathy (14).

Currently, the mechanisms of coagulopathy in COVID-19 have not yet been elucidated. Inflammatory cytokines, lymphocyte cell death, hypoxia, and irregular immune responses caused by endothelial damage may play roles in this respect. The bleeding tendency is rare even in cases with prolonged coagulation tests (14, 15). Certain studies found D-dimer levels to be associated with increased mortality. These studies recommended heparin at low molecular weight to prevent thromboembolic complications (16, 17). We found in our research that the PT and APTT values were slightly prolonged, and the platelet values did not show any significant changes. Moreover, the finding of high D-dimer levels is compatible with the literature data. The ROC curve shows that D-dimer is associated with mortality as a biomarker in COVID-19 patients in invasive mechanical ventilation.

Certain publications reported the optimum cut-off value of some serum biochemical parameters as the indication of prognosis in COVID-19 by using the severe disease ROC curve (4, 18). D-dimer, GGT, D. Bil, LDH, NLR and AST had the highest AUC in the ROC Analysis. The AUC values of the AST, GGT, D. Bilirubin, and LDH, which show the liver functions, are crucial in revealing the severity of COVID-19 infection. These tests are significant compared to the ROC Analysis in showing the prognosis.

Elevated D-dimer, GGT, D. Bil, LDH, NLR and AST levels are associated with mortality in COVID-19 patients in invasive mechanical ventilation.

Conflict of interest

The authors declare that there is no conflict of interest between them.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 699-705
doi: 10.52142/omujecm.39.3.20

The efficacy of computed tomography-guided percutaneous microwave ablation in patients with osteoid osteoma according to nidus location in long bones: A single-centre initial experience

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Received: 19.01.2022 • **Accepted/Published Online:** 15.02.2022 • **Final Version:** 30.08.2022

Abstract

This study aims to assess the efficacy of microwave ablation (MWA) treatment in painful extra-articular osteoid osteoma (OO) in long bones in a consecutive series of patients and to compare the technical and clinical success in terms of pain scores according to their location. A total of 23 patients who were diagnosed with OO in long bones and treated with MWA between January 1, 2016, and June 1, 2020 were enrolled in this study. Medical data and images were reviewed for age, gender, size of the nidus and duration of pain. Patients were then categorized into three groups according to the location of nidus (pertrochanteric, metaphyseal or diaphyseal). Operation length and technical and clinical success rates were compared. Technical success was achieved in 100% of patients. Primary clinical success following MWA was 82.6%. The most common location was the pertrochanteric region (34.8%). In comparing the pain scores with the locations of the lesions, no statistically significant difference was found between the first-day ($p = 0.504$) and first-week ($p = 0.648$). However, significant difference was present between the first month ($p = 0.016$), third month ($p < 0.001$) and sixth month ($p = 0.001$). The statistically significant differences between the sites were due to the difference between the pertrochanteric and diaphyseal lesions. CT-guided percutaneous MWA is safe and effective in the treatment of OO located in long bones without any recurrence. However, management of pertrochanteric OOs requires specific expertise and follow up. Further studies are expected in the future to assess the long-term efficacy and safety of MWA for the treatment of OO, especially for cases in pertrochanteric locations of the femur, which have an increased risk of local recurrence.

Keywords: microwave ablation, osteoid osteoma, bone tumor, clinical efficacy

1. Introduction

Osteoid osteoma (OO) is a benign inflammatory bone tumour of unknown etiology that accounts for 2–3% of all primary bone tumours, with an incidence of 10–12% among all benign bone tumours. It was first described by Jaffe in 1935 (1). Its most common symptom is bone pain with an immediate response to salicylates or other nonsteroidal anti-inflammatory drugs (NSAIDs). Cases of spontaneous remission have been reported in studies on the natural history of lesions, but treatment is usually required to provide relief (2). Most lesions are located in lower extremities, especially metadiaphyseal parts of the femur and tibia (3). Around the circumference of the femoral neck and intra-articular regions are the most frequently involved areas. It is also common in the spine, hands and feet. Radiographically, an OO is observed as an intracortically located radiolucent nidus with a dense sclerosis surrounding it, but it may rarely show the intramedullary location. In computed tomography (CT), it appears as hypodense areas in the form of central calcification and perinidal sclerosis (4).

The gold standard treatment method for OO was surgery in the past. However, due to the difficulties in identifying the lesion intraoperatively and the need for long-term rehabilitation, treatment with percutaneous methods has become the first preferred treatment option today (5). Radiofrequency ablation (RFA) has been the first preferred minimally invasive method since its use, but in recent years, the number of studies reporting that microwave ablation (MWA) is also an effective and safe treatment method has been increasing. The main clinical advantages of MWA are higher temperatures and faster heating, shorter ablation times, larger ablation volumes and less heat sink effect compared to RFA (6,7). Several studies have been published describing the outcomes and side effects of MWA (8-10). However, only a few studies have investigated the effectiveness of the treatment according to the location of the nidus in the long bones (11,12). Proximal femur is one of the most common sites of primary benign bone tumors such as OO, and it becomes an important anatomical region due to its unique anatomy and biomechanics. Also, there is a high risk of pathological fracture after surgery due to anisotropy in this localization (13). In

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addition, lesions seen in the juxta-articular region of the femur may show different clinical and radiological symptoms from those seen in other regions. On the other hand, painful joint limitation of motion was observed in some cases after RFA treatment. In proximal femoral OOs, the combined effects of periarticular muscle contracture and synovitis may induce more complex skeletal deformity (14). Also, some studies have hypothesized that medications used for pain relief would be less effective due to secondary synovitis (15). In the light of all these facts, the management of OOs located in the proximal femur requires special expertise and follow-up.

The efficacy of MWA treatment of intra-articular and extra-articular OOs has previously been identified in some studies (16), but to our knowledge, no studies have yet compared the effectiveness of treating extra-articular lesions located in the long bones especially between proximal femur and the other regions of long bones. Therefore, the aim of our study was to assess the efficacy of MWA in the treatment of painful extra-articular OOs in long bones in a consecutive series of patients and to compare the technical and clinical success and complications according to their location.

2. Materials and Methods

Institutional review board approval was obtained for this retrospective study for all OOs treated with MWA between January 1, 2016, and June 1, 2020 at our single-centre tertiary care hospital. Thirthy consecutive MWA examinations were performed during the study period. In each OO cases, diagnosis was established by demonstrating a definite nidus along with perilesional cortical thickening on CT or bone marrow edema on magnetic resonance imaging (MRI) in patients with severe bone pain who clinically required treatment with NSAIDs almost every day to relieve pain. The decision to continue with MWA was made by a team including a radiologist and an orthopedic specialist. Patients who had high-risk anatomical lesions were excluded from ablation therapy. For example an osseous lesion in close proximity to the main vascular structures or having a nerve trace adjacent to the lesion. Additionally, patients who had insufficient information in the hospital information archive and who could not be reached by telephone were excluded from the study. Consequently, 23 patients (mean age 16.22 ± 7.89, range 2–35) were enrolled in the study. There were 14 women (60.9%) and 9 men (39.1%). We divided the patients according to the location of nidi into three groups as pertrochanteric, metaphyseal or diaphyseal regions in long bones.

All patients and/or their guardians were informed about the technique and its possible benefits and complications, and they signed a written consent form to undergo the procedure. CT was used as an imaging modality to locate the lesion, follow the needle trace, and reveal the final position of the probe. For all patients, general anaesthesia was applied in addition to local lidocaine at the ablation sites. All patients were treated using the Helios Microwave Ablation System H-1 (Canyon Medical, Jiangsu, China). In all cases, after the bony cortex was passed using an 11-gauge cortical drill, the 13-gauge core needle was advanced coaxially into the lesion nidus with imaging guidance. After inserting the microwave probe into the OO through a coaxial sheath, the sheath was retracted as far as possible from the probe tip (Fig. 1, 2). A minimum of three ablation cycles targeting 80°C were performed at 30 to 50 W for 30 seconds at 1-minute intervals. Patients who did not develop any complications were discharged on the same day of the procedure. For antibiotic prophylaxis, 2 g cefazoline was administered intravenously before MWA ablation and for the first 2–3 days after the procedure.

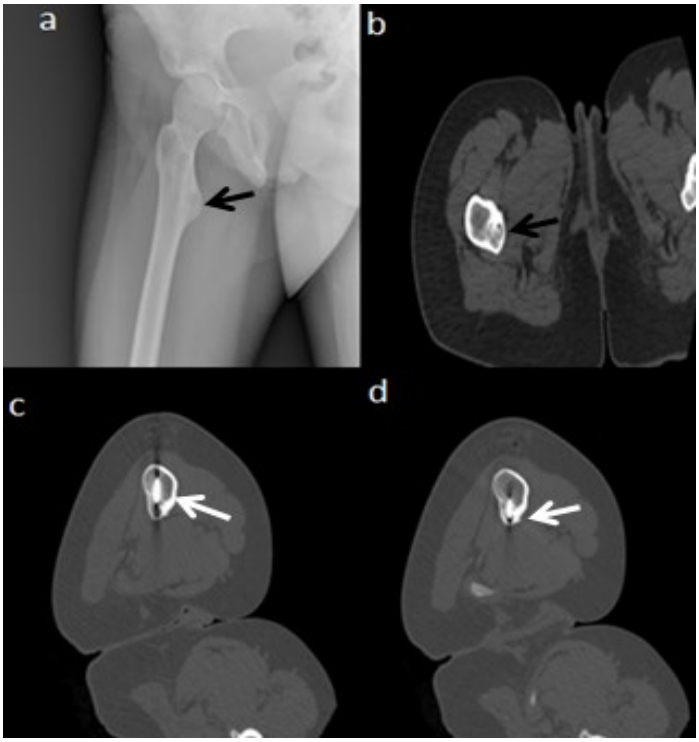

Fig. 1. 18-year old girl wtih osteoid osteoma of the right femur, (a) The AP plain radiographs and (b) Axial CT image shows the nidus at lesser trochanter (black arrow) with adjacent cortical thickening (c-d) The intraprocedural axial CT image shows the MWA antenna in the center of the lesion (white arrows)

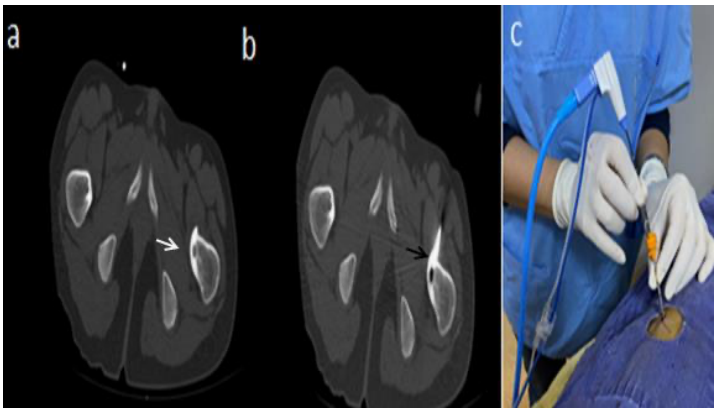

Fig. 2. Ablation of an osteoid osteoma of the left femur intertrochanteric region (a) CT image demonstrates the nidus (white arrow) (b-c) The intraprocedural axial CT image shows the MWA ablation probe being inserted into the nidus through a needle (black arrow)

Medical data and images were analyzed for age, gender, nidus size, date, duration, and location of the lesion in the pertrochanteric, metaphyseal, or diaphyseal regions of the long bones. A follow-up appointment is planned for all our patients for 1 and 6 months after the procedure. In the vast majority of patients, we prefer CT as a follow-up imaging modality to determine if ablation has been completed. However, in some cases where we want to show bone marrow edema or suspect infective conditions, we resort to MRI. Numerical pain scores (0–10) of the patients were recorded using a visual analog scale (VAS). Patients were grouped into three categories based on location of in long bones and treatment with MWA. Operation length, recurrence and success rates were compared between three groups. The success of each ablation was evaluated both clinically and technically.

Technical success was defined as the application of the target ablation temperature by reaching the ablation probe distal to the nidus. Clinical success was defined as the complete relief of pain without the use of painkillers in the sixth month follow-up of the patients. The complications were defined according to the Society of Interventional Radiology (SIR) guidelines (17). Patients were also called one year after the procedure for follow-up on any complications or recurrence of pain.

The study data were evaluated using SPSS for Windows 22.0 software (SPSS Inc. Chicago, IL). The compatibility of the variables with a normal distribution was examined using the Shapiro-Wilk test.

For analysis frequency, percentage, mean value, standard deviation and data range (min–max) were used. Categorical variables were indicated by number and percentage (%). For dependent groups, a comparison was conducted using the Wilcoxon signed-rank test; for independent groups, the independent samples t-test was used. In the comparison of continuous measurements between groups (three groups), by controlling the distributions, one way analysis of variance (ANOVA) was used for variables conforming to the normal distribution, and the Kruskal Wallis test was used for variables that did not fit the normal distribution. Pairwise comparisons were made using the Mann-Whitney U test and were evaluated using post-hoc Bonferroni correction. Spearman test was used for correlation analysis. Statistical significance was recognized as $p < 0.05$ for all tests.

3. Results

We included 23 patients with OOs in long bones. All procedures were completed successfully. Demographic and clinical data are summarized in Table 1.

The youngest patient in our series was 2 years old and the oldest was 35 years old. Of the lesions, 16 (69.6%) were located on the femur: 8 on the right side and 8 on the left. The most common location of tumours in the femur was the pertrochanteric region (34.8%). The average ablation duration was 48.04 (30–65) minutes.

Table 1. Patient demographic and clinical data (n=23)

Characteristic	Value
Age (years)	
Mean	16.22
Range	2-35
SD	7.89
Gender	
Male	14(60.9%)
Female	9 (39.1%)
Preprocedural pain (months)	18.78
Mean	2-72
Range	16.62
SD	
Nidus size (mm)	
Mean	5.61
Range	2-10
SD	2.17
Nidus location	
Pertrochanteric femur	8 (34.8%)
Diaphysial femur	5 (21.7%)
Metaphysial femur	3 (13%)
Metaphysial Tibia	4 (17.3%)
Diaphysial Tibia	2 (8.6%)
Metaphysial humerus	1 (4.3%)

SD: Standard deviation

In a two-year-old paediatric patient with a lesion in the tibia diaphysis, osteomyelitis developed in addition to the wound discharge and infection findings two weeks after the procedure. It was the only serious complication encountered in all procedures. The patient was hospitalized for osteomyelitis treatment and received intravenous antibiotics. The wound site was cleaned and he was discharged in a healthy condition. Another complication was mild femoral reversible postprocedural neurapraxia releated with lateral femoral cutaneous nerve in a patient with osteoid osteoma of the femur. Patient reported numbness and burning sensation on the anterolateral thigh for 6 months. Permanent neurological deficits or intervention-related mortality were not observed in any of the patients included in this study.

A nine-year-old patient with a lesion in her lesser trochanter developed bone marrow oedema after MWA. There was no osteomyelitis or skin lesion. The patient had only moderate pain for six months after the ablation. Then, by the one-year follow-up, her symptoms had resolved completely.

The results of 23 patients who underwent MWA are summarized in Table 2. The difference between preintervention and postintervention pain scores was statistically significant ($p < 0.001$). At the last one-year follow-up, OO-related pain had completely disappeared in all patients. Therefore, no additional imaging was required. Clinically, no recurrence was observed in any of the patients in the study

In comparing the pain scores by the location of each lesion,

no statistically significant difference was found between the first-day (p = 0.504) and first-week (p = 0.648) pain scores. However, there was a statistically significant difference between the first-month (p = 0.016), third-month (p < 0.001) and sixth-month (p = 0.001) pain scores.

Table 2. Outcome measurements, correlation between nidus location and clinical success

	Mean	Range	SD	P value	Post-hoc p value***
VAS score pre procedure					
Pertrochanteric[a]	9.75	9-10	0.463		
Metaphsial[b]	9.88	9-10	0.354	0.735*	-
Diaphysial[c]	9.71	9-10	0.488		
VAS score 1 day post procedure					
Pertrochanteric[a]	6.88	5-8	0.991		
Metaphsial[b]	7.75	5-10	1.699	0.504**	-
Diaphysial[c]	7.43	5-10	1.718		
VAS score 1 week post procedure					
Pertrochanteric[a]	4.5	4-6	0.756		
Metaphsial[b]	5.13	3-7	1.458	0.648*	-
Diaphysial[c]	4.86	2-8	1.864		
VAS score 1 month post procedure					
Pertrochanteric[a]	3	2-4	0.756		a-b 0.108
Metaphsial[b]	1.75	0-5	1.909	0.016*	a-c 0.003
Diaphysial[c]	0.86	0-2	1.069		b-c 0.384
VAS score 3 month post procedure					
Pertrochanteric[a]	2.75	2-6	1.389		a-b 0.001
Metaphsial[b]	0.25	0-2	0.707	<0.001*	a-c <0.001
Diaphysial[c]	0	0	0		b -c 0.350
VAS score 6 month post procedure					
Pertrochanteric[a]	1.5	0-4	1.309		a-b 0.004
Metaphsial[b]	0	0	0	0.001*	a-c 0.006
Diaphysial[c]	0	0	0		b-c 1

SD: Standard deviation. VAS: Visual analog scale. *Kruskal-Wallis test. **One-way ANOVA. *** We performed Bonferroni correction to compare groups as a post-hoc test. Accordingly. p<0.017 is statistically significant in paired comparisons.

Table 3. Correlation between pre and post-procedural pain and nidus size

	Nidus size ≤5 mm		Nidus size >5 mm		
	Mean±SD	Range	Mean±SD	Range	P value
VAS score pre procedure	9.8±0.42	9-10	9.7±0.43	9-10	0.862*
VAS score 1 day post procedure	7.1±1.19	5-9	7.54±1.66	5-10	0.49**
VAS score 1 week post procedure	4.8±0.78	4-6	4.85±1.72	2-8	0.796*
VAS score 1 month post procedure	2±1.41	0-4	1.85±1.72	0-5	0.821**
VAS score 3 month post procedure	1.1±2.02	0-6	1±1.15	0-3	0.695*
VAS score 6 month post procedure	0.7±1.33	0-4	0.38±0.76	0-2	0.658*

* Mann-Whitney U test, ** Independent samples t-test

Bonferroni correction was conducted as a post-hoc test in the paired comparisons between the three differently located groups. Accordingly, comparisons with p < 0.017 were considered statistically significant. It was determined that the statistically significant differences between the sites at the first, third and sixth months were due to the difference between the pertrochanteric lesions and the diaphyseal lesions.

The mean durations of the MWA procedures were found to be 57.50 + 4.62, 50 + 8.01 and 35 + 2.88 minutes for pertrochanteric, metaphysial and diaphyseal lesions,

respectively. While there was no statistically significant difference between the pertrochanteric and metaphysial groups in terms of procedure time (p = 0.059), there was a statistically significant difference between pertrochanteric lesions and diaphyseal lesions in procedure time (p = 0.001). There was also a statistically significant difference between the metaphyseal and diaphyseal groups (p = 0.001). Therefore, it was concluded that the difference between the operation time and the groups was likely due to the lesions in the diaphysis.

When nidi with a diameter of 5 mm or less and nidi larger than 5 mm were compared in terms of pain scores, no statistically significant differences were found between the scores at the first day, first week, first month, third month and sixth month (Table 3).

A statistically significant negative correlation was found between the duration of pain before the procedure and the first-day pain scores (p = 0.003, r = –0.592). A statistically significant negative correlation was also found between the duration of pain before the procedure and pain scores in the first week (p < 0.001, r = –0.721). However, correlations found with other pain scores are not statistically significant (Table 4).

Table 4. Correlation between preprocedural pain, VAS score 1 day post procedure and VAS score 1 week post procedure

	rho*	P value
Preprocedural pain (months) – VAS score 1 day post procedure	-0.592	0.003
Preprocedural pain (months) – VAS score 1 week post procedure	-0.721	< 0.001

* rho :Spearman's correlation coefficient

4. Discussion

Osteoid osteoma is a benign osteogenic tumor that accounts for 13.5% of all benign tumors and predominantly affects children and young adults (18). Although surgical removal of the nidus was used as the gold standard treatment method among the treatment options used for OO in the past, modern interventional methods such as RFA and MWA have lower complication rates and shorten the duration of hospital stay (19).

Our results confirm that percutaneous ablation is the treatment of choice for OOs, suggesting that there is little difference between procedure time, clinical presentation, follow-up and outcomes in terms of OO location in long bones. Our overall technique and primary clinical success (in terms of complete pain relief following MWA at 6 months) were 100% and 82.6%, respectively. These rates are in line with the findings of prior studies (3,5,9,12).

Most treatment failures are due to recurrent disease and are reported at a rate of 5.6% for all methods. Cantwell et al. (20) and Lee et al. (21) reported the success rate of surgical interventions as approximately 88-100% and the recurrence rate as 4.5-25% in their study. According to the results of a literature review (22), percutaneous thermal ablation of OOs with an overall success rate of 91.9% and an overall complication rate of 2.5% has been proven to be a safe and effective therapeutic alternative to open surgery.

Studies have reported that the recurrence rate decreased significantly after six months and reached very low rates, especially after two years (3). In our study, when we determined according to the symptoms of the patients in the mean follow-up period of one year, there was no recurrence in any of the cases.

Studies have concluded that even if there is a possibility of thermal risk, OOs can be safely treated with RFA. Vanderschueren et al. (23) reported that the only complication among the 97 participants in their study was skin necrosis, which resulted in a fistula in one patient. In their study, Lanza et al. (24) evaluated 27 articles including 1,772 patients and reported that 12 of 44 patients with complications had skin burns. Since RFA energy is transmitted by resistive heating, it spreads to the surrounding tissue, while heat production in MWA occurs by the rapid agitation of water molecules. It is important to minimize the procedure time to reduce the cumulative effect of anesthesia in pediatric patients. Therefore, it is possible to reach the target temperature in a shorter time with MWA, which allows faster tissue warming compared to RFA (25). There is also no potential for skin burns associated with pad placement in MWA (26). There were no skin burn complications in our series either. Our only serious complication was osteomyelitis in a two-year-old patient.

The proximal femur is one of the most common locations of primary benign bone tumours. Since tumours in this area are more commonly associated with clinical symptoms and serious complications as compared to other regions, numerous studies have focused on exposing the hip joint capsule or femoral neck (27). In addition, according to Campanacci (1), OOs were frequently encountered in the proximal femur in 25.4% of cases, while this rate was 40% in our series. The frequent localisation of the lesions in this region confirms the value of this study. In most studies (2,4,20,28,29) previously conducted on percutaneous treatment by drilling or thermoablation, no differentiation was made between the proximal femur and lesions located in other regions. The advantage of our range is that it focuses specifically on proximal femoral lesions.

Vanderschueren et al. (23) could not find any relationship between location and unsuccessful outcomes in their study, in which they grouped lesions as intra-articular or extra-articular and intracortical or extracortical according to their location. Rosenthal et al. (29), on the other hand, found that there was no statistically significant relationship between location (femur, tibia and other) and clinical outcome.

In this study, we classified the location of the osteoid osteomas as pertrochanteric, metaphyseal or diaphyseal in long bones. We found that the time it took for pain to disappear completely in pertrochanteric tumours was longer than in the

other groups. This difference is statistically significant and has not been previously described.

We can associate the presence of a more pronounced cortical reaction in lesions located in the diaphysis with a faster disappearance of pain. Heat insulation is easier in the ablation treatment of lesions with a better cortical surrounding. In lesions located in other regions (e.g. non-diaphyseal), since there is less accompanying cortical thickening, we predict that local ablation may be less effective, increasing the risk of local recurrence. However, no recurrence was observed in our series at one year.

We acknowledge some limitations in our study. First, although our study was the largest case series in the literature comparing lesions by location in long bones treated with MWA, it consisted of a relatively small number of patients. Second, we did not have histopathological confirmation; instead, we described lesions based on characteristic clinical presentation and imaging findings. However, as noted in previous studies in the literature, percutaneous pre-ablation bone biopsy may not be necessary in typical cases, as there is a significant percentage of non-diagnostic biopsy findings in OO, and most lesions that are similar to OO can be safely and successfully treated with thermal ablation (30). Finally, long-term follow-up data were not available in our study.

In conclusion, our study demonstrates that the CT-guided percutaneous MWA method is effective in the treatment of OOs located in long bones without recurrence. However, the management of pertrochanteric OOs requires special expertise and follow-up. In the future, more studies are needed to evaluate the long-term efficacy and safety of MWA for OO therapy, particularly in cases in pertrochanteric areas where the risk of local recurrence is heightened.

Conflict of interest

The authors declared no conflict of interest.

Ethical Approval: Ethical approval of this study was obtained from Ondokuz Mayıs University Hospital Institution Review Board and Ethics Committee prior to initiation this research study. Local Ethics Committee approval (date: 25/05/2021; approval number: 2021/163).

Funding

No funding was used for the study.

Acknowledgments

None to declare.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 706-709
doi: 10.52142/omujecm.39.3.21

Radiological evaluation of sella turcica dimensions in patients with empty sella

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Received: 20.01.2022 • **Accepted/Published Online:** 05.06.2022 • **Final Version:** 30.08.2022

Abstract

Empty Sella (ES) occurs when the subarachnoid space is herniated into the Sella Turcica (ST). ES may be radiologically determined randomly, and patients with ES are usually asymptomatic. However, approximately 20% of partial ES (PES) cases can be symptomatic. Therefore, it is essential to diagnose patients with ES accurately for treatment. We studied whether there was a difference in ST dimensions between patients with ES and healthy individuals using magnetic resonance imaging (MRI), and we compared a group of measurements using computed tomography (CT). 212 patients with ES and 98 healthy individuals participated in this study and underwent cranial 3T MRI. We divided the study population into three groups: the PES, total ES (TES), and control groups. We placed the patients who underwent both cranial MRI and paranasal CT in a separate group. The aperture, height, and length of the ST of all subjects were measured. MRI and CT showed that the length, height, and aperture diameters of the ST were statistically significantly different between the PES, TES, and control groups ($p<0.05$). In receiver operating characteristic analysis, the cut-off values for the length, height, and aperture measurements were 12.05 mm, 8.35 mm, and 9.65 mm, respectively. The dimensions of the ST expand in patients with ES, and we found a reliable threshold value for this expansion. CT taken for unrelated reasons may be used in the diagnosis of ES by measuring the dimensions of the ST.

Keywords: radiological evaluation, sella turcica, dimensions, empty sella

1. Introduction

Empty Sella (ES) occurs when the subarachnoid space is herniated into the Sella Turcica (ST), causing the pituitary gland to flatten in various degrees (1–3). ES has primary or secondary causes (4). Primary ES is less common than secondary ES (5, 6). The etiology of primary ES is unknown (3, 4). However, researchers have explained several causative mechanisms, including incompetence or complete absence of the diaphragma sellae, chronic intracranial hypertension (pseudotumor cerebri, brain tumors, intracranial thrombosis, and hydrocephalus), and temporary expansion followed by regression of the pituitary gland (e.g., expected increase in pituitary volume during pregnancy and lactation and spontaneous regression in pituitary volume during menopause) (5,6). Trans-sphenoid treatments, infections, bleeding, infarction, trauma, and pituitary autoimmune diseases cause secondary ES (3,4).

Primary ES may be radiologically determined randomly and patients with primary ES are usually asymptomatic (3,7). However, in approximately 20% of primary ES cases, endocrine, neurological, ophthalmological, and psychiatric symptoms may occur (7). Headache, menstrual irregularities, galactorrhea, hirsutism, and sterility are the most common clinical findings in patients with primary ES (7). In particular, headache and obesity are considered the most common clinical symptoms in men and women, respectively (3, 4). Therefore, it is crucial to diagnose patients with ES accurately for treatment.

ES is diagnosed using magnetic resonance imaging (MRI) of the sellar and suprasellar regions. Computed tomography (CT) can be used in patients with MRI contraindications. In CT and MRI, typical findings of ES (8) are intrasellar CSF filling in continuity with overlying subarachnoid spaces, residual pituitary gland with a semi-lunate shape flattened against the sellar floor, and enlarged bony sella (1–4, 6–8). CT and encephalograms have low sensitivity in diagnosing ES due to their low soft-tissue resolution. However, MRI is highly sensitive.

This study determined whether there was a quantitative difference in ST measurements between patients with ES and healthy individuals.

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2. Materials and Methods

2.1. Patient Population

212 patients with ES and 98 healthy individuals who met the study criteria participated in this retrospective study between January 2018 and June 2020 and underwent cranial MRI and paranasal CT. Ethics committee of Faculty of Medicine of Muğla Sıtkı Koçman University approved this study. We included patients aged over 18 and under 70 with nonspecific symptoms such as headache and dizziness who underwent MRI. We excluded patients with any anatomic malformation, trauma, history of surgery, and non-diagnostic MR images. We conducted this study the World Medical Association's Declaration of Helsinki principles.

2.2. MR and MDCT Imaging Protocol

We obtained MR images using a 3T scanner (MAGNETOM Skyra; Siemens Healthineers, Berlin, Germany) and performed ST measurements on sagittal T2-weighted brain MR images. We obtained images using a protocol of 384×278 matrix, a field of view (FOV) of 23.5 cm, repetition time of 3360 milliseconds (TR 3360 msec), echo time of 75 milliseconds (TE 75 msec), number of excitations of 1 (NEX), and slice thickness of 3.5 mm.

We obtained the paranasal CT images using the following protocol: FOV, 180 mm; slice thickness, 3 mm; kvp, 120; and mAs, 150. After the images were obtained, multiplanar reconstructions were performed and measurements were made from reconstructed images. We took sagittal images of the ST and performed, the length, height, and aperture measurements of the ST on the sagittal images.

2.3. Image Analysis

A radiologist with ten years of experience, blinded to the subjects, evaluated the ST length, height, aperture and shape. We divided the study population into three groups: the partial empty sella (PES), total empty sella (TES), and control groups. On MRI, PES is defined as less than 50% of the sella filled with cerebrospinal fluid (CSF) and pituitary size of 3 mm, and TES is defined as more than 50% of the sella filled with CSF and pituitary size of 2 mm (7, 9–11). Furthermore, we grouped participants according to gender.

We placed thirty-five patients who underwent both cranial MRI and paranasal CT in a separate group. We compared ST measurements based on compliance in patients who underwent MRI and multidetector CT examination.

We named the distance between the dorsum sellae and tuberculum sellae ends as the aperture, the height was the line extending from the deepest point of the sellar base to the aperture, the length was the distance between the top of the tuberculum sellae and the deepest point of the posterior wall of the dorsum sellae. We measured these parameters (Fig. 1), and classified the shape of the ST on the sagittal images as type 1, round; type 2, oval; and type 3, flattened (Fig. 2).

2.4. Statistical Analysis

We performed the statistical analyses of the data using Statistical Package for the Social Sciences, version 20.0 (IBM Corp., Armonk, NY, USA) and evaluated the conformity of the data to a normal distribution using the one-sample Kolmogorov–Smirnov test. We performed the paired sample t-test to compare the measurement compliance of the patients who underwent MRI and multidetector CT and recorded the patient's demographic data. We presented the ST's length, height, aperture as mean ± standard deviation (SD) and conducted and independent-samples t-test to evaluate statistical differences between men and women and One-way ANOVA to detect differences between the patient and control groups. We compared the groups using the Tukey test and calculated cut-off values using receiver operator characteristics (ROC) curve analysis between the patient and control groups. We presented the ST shape as a percentage (%) and used P values of 0.05 to denote statistical significance.

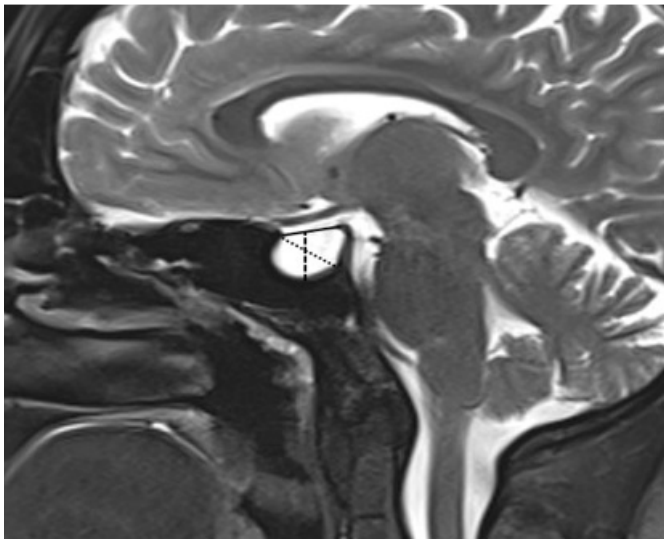

Fig. 1. On sagittal MR images, the length, height and apertura of sella turcica

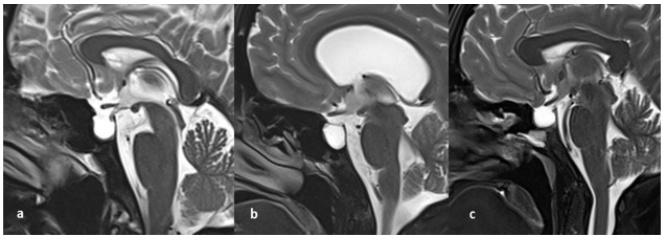

Fig. 2. The shape of the sella turcica on the sagittal MR images. (a) round, (b) oval, (c) flattened

3. Results

The total study population comprised 59 men and 153 women with a mean age of 54.21 ± 12.60 years (18–69 years). The MRI and multidetector CT examination perfectly agreed with the ST measurements ($p>0.05$). Table1 shows the length and height values of the ST according to gender. The aperture was higher in men than in women ($p<0.034$).

Table 2 shows the ST's length, height, and aperture values according to the groups. The ST's length, height, and aperture were significantly different between the PES, TES, and control

groups (Table 2). Table 3 shows the percentage distribution of the ST shape.

Table 1. The mean dimensions of Sella Turcica in patients with empty sella by gender

	Female (n=153) Mean±SD	Male (n=59) Mean±SD	p
Length (mm)	13.46±2.63	12.94±2.23	0.102
Depth (mm)	9.40±2.08	8.83±1.78	0.297
Apertura	10.52±1.96	10.60±1.94	0.034

Table 2. The mean dimensions of Sella Turcica in empty sella patients and control groups

	Partial ES Mean±SD	Total ES Mean±SD	Control Mean±SD
Length (mm)	14.10±1.60[a,b]	15.03±2.81[a]	10.83±0.96
Depth (mm)	9.73±1.56[a, c]	10.34±2.39[a]	7.66±1.03
Apertura	11.06±1.36[a,d]	11.73±2.35[a]	8.88±0.93

Data are n of participants, mean±SD.
a P<.001 compared with control group (One way ANOVA-Tukey test).
b P<.001 compared with TES group (One way ANOVA-Tukey test).
c P<.05 compared with TES group (One way ANOVA-Tukey test).
d P<.01 compared with TES group (One way ANOVA-Tukey test).

Table 3. The shapes of Sella Turcica in patients with empty sella

	PES (n=132)	TES (n=80)	Control (n=98)
Round	70 (53.03%)	34 (42.50%)	49 (%50%)
Oval	20 (15.15%)	25 (31.25%)	23 (23.47%)
Flattened	42 (31.82%)	21 (26.25%)	26 (26.53%)

We calculated cut-off values in ROC analysis for the length, height, and aperture measurements (Table 4).

Table 4. Sensitivity and specificity values in patients with empty sella according to ROC analysis

	Cut-off values	Sensitivity (%)	Specificity (%)	p
Length	12.05	92.9	91.8	0.001
Depth	8.35	81.1	81.6	0.001
Apertura	9.65	87.3	81.6	0.001

4. Discussion

This study, evaluated changes in the ST sizes in patients with PES and TES compared with the healthy control group. This study bore some significant findings. First, the sizes of the ST in the TES group were significantly larger than the PES and control groups. Second, the sizes of the ST in the PES group were significantly larger than in the control group. Third, the values of 12.05 mm, 8.35 mm, and 9.65 mm for the ST's length, height, and aperture respectively, are reliable thresholds to distinguish ES.

The size of the ST varies in different populations. The dimensions were smaller in the control group of this study than those in the Saudi and Caucasian populations but similar to those in the Norwegian, Iraqi, and Greek populations (12–17). The aperture values of the ST were higher in men with ES than those in women in our study ($p<0.034$), and we found no significant difference in other measurements. However, other studies reported no statistically significant differences between men and women regarding the ST's length, height, and aperture in their normal population (12–19).

If there are no contraindications, diagnosing ES is achieved using MRI (3). However, the literature measured ST bone sizes using direct radiography and CT (12–19). We performed the measurements in this study using MRI. We found that the patients who underwent both MRI and CT had high conformity by comparing MRI and CT measurements. We found in the literature no study investigating this type of correlation. Although CT is preferred in evaluating bone structures, our measurements using MR images indicate that the dimensions of the sella can be measured using MRI similar to CT.

Enlargement of the ST has been reported in patients with ES (1–8). This has been associated with the formation mechanisms. Incompetent diaphragma sellae and intracranial hypertension allow the accumulation of CSF into the ST, causing its enlargement. Initially, the pituitary gland enlarges, followed by a later decrease in gland size, which creates a space in which CSF can accumulate. Examples include an expected increase in pituitary volume during pregnancy and lactation and then spontaneous regression during menopause (5, 6, 20, 21). However, the threshold of this expansion has not been reported. We calculated the threshold value that would suggest the presence of enlarged sellae in patients who applied at our clinic. The values of 12.05 mm, 8.35 mm, and 9.65 mm for the sellar length, height, and aperture, respectively, are reliable thresholds to distinguish ES. As such, MRI and clinical evaluation could help diagnose ES in patients with increased sellar sizes who underwent CT and X-ray examinations involving the sellar region for any reason.

The shape of the ST can be round, oval, or flat. The most common types are round and oval (18,22). These classifications are based on the contours of the sellar floor and the angles formed by the contours of the anterior and posterior clinoid processes and the tuberculum sellae (23, 24). We used in this study, the basic shapes (oval, round, and flattened) to classify the ST and found round to be the most frequent shape in all groups (the control, PES, and TES groups). Although the size of the ST increased in patients with ES, it seemed that there was no change in shape. Many studies have investigated the shape of the ST in normal populations; however, we could not find any recent research examining the shape of the ST in patients with ES.

This study has some limitations. First, patient randomization may be impaired due to the study' retrospective nature. Second, a single radiologist made the measurements. Third, the results revealed the values of the patients evaluated in our hospital. However, the results, especially the threshold value findings, should be compared with multi-center studies or studies from different regions.

In conclusion, we have determined that the dimensions of the ST expand in patients with ES and found a reliable threshold value for this expansion. We believe that the maxillofacial and cranial CT taken for unrelated reasons can be used to diagnose ES by measuring the dimensions of the ST

and can be used to refer the patient for further clinical evaluation.

Conflict of interest

None to declare.

Funding

None to declare.

Acknowledgments

None to declare.

Authors' contributions

Concept: R.M.K., Design: R.M.K., Ö.Y., Data Collection or Processing: Ö.Y., M.Y.Ö., Analysis or Interpretation: Ö.Y., M.Y.Ö., Literature Search: R.M.K., N.Ç., Writing: R.M.K., N.Ç.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 710-713
doi: 10.52142/omujecm.39.3.22

Factors affecting the result of a conservative treatment in post-collapse osteonecrosis of the femoral head

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Received: 27.01.2022 • **Accepted/Published Online:** 17.07.2022 • **Final Version:** 30.08.2022

Abstract

Osteonecrosis of the femoral head (ONFH) affects patients' quality of life. In our institute, some patients in the post-collapse state denied having an operation and considered continuing conservative treatment. Hence, the purpose of this study was to compare patient characteristics between patients who had total hip arthroplasty and those still on conservative treatment. We collected the data of seventy patients who had osteonecrosis of the femoral head with femoral head collapse from the hospital electronic database. All patients underwent the same approach in consideration of THA. We advised patients to shift to a THA treatment plan from a conservative one if the pain did not decrease by conservative treatment or improvement did not meet the patient's lifestyle and daily living requirements. We recorded the patient's demographic data, physical examination and hip radiographic. Twentynine patients (40 hips) had THA, while 31 patients (45 hips) were still undergoing conservative treatment. There were no differences in the patients' base demographic data. However, patients with THA had a lower rate of history of previous core decompression before head collapse than patients still undergoing conservative treatment (90.3% and 37.9%, respectively, $p<0.01$). In the conservative treatment group, most cases had femoral head collapse at the superolateral head, while patients in the THA group had an increased number of patients with collapse at the superomedial part of the femoral head. This study revealed that patients considering the continuation of conservative treatment had a higher rate of history of core decompression and a lower rate of supermedial femoral head collapse. Thus, these factors might be considered in the discussions with patients concerning the prognosis of the disease.

Keywords: osteonecrosis, hip, conservative treatment, total hip arthroplasty

1. Introduction

Osteonecrosis of the femoral head (ONFH) is a common hip condition affecting the quality of life in adult patients worldwide. In the early 2000s, the incidence in the general population in the United States was estimated to be between 300.000 and 600.000 cases (1). The disease has been divided into two major groups: traumatic and non-traumatic ONFH. In the non-traumatic ONFH group, risk factors were studied and shown to be: alcohol intake (2), hypercoagulation disorder (3), and high-dose corticosteroids (4). However, 30% of non-traumatic ONFH were idiopathic (5).

The natural history of the disease begins at the non-collapsed femoral head, with or without pain, then the disease progresses to the post-collapsed femoral head in some patients, with arthritis change of acetabulum at the end-stage (6). Ficat and Arlet et al. classified the disease into four states by clinical and radiographic appearance (7). Besides, this ONFH has been categorized into pre-collapsed and post-collapsed stages. In the pre-collapse stage, the primary treatment is hip preservation, consisting of conservative treatment or core decompression, whereas in a post-collapse state, the standard treatment is total hip arthroplasty (THA) (8). Mohammad et al. reported that THA improved quality of life in patients with post-collapse non-traumatic ONFH (9).

However, in our institute, some patients in the post-collapse state denied having an operation and considered continuing conservative treatment. Hence, the purpose of this study was to compare patient characteristics between patients who had total hip arthroplasty and those still on conservative treatment.

2. Material and Methods

This study was retrospective. We collected the data from the hospital electronic database (database 2011-2019) and included patients who had osteonecrosis of the femoral head with femoral head collapse. The inclusion criteria were being diagnosed with post-collapse stage ONFH and followed up in our center for at least six months. All patients underwent the same approach in consideration of THA. We advised patients to shift to a THA treatment plan from a conservative one if the pain did not decrease by conservative treatment or improvement did not meet the patient's lifestyle and daily living requirements. All patients considered the choice themselves at each follow-up visit. Patients who chose

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conservative treatment would be treated with non-steroidal anti-inflammatory drugs, activity modification, weight reduction (if overweight), and hip strengthening exercises.

We recorded the demographic data, occupation, working level, underlying diseases, history of hip core decompression, range of motion on physical examination, hip radiographic data, and THA requirement. We classified occupation and working levels according to the International Standard of Occupations (10,11) and measured basic radiographic parameters of both hips in anteroposterior (AP) and frog-leg view to evaluate the location of femoral head collapse. The local Ethics Committee and Institutional Review Board approved this report.

We performed statistical analysis using R-program version 3.2.1 software (R Foundation for statistical computing, Vienna, Austria). We used the independent sample t-test to compare age, height, weight, body mass index (BMI), and the total motion range between the groups. We compared the difference in the motion range in each direction with the Wilcoxon rank-sum test. We analyzed gender, occupation, working level, site, history of previous core decompression, underlying diseases, risk etiology of osteonecrosis, and femoral head collapse data location with Pearson's chi-square to compare the groups. We defined statistical significance as $p<0.05$.

3. Results

We included 70 patients in the post-collapsed ONFH group. Twenty nine patients (40 hips) had THA, while 31 patients (45 hips) were still undergoing conservative treatment. There were no differences in the patients' base demographic data between the two groups regarding age, gender, body mass index (BMI), occupation and working level. (Table 1)

The data relating to osteonecrosis found that unilateral or bilateral inflammatory joint disease (RA, SLE) and risk etiology of osteonecrosis was not different between the groups. (Table 2) However, patients with THA had a lower rate of history of previous core decompression before head collapse than patients still undergoing conservative treatment.

The physical examination and radiographic data found that patients in the conservative treatment group had a higher range of motion in all directions compared with the THA group. (Table 3) Additionally, the location of femoral head collapse had different patterns in both groups. In the conservative treatment group, most cases had femoral head collapse at the superolateral head, while patients in the THA group had an increased number of patients with collapse at the superomedial part of the femoral head.

Table 1. Demographic data

Characteristic	Conservative group (n = 31)	THA group (n = 29)	p-Value
Age (years)	48.4 ± 12.8a	47 ± 13.1a	0.68
Gender			0.80
Male	16 (51.6%)	13 (44.8%)	
Female	15 (48.4%)	16 (51.7%)	
Weight (kg)	60.6 ± 12.3a	63.4 ± 13.6a	0.40
Height (cm)	162.2 ± 8.0a	160.2 ± 8.2a	0.36
BMI (kg/m2)	22.9 ± 3.6a	24.7 ± 4.8a	0.10
Occupation			0.60
Manager	6 (19.4%)	6 (20.7%)	
Technician	8 (25.8%)	5 (17.2%)	
Service	5 (16.1%)	6 (20.7%)	
Farmer	7 (22.6%)	10 (34.5%)	
Craft worker	0 (0%)	1 (3.4%)	
Operator	2 (6.5%)	0 (0%)	
Housewife	3 (9.7%)	1 (3.4%)	
Working level			0.71
Sedentary	21 (67.7%)	17 (58.6%)	
Light	5 (16.1%)	7 (24.1%)	
Medium	5 (16.1%)	5 (17.2%)	

THA: Total Hip Arthroplasty; BMI: Body Mass Index.
aMean values with standard deviations.

Table 2. Medical history data

Characteristic	Conservative group	Characteristic	Conservative group
Affected side			0.76
Bilateral	14 (45.2%)	11 (37.9%)	
Unilateral	17 (54.8%)	18 (62.1%)	
Contralateral hip problem			0.70
Yes	3 (9.7%)	4 (13.8%)	
No	28 (90.3%)	25 (86.2%)	
Previous core decompression			<0.01
Yes	28 (90.3%)	11 (37.9%)	
No	3 (9.7%)	18 (62.1%)	
Underlying disease			0.97
No	9 (29%)	8 (27.6%)	
RA	0 (0%)	1 (3.4%)	
SLE	13 (41.9%)	11 (37.9%)	
Other	9 (29%)	9 (31%)	
Risk etiology of osteonecrosis			0.27
Undetectable risk	3 (9.7%)	7 (24.1%)	
Corticosteroid	20 (64.5%)	17 (58.6%)	
Alcohol	6 (19.4%)	2 (6.9%)	
Hemoglobinopathy	0 (0%)	1 (3.4%)	
Irradiation	0 (0%)	1 (3.4%)	
Other	2 (6.5%)		

THA: Total Hip Arthroplasty; BMI: Body Mass Index; SLE: Systemic Lupus Erythematosus; RA: Rheumatoid Arthritis

Table 3. Physical examination and radiographic data

Characteristic	Conservative group	Characteristic	Conservative group
Total ROM	255.4 ± 33.9a	178.8 ± 43.5a	<0.01
Flexion	110 (110,120)b	90 (78.8,100)b	<0.01
Adduction	30 (30,30)b	15 (10,21.2)b	<0.01
Abduction	45 (30,45)b	30 (27.5,30)b	<0.01
External rotation	46.7 (12.7)b	36.1 (15.8)b	<0.01
Internal rotation	15 (15,30)b	10 (0,15)b	<0.01
Location of femoral head collapse			< 0.01
Superomedial	0 (0%)	5 (12.5%)	
Superolateral	44 (97.8%)	23 (57.5%)	
Both	1 (2.2%)	12 (30%)	

4. Discussion

Total hip replacement is one of the standard treatments for post-collapse osteonecrosis of the femoral head (10). However, some patients consider undergoing conservative treatment instead of surgery. Our study aimed to evaluate the difference in patient characteristics between patients with post-collapse osteonecrosis of the femoral head who had THA and those who considered continuing conservative treatment. This study found that the group of patients who considered continuing conservative treatment had a higher rate of history of core decompression, a higher range of motion, and a lower rate of patients who had a collapse in the superomedial part of the femoral head.

Core compression is the treatment for pre-stage collapse and is accomplished by reducing intraosseous pressure to reduce pain and delay the progression of the disease (11-13). This study found that patients who considered continuing conservative treatment had a higher rate of history of core decompression in pre-stage collapse. We hypothesized that patients who had previous core decompression might have lower intraosseous pain than those who did not. Hence, the primary source of pain in such patients might stem from articular pain following the femoral head collapse, while patients who did not have core decompression had combined pain of intraosseous and articular pain.

The location of the femoral head collapse was one factor that may predict the requirement for THA. Our study found that the group of patients who underwent THA had a higher rate of patients with femoral head collapse at the superomedial part. A previous study by Sugano et al. reported that osteonecrosis lesions that involved more than the medial one-third of the weight-bearing surface from anteroposterior radiographic had a poor prognosis for massive collapse (14). We hypothesized that patients suffering from the superomedial part of femoral head collapse might have higher pain and more limited function because the superomedial part of the head has a higher contact surface with the acetabulum throughout the whole range of motion.

There were some limitations in this study. First, the limited number of patients could not demonstrate any significant differences for some characteristics, and due to the mixing of categories, some biases may have occurred. Second, there was no data on the duration of the disease nor treatment after the femoral head had collapsed in each group, which is a factor affecting the treatment results. Finally, this study used only plain radiographic to evaluate hip lesions, which are not as accurate as magnetic resonance imaging (MRI). Therefore, further prospective studies should address this limitation.

In conclusion, this study revealed that patients considering the continuation of conservative treatment had a higher rate of history of core decompression, a higher range of motion, and a lower rate of supermedial femoral head collapse. Thus, these factors might be considered in the discussions with patients for the prognosis of the disease and might be used as evidence for the choice of treatment.

Acknowledgements

We would like to express our gratitude to Andrew Jonathan Tait of the International Affairs Department for proofreading this report's English.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 714-718
doi: 10.52142/omujecm.39.3.23

Evaluation of the effect of Covid-19 pandemic on hand washing habits in pregnant women

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Received: 29.01.2022 • **Accepted/Published Online:** 18.04.2021 • **Final Version:** 30.08.2022

Abstract

We aimed to investigate the change in hand washing habits of pregnant women before and during the COVID-19 pandemic. This study was designed prospectively and observationally. Two questionnaire forms were administered to each participant to determine their pre-pandemic and during pandemic habits relating to handwashing. The total score of handwashing attitude before and during the pandemic was calculated by summing the answers to the 25 questions in the survey. Independent t-tests were performed for data. P-values of <0.05 were considered statistically significant. The mean age of the 392 pregnant women participating in the study was 30.29±5.92. The values of gravidity, BMI and gestational age were estimated as 1.89±1.05, 27.08±4.26 kg/m^2, 27.08±4.26 weeks, respectively. The frequency of the participants washing their hands more than 10 times a day increased from 32.2% before the pandemic to 75.8% during the pandemic. While the answer to the question "Is hand washing important in the prevention of diseases causing pandemics?" was 65.1% before the pandemic, it was 93.7% during the pandemic. Although the answer to the question on "How many seconds should the hand washing time be at least?" was 20 seconds at a rate of 42.9% before the pandemic, it was 84.1% during the pandemic. The total handwashing habit score was calculated as 79.25±11.92 before the COVID-19 pandemic and 94.42±5.58 during the pandemic (p<0.05). This study demonstrated that the pregnant population was affected by the COVID-19 pandemic and experienced a great change in hand hygiene.

Keywords: Covid-19 pandemi, hand hygiene, handwashing habit score, pregnant population

1. Introduction

The new type of coronavirus was first detected towards the end of 2019 in Wuhan, Hubei Province, China. COVID-19 stands for “2019 coronavirus disease” and is used to refer to an acute respiratory infection epidemic caused by a novel coronavirus. It was originally called 2019-nCoV (2019 novel coronavirus). The coronavirus strain recently started to be called SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2). Coronavirus spread rapidly to neighboring countries (South Korea, Japan, and Iran) after being first detected in Wuhan, China (1). Later, due to the fact that the disease continued to spread to Europe and the United States, the World Health Organization (WHO) declared COVID-19 disease a global epidemic in March 2020. Countries were encouraged to take effective measures to reduce transmission (2).

While there was no vaccine at the beginning of the Covid-19 pandemic, vaccines have been developed recently. However, due to the presence of new mutant variants and the fact that the entire world population has not yet been vaccinated, the epidemic has still not been brought under control (3). In this case, personal protective measures, non-pharmaceutical measures such as wearing surgical masks and washing hands are important to reduce transmission risk by creating a barrier to prevent aerosol spread and protect susceptible populations (4, 5). In this context, public health action to prevent transmission is critical in slowing the spread of the disease. One of the main recommendations published by the WHO for communities is to wash their hands frequently and correctly.

Evidence from the literature has shown that frequent handwashing can reduce the risk of viral transmission by 55% (6, 7). Given the widespread population vulnerability to COVID-19 infection, hand hygiene is repeatedly emphasized in the entire population (8). Pregnant women constitute one of the most vulnerable groups in society. In this study, we aimed to investigate the change in handwashing habits of pregnant women at risk of epidemic diseases.

2. Materials and Methods

This study was designed prospectively and observationally. It

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was conducted between April 2020 and June 2020, at the Gynecology and Obstetrics Polyclinics of Kanuni Training and Research Hospital in Trabzon, Turkey. All participants signed informed written consent before being enrolled in the study. The study was reviewed and approved by the ethics committee of Trabzon Kanuni Health Practice and Research Center (Ethics approval reference number: 2020/36 date 23.07.2020). All procedures were performed according to the Declaration of Helsinki.

As a result of the sample size analysis, 400 pregnant women were included in the study. Pregnant women who consulted antenatal outpatient clinics were included in the study. Pregnant women who could not give importance to personal hygiene, who were mentally ill, or who were allergic to personal cleaning agents were not included in the study.

Demographic data of all participants included in the study were recorded. These are age, BMI, gravida, parity, personal history, family history, operation history, gestational age, and education level. Detailed physical and obstetric examinations were performed. The Ministry of Health study was taken as a reference to measure hand washing habits, and a total of 25 questions were used in the questionnaire forms. Its validation and verification has been done in the Ministry of Health study. (9). Each participant received two questionnaire forms, one containing pre-pandemic habits and the other questioning during pandemic habits. Hand washing habit survey questions are shown in Table 1. In addition, the participants were asked in the following five questions.

Table 1. A total of 25 questions were asked before and during the pandemic to measure the hand washing habit score

	Before COVID-19 pandemic	**During COVID-19 pandemic**
	Never (0) / Rarely (1) / Sometimes (2) / Often (3) / always (4)	Never (0) / Rarely (1) / Sometimes (2) / Often (3) / always (4)
I wash my hands before meals		
I wash my hands after meals		
I wash my hands before using the toilet		
I wash my hands after using the toilet		
I wash my hands after coming home from outside		
I wash my hands after shaking hands with people		
I wash my hands before going to bed		
I wash my hands after touching animals		
I wash my hands when I get up in the morning		
I was my hands before and after changing babies' diapers		
I wash my hands before eating anything		
I wash my hands when I see them dirty		
I wash my hands before preparing food		
I wash my hands after exchanging money		
I wash my hands after cleaning my nose		
I wash my hands after touching garbage		
I wash my hands before touching a sick person		
I wash my hands after touching a sick person		
I wash my hands after combing my hair		
I wash my hands after cleaning the house		
I wash my hands after washing the dishes		
I wash my hands after doing some cleaning		
I dry my hands after washing them		
When I wash my hands, I remove my ring.		
I wash my hands after touching something commonly used like a doorknob.		
Total Hand Washing Habits Score		

Are you using a hand sanitizer? How many seconds should the hand washing time be at least? Is hand washing important in the prevention of diseases causing pandemics? How often do you wash your hands? What do you often use during the hand cleaning process?

The answers to the 25 questions in the survey were taken as 5-point Likert (never, rarely, sometimes, often, and always) in order of importance. The scoring was assigned in order of importance as 0, 1, 2, 3, 4. The total score of hand washing attitude before and after the pandemic was determined by summing the answers to these 25 questions. The lowest 25 and the highest 100 points can be obtained (9).

Statistical analysis was performed using SPSS version 23.0 (IBM Corp., Armonk, NY, USA). Means, medians, and modes were determined, and the Kolmogorov-Smirnov test was performed to determine normality. To compare responses from before and during the pandemic, normally distributed data were analyzed with independent t-tests. Pearson's correlation analysis was applied to the data conforming to a normal distribution for the relationship between education

level and handwashing habits. P-values <0.05 were considered statistically significant.

3. Results

Altogether, 400 patients were initially planned for inclusion in the study. However, the study was completed with 392 pregnant women due to 8 incomplete or incorrectly filled questionnaires. The mean of the demographic data of the participants were as follows; the mean age was found as 30.29 ± 5.92, gravida as 1.89 ± 1.05, BMI as 27.08 ± 4.26 kg / m2, and gestational age as 27.08 ± 4.26 weeks. Education level ratios were 22.4% for primary education, 38.3% for high school, and 39.3% for university (Table 2). In parallel with the increase in the education level, the handwashing habit score increased significantly.

Table 2. Demographic variables of the study population

		Mean	P
Age (year)		30,29±5,92	
Gravida		1,89±1,05	
BMI (kg/m^2)		27,08±4,26	
Gestational age (week)		24,37±11,74	
Education (%)	Elementary scohool	22,4	
	High school	38,3	
	University	39,3	
Total hand washing habits score	Before Covit-19 pandemic	79,25±11,92	**0.001**
	During Covit-19 pandemic	94,42±5,58	

Independent t test, data is presented as frequency and percentages or mean ± SD, p<0.05

It was determined that the frequency of hand washing 1-2 times a day before the pandemic was 3.2%, while this value decreased to 1.6% during the pandemic, and the frequency of washing more than 10 times per day increased from 32.2% before the pandemic to 75.8% during the pandemic.

While the answer to the question "Is hand washing important in the prevention of diseases causing pandemics?" was 65.1% before the pandemic, it was 93.7% during the pandemic. Although the answer to the question "How many seconds should the hand washing time be at least?"' was 20 seconds before the pandemic at a rate of 42.9%, it was at 84.1% during the pandemic. Most participants (72.1%) answered "liquid soap" to the question "What do you use frequently in hand washing?". The use of hand disinfectant increased during the pandemic (Table 2). The total hand washing habit score was 79.25±11.92 before the COVID-19 pandemic and 94.42±5.58 during the pandemic. This difference was statistically significant (p=0.001) (Table 3).

4. Discussion

Hand hygiene is a widely accepted principle in preventing disease transmission because proper hand hygiene is likely to reduce the spread of infectious diseases by 24-31% (10). According to the Center for Disease Control (CDC), the virus is currently believed to be spreading through direct contact, indirect contact, and droplet contact. To avoid virus transmission, the CDC recommends washing hands frequently for 20 seconds with soap and water; alternatively, if soap and water are not available, a hand sanitizer containing at least 60% alcohol can be used (11). Although hand washing is very important, it is very difficult to maintain the habit. Due to different populations and contexts, compliance with hand washing ranges from 1.80% to 78.00% (12).

Table 3. The answers of pregnant women about COVID-19 and hand washing before and during the pandemic.

		Before COVID-19 pandemic (%)	During COVID-19 pandemic (%)
Count of hand washing per day.	1-2	3,2	1,6
	3-5	24,2	3,2
	6-10	40,3	19,4
	More than 10	32,3	***75,8***
Is hand washing important in the prevention of diseases causing pandemics?	Little matter	7,9	3,2
	Important	15,9	0,0
	ghly important	11,1	3,2
	Very highly important	65,1	***93,7***
Minimum hand washing (sec.)	5	1,6	0,0
	10	36,5	7,9
	15	19,0	7,9
	20	42,9	***84,1***
What do you use often for hand cleaning?	Water	6,6	3,3
	Solid soap	18,0	13,1
	Liquid soap	72,1	65,6
	Hand Sanitizer	3,3	***18,0***

Data are given as percentages.

The pandemic is still not under control, as the entire world is unvaccinated and new mutant viruses are emerging (3). For these reasons, hand washing is very important in preventing COVID-19 infection (13). There are serious campaigns all over the world about the importance of hand washing. In fact, it would be very appropriate to name 2020 as "the year in which human beings reinvented hand washing". Special attention should be paid to pregnant women, who are among the most vulnerable populations to the COVID-19 pandemic. This is why hand washing is so important for this group. For this reason, in this study, we aimed to investigate the extent to which pregnant women view the COVID-19 epidemic and their hand washing attitude during the pandemic period.

The results of this study demonstrated that the hand-washing habit of the pregnant population was in good condition in the pre-epidemic period. It was determined that the total hand washing score was 79.25±11.92 before the pandemic and that this score increased to 94.42±5.58 during

the pandemic. A maximum of 100 points can be obtained from the hand-washing questionnaire. Accordingly, the hand washing score of 79.25 before the pandemic was considered good. The hand washing score measured during the pandemic was at a much better level.

According to the Turkey Hand Washing Habits 2009 study, hand washing habits from 6854 people showed that; the hand washing score was higher among females, increased with education level, and a difference was found between staying in the countryside and staying in the city (9). Similarly, in our study results, as the level of education increased, the handwashing habit score also increased significantly.

Under ideal conditions, the virus can stay on different surfaces for hours or days. The surfaces most exposed to this type of transmission include door handles; the handles of public transport, light switches, and mobile phones (14). In this study, it was found that the average number of daily hand washes was 6-10 times (40.3%) most frequently before the pandemic, but this frequency changed to more than 10 times (75.8%) during the pandemic. "After touching the door handle, after shaking hands with people, before going to the toilet", it is seen that the habits of handwashing are the most striking attitudes compared to the pre-pandemic. This change can be said to occur after it has been proven that it is possible to become infected by touching surfaces or objects with the COVID-19 virus and then bringing the hands towards the mouth, nose, or eyes (15).

The answers given by pregnant women before and during the epidemic (65.1% vs. 93.7) to the question "Is hand washing important in preventing an epidemic disease such as COVID-19?" are at a satisfactory level. It has been known for many years that hand washing is effective in preventing epidemic diseases. The answer to the question "How many seconds should the hand wash be at least?" was "20 seconds" at a rate of 42.9% before the pandemic. During the pandemic, this rate increased to 84.1%. These rates show that the pregnant population in this country seriously cares about this situation. In fact, pregnant women applied to a doctor's examination to find out if the pregnancy was on the way, even on the days when curfews were imposed during a pandemic transmitted from person to person. This is the protective instinct found in all living beings. Hormonal changes during pregnancy and the instinct to protect their baby may be the reason for the importance pregnant women give to hand washing.

Hand hygiene products include liquid or bar soaps, synthetic detergents, antiseptic hand washing wipes, and alcohol-based hand sanitizers. These (alcohol-based hand lotion, povidone iodine) are highly effective against enveloped corona viruses and other agents. If alcohol-based hand rubs (gel or foam) or povidone iodine are not available, a 70% ethanol solution can be used. Correct use is very important for these to be effective. While each formulation can be effective against COVID-19, it can increase the risk of hand dermatitis by altering the skin barrier integrity and function (15). In this study, liquid soap was highly preferred in hand hygiene products before and during the pandemic. At the same time, the use of hand disinfectants that we encounter in almost every public space after the pandemic has increased.

Considering publications on handwashing in the prevention of the COVID-19 pandemic, none report on the handwashing habits of pregnant women. The publications found are generally related to hand dermatitis due to frequent hand washing of healthcare workers and disinfectant formulations effective against COVID-19 infectious viruses (16-19). While the habit of handwashing decreased in the pre-pandemic period, it increased during the pandemic period. Although the importance of handwashing has been known for many years, in the 21st century, it is the most prominent method of protection against the pandemic in the vaccine-free period. Studies on the importance of handwashing and its correct application should always be done.

The limitations of the study are the fact that the study is a questionnaire based on subjective answers, the pregnant female population was preferred in the selection of a sensitive population, and that men and non-pregnant women were not included in the study. This work is supported by all community-based studies.

Consequently, the most serious epidemic of our century, which still affects the whole world, is the COVID-19 outbreak. While all members of the society can be affected, the most vulnerable groups are immunodeficiencies, chronic patients, the elderly and special groups like pregnant women. No studies have reported the change in hand hygiene attitudes of pregnant women on the COVID-19 outbreak. This work is the first in this area. In this study, we have demonstrated how the pregnant population has been affected by the epidemic in this period when the world experienced a serious change in attitude and hand hygiene.

Conflict of interest

The authors declare that they have no conflict of interests**.**

Funding

No funding was used for the study.

Acknowledgments

We would like to thank to SBÜ Kanuni Education and Research Hospital management for their support to our work. We also acknowledge the help of Prof. Dr. Sevim Kose in revising the English of this manuscript.

Authors' contributions

Concept: R.E., Y.B.T., Design: R.E., Data Collection or Processing:R.E., K.B.E., D.K, Analysis or Interpretation: R.E., Literature Search: R.E., D.K., Writing: R.E., D.K., K.B.E

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 719-722
doi: 10.52142/omujecm.39.3.24

Evaluation of ovarian reserve at late reproductive age

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Received: 01.02.2022 • **Accepted/Published Online:** 17.07.2022 • **Final Version:** 30.08.2022

Abstract

Although follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), antimullerian hormone (AMH), and ovarian volume are commonly used to assess ovarian reserve and ovarian response to stimulation, no test directly indicates this. We aimed to observe how ovarian reserve tests help us in the diagnosis and treatment of women of late reproductive age. We included 190 patients in this prospective cohort study. We took FSH, LH, E2 and AMH blood samples on the third day of menstruation to biochemically evaluate the ovarian reserve of the patients who were planned for hysterectomy and bilateral salpingo-oopherectomy at late reproductive age. We recorded antral follicle counts (AFC) and ovarian volumes by transvaginal ultrasonography for ultrasonographic evaluation and weighed ovarian weights postoperatively. The data of 190 women aged 39-44.9 years revealed that AMH and ovarian volume, ovarian volume and AFS show significant positive correlations, whereas FSH, LH, and E2 levels do not indicate a significant relationship with AFS. AMH, ovarian volume and ovarian weight, and AFC may show us the ovarian reserve with the highest reliability in women of late reproductive age.

Keywords: Antimullerian hormone, antral follicle, infertility, ovarian reserves

1. Introduction

The purpose of ovarian reserve tests is to determine the number of eggs remaining in the ovaries and thus the ovarian response to ovarian stimulation. Antimullerian hormone (AMH), a dimeric glycoprotein that suppresses the development of Müllerian ducts, was first described by Josso (1). Various studies revealed that granulosa cells in the ovaries produced moderate AMH amounts after birth (2). AMH plays a role in the negative regulation of aromatase and luteinizing hormone (LH) receptor genes, increasing androgen production by theca cells while inhibiting the stimulatory effects of follicle stimulating hormone (FSH) on small and preantral follicle growth in postnatal ovarian tissue (3). Although antimullerian hormone (AMH) has been used as the greatest helper in recent years, it should be kept in mind that it may change with the menstrual cycle and may yield different results when studied with different methods (4).

FSH, inhibin B, and estradiol (E2) levels measured on the 3rd day of the cycle were other tests used to evaluate ovarian reserve (5). Basal FSH level was expected to increase with age, possibly correlated with decreased inhibin-mediated feedback to the pituitary (6). Since inhibin B production was mainly from small antral follicles, its level decreased with age (7). Evaluation of E2 level together with FSH gave information about ovarian follicles (5).

Transvaginal ultrasound was also an observer-dependent method, helping directly determine ovarian reserve by measuring ovarian volume or antral follicle count (8). To determine the number of antral follicles, FSH, and AMH levels; ovarian volume was independent of the menstrual cycle, although it was easier to determine ovarian volume with two-dimensional (2D) transvaginal ultrasound (8). Evaluating the relationship between ovarian weight and ovarian volume, Rosendahl et al. stated that one cubic centimeter equaled one gram by measuring with a microbalance (8). Although no research revealed a relationship between smoking and ovarian reserve, various studies claimed the opposite (9–11).

The present study aimed to show the relationship between ovarian weight, volume, and number of follicles, AMH, LH, FSH, and E2. The primary aim was to observe to what extent we could rely on ovarian reserve testing for late fertility.

2. Material and Methods

We included in this study 190 patients scheduled for

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hysterectomy in a tertiary hospital in Northern Turkey and excluded patients in the postmenopausal period, genital tract cancer patients, patients using hormones and patients scheduled for surgery because of ovarian mass. We recorded patients' age, number of pregnancies, smoking habits, and BMI (weight/height squared) and evaluated FSH, LH, AMH, E2, endometrial thickness, right ovarian volume and left ovarian volume on the third day of menstruation.

We carried out all hormone tests using a chemiluminescence immunoassay method (Abbott®); its normal ranges were 3.03-8.8 mIU/ml for FSH, 1.8-11.78 IU/L for LH, and 21-251 ng/L for E2 in the follicular phase. Another chemiluminescence immunoassay method (Beckman-Coulter®) determined the AMH levels; its normal ranges were 0.07 -7.35 ng/ml. We conducted ultrasonographic measurements transvaginally by ESAOTE S.p.A./Italy model EA720 and measured endometrial thickness in mm. By taking the width, height and depth measurements of both ovaries in three dimensions, we made volume calculations with the formula D1xD2xD3x0.523 (Fig. 1). We scanned both ovaries systematically and counted follicles 2 to 10 mm in diameter to determine the number of antral follicles. We measured the ovarian weights in grams with a microbalance after surgery (Denver Instrument APX-200®).

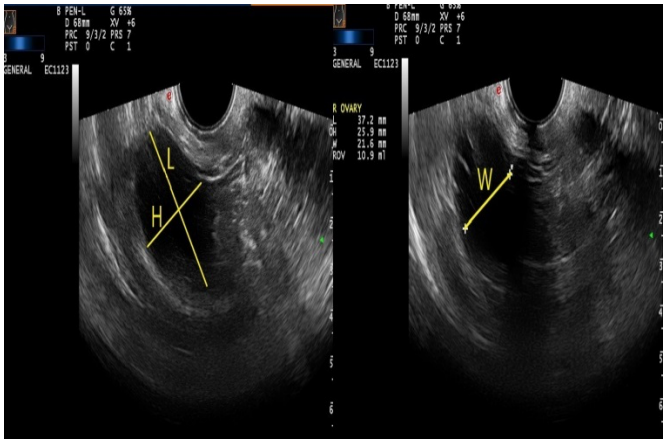

Fig. 1. Calculation of ovarian volume ultrasonographically

We obtained ethics approval number 2018/40 from the Clinical Research Ethics Committee of the University of Health Sciences Kanuni Training and Research Hospital. After informing all patients about the study, we obtained informed consent.

For power analysis, we reached the requirement of 120 patients to obtain a medium effect size (d = 0.5) with 80% power using version 3.01 of the G* Power software (Franz Faul, Kiel, Germany) at a significance level of 0.05. We performed the Kolmogorov-Smirnov test for conformity of the data to see the normal distributions. We used the Mann-Whitney U test to analyze the data to determine the differences between groups and the Pearson correlation test as the basis for the regression analysis. We analyzed all data obtained with IBM SPSS Statistics for Windows, Version 23.0, and $p \leq 0.05$ was significant.

3. Results

We included in this study 190 patients aged between 39 and 44.9 years whose number of pregnancies varied between 1 and 6. 72 were smokers while 118 were non-smokers. Table 1 shows the ovarian reserve test and age and BMI results. The mean endometrial thickness was 10.6 ± 1.9 cm. BMI, E2, right ovarian and left ovarian volumes showed normal distribution in the Kolmogorov-Smirnov statistical analysis. AMH, FSH, LH, E2, the number of follicles in the ovaries, ovarian volumes, and ovarian weights seemed to be statistically unrelated to age. Endometrial thickness and BMI, E2, FSH, LH, AMH were statistically unrelated. AMH levels decreased with an increase in BMI (r=-0.125). No statistical differences were present between FSH, LH, E2, AMH, follicle counts and ovarian volumes according to the smoking status by the Mann-Whitney U test.

Table 1. Biochemical and ultrasonographic ovarian reserve tests obtained on the third day of menstruation and age and body mass index data of patients

	Mean ± SD
Age (years)	42.2 ± 1.6
BMI (kg/m2)	29.4 ± 2.8
Follicle stimulating hormone (FSH) (mU/mL)	7.4 ± 2
Luteinizing hormone (LH) (mU/mL)	10.4 ± 4.4
Estradiol(E2) (ng/L)	75.3 ± 20.2
AMH (ng/ml)	1.2 ± 0.6
Right ovarian volume (cm^3)	6.9 ± 0.5
Left ovarian volume (cm^3)	6.8 ± 0.5
Right ovarian weight (grams)	6.9 ± 0.6
Left ovary weight (grams)	6.9 ± 0.6
Number of antral follicles in the right ovary	2.5 ± 0.8
Number of antral follicles in the left ovary	2.5 ± 0.9

AMH level was statistically unrelated to the number of pregnancies, FSH, LH, and E2. The number of follicles in the ovaries (r=0.982), ovarian volumes (r=0.817) and ovarian weights (r=0.798) were positively correlated with AMH levels. There was a significant positive correlation between ovarian volumes and ovarian weights and the number of follicles in the ovaries (r=0.912 and r=0.874, respectively). There was a significant positive correlation between ovarian volumes and ovarian weights (r=0.932).

4. Discussion

In recent years, the incidence of infertility has increased with an increase in the first gestational age (12). Therefore, we planned the patients included in this study to be women of late reproductive age. By resecting the ovaries, we could also evaluate their volumes and weights.

Ovarian reserve defines primordial follicles that increase in quality and quantity and decrease with age (13). Although no serum marker can directly measure the number of primordial follicles, AMH is an important helpful marker and decreases with age (14). AMH levels plateau around the age of 25 (15). Cui et al. reported median levels of AMH as 2.35 ng/mL for 20-31 years old, 1.58 ng/mL for 32-34 years old, 1.30 ng/mL, 0.96 for 35-37 years old. ng/mL for 38-40 years old, 1.05 ng/mL for 41-43 years old and 0.67 ng/mL for 43 years and older (16). The current study revealed the AMH level as 1.2 ± 0.6 ng/ml. While the AMH results were similar, they were slightly different. This difference may be due to the menstrual cycle and the patient profile included in the study.

AMH occurs in the 36th week of pregnancy and cannot be detected at menopause (17). Fanchin et al. found the AMH level to be more associated with antral follicle number than inhibin B, FSH, and E2 levels in infertile patients (18). The data in the present study supported the literature.

Sova et al. found higher AMH levels in obese polycystic ovarian syndrome (PCOS) patients than normal-weight patients while finding a negative correlation between serum AMH levels and BMI in the normal weight group (19). The current study revealed a negative correlation between the increase in BMI and AMH levels.

Although serum AMH is an important indicator of ovarian reserve, the lack of an international standard for AMH and multifactorial variability are the primary handicaps of the test (20). Furthermore, this review shows that AMH and the number of ovarian follicles are correlated in many studies, but results may vary depending on the population and technique chosen (20). The present study revealed a positive and significant correlation between follicles numbers and the AMH level.

The present study found no significant relationship between FSH, LH, E2 and AMH and smoking. Dafopoulos et al.'s research with 137 women found no significant relationship between smoking in FSH, LH, E2 progesterone and AMH levels (21).

The current study also found no relationship between endometrial thickness and FSH, E2, AMH and BMI. This may be explained by the multifactorial variability of the endometrial thickness (22). FSH and LH were biochemical markers reflecting aging in women, and they deteriorated significantly after the age of 35 due to the decrease in ovarian reserve (23). The Buyalos and Danesmand studies showed that the E2 level in infertile patients entered the abnormal range within two years of observation (22). Although FSH, LH, and E2 levels are consistent with the current literature (24), they did not change with age in the present study. The homogeneity of our study group and the narrow age range of the patients have an important effect on this.

In line with our findings, one cm^3 ovarian volume was equal to one gram ovarian weight in Rosendahl et al.'s study (8). Although a positive relationship was present between the number of antral follicles and ovarian volumes, the role of the ovarian volume in determining ovarian reserve has not been fully defined yet (25,26). The present study revealed a significant positive correlation between ovarian volumes and the total ovarian weight and follicle count.

AMH, ovarian volumes and ovarian weights, and AFC were crucial data for us in evaluating the ovarian reserve in women of late reproductive age.

Conflict of interest

The authors declared no conflict of interest.

Funding

No funding was used for the study.

Acknowledgments

The authors thank Prof Dr Suleyman GUVEN for their assistance in editing the study.

Authors' contributions

Concept: M.U., T.A., Design: M.U., T.A., Data Collection or Processing: M.U., T.A., Analysis or Interpretation: M.U., T.A., Literature Search: M.U., T.A., Writing: M.U., T.A.

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Research Article

J Exp Clin Med
2022; 39(3): 723-727
doi: 10.52142/omujecm.39.3.25

The evaluation of accesses for febrile and afebrile seizures in the pediatric emergency department during COVID-19 lockdown

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Received: 08.02.2022 • **Accepted/Published Online:** 01.07.2022 • **Final Version:** 30.08.2022

Abstract

We aimed to evaluate the admissions to the Pediatric Emergency Department (PED) due to seizures one year before and one year after the first COVID-19 case in Turkey. We divided the admission time into two: The prelockdown period (March 2019-February 2020) and the lockdown period (March 2020 – February 2021). We divided the seizures into six parts: First simple febrile convulsion (FK), first complex FK, recurrent simple FK, recurrent complex FK, first non-febrile seizure, and recurrent non-febrile seizure. We compared the prelockdown and lockdown periods in terms of demographic and clinical characteristics and monthly admission of patients. The total number of patients admitted to PED was 37.323 and 10.191 during the prelockdown and lockdown period, respectively. While seizure-related PED accesses were 918 during the prelockdown period, 508 patients were admitted to PED during the lockdown period. Monthly average admissions decreased in all seizure types during the lockdown period. The ratio of first simple febrile seizures to total seizures decreased from 22.4% (206/918) to 16.3% (83/508), while the ratio of first afebrile seizures to total seizures increased from 29.6% (272/918) to 36.8% (186/508). While the rate of first febrile seizures decreased, we observed a significant increase in the rate of first afebrile seizures. The findings suggested that risk factors such as depression/anxiety or screen exposure may have caused seizures in patients with a predisposition to seizures.

Keywords: pediatric emergency department, children, COVID-19, pandemic, seizure, lockdown

1. Introduction

As of mid-December 2019, a new type of coronavirus infection was identified in Wuhan, China, and spread rapidly to most world countries (1). It reached pandemic status on March 12, 2020 (2).

The first patient in Turkey was reported on March 11, and schools were closed on March 16, 2020, to control transmission, then all non-essential businesses and services were closed. On May 23, 2020, the country closed completely. After the closure of schools in Turkey, students continued the academic year through distance education and remained isolated at home.

Since then, according to the literature, there has been a worldwide decrease in pediatric admissions to the Pediatric Emergency Department (PED), both because of the mild clinical course in childhood, the higher incidence of COVID-19 in advanced age groups, and the fear of contagion (3, 4). Parents' concerns, such as the increased risk of transmitting the infection to their children in the hospital setting, delayed the diagnosis of severe acute diseases that needed to be treated. Other viral infections have also decreased due to social distancing measures and isolation (5).

At the same time, lockdown, contact restrictions, isolation, and financial closure have changed the psychosocial environment, and increasing mental health disorders such as anxiety and depression have been observed in children (6).

This new condition has pressured children and adolescents, including sleep disturbances and limited access to street playgrounds. As a result, the use of smartphones, television, tablets and video consoles has increased (7).

Patients with epilepsy are susceptible to many factors, such as physical or emotional disorders or lifestyle and environmental changes. Many factors such as illness and fever, sleep deprivation, stress and disruption in antiepileptic drug treatments can increase the risk of seizures (8). The admissions of these patients to ED were evaluated in short periods (2-4 months) in the literature (7, 8). We aimed to evaluate the effects of life changes on the admissions to the PED due to febrile or afebrile seizures as one year before COVID-19 quarantine (prelockdown period) and one year during COVID-19 quarantine (lockdown period).

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2. Materials and methods

2.1. Patients and data collections

To evaluate the accesses to PED due to seizures during the COVID-19 quarantine, we divided the admission time into the prelockdown period (March 11, 2019-March 11, 2020) and lockdown period (March 11, 2020-March 11, 2021). We included children less than 18 years who applied to the Ondokuz Mayıs University Medical Faculty Pediatric Emergency Department during these periods due to febrile and afebrile seizures and excluded those with non-epileptic conditions. We received ethics committee approval from the Ethics Committee of Ondokuz Mayıs University (2021/324, Date: 16/07/2021).

We divided the patients into two groups: Febrile and afebrile seizures, and further divided these two groups into two: First seizure and recurrent seizure. We subgrouped febrile convulsions into simple febrile convulsions and complex febrile convulsions. Definition of a febrile seizure: A febrile seizure is an event associated with fever, usually occurring between the ages of six months and five years, but without intracranial infection or an identified cause. Febrile seizures that develop after afebrile seizures are excluded from this definition. Febrile seizures are not considered a type of epilepsy characterized by recurrent non-febrile seizures (9).

The febrile seizures criteria include (10):

- The convulsion that occurs at a temperature higher than 38°C
- Children over six months and under five years old
- No infection of the central nervous system
- Absence of underlying metabolic disease
- No previous history of afebrile seizures

Febrile seizures are divided into simple and complex categories (11).

- Simple febrile seizures, the most common type, are characterized by generalized seizures that last less than 15 minutes and do not recur within 24 hours.
- Complex febrile seizures are characterized by focal-onset episodes lasting longer than 15 minutes or occurring more than once in 24 hours.

2. 2. Statistical analysis

We conducted statistical analyses using the SPSS software (v22.0, IBM Corp., Armonk, NY, USA) and expressed all data as mean±standard error of the mean (SEM). We used the Shapiro-Wilk test to determine the normal distribution of the data. We used the unpaired t-test or Mann-Whitney U test as applicable to determine the differences between the groups and the Chi-squared and Fisher's exact tests for testing the relationships between categorical variables. We defined significance as $p<0.05$.

3. Results

The total number of patients admitted to PED between March 2019-March 2020 (prelockdown period) was 37.323, whereas it was 10.191 between March 2020 and March 21 (lockdown period).

Seizure-related PED applications were n=918 and n=508, and the mean age was 5.59±0.16 and 6.06±0.24 years during the prelockdown and lockdown periods, respectively. Male patient admission was predominant during both periods.

The ratio of the number of patients presenting with any seizure to the total number of patients during the prelockdown period was 2.45% (918/37323), while it was 4.98% (508/10191) during the lockdown period.

The ratio of first simple febrile seizure to total seizure decreased from 22.4% (206/918) to 16.3% (83/508), while the rate of first afebrile seizure to total seizure increased from 29.6% (272/918) to 36.8% (186/508). We found no statistically significant difference in other seizure types (Table 1).

There was no difference in terms of the gender of the children admitted between the prelockdown and lockdown periods (p=0.5402).

There was no difference between the two periods in terms of age, except for recurrent non-febrile seizures. We determined that the mean age of children presenting with recurrent afebrile seizures increased during the lockdown period (7.45±0.27 vs 8.51±0.40) (Table 1).

Table 1. Demographic and clinical characteristics of patients admitted to the emergency department during the one year before and after the pandemic

Demographic and clinical characteristics	2019 Mar-2020 Feb	2020 Mar-2021 Feb	*P value*
Total admission (All patients)	37.323	10.191	-
Total admission with seizure	918 (100 %)	508 (100 %)	-
First simple FK*	206 (22.4 %)	83 (16.3 %)	**0.0059**
First complex FK*	29 (3.3 %)	17 (3.3 %)	0.8762
Recurrent simple FK*	50 (5.4 %)	25 (4.9 %)	0.7117
Recurrent complex FK*	4 (0.4 %)	1 (0.2 %)	-
First afebrile seizure	272 (29.6 %)	186 (36.8 %)	**0.0077**
Recurrent afebrile seizure	357 (38.8 %)	196 (38.4 %)	0.9548
Gender (male)	512 (55.8 %)	292 (57.5 %)	0.5402
Age (year)	5.59 ± 0.16	6.06 ± 0.24	0.2517
First simple FK*	2.05 ± 0.18	2.33 ± 0.13	0.1541
First complex FK*	2.18 ± 0.30	2.28 ± 0.34	0.3305
Recurrent simple FK*	3.14 ± 0.26	3.11 ± 0.41	0.4767
Recurrent complex FK*	1.56 ± 0.35	0,66	-

First afebrile seizure	6.50 ± 0.31	6.03 ± 0.39	0.1008
Recurrent afebrile seizure	7.45 ± 0.27	8.51 ± 0.40	**0.0206**
Mean hospitalization day	1.13 ± 0.03	1.26 ± 0.08	0.2788
First simple FK*	1.10 ± 0.04	1.18 ± 0.07	0.2637
First complex FK*	1.10 ± 0.10	1.17 ± 0.17	0.4327
Recurrent simple FK*	1.07 ± 0.07	1.15 ± 0.10	0.3495
Recurrent complex FK*	0.25 ± 0.25	0	-
First afebrile seizure	1.13 ± 0.05	1.31 ± 0.16	0.3182
Recurrent afebrile seizure	1.20 ± 0.06	1.13 ± 0.07	0.2062
Arrival by ambulance	152 (16.6 %)	53 (10.4 %)	**0.0016**

*FK: febrile convulsion

Table 2 reveals that the average monthly seizure admission decreased in the lockdown period compared to the prelockdown period.

Table 2. Average number of patients admitted to the emergency department per month during the one year before and after the pandemic

Monthly average admission	**2019 Mar-2020 Feb**	**2020 Mar-2021 Feb**	***P value***
Total admission	76.50 ± 3.30	42.33 ± 2.93	**<0.0001**
Febrile seizures	24.17±3.33	10.42±1.45	**<0.0001**
*First simple FK**	17.17±2.99	6.92 ± 1.05	**<0.0003**
***First** complex FK**	2.5 ± 0.51	1.42 ± 0.31	**<0.0430**
***Recurrent** simple FK**	4.17 ± 0.68	2.08 ± 0.50	**<0.0111**
*Recurrent complex FK**	0.33 ± 0.19	0.08 ± 0.08	-
Nonfebrile seizures	52.33±1.92	31.92±2.21	**<0.0001**
First nonfebrile seizure	22.67±1.31	15.58±1.56	**0.0016**
Recurrent nonfebrile seizure	29.67±1.84	16.25±1.40	**<0.0001**

*FK: febrile convulsion

Fig. 1 shows the total number of accesses to PED in all types of seizures.

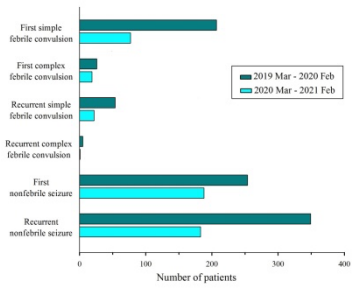

Fig. 1. Total number of visits by seizure type

Fig. 2 shows the monthly PED visits during the lockdown and prelockdown periods, while Figs. 3 and 4 demonstrate the monthly numbers of febrile and afebrile seizure-related visits, respectively.

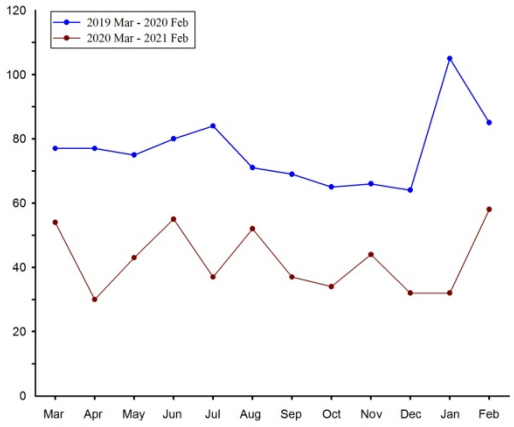

Fig. 2. Monthly number of seizure-related visits

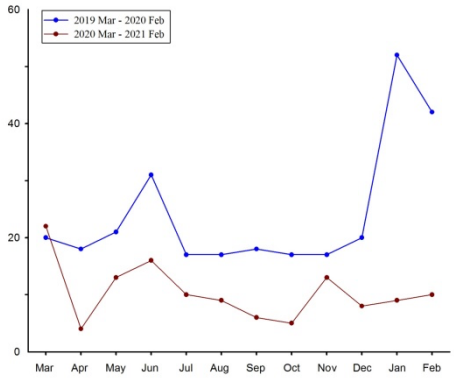

Fig. 3. Monthly number of febrile seizure-related visits

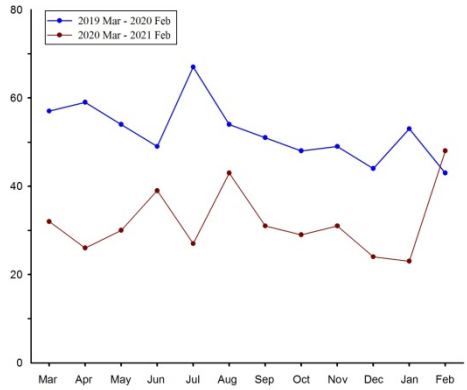

Fig. 4. Monthly number of afebrile seizure-related visits admitted to the emergency department during the one year before and after the pandemic

4. Discussion

The impact of the lockdown on healthcare was evident, with the suspension of routine outpatient services and the postponement of all non-emergency care (5). There has been a decrease in PED admissions for many diseases such as asthma and acute respiratory tract infections (12). However, the access frequency to PED for severe medical illnesses such as seizures is not expected to change.

Seizures are one of the most common reasons for PED

visits in children (13). Hartnett et al (14) observed reductions in PED visits in patients seeking medical care for reasons other than COVID-19, including patients with epilepsy. In this study, while the total number of patients admitted to PED during the prelockdown period was 37.323, it decreased more than three times to 10.191 during the lockdown period, consistent with the literature. Furthermore, the number of pediatric patients with total seizures was 918 in the prelockdown period and decreased to 508 in the lockdown period in the current study. The decrease in seizure admissions was not as dramatic as total admissions.

The literature reported increasing depression and sleep disorders in children during the pandemic (15). It also led to children forming unwanted habits about food, screen time and physical activities due to social restrictions (16). Children were exposed to social media through tools such as tablets, TVs and smartphones at home (17).

The COVID-19 pandemic can exacerbate existing mental health issues due to the unique combination of anxiety, uncertainty, fear of getting sick or losing our daily routines, seeing a loved one sick and life's challenges (18). Segre et al. (19) showed that 78% of children aged 6-14 years developed anxiety symptoms due to the COVID-19 pandemic quarantine measures, and most of the participants in this study stated they had difficulty adapting to learning at home and changed their eating and sleeping habits. It is commonly known that patients with epilepsy are susceptible to physical or emotional deterioration or lifestyle and environmental changes, and these changes may trigger seizures in patients with a predisposition to seizures (8).

We evaluated the number of patients with febrile and afebrile seizures admitted to the pediatric PED before and after lockdown for one year each. We found the first simple febrile seizure rate to be significantly lower after closure, which may be the result of a decrease in other infections due to complete closure and the importance given to hygiene. Although not statistically significant, there was a slight decrease in recurrent febrile seizures.

Smarrazzo et al. (20) reported that the percentage of febrile seizures admitted to PED during the lockdown period between March 1 and May 31 increased from 2.06% to 11.62%. Only 2 of 25 febrile seizure patients tested positive for COVID-19 during this period. In our study, 4 of 126 patients presenting with febrile seizures tested positive for COVID-19.

This study evinced a significant increase in the rate of first afebrile seizures during the lockdown period. These findings confirm the view that changes in living habits, including mobile device use and sleep changes, and possible stressors may have promoted the occurrence of epileptic seizures in a child population during the COVID-19 quarantine.

We also found there was no increase or decrease in recurrent afebrile seizures, although the rate of first afebrile seizures increased. These patients might not have applied to the hospital even if they had seizures. It also showed that they used the seizure drugs regularly despite the lockdown.

Cheliet al (21) reported a decrease in the number of patients presenting to PED for seizures during the pandemic, but the decrease was not as dramatic as the decrease in overall ED attendance. They also reported an increase in the number of first seizures despite this decrease, similar to the current study.

Palladino et al. (7) found a significant increase in the prevalence and incidence of admissions to PED due to seizures in the first three months of lockdown. In the study of Sanchez-Larsen et al. (8) conducted in adults, 27% of patients reported a >50% increase in seizure frequency and this increase in seizures was associated with stress/anxiety during lockdown period.

A study from Italy (22) reported a 72% decrease in all pediatric PED accesses during the three-month quarantine period compared to the corresponding 2019 period and a 38% reduction (n=41 vs. n=66) in the number of patients presenting to ED with seizures. The observed reduction in seizure-related ED access was not accompanied by significant changes in age, gender, or hospitalization day (22). Huang et al. (23) showed that a small proportion of adult patients with epilepsy experienced increased seizures during the outbreak of COVID-19 and suggested that stress was an independent factor for triggering seizures in these patients. Chiu et al. (24) observed a significant decrease during the first four months of closure in the rate of patients who applied to PED with seizures; especially, the decrease in the 0-6 age group was more significant than the 7-18 age group. This study evinced a slight increase in the mean age of recurrent afebrile seizures in the lockdown period. While the mean age was 7.45±0.27 years in the prelockdown period, it increased to 8.51±0.40 years in the lockdown period (p=0.0206). There was no difference between the two periods in terms of the mean age of other seizures. The mean age in the first afebrile seizure group, which had an increased rate during the lockdown period, was 6.03±0.39 years, and this was the age when there was no online education.

In conclusion, we observed a significant decrease in the number of admissions to PED in febrile seizures patients. While observing no significant change at admission with recurrent afebrile seizures, there was a significant increase in the rate of first afebrile seizures. This suggested that risk factors such as depression/anxiety or screen exposure may have caused seizures in children who had a predisposition to seizures. The limitation of this publication is that it is a single-center study; it can be carried out in a larger population with several centers.

Conflict of interest
The authors declared no conflict of interest.

Funding
No funding was used for the study.

Acknowledgments
We received Ethics committee approval for the study from Ondokuz Mayıs University Ethics Committee (2021/324) on June 10, 2021.

Authors' contributions
Concept: E.A.Ö, G.A., T.A.D., Design: E.A.Ö., G.A., Data Collection or Processing: E.A.Ö., T.A.D., Analysis or Interpretation: G.A., Literature Search: E.A.Ö., Writing: E.A.Ö.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 728-732
doi: 10.52142/omujecm.39.3.26

Comparison of the clinical outcomes of chronic rupture, arthroscopic tenotomy and tenodesis of proximal biceps tendon

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Received: 08.02.2022 • **Accepted/Published Online:** 17.05.2021 • **Final Version:** 30.08.2022

Abstract

Biceps tendon disorders are substantial cause of intense shoulder pain, and thus they require a careful medical attention. We compared the clinical outcomes of chronic rupture, tenotomy and tenodesis of proximal biceps tendon of patients underwent arthroscopic shoulder surgery. We included 98 patients over 50 years-old who underwent arthroscopic surgery for rotator cuff tears and grouped as tenotomy, tenodesis and chronic rupture of biceps tendon. Preoperative and postoperative 24-months follow-up Visual Analog Scale (VAS) score and the University of California–Los Angeles (UCLA) Shoulder Scale scores were compared. Chronic biceps rupture was the most common pathology in massive rotator cuff rupture group (75.9%). As tenotomy, tenodesis and chronic rupture groups were compared in terms of VAS and UCLA score improvements (preoperative and postoperative change); tenotomy showed better outcomes and there was a statistically significant difference between tenotomy and tenodesis groups in terms of improvements in VAS and UCLA scores (p = 0.036, p = 0.010, respectively). No statistically significant difference between chronic rupture and tenodesis in terms of VAS and UCLA scores improvements were observed (p = 1.000, p = 0.250, respectively). Also, tenotomy showed superior VAS score improvement than chronic tendon rupture (p=0.024) however no statistically significant difference was observed in UCLA score improvement between tenotomy and chronic rupture (p = 0.527). According to our study, tenotomy seems to be a more appropriate surgical method than tenodesis in massive and full-thickness rotator cuff tears with severe biceps tendon degeneration, and also it is not necessary to perform tenodesis in cases with chronic biceps rupture.

Keywords: chronic proximal biceps tendon rupture, biceps tenodesis, biceps tenotomy, rotator cuff tear, shoulder, shoulder arthroscopy

1. Introduction

The functional role of the long head of the biceps tendon (LHBT) is still not fully established. However, biceps tendon disorders are substantial cause of intense shoulder pain and range of motion (ROM) limitation, and thus they require a remarkable medical attention. It is important to reveal the relationship between biceps tendon disorders and other shoulder lesions since the treatment of a local shoulder lesion may not solve all shoulder complaints of the patient. The LHBT originates from the superior glenoid tubercle and superior labrum, extends through the intra-articular space and passes distally through the intertubercular sulcus (1). The close relationship of proximal biceps tendon with the shoulder ligaments and rotator cuff muscles causes high stress exposure and wear due to function. In this way, LHBT disorders are seen in a wide spectrum; from mild inflammation to complete chronic rupture.

Chronic rupture of the LHBT may occur due to inflammatory arthritis, osteophyte formation, subacromial impingement syndrome, rotator cuff tear, local injections applied to biceps tendon sheath and high-dose corticosteroid therapy (2). It occasionally develops in patients over the age of 50 and is usually associated with intrinsic tendon degeneration at the superior labrum insertion or in the bicipital groove (3, 4). There is yet no consensus on the treatment modalities of biceps tendon ruptures however, acute ruptures in high demanding physically active patients may require surgical interventions while non-operative treatment may be sufficient in sedentary patients with chronic degeneration (5). Nevertheless, each patient should be evaluated separately, and the treatment decision should be made individually.

Although there are many studies in the literature comparing tenotomy and tenodesis performed in patients underwent shoulder arthroscopy (6-8), to the best of our knowledge, there is no study that compares the clinical outcomes between chronic biceps tendon rupture and tenotomy or tenodesis. In this study, we aimed to compare the postoperative 24-months clinical results of patients with chronic rupture of the LHB and patients who underwent tenotomy or tenodesis. By comparing the preoperative and postoperative VAS and UCLA score improvements of the patients which we divided into three groups (chronic rupture, tenotomy and tenodesis), we investigated the following research questions:

1- Is there a statistically significant difference between

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preoperative and postoperative clinical outcomes of tenodesis, tenotomy and chronic biceps rupture?

2-Is tenodesis a necessity in patients with severe biceps tendon degeneration? Is tenotomy sufficient?

2. Materials and Methods

A retrospective study was carried out with the approval of our institutional review board in accordance with the Declaration of Helsinki. 179 patients who underwent arthroscopic shoulder surgery between January 2016 and January 2021 in our institute were analyzed. Patients over the age of 50 who underwent arthroscopic shoulder surgery and who were compatible with the standard follow-up program and at least 24 months follow-up in our institute were included in this study. Exclusion criteria were follow-up duration less than 24 months, incomplete follow-up data, previous surgery of the same shoulder, systemic inflammatory diseases and patients who did not have biceps disorders (Fig.1).

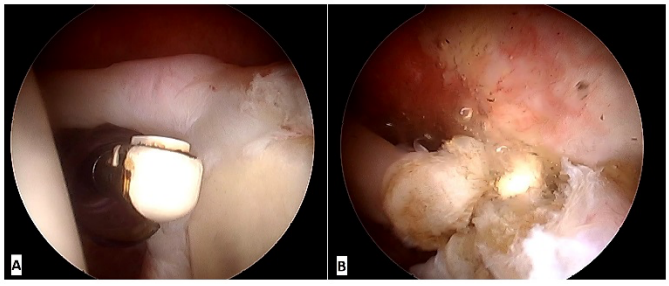


Fig.1A-1B. Utilization of the tenotomy process

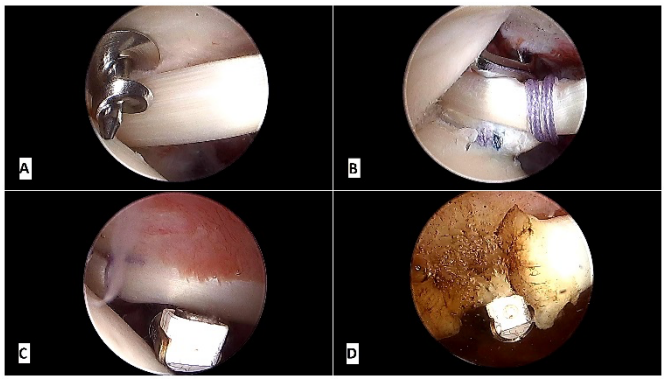
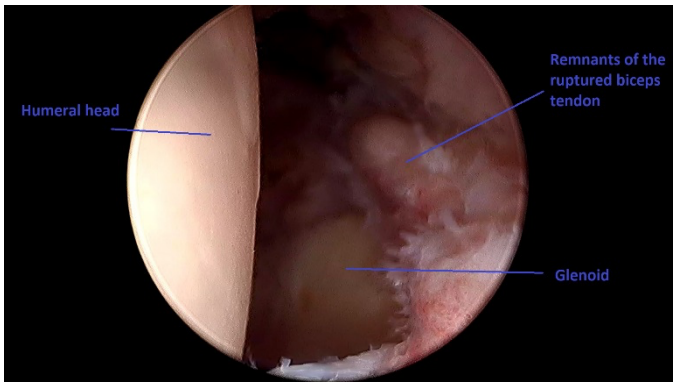


Fig. 2A: Anchor application to the humeral head for tenodesis, **2B**: Suture application to the long head of biceps tendon, **2C**: Identification of the tenotomy level of biceps tendon, **2D**: Application of the tenotomy process

Tenotomy or tenodesis was preferred by the surgeon in response to tendon tears, injury or inflammation and according to biceps tendon degeneration severity. All surgeries were performed by the same surgeon in beach chair position. Arthroscopic tenotomy or tenodesis was performed to biceps tendon if significant degeneration was observed (Fig.2). Tenodesis was performed using suture anchor technique (Fig.2). The rotator cuff tears were repaired with either tendon-tendon, tendon-bone or both with suture anchors. Debridement was only performed to the superior glenolabral insertion area of biceps tendons with chronic rupture (Fig.3). Velpeau Bandage was applied to all patients postoperatively. After 1 week of immobilization, passive ROM exercises were performed. Active ROM exercises were allowed after 6 weeks. Clinical follow-ups were routinely performed. In our clinical follow-ups, we routinely record UCLA and VAS score analysis in the preoperative and postoperative periods in order to assess the effectiveness of our surgical treatment. The preoperative and postoperative 24-months UCLA and VAS score records of all patients included in our study were compiled and analyzed. The cases were analyzed retrospectively, with video images recorded during surgery, medical records and preoperative and postoperative scale forms of each scoring systems. The preoperative and postoperative differences of UCLA scale and VAS scoring systems for all three groups that we divided into three groups as tenotomy, tenodesis and chronic biceps rupture were compared.

Fig. 3. Chronic rupture of long head of biceps tendon. The glenolabral insertion are is degenerated and the tendon which cannot be visualized in the picture, is retracted within the bicipital groove.



2.1. Statistical analysis

Statistical analysis was carried out with SPSS v.22.0 software (SPSS Inc., IBM Corporation, Armonk, New York, USA). For this study, due to the data not being normally distributed ($p < 0.05$, Shapiro-Wilk test), non-parametric tests were used to compare the independent groups and pairwise comparison tests were used to determine the particular differentiating group. Preoperative and postoperative scores were compared using a repeated-measure Wilcoxon signed rank test. The study was carried out at 95% confidence level and $p < 0.05$ was considered statistically significant.

3. Results

Of the 98 patients (mean age 61.2 ±8.44; range 50-83, 41 male); 31 (31.6%) had tenotomy, 31 (31.6%) had tenodesis and 36 (36.7%) patients had chronic tendon rupture. When the cuff pathologies of the patients were examined, partial rotator cuff tear was observed in 33 (33.7%) cases, full-thickness in 36 (36.7%) cases, and massive rotator cuff tear in 29 (29.6%) cases. Within the tenotomy group, patients with partial (16.6%) and full thickness rotator cuff tears (46.7%) were in the majority; while in chronic biceps tendon rupture group, massive rotator cuff tears were mostly observed (75.9%).

Table 1. Preoperative and Postoperative VAS and UCLA score values (minimum, maximum, median)

		Preoperative_VAS Score			Postoperative_VAS Score			Preoperative UCLA_Score			Postoperative_UCLA_Score		
		Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
Rotator Cuff_Pathology	Partial	3.00	7.00	4.94	2.00	6.00	3.55	9.00	24.00	16.52	13.00	32.00	24.15
	Full-thickness	3.00	8.00	5.17	2.00	7.00	3.67	6.00	26.00	18.06	14.00	33.00	25.81
	Massive	3.00	9.00	5.31	1.00	7.00	3.72	8.00	26.00	17.17	10.00	33.00	24.83
Biceps Tendon Disorder	Tenotomy	3.00	7.00	5.13	1.00	5.00	3.30	6.00	26.00	17.50	14.00	33.00	26.77
	Tenodesis	3.00	8.00	5.23	2.00	7.00	3.94	9.00	24.00	17.84	15.00	31.00	24.13
	Spontaneous Rupture	3.00	9.00	5.05	1.00	7.00	3.68	8.00	24.00	16.62	10.00	33.00	24.19

When the preoperative and the postoperative UCLA and VAS scores of the patients were compared according to Wilcoxon Signed Rank Test, it was observed that the postoperative scores significantly improved in all three groups (p = 0.001). The minimum, maximum and average values of VAS and UCLA scores measured in the preoperative and postoperative period are shown in Table 1. As a result of comparing the improvements of UCLA and VAS scores in the postoperative period, it was observed that the tenotomy group showed better improvement compared to the tenodesis group (p = 0.036, p = 0.010, respectively). There was also a statistically significant difference between tenotomy and chronic biceps rupture in terms of VAS score (p = 0.024). However, no statistically significant difference between tenodesis and chronic biceps rupture in terms of improvement in VAS scores (p=1.000) was observed. As the UCLA score improvements were compared, tenotomy was found to have a statistically significant difference compared to tenodesis (p=0.010) however, when compared with chronic biceps rupture, there was no significant difference between them (p=0.527). When chronic biceps rupture was compared with tenodesis, there was also no statistically significant difference between them in terms of UCLA score development (p=0.250). The minimum, maximum and mean values of the improvement between preoperative and postoperative UCLA and VAS scores for each biceps tendon procedure is given in Table 2.

When postoperative total VAS and UCLA scores were compared between tenotomy, tenodesis, and chronic biceps rupture groups, no statistically significant difference was observed (p=0.620, p=0.247, respectively) although significant differences in terms of score improvements between preoperative and postoperative periods were observed.

Table 2. UCLA and VAS score improvement in each biceps tendon disorder group

		VAS score_total_change				UCLA_score total_change			
		Min	Max	Median	Mean	Min	Max	Median	Mean
Biceps Disorder	Tenotomy	.00	6.00	2.00	2.50	4.00	19.00	9.00	9.27
	Tenodesis	.00	6.00	1.00	1.52	.00	13.00	6.00	6.29
	Rupture	.00	6.00	1.00	1.70	-1.00	14.00	8.00	7.57

4. Discussion

In the context of rotator cuff tears, 90% of patients demonstrates accompanying LHBT disorders and 45% of cases has also LHBT instability (9, 10). During the surgical treatment of shoulder lesions such as rotator cuff tears and labral tears, if biceps tendon disorders are not given full consideration, the pain may not completely vanish and the patient's satisfaction will also decrease (11). In the literature, there are different views about the superiority of the surgical methods that can be performed in biceps tendon disorders. Although both biceps tenotomy and tenodesis have shown successful outcomes, there is still no consensus on the superiority of these two procedures (12-15). In a study performed by Belay et al. (2019), tenotomy and tenodesis were compared and the researchers stated that VAS scores at the 3rd month follow-up were lower in the tenotomy group; however, they did not find a significant difference between tenodesis and tenotomy at the 2nd year follow-ups. The researchers also declared that these patients needed less pain killers at the 2nd week follow-up than those who underwent tenodesis (16). Franceschi et al. (2007) reported that patients underwent tenotomy had significantly better functional shoulder outcomes and higher satisfaction levels than the tenodesis group did. (17). In a systematic review presented by Frost et al. (2009), the failure rates and clinical outcomes of tenotomy and tenodesis were searched in various studies, both procedures present a similar number of good to excellent results, ranging from 65% to 90% in the tenotomy group, and 40% to 93% in the tenodesis group. The failure rates for tenodesis varied from 5% to 48% and 13% to 35% for tenotomy (18). In the same review, it is mentioned that patients underwent tenotomy are occasionally allowed to return to daily normal activities immediately after the operation while tenodesis generally requires a strict

postoperative rehabilitation protocol (18).

In a study conducted by Castricini et al. (2018), tenotomy and tenodesis were compared in 69 patients with rotator cuff tears, and the superiority of tenodesis over tenotomy was not observed in terms of postoperative Constant and Murley scores, quality of life, pain, and muscle strength (6). Gill et al. and Kelly et al. compared biceps tenotomy and tenodesis and found no differences in terms of clinical outcomes between them (19, 20). In a study conducted by Hufeland (2019), one-year clinical outcome scores of tenodesis and tenotomy were compared. No significant difference was found between the two methods in terms of clinical scores, however, they observed higher muscle strength with tenodesis 6 months after surgery (21). In a randomized controlled trial performed by MacDonald et al. (2020), 114 patients were compared in terms of the clinical results such as pain, cramps and muscle strength of tenotomy and tenodesis. According to their study, although both tenotomy and tenodesis provided satisfactory clinical improvements, there was no difference between both procedures (22). In a systematic review performed by Slenker et al (2012), sixteen studies were included to the review and the clinical outcomes of tenodesis and tenotomy were compared. In this review, 74% of 433 patients who underwent tenodesis had good / excellent results; while in 699 patients who underwent tenotomy this rate was found to be 77%. However, they added that tenotomy or tenodesis in general were not superior to each other (23). In a study performed by Friedman (2015), functional results of 42 patients who underwent tenotomy or tenodesis were compared, and DASH, ASES and VAS scores were found to be similar across the groups. The researchers also indicated that the frequency of cramping complaints was higher in the tenotomy group while pain was encountered more in the tenodesis group (5).

Although the LHBT is thought to play an important role in the stability of the shoulder joint (24), the complete mechanism of the process has not been fully elucidated. In the literature, a statistically significant difference between biceps tenotomy and tenodesis was not detected therefore, preservation of the degenerated tendon may not be necessary. Indeed, especially in arthroscopic surgery of massive and full-thickness rotator cuff tears, the LHBT is generally found ruptured or grossly shredded, yet it is still observed that the cuff repair outcomes are satisfying. We also observed that the clinical outcome improvements of our patients who underwent arthroscopic rotator cuff repair showed very good/excellent results in our clinic although no repair was performed to a complete chronic LHBT rupture. Therefore, we conducted this study to compare the clinical outcomes of chronic LHBT rupture, tenodesis and tenotomy which yielded significant results. UCLA and VAS shoulder scores were compared in terms of preoperative and postoperative period, and also improvement after surgery.

Rupture of LHBT is usually a sequela of a massive and full-thickness rotator cuff rupture and develops by the loss of the protective soft tissue cover (9, 25). The biceps tendon is usually progressively injured, flattened, torn and eventually ruptured as the rotator cuff tear extends (26). The vast majority of LHBT ruptures are confronted over the age of 50 and commonly comprises intrinsic tendinous degeneration (3, 4). Isolated ruptures which are not associated with rotator cuff tears are much rarer and tend to occur in middle-aged patients as a result of traumatic injuries (27). Isolated rupture of LHBT has also been reported in non-traumatic conditions such as hypothyroidism (28), and steroid injection (29) etc. nevertheless, these were not addressed in our study.

We particularly analyzed “chronic” proximal biceps tendon ruptures that occur over time due to the degenerative process in patients with massive and full-thickness rotator cuff rupture and excluded acute spontaneous traumatic ruptures. The incidence of chronic rupture of proximal biceps tendon has been reported to be approximately 5% (26). The possible reason for higher rate in our study may be that the majority of patients who underwent surgery had massive rotator cuff tears.

There are some limitations in our study. First, there exists an inevitable potential failure to demonstrate the superiority of one technique over the other secondary to several factors such as concomitant shoulder lesions which should also be repaired. Since the number of patients who have been operated for biceps tendon alone is low, it seems necessary to perform this study in patients with accompanying rotator cuff ruptures. Also, as seen in literature, many of the studies included patients undergoing either a tenotomy or a tenodesis with a concomitant surgical intervention such as rotator cuff repair. The comparison between the procedures is difficult and their reliability is questionable because of this potential influence of aforementioned other shoulder lesions. The other limitation is the retrospective nature of our study.

We believe that tenotomy is a more appropriate surgical method than tenodesis in massive and full-thickness rotator cuff tears with severe biceps tendon degeneration, and also it is not necessary to perform tenodesis in cases with chronic biceps rupture**.**

Conflict of interest

The author declares that there is no conflict of interest.

Funding

The author declares that there is no funding.

Acknowledgments

None.

Authors’ contributions

Concept: F.İ.C., Design: E.G., Data Collection or Processing: İ.G.Ş, Analysis or Interpretation: F.İ.C., C.Y.K., Literature Search: F.İ.C., Writing: F.İ.C.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 733-737
doi: 10.52142/omujecm.39.3.27

Imaging of the right ventricle in predicting the development of chronic thromboembolic pulmonary hypertension (CTEPH)

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Received: 16.02.2022 • **Accepted/Published Online:** 14.08.2022 • **Final Version:** 30.08.2022

Abstract

There is increasing evidence in the literature emphasizing the importance of right ventricular (RV) imaging in the prognosis of pulmonary hypertension. We aimed to investigate the predictive role of RV dysfunction parameters assessed by echocardiography (ECHO) and thorax computed tomography (CT) in developing CTEPH. We included prospectively patients diagnosed with pulmonary embolism (PE). All patients underwent ECHO and CT within 24 hours after admission. We repeated CT and ECHO after six months and one year to assess the incidence of CTEPH and the predictive role of RV dysfunction factors in the development of CTEPH. We included twenty-two patients (seven male) with a mean age of 53.9±17.9 years; CTEPH developed in two patients during the follow-up. Baseline PO2 levels were significantly lower in patients with CTEPH (61.5±11.4 vs 77.8±25.2, $p<0.05$). The baseline RV diameter, RV EF, and systolic PAP levels evaluated by ECHO differed significantly in two patients who developed CTEPH. Two patients that developed CTEPH had the lowest RVS (-10.3% and -11.7%). This study claims that hypoxemia, decreased RV EF, RVS, increased systolic PAP values in ECHO, and increased RV/LV ratio evaluated in thorax CT indicate the severity of RV dysfunction in acute PE and may predict CTEPH development.

Keywords: Hypertension, echocardiography, pulmonary circulation, right ventricle

1. Introduction

Chronic thromboembolic pulmonary hypertension (CTEPH) is a rare pulmonary vascular disease caused by clot formation that occludes the vascular bed and may occur after a symptomatic acute pulmonary embolism (PE) event. There is an expected natural course following acute PE. The clot's complete or significant resolution is marked by improved pulmonary hemodynamics, gas exchange, and exercise tolerance. However, CTEPH may occur after a recurrent PE event and inadequate resolution of thrombus. CTEPH frequency was reported as 1-4% in PE survivors (1,2).

Clinical symptoms and signs of CTEPH are absent in early disease and may resemble acute PE or idiopathic pulmonary hypertension. Early diagnosis is essential in CTEPH because it is a disease that can be cured in the majority of patients who are eligible for pulmonary endarterectomy. However, routine screening for CTEPH after PE is not supported by current evidence (3).

Several risk factors have been reported in previous studies considered associated with the development of CTEPH (4). These risk factors mainly include comorbid conditions such as ventriculoatrial shunt, infected cardiac pacemaker, splenectomy, thyroid replacement therapy, and malignancy (5). Identifying simple, non-invasive, reproducible parameters that predict the development of CTEPH during PE evaluation is vital for the early diagnosis of the disease. There is increasing evidence in the literature emphasizing the importance of right ventricular (RV) imaging in the prognosis of pulmonary hypertension. However utility of these factors in the development of CTEPH after PE event is not evaluated (6-8). This study aimed to investigate the predictive role of RV dysfunction parameters assessed by echocardiography (ECHO) and thorax computed tomography (CT) in developing CTEPH.

2. Materials and Methods

According to thorax CT findings, we included prospectively patients admitted to our hospital and diagnosed with PE in this study while excluding the patients with different diseases such as scleroderma, COPD, left heart failure, sleep apnea, and connective tissue disease that may cause pulmonary

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hypertension (PH). We recorded patients' demographic characteristics, arterial blood gas analysis, D-dimer, and NT-proBNP levels. All patients underwent thorax CT and ECHO within 24hours after admission. We repeated thorax CT and ECHO after six months and one year to assess the incidence of CTEPH and evaluate the predictive role of RV dysfunction factors in the development of CTEPH. The same physician performed the ECHO examination within 24 hours after application using a 3.0 MHz transducer on a General Electric VingmedVividSystem7 device (Horten, Norway). We determined the right ventricular (RV) diameter, ejection fraction (EF), systolic pulmonary artery pressure (sPAP), acceleration time (ACT), tricuspid annular plane systolic excursion (TAPSE), right ventricular strain (RVS), the rate of change in myocardial diastolic and systolic peak.

The same radiologist performed thorax CT by intravenous opaque material in 0.5mm sections from the apex of the lung through Toshiba Aquilion (Tokyo, Japan) tomography on all study patients. We evaluated the primary PA diameter, RV diameter, right atrium diameter, RV wall thickness, septum wall thickness, and RV/LV ratio by CT.

In follow-ups, we suspected CTEPH in patients with a systolic PAP greater than 50 mmHg in ECHO and with chronic, organizing, and occlusive thrombus in the pulmonary arteries (PA) on thorax CT despite effective anticoagulant therapy[3]. We performed right heart catheterization to confirm the CTEPH diagnosis. The ethical committee of Kocaeli University approved the study, and we obtained written informed consent from all participants.

2.1. Statistical analysis

We conducted the statistical analysis of the data obtained in the study in the SPSS 16.0 program and expressed the results as mean ± standard deviation (SD). We used descriptive statistics and Chi-square tests to compare the demographic characteristics of the patients while using the non-parametric Wilcoxon and Friedman tests to compare the initial and control parameters and the Mann-Whitney U test to compare the intergroup meanings. The Pearson correlation test assessed the correlation between variables. We considered a p-value of less than or equal to 0.05 significant.

3. Results

Twenty-two patients (seven male) with a mean age of 53.9±17.9 years were diagnosed with PE. CTEPH developed in two patients during the follow-up, and both patients underwent pulmonary endarterectomy. We observed a significant improvement in clinical and functional parameters in the postoperative follow-ups.

The leading symptom was dyspnea in most patients (15 patients, 68.2%). Baseline demographic characteristics were not different in patients diagnosed with CTEPH compared to others; however, baseline PO2 levels were significantly lower in patients with CTEPH (61.5±11.4 versus 77.8±25.2 p <0.05) (Table 1).

Table 1. Comparison of laboratory findings between CTEPH and non-CTEPH patients

	CTEPH (+)	CTEPH (-)	p
D-dimer	0.95±0.4	3.89±3.53	>0.05
NT-ProBNP	165±26.8	159±281	>0.05
Troponin	0.05±0.01	0.06 ± 0.03	>0.05
PO2	61.5±11.4	77.8±25.2	<0.05

CTEPH: Chronic thromboembolic pulmonary hypertension, NT-ProBNP: NT-pro brain natriuretic peptide, PO2: Partial oxygen pressure

Control ECHO was available only in 18 patients because two patients died and two did not come to their follow-ups. Three patients had tricuspid regurgitation (TR) at the advanced stage, while no TR was present in seven patients. Systolic pulmonary artery pressure was higher than 50 mmHg in three patients, two of whom developed CTEPH in follow-ups. After treatment, sPAP and RV diameters decreased significantly. Furthermore, compared to baseline parameters, RVS, RV EF, and TAPSE values significantly increased in the 6th and 12th-month follow-up ECHO (Table 2).

Table 2. Baseline and follow-up echocardiographic characteristics of patients

	Baseline	6 month	12 month	p-value
RVS (%)	-16.88±3.76	-18.62±3.96	-19.16±3.16	0.001
RV diameter (mm)	28.58±3.32	28.31±7.85	25.72±3.67	0.001
RV EF, %	58.11±15.64	63.77±12.84	66.27±10.47	0.001
PAPMax (mmHg)	33.48±18.75	30.53±19.70	25.51±17.86	0.011
ACT (msn)	72.45±17.41	75.83±15.43	78.33±16.38	0.245
TAPSE (mm)	17.95±3.53	19.50±2.87	20.66±3.18	0.001

RVS: right ventricular strain, RV: right ventricle, PAPmax: systolic pulmonary artery pressure, ACT: acceleration time, TAPSE: tricuspid annular plane systolic excursion

In thorax CT, we detected thrombus originating from the right system in five, from the left system in four, and bilateral involvement in 13 patients. In the 6th and 12th months, control CT examination was available in 19 and 18 patients, respectively. Compared to baseline measurements, the main pulmonary artery (PA), RA, RV diameters, and RV/LV ratios significantly decreased on the 6th and 12th-month control CT (Table 3). We found a significant correlation between RV EF detected in ECHO and RV diameter detected in thorax CT (p:0.001). In thorax CT, RV strain in ECHO significantly correlated with septal flattening (p:0.035) and RV diameter (p:0.008). We observed that the RV/LV ratio evaluated in CT was correlated with all follow-up ECHO parameters (RV diameter, p:0.003; RV EF, p:0.007; PAP max, p:0.044; and RVS, p: 0.02).

Table 3. Baseline and follow-up thorax computed tomography characteristics of patients

	Baseline	6 month	12 month	p-value
Main pulmonary artery diameter (mm)	28.81±5.19	26.78±5.38	25.61±5.78	0.006
RV diameter (mm)	42.86±8.10	40.05±5.97	38.88±6.89	0.005
RA diameter (mm)	49.04±9.06	44.89±14.27	42.11±8.69	0.002
RV wall thickness (mm)	3.41±1.64	3.01±1.02	2.71±1.00	0.282
Septum wall thickness (mm)	10.75±3.10	10.65±1.89	11.05±2.04	0.740
RV/LV ratio	1.26±0.45	1.09±0.25	1.00±0.20	0.002

RV: right ventricle, RA: right atrium, LV: left ventricle

The baseline RV diameter, RV EF, and sPAP levels evaluated by ECHO were significantly different in two patients who developed CTEPH compared to others. We detected the lowest RVS values in these patients (-10.3% and -11.7%).

4. Discussion

This study suggests that hypoxemia, decreased RV EF, RVS, increased systolic PAP values in ECHO, and increased RV/LV ratio evaluated in thorax CT indicate the severity of RV dysfunction in acute PE and may predict CTEPH development.

The actual incidence and prevalence of CTEPH have not yet been fully defined and remain a controversial topic (3, 5). In recent studies, the incidence of CTEPH after acute PE has been reported to be around 4-5%. However, most experts believe real CTEPH varies from 0.5% to 2% after acute PE (1, 5, 9). In a study conducted by Ribeiro et al., 78 patients (5.1%) who had survived acute PE developed CTEPH at the end of a 1-year follow-up (2). Kayaalp et al. showed that CTEPH developed in five of 90 (5.5%) patients followed up for 1-2 years after acute PE (10). In another study, symptomatic CTEPH frequency in the first two years after a critical PE event was 3.8% in 223 patients followed up for an average of 7.8 years, and there were no new cases diagnosed with CTEPH after the first two years (1) . Dentali et al. included 92 patients with acute PE with a median follow-up time of 6 to 12 months and showed that CTEPH developed in 8 patients while half of these patients were asymptomatic (9). Our study included a relatively low number of patients; however, two patients (9%) out of 22 patients developed CTEPH. This data suggests that the prevalence of the disease might be higher than reported in the literature, and active screening after acute PE events may be beneficial on an individual basis by increasing the number of early diagnosed patients. However, it is clear that this is not a cost-effective method and is not supported by the current evidence. Therefore, it is necessary to identify the high-risk group and improve the follow-up parameters used in the early diagnosis of these patients.

Echocardiography (ECHO) is a non-invasive method widely used in screening PH. In the diagnosis of PH, the sensitivity of ECHO varies between 63-100%, and specificity ranges between 60-98% (11). Echocardiography should always be performed when PH is suspected; however, it is not sufficient to make a specific diagnosis and decide on treatment, and cardiac catheterization is required (3).

CTEPH is limited as it allows diagnostic evaluation only in symptomatic patients, carrying the risk of diagnosing disease in advanced stages. On the other hand, routine screening for CTEPH in asymptomatic survivors of PE is not cost-effective and is not recommended by current guidelines (3). Therefore, we need to determine predictive factors for CTEPH from the routine diagnostic workup in acute PE besides the above-mentioned clinical risk factors to compose a high-risk population in which follow-up for CTEPH is reasonable.

Previous studies evaluated several echocardiographic parameters to predict CTEPH development, such as PA pressure, RV diameter, RV EF, ACT, and TAPSE (12). However, assessment of RV function is complex because of the complexity of RV anatomy. Recently RV strain analysis by 2D speckle tracking ECHO has been introduced to evaluate RV function in PH. Shiino et al. demonstrated that RVS had a significant correlation with an invasively determined pulmonary hemodynamic parameter. It was a valuable indicator to detect increased mean PAP in patients with CTEPH (13). Strain is a parameter of RV myocardial deformation, and it has an excellent diagnostic and prognostic accuracy in patients with PH (14). Previous studies have shown that RVS markedly decreased in patients with PE compared to healthy controls and improved on the 15th day after treatment (14, 15). In our study, lower RVS values in patients with CTEPH suggest that ECHO follow-up should be planned in patients with low strain values, and CTEPH should be suspected if there is no improvement in RVS after the treatment. However, RVS measurement is still not widely used in routine clinical practice.

Nowadays, multislice thorax CT is the most commonly used imaging technique in diagnosing PE. It has been reported that thorax CT can evaluate RV structure and function and diagnose PE or CTEPH, which correlates with disease severity and ECHO follow-up parameters (16, 17). It has been shown that the ratio of RV/LV above 0.9 in thorax CT is associated with a poor prognosis in PE (18, 19). The study by Ende-Verhaar et al. showed that the risk of

developing CTEPH after pulmonary embolism is higher in patients with RV/LV ratio greater than 1 in thorax CT (20). In this study, both patients diagnosed with CTEPH had an RV/LV ratio greater than 1.0 in baseline thorax CT evaluation. In addition, we determined that this parameter was the best-correlated variable with the follow-up parameters in ECHO.

The most important limitation of our study was the relatively low number of patients. In addition, some follow-up parameters in patients with and without CTEPH do not differ in the statistically significant dimension and are attributed to the small number of patients. We believed that the number of patients was influential in elucidating the difference in variables such as pro-BNP, D-dimer, troponin, and CT findings. Nevertheless, we consider early detection of two CTEPH cases, appropriate surgical intervention, and improvement of their prognosis significant despite the small number of patients. Limiting the follow-up period to one year can constitute another limitation. Prolonging the follow-up time may allow early detection of new patients (1, 21).

CTEPH is a severe disease that may result in progressive right heart failure and death. It may follow acute PE events; however, routine screening of every asymptomatic survivor after acute PE is not practical and not recommended. This study suggests that hypoxemia, decreased RV EF, RVS, increased systolic PAP values in ECHO examination and increased RV/LV ratio evaluated in thorax CT indicate the severity of RV dysfunction in acute PE events and may predict CTEPH development. Control ECHO examination on the third or sixth month after the treatment in patients with one or more of these risk factors during acute PE events may provide an early diagnosis of CTEPH.

Conflict of interest

The authors declared no conflict of interest.

Funding

No funding was used for the study.

Acknowledgments

Ethical Approval: The Ethics Committee of Kocaeli University approved this study (date: 25.07.2011, No: KAEK 10/11). We conducted the study per the principles of the Declaration of Helsinki and obtained written informed consent from all participants.

Authors' contributions

Concept: İ.B., F.Y., Design: Y.T.G., İ.B., Data Collection or Processing: Y.T.G., İ.B., Analysis or Interpretation: İ.B., Y.T.G., F.Y., H.B., T.Ş., S.D., Literature Search: Y.T.G., İ.B., S.B., T.Ş., S.D., Writing: Y.T.G., İ.B., H.B.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 738-742
doi: 10.52142/omujecm.39.3.28

Diagnostic accuracy of clinical gestalt of doctors with different experiences in COVID-19 suspected patients

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Received: 20.02.2022 • **Accepted/Published Online:** 05.06.2022 • **Final Version:** 30.08.2022

Abstract

This study aims to evaluate the ability of physicians' predictions to predict mortality in COVID-19 patients and compare physician predictions with scores developed for COVID-19 patients in predicting mortality and patient worsening. This study was conducted prospectively in the emergency department. Patient data were collected between 20.03.2021 and 20.06.2021. Patients who applied to our hospital with COVID-19 symptoms and were confirmed to be COVID-19 by rt-PCR results were included in our study. Patients aged 18 years and over who were tr-PCR positive were included in the study. Quick COVID-19 Severity Index (qCSI), Brescia-COVID Respiratory Severity Scale (BCRSS), and CURB-65 scale were calculated and recorded by a researcher. A total of 176 patients were included in our study. There was no significant relationship between physicians' gestalt and 28-day mortality (p=0.121, p=0.282, Mann-Whitney U Test, respectively). Physicians' gestalt was found to be insufficient to predict mortality in COVID-19 patients. There was a significant difference between the CURB-65 short-term mortality group and the survivors.

Keywords: COVID-19, gestalt, CURB-65, mortality

1. Introduction

The coronavirus disease COVID-19 started in Wuhan in December 2019. It spread rapidly around the world and was declared a pandemic. It causes severe acute respiratory syndrome in patients. Reverse transcription-polymerase chain reaction (RT-PCR) is used in the diagnosis of COVID-19 (1). The symptoms of the disease are very extensive. The most common symptoms are cough, shortness of breath, weakness, joint-muscle pain, and loss of smell and taste. Risk factors for the progression of the disease and death include old age, hypertension, diabetes, cardiovascular disease, lung disease, chronic kidney disease, malignancy, and immune system diseases (2). The mortality of the disease varies between 0.4% and 7%. It has been found that biomarkers associated with mortality were found to be a low amount of lymphocyte, high d-dimer, high C-reactive protein, high lactate dehydrogenase enzyme, and high interleukin-6 (3, 4).

In many countries, the number of COVID-19 patients exceeded the current health capacities of the countries in a short time. The high number of COVID-19 patients made it necessary to apply triage to these patients. Estimating the need for intensive care support or the need for a ventilator created a serious problem. Various scoring systems are used to predict the conditions such as hospitalization, need for ventilators, and mortality of COVID-19 patients (5). Quick COVID-19 Severity Index (qCSI), Brescia- COVID-19 Respiratory Severity Scale (BCRSS), and CURB-65 scale are some of them These scores are used in patient triages in many emergency departments (6). Until sufficient data on COVID-19 patients were published, all physicians decided to hospitalize or discharge patients with their own foresight. Physicians working in the emergency department used their experiences from lung infections and critical patients in the triage of COVID-19 patients. In this study, our aim is to evaluate the ability of physicians' predictions to predict mortality in COVID-19 patients and compare physician predictions with scores developed for COVID-19 patients in predicting mortality and patient worsening.

2. Materials and Methods

This study was conducted prospectively in the emergency department of Ümraniye Training and Research Hospital. Patient data were collected between March 20, 2021 and June 20, 2021. Approval was obtained from the local Ethics

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Committee. Our hospital worked as a tertiary pandemic hospital from 2020 to 2021. There were COVID-19 patients in all of the hospital wards and intensive care units during the study period. Patients who applied to our hospital with COVID-19 symptoms and were confirmed to be COVID-19 by rt-PCR results were included in our study. Patients aged 18 years and over who were rt-PCR positive were included in the study. Outpatients, patients who refused to participate in the study, and patients whose data could not be reached in the national death notification system were excluded from the study. Data in the study were obtained from three sources: the study form, the hospital computer-based data system, and the national death notification system. After the physical examination, the form for the same patient was filled out by two different physicians. The study form included age, gender, vital parameters, comorbidities and physician gestalt.

Vital parameters were recorded as saturation, pulse rate, systolic and diastolic blood pressure, state of consciousness, fever, respiratory rate, and oxygen support. Comorbidities were recorded as chronic obstructive pulmonary diseases, hypertension, diabetes mellitus, congestive heart failure, chronic kidney disease, active malignancy, cerebrovascular disease, and coronary artery disease. Laboratory parameters at admission and 28-day mortality information were recorded from the hospital information system. Blood urea nitrogen and C-reactive protein were recorded from laboratory parameters.

Brescia- COVID-19, qCSI, BCRSS, and CURB-65 scales were calculated and recorded by a researcher (A. Ö.) from the hospital's computerized information system. For the calculation of BCRSS, parameters such as wheezing or inability to speak comprehensively inability to form sentences with minimum effort while resting, respiratory rate >22, oxygen saturation (SpO_2) <90%, and deterioration in lung imaging were used. Patients were then classified into five risk levels by BCRSS. Nasal cannula flow rate, respiratory rate, and minimum fingertip oxygen level parameters were used to calculate qCSI. Parameters of confusion, blood urea nitrogen >19 mg/dl, respiratory rate, systolic blood pressure 90 mmHg or diastolic blood pressure below 60 mmHg, and age over 65 were used to calculate CURB-65. The primary outcome of our study was 28-day mortality. Data were analyzed using Jamovi (Version 1.6.21.0; The Jamovi Project, 2020; R Core Team, 2019). Categorical variables were expressed as a percentage. It has been calculated as the median (interquartile range (IQR)) for continuous variables. The patients were grouped as survivor and non-survivor. The relationship between clinicians' gestalt and mortality was evaluated. Variables that did not fit the normal distribution were compared using the Mann-Whitney U test. The receiver operating characteristic (ROC) curve was used to determine the accuracy of the regression model in predicting short-term mortality. The area under the curve (AUC) and a 95% CI were calculated for the short-term mortality prediction of the gestalt. A p-value less than 0.05 was considered statistically significant.

3. Results

Between March 20, 2021 and June 20, 2021, 393 patients applied to the emergency department of our hospital due to COVID-19. Two hundred seventeen of those were excluded from the study (Fig. 1.). A total of 176 patients were included in our study. The median (25th-75th percentile) age was 8.5 (48-68) years, and 51.1% of the patients were female. The mortality rate in our study was 15.9%. The most common comorbidity was hypertension 71 (40.3%). According to their frequency, other comorbidities are diabetes mellitus 45 (25.6%). Congestive heart failure is 13 (7.4%). Coronary artery disease is 12 (6.8%). Baseline characteristics of the enrolled patients and a comparison of the characteristics between the survivor and non-survivor groups are shown in Table 1. The 28-day mortality values of the other scores in which the physician's predictions were compared are given in Table 2. AUC values were calculated to measure how different scores predicted mortality. The AUC value for qCSI was 0.567, 0.503 for BCRSS, and 0.656 for CURB-65 Score (p=0.210, p= 0.966, p=0.004, respectively) (Table 3). ROC curves for the scores are shown in Fig. 2. There was no significant relationship between physicians' gestalt and 28-day mortality (p=0.121, p=0.282, Mann-Whitney U Test, respectively).

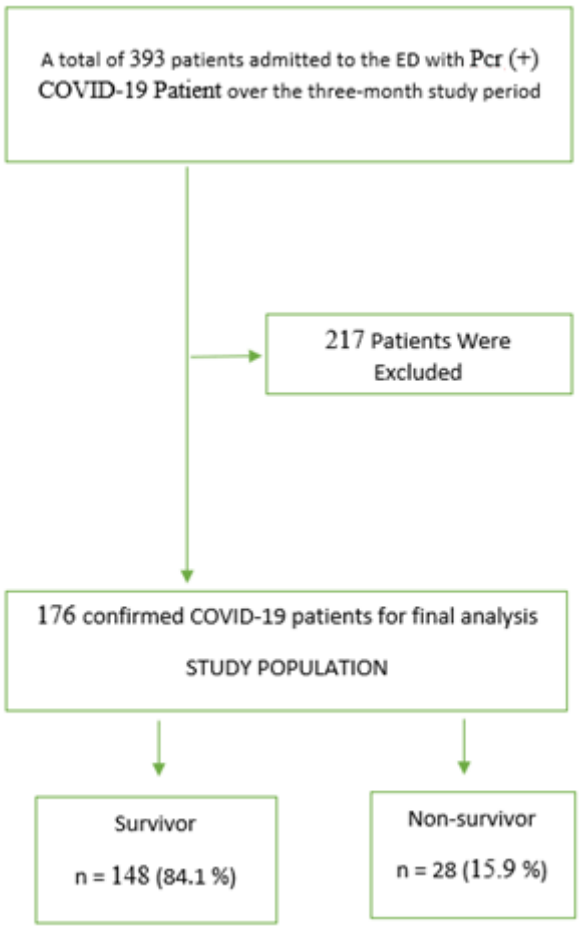

Fig. 1. A flow diagram of the study population

Table 1. Baseline characteristics of the enrolled patients and comparison of the characteristics between the survivor and non-survivor groups

Variables	Total	Survivor	Non-survivor	p values
	n=176 (%, 25th –75th percentiles)	**n=148** (%, 25th – 75th percentiles)	**n=28** (%,25th – 75th percentiles)	
Age, years	58.5 (48-68)	57.5 (47-67.3)	63.7 (51.0-71.8)	0.094
Gender				
Male	86 (48.9%)	72 (48.6%)	18 (64.3%)	
Female	90 (51.1%)	76 (51.14%)	10 (35.7%)	
Comorbidities (%)				
Chronic obstructive pulmonary diseases	6 (3.4%)	4 (2.7%)	2 (7.1%)	0.239
Hypertension	71 (40.3%)	61 (41.2%)	10 (35.7%)	0.589
Diabetes mellitus	45 (25.6%)	42 (28.4%)	3 (10.7%)	**0.050**
Congestive heart failure	13 (7.4%)	10 (6.7%)	3 (10.7%)	0.467
Chronic kidney disease	6 (3.4%)	3 (2%)	3 (10.7%)	**0.021**
Active malignancy	2 (1.1%)	2 (1.4%)	0 (0%)	0.545
Cerebrovascular disease	6 (3.4%)	6 (4.0%)	0 (0%)	0.079
Coronary artery disease	12 (6.8%)	12(8.1%)	0 (0%)	0.945
Comorbidity	109 (61.9%)	90(60%)	19 (67.8%)	0.484
Vital parameters				
Systolic blood pressure	128 (114-140)	128 (114-140)	132(118-144)	0.270
Diastolic blood pressure	76.5 (69.0-84.0)	76.3 (69.0-84.0)	77.2 (69.8-84.5)	0.453
Pulse pressure	90 (79.0-100)	89.2 (78.0-100)	94.2 (82.0-104)	0.106
Respiratory rate	21.9 (18.0-25.0)	21.6 (18.0-24.0)	23.8(18.8-28.5)	0.068
Oxygen saturation	88.0 (86-93)	89.2 (87.0-93.0)	86.3 (83.0-93.3)	0.535
Oxygen supplement	3.40 (2-4)	3.11 (2-4)	4.89 (2-6.5)	0.086
Lactate	1.62 (1.20-1.98)	1.68 (1.20-2.0)	1.38 (1.00-1.60)	0.132
Blood urea nitrogen	37.6 (23.0-45.0)	36.1 (23.0-42.0)	45.3 (29.5-53.3)	**0.011**
C-Reactive Protein (mg/L)	94.3 (45.8-131)	93.5 (45.8-133)	98.6 (46.3-121)	0.921

Table 2. comparison of the scores between the survivor and non-survivor groups.

		n (%)	Survivor	Non-survivor	p
CURB-65	**0**	1 (0.6 %)	1	0	0.003
	1	25 (14.2 %)	24	1	
	2	104 (59.1 %)	90	14	
	3	38 (21.6 %)	28	10	
	4	8 (4.5 %)	5	3	
BCRSS	**0**	31 (17.6 %)	25	6	0.955
	1	64 (36.4 %)	55	9	
	2	48 (27.3 %)	41	7	
	3	33 (18.8 %)	27	6	
qCSI	**1**	81 (46.0 %)	71	10	0.232
	2	21 (11.9 %)	17	4	
	3	30 (17.0 %)	25	5	
	4	44 (25.0 %)	35	9	

Table 3. Sensitivity, specificity, positive predictive value, negative predictive value, and likelihood ratio for different CURB-65, qCSI and BCRSS scores for predicting death

	Cut point	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC	LR		p
qCSI	1	100%	0%	15.91%	NaN%	0.567	1	1-1	0.381
	2	64.29%	47.97%	18.95%	87.65%		1.24	0.9- 1.7	
	3	50%	59.46%	18.92%	86.27%		1.23	0.81- 1.87	
	4	32.14%	76.35%	20.45%	85.61%		1.36	0.74- 2.51	
BCRSS	1	100%	0%	15.91%	NaN%	0.503	1	1-1	0.733
	2	78.57%	16.89%	15.17%	80.65%		0.95	0.77- 1.17	
	3	46.43%	54.05%	16.05%	84.21%		1.01	0.65- 1.56	
	4	21.43%	81.76%	18.18%	84.62%		1.17	0.53- 2.57	
CURB-65	1	100%	0%	15.91%	NaN%	0.656	1	1-1	0.014
	2	100%	0.68%	16%	100%		1.01	1- 1.02	
	3	96.43%	16.89%	18%	96.15%		1.16	1.05- 1.28	
	4	46.43%	77.7%	28.26%	88.46%		3.17	0.8- 12.51	
	5	10.71%	96.62%	37.5%	85.12%		2.08	1.26- 3.43	

AUC: area under the curve; PPV: positive predictive value; NPV: negative predictive value; LR: likelihood ratio.

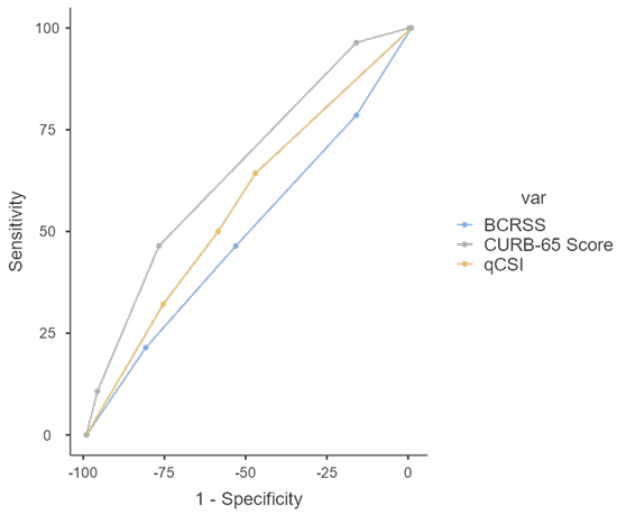

Fig. 2. ROC curves for mortality for the CURB-65, qCSI and BCRSS

4. Discussion

In our study, the role of emergency physicians' gestalt in predicting mortality in COVID-19 patients was investigated. Physicians' gestalt was found to be insufficient to predict mortality in COVID-19 patients. Moreover, when the compatibility of physicians with different experiences was evaluated, a statistically significant agreement was observed among physicians. (Kendall's Tau B: 0.617 p: 0.001). To the best of our knowledge, our study is the first to evaluate the compliance of physicians' gestalts in patients with COVID-19.

Clinical gestalt means that clinicians are capable of making clinical decisions indirectly in the absence of complete information and are able to generate solutions characterized by generalizations that allow transfer from one problem to another. In other words, clinical gestalt is pattern recognition and a heuristic approach to decision making (7). Various clinical scenarios have been studied on the gestalt of clinicians (8). In the COVID-19 pandemic, clinicians had to make vital decisions, given the burden on the healthcare system, especially in the early stages of the disease (9). Many clinicians have had to manage clinical condition of COVID-19 patients they have not encountered before. Soto-Mota et al. evaluated clinicians' gestalts in clinical scenarios in the management of the disease in their study during the first peak period. They found the diagnostic value of gestalt to be 0.680, a value that could be reported as insufficient (10). In our study, similar to the study of Sota-Mota et al., we found the gestalt to be insufficient in predicting mortality. A plausible explanation for this may be that the COVID-19 Disease does not resemble the course of pneumonia clinicians previously knew. Mortality in COVID-19 occurs in three ways. These are cytokine storms thromboembolic processes, and respiratory failure caused by viral pneumonia. These processes may follow clinical courses contrary to the previous experience of clinics with viral pneumonia. These new pathogeneses may have caused gestalt insufficiency. There was agreement between the different clinician gestalts, confirming this explanation.

The disease, which spread rapidly during the pandemic, caused an overload in the health system. Identifying patients in need of medical support was essential to use health capacity effectively (11). Bradley et al. reported in their study that CURB-65 can predict short-term mortality of COVID-19 (12). Akça et al. investigated the relationship between PSI, CURB-65, CALL and BCRSS and short-term mortality in their study. They showed that there was a significant difference in scores between the mortality group and the survivors (13). In our study, there was a significant difference between the CURB-65 short-term mortality group and the survivors.

Results of this study showed that the qCSI could not be a predictor of mortality in COVID-19 (AUC 0.567, p:0.232). Covino et al. found qCSI to have the highest PPV in predicting hospital mortality (14). However, this AUC could not rise above the strong correlation of 0.8 (AUC: 0.749) (15). According to results of current study, BCRSS is a scale used to determine the respiratory severity of COVID-19 pneumonia, showing the patient's need for oxygen and mechanical ventilation. While providing information about the clinical course of the patients, it could not predict mortality (p 0.955). This may be the reason why qCSI, BCRSS, and clinician gestalt fail to predict mortality. The disease has a mild infection phase, pulmonary involvement phase, and cytokine storm phase (16). The vast majority of patients admitted to the hospital are in the stage of pulmonary involvement. These scorings cannot predict the cytokine storm that is thought to cause the death of COVID-19 patients. The cytokine storm is generally blamed for mortality during intensive care or service admission. There is no suggestion or scoring system that will help us understand when the cytokine storm will start and its severity (17). None of the scores we evaluated can predict the cytokine storm (18). Other reasons why the scoring done in the emergency department could not predict mortality may be: Arterial and venous thrombotic conditions which are causes of mortality and morbidity, formation of disseminated intravascular coagulopathy, long-term hospitalization, admission to the intensive care unit, and the necessity for mechanical ventilation's being after hospital admission (19). Scorings made in the emergency services are made at the time of application. Therefore, the inability to predict mortality can be attributed to these reasons. We think that more research is needed on this subject.

Our study had several limitations. Firstly, vaccine applications had just started in our country at the time of our study. Therefore, it can be said that our study cohort was unvaccinated. Considering that vaccination programs have become widespread throughout the world, we believe that the generalizability of our study to the vaccinated population is limited. Secondly, although agreement between clinicians was

observed in our study, our study was conducted with emergency specialists. We believe that its generalizability to other specialists may be limited. Finally, the fact that our study was single-centered is another factor that will affect its generalizability. We recommend that our results be validated with multicenter studies involving other specialists.

In conclusion, according to the results of our current study, the clinical gestalt of emergency medicine specialists is not sufficient to predict short-term mortality in COVID-19 patients. We recommend that clinicians use scoring systems such as CURB-65 instead of gestalt when making clinical decisions regarding COVID-19 patients. Evaluation of different scoring systems in patients hospitalized for COVID-19 can be cited as one of the strengths of this study.

Conflict of interest

We declare no conflict of interest.

Funding

We received no financial support for the research, authorship, or publication of this article.

Acknowledgments

None to declare.

Authors' contributions

Concept: A. Ö., Design: A. Ö., Data Collection or Processing: A. Ö., Analysis or Interpretation: A. Ö, Literature Search: A. Ö, Writing: A. Ö.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 743-748
doi: 10.52142/omujecm.39.3.29

Effects of beta-1,3-D glucan on systemic bortezomib treated rat pancreas

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Received: 22.02.2022 • **Accepted/Published Online:** 05.06.2022 • **Final Version:** 30.08.2022

Abstract

Bortezomib, selective inhibitor of the 26S proteasome, is used for treatment of some types of cancer and immunosuppressive therapies. B-1,3-(D)-glucan, a synthetic antioxidant is used complementary medical treatment for human. This study was conducted to investigate the effects of the antioxidant Beta-1,3-D glucan on rat pancreas treated with systemic bortezomib. In the study, 36 Sprague-Dawley adult male rats were divided into four groups: control (C), bortezomib (BZ), β-1, 3-D-glucan (BD) and bortezomib + β-1,3- (D) –glucan (BZ+BD). Each group was divided into two subgroups (48 or 72 hours), depending on the time of scarification. After experiments, immunohistochemical, stereological and histopathological changes in all rat pancreatic tissues were examined. It was determined increased degenerative, vacuolated serous acini cells and inflammatory cell infiltrations in the groups of BZ and BZ+BG. In immunohistochemical analysis, densities of insulin positive cells were decreased in the groups of BZ and BZ+BG. Furthermore, in stereological mean volume of serous acinus analysis, significantly increases were detected in the groups of BZ and BZ+BG ($p<0.05$). BZ treatment had the detrimental effects on pancreas tissues. Also, administration of BG was insufficient to prevent injury induced by BZ treatment in the pancreas tissues.

Keywords: bortezomib, beta glucan, pancreas, insulin, oxidative stress

1. Introduction

Proteasomes are multi-catalytic enzyme complexes that reduce oxidized and misfolded proteins (1, 2). The ubiquitin-proteasome pathway produced by proteasomes is responsible for misfolding and degradation of intracellular proteins that control processes such as cell proliferation, cell cycle, transcription, and apoptosis. Many studies showed that the proteasome system prevented the post-translational changes and accumulation of misfolded/oxidized protein induced by oxidative stress (2). The uncontrolled and rapid proliferation of cancer cells is a major problem. Inhibiting the proteasome system provides an accelerating effect on cell death (3). Using proteasome inhibitors, such as bortezomib, is one of the methods to prevent the uncontrolled and rapid proliferation of cancer cells. Bortezomib (BZ), a selective inhibitor of the proteasome, is a drug is used for the treatment of cancer and immunosuppressive therapies (2,4). 26S Proteasome inhibition may inhibit pro-apoptotic degradation dynamics, allowing the initiation of apoptosis in neoplastic cells by suppressing pro-apoptotic pathways. Many studies showed the therapeutic effects of BZ in cancer therapies. Although there are many studies reported that this drug is effective only in the treatment of target cancer cells (5), recent studies reported that BZ could cause changes in the intracellular peptide levels that might cause cell damage through many basic metabolic processes in other tissues (2, 6) and contribute to the biological and/or side effects of the drug (7) like neuropathy and myelosuppression (8-10). In some studies, it was revealed that oxidative stress might be responsible for a chemotherapeutic drug such as bortezomib-induced non-target tissue damage (11).

Oxidative stress is a state meaning an inequality in the balance between free radicals production and antioxidants. Inhibition of proteasome disrupts protein homeostasis that causes overproducing reactive oxygen species (ROS) (12). High levels of free radicals lead to damage to cellular proteins, membrane lipids, and nucleic acids (3). Moreover, this current state decreases different metabolic processes and induces cell deaths (1-3). Research has shown that diseases such as diabetes, pancreatitis, hepatitis C infection, and chronic kidney diseases are associated with increased oxidative stress to all the bimolecular (13-15). The elimination of ROS by protective

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mechanisms is mentioned as antioxidants (16).

Antioxidants are substances that prevent damage caused by unstable molecules known as ROS (3). They inhibit cell injury via interacting with and stabilizing free radicals (17). In many chemotherapeutic treatments to reduce non-target tissue damages, antioxidants are used clinically, and it has known that these antioxidants also have anti-tumor effects on cancer cells (18). Although initial studies showed that antioxidant substances might promote health, recent clinical trials showed that some synthetic antioxidant substances do not offer adequate protection against oxidative damage and may cause an increase in tissue damage (19). One of the examples of these antioxidants includes beta-glucans (BGs) (20). BGs are antioxidant supplements that join in the processes of metabolism, repair, and detoxification. Also, it has anti-tumor activity (21) and has also been used clinically in Japanese and Chinese medicine. In addition to neutralizing the pathological effects caused by oxidative stress, some studies have shown that the curative effect of this substance is insufficient (20, 22).

The purpose of this study is to investigate the effects of BGs on systemic BZ-treated rat pancreas. In addition to our knowledge, this is the first study on the effects of BGs on BZ-treated pancreas tissue by immunohistochemical and stereological methods.

2. Materials and Methods

2.1. Animals

36 adult 12 weeks male Sprague-Dawley rats weighing between 230 g and 250 g were used in this study. The rats were housed in a light-controlled room with a constant temperature and fed standard laboratory water and chow. Ethics committee approval of this experiment was given by Aksaray University Institutional Animal Care and Use Committee. (2021/3-8). Chemicals Bortezomib (Velcade®) as a lyophilized powder (Velcade; Janssen-Cilag, Beerse, Belgium) dissolved in a solution of sterile saline at final concentration and β-1,3-D-glucan (SigmaAldrich (Steinheim, Germany) were used. All the other chemicals were purchased from Sigma-Aldrich (Germany) for laboratory experimentation.

2.2. Experimental procedure

For this study, the rats were divided into four groups: Control (sham), β-1,3-D-glucan, bortezomib, and bortezomib + β-1,3-D-glucan. Each group was divided into two subgroups according to the time they would be sacrificed (48 and 72 hours after drug administration) (n=6). The rats in the bortezomib and bortezomib + β-1,3-D-glucan group were injected only with 0.2 mg/kg bortezomib subcutaneously (sc) on the first day of the study, and no further treatment was applied to these rats in the following days. Rats in the bortezomib + β-1,3-D-glucan group were injected with bortezomib. Then, 75 mg/kg of beta-glucan was injected intraperitoneally until the end of the experiment. Rats in the β-1,3-D-glucan group were injected daily with 75 mg/kg β1,3-D-glucan intraperitoneally until the day they were sacrificed.

2.3. Histopathological procedures

Pancreas tissues were fixed in 10% neutral-buffered formalin solution for 72 h. The specimens were dehydrated in graded alcohol series, cleared with xylene, and embedded in paraffin wax. 4-µm thick histological sections were obtained using a Leica RM2125RT microtome (Leica Microsystems, Wetzlar, Germany). Sections were mounted onto glass slides. Samples were evaluated under a light microscope (Leica DM) after being stained with Hematoxylin & Eosin (H&E) and Mallory's triple modified by Crossman.

2.4. Immunohistochemical procedures

Pancreas tissues were examined by immunohistochemistry using the streptavidin-biotin peroxidase method (DAKO-Universal LSAB Kit-K0690) and 3,3'-diaminobenzidine tetrahydrochloride (DAB, SIGMA-D5905). Sections were held in xylene series and a decreasing series of ethanol. Then, they were incubated with 3 % H2O2 for blocking endogenous peroxidase activity and with normal bovine serum for blocking nonspecific binding sites of antibodies. Later primary antibody (anti-insulin IgG -dilution: 1/150-Polyclonal Guinea Pig Anti-Swine Insulin, DAKO-A0564) for beta cells and biotinylated secondary antibody (DAKO-Universal LSAB Kit-K0690) were respectively used for 30 min. Subsequently, specimens were incubated with streptavidin-Horseradish Peroxidase (DAKO Universal LSAB Kit-K0690), and binding sites of antibody were made visible with DAB and rinsed with PBS. Harry's hematoxylin was used to stain nuclei. After the samples were dehydrated with gradually increasing alcohol series, they were cleaned in xylene series and covered using entellan.

2.5. Semi-quantitative analysis for histopathological and immunohistochemical changes

Histological and immunohistochemical assessments and scores were done semi-quantitatively using light microscopy on sections from each animal. The 100 square micrometer area was determined by means of a micrometer slide (almost in 1 mm2) for the X 10magnification. The endocrine and exocrine parts of specimens were counted in 5 randomly selected microscopic areas at X 10 objective in each section, and the arithmetic mean was scored semi-quantitatively. Insulin-positive cells in immunohistochemical assessment, Intracytoplasmic vacuolization, PMNL infiltration, and degenerative cells in the histopathological assessment were scored. The scoring was reported as follows: none = –, mild = +, moderate = ++, severe = +++ (Table 2).

2.6. Stereological procedures and analysis

Sections were placed under the microscope, and serous acini were projected onto the monitor at 40X magnification via the camera. Each section was analyzed by systematic sampling.

The mean serous acinus volume was evaluated using the software Stereo Investigator (Microbrightfield) with the "nucleator method" described by Gundersen (23).

2.7. Statistical analysis

The statistical analysis was performed using SPSS (IBM SPSS

Statistics 18.0, IBM Corporation, Somers, NY, USA). Groups were compared using one-way analysis of variance (ANOVA) followed by an LSD test. (p-value less than 0.05 was considered significant ($p<0.05$). Values were also expressed as means ± standard deviation. [abc] the footnote letters in the same column indicate significant differences between groups.

3. Results

3.1. Histopathological examinations

Histopathological analysis of H&E and Mallory's triple modified by Crossman staining of pancreas tissues revealed that the control group's sections were seen as normal histologic structure. The experimental group's histologic analysis revealed that the quantity and distribution of the zymogen granules were altered when compared to the control group. BG group was similar to the control group than the other experimental groups except for a few polymorphonuclear leukocyte (PMNL) infiltrations and a few cellular degenerations, and the acinar cells showed either central distribution like the control group. BZ 48 h group sections showed a little vacuolization in acinar cells. Increased cellular degeneration and PMNL infiltration were more pronounced than the control and BG group in BZ 48 h group. BZ 72h group sections were near to BZ 48 h group except for vacuolization of the striated duct cells. In the sections of the group BZ 48h+BG, in addition to the other experimental groups, it was seen some capillaries were dilated and congested. BZ 72h+BG results showed the vacuolation and degeneration of the acinar cells were more intense than the other experimental groups. Furthermore, there was interstitial edema in this group. In all groups, collagen depositions were nearly similar. (Fig. 1) (Table 1).

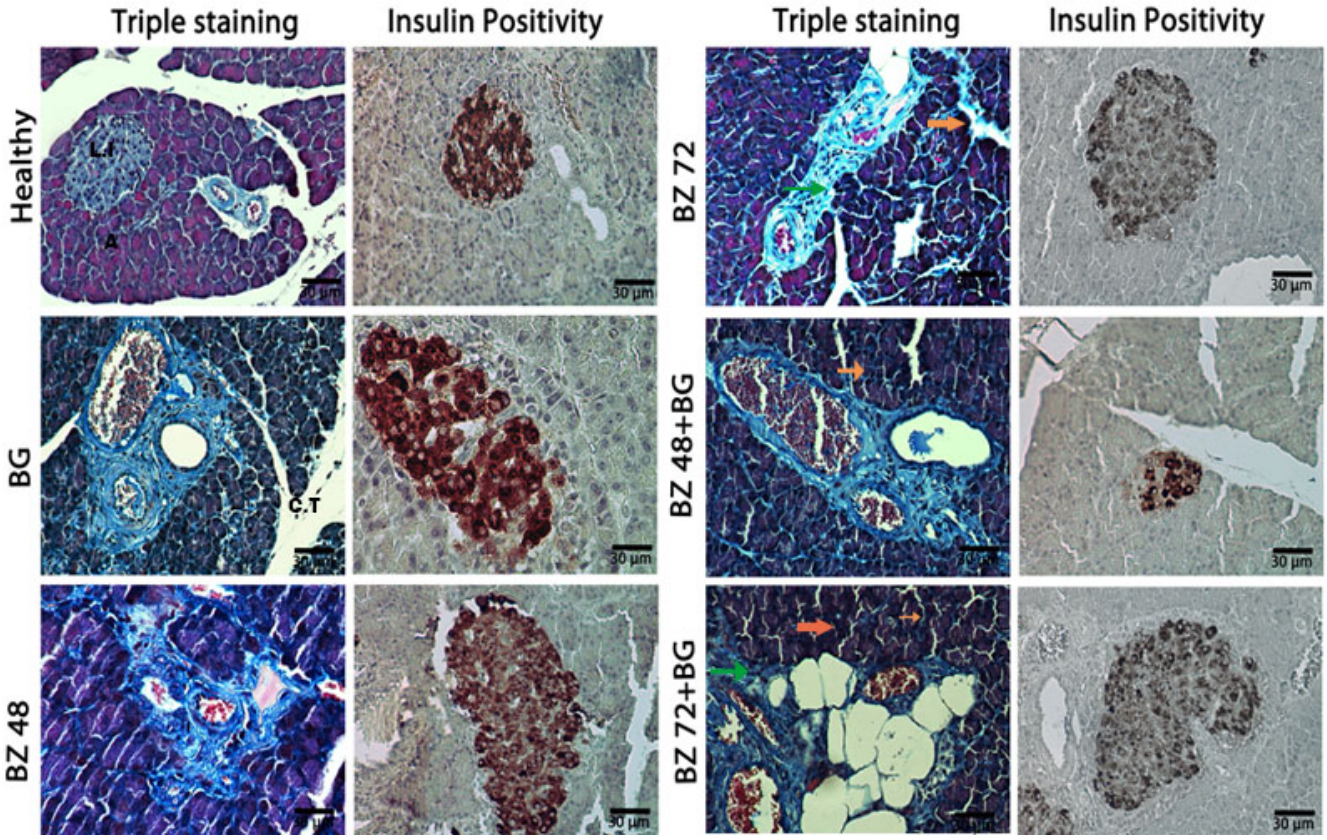

Fig. 1. Serous acinus (A), connective tissue (C.T), Langerhans islands (L.I). Degenerative acinar cells (thick orange arrow), vacuolization (thin orange arrow), interstitial edema (green arrow)

Table 1. The assessments of the mean serous acinus area of all groups. [abc] the footnote letters in the same column indicate significant differences between groups

Groups	Mean serous acinus area	Mean amylase level
C group	1001.66±110.06[a]	1730±153.84
BG group	913.77±68.66[a]	1901±410.54
BZ 48h group	1015.54±140.12[b]	2058±389.97
BZ 72h group	1100.46±72.48[b]	2139±83.18
BZ 48h+BG group	1025.82±62.73[c]	1487±121.19
BZ 72+BG group	1130.64±97.36[c]	1274±22.80

3.2. Immunohistochemical examination

Immunohistochemical analysis of positive beta cells revealed that BG groups results were close to the control group. Insulin (+) results of cells in BZ 48h and BZ 48h+BG groups were found to be decreased compared to control and BG groups. BZ 72h and BZ 72h+BG groups have the least insulin (+) cells compared to other groups (Fig. 1) (Table 1).

3.3. Stereological examinations

Stereological examinations showed that BG group results were nearly similar to the control group. BZ 48h and BZ 48h+BG groups have increased mean serious acinus area and significant differences when compared to control and BG groups. BZ 72h and BZ 72h+ BG group mean serous acinus area results were

higher than BZ 48h and BZ 48h+ BG groups and have significant differences from the other groups. (Table 2).

Table 2. Semi-quantitative analysis of histopathological and Immunohistochemical assessments

Groups	Insulin	Intracytoplasmic vacuolization	PMNL infiltration	Degenerative cells
C	+++	-	-	-
BG	+++	-	+	+
BZ 48h	++	+	+	++
BZ 72h	+	++	++	++
BZ 48h+BG	++	++	++	++
BZ 72h+BG	+	+++	+++	+++

4. Discussion

The ubiquitin-proteasome pathway is responsible and very important for the eukaryotic intracellular protein turnover. This function is crucial to many cellular processes, comprising cell growth, activation, differentiation, and signaling. The proteasome inhibitors are used for inhibition of these pathways in cancer therapies (24). Although they are effective in preventing cancer cell proliferation, they can be harmful to some healthy tissues via mediated oxidative stress (2, 6, 12). The antioxidants have protective effects on damaged tissues mediated oxidative stress, and the positive effects of glucans have been reported to modulation of immune function and antioxidant effects (22, 25). Contrary to this information, insufficient or adverse effects of BGs have been reported recently (20, 22, 26) Tsiapali et al. (26) observed an increase in free radical levels in BG treated groups and reported the modulatory effects of BGs could not be sufficient in inflammatory components comprising tissue culture and/or disease models. In this study, the effects of BZ, which is a selective inhibitor of 26S proteasome, and B-1,3-(D)-glucan (BG), a synthetic antioxidant, on rat pancreatic tissues were examined according to dose and time.

In the immunohistochemical analysis, immune-positive beta cells densities were decreased in the BZ and especially in the BZ+BG groups. Several studies (27, 28) reported that proteasome inhibition by bortezomib might cause hyperglycemia. Tsiapali et al. (26) determined an increase in free radical levels in BG treated groups, and many studies (29-31) showed that oxidative stress played a major role in the pathogenesis of type 1 and 2 diabetes. In our study BG alone showed no effect on insulin levels. On the contrary, the lower insulin levels of BZ+BG groups can be attributed to the effects of BZ.

According to histopathological evaluation, the BG group was similar to the control group. Degenerative changes, intracytoplasmic vacuolizations, and PMNL infiltrations were observed in the acini and duct cells of the BZ-treated groups. It was determined that BG treatments did not reduce the pathological changes in pancreatic tissue after BZ application.

In recent years in some case reports, it was reported BZ induced acute pancreatitis (32, 33), and BZ treatment can cause morphological alterations like intracytoplasmic vacuolization (34-36) and degenerations (37) on the cellular level, and in some researches (38) it was suggested that there could be an increase in ROS levels in BG treated groups and combined BG therapies with some drugs can cause activation of immune responses and inflammation.

In stereological examinations, the mean acinus volume of BG group sections was near to the control group. But there were increases in acinus volumes of BZ groups and more in BZ + BG groups. It has known BZ may cause hyperglycemia. The pancreas exocrine and parotid gland are only serous glands. Parlak et al. (39) detected that diabetes caused vacuoluzations in the parotid gland. Tsiapali et al. (26) determined in BG treated groups an increase in free radical levels.

ROS stimulated various hypertrophic transcription factors, myocardial growth-related signals, cellular dysfunction and matrix remodeling (40). In our study, it was suggested that BG application may be protective against degenerations and hypertrophied acinus cells. This inference is consistent with our result of decreased amylase levels. In addition, it was thought that increased amylase levels in BZ groups might be related to changes in molecular level, not morphological level.

In conclusion, the study results revealed that treatment with BZ causes pancreatic damage, and administration of BG have insufficient effects against BZ-related degenerations. The related molecular mechanism of BZ toxicity on pancreas tissue requested further investigation.

Conflict of interest

None to declare.

Funding

None to declare

Acknowledgments

None to declare.

Authors' contributions

Concept: N. A., SNP., Design: N. A., SNP., Data Collection and Processing: N. A., SNP., Analysis and Interpretation: N. A., SNP., Literature Search: N. A., SNP., Writing: N. A., SNP.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 749-754
doi: 10.52142/omujecm.39.3.30

Malignancy rates and risk factors in Bethesda category IV thyroid nodules: Is lobectomy enough in an endemic region?

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Received: 23.02.2022 • **Accepted/Published Online:** 05.06.2022 • **Final Version:** 30.08.2022

Abstract

In this paper, we determined the rates of Bethesda IV thyroid nodules and calculated the malignancy rates of these nodules in a university hospital located in an endemic area for thyroid diseases. We aimed to define the predictive factors for malignancy to select patients who need surgery. We included in the study 221 patients who had a preoperative biopsy for follicular neoplasm or because of suspicion for follicular neoplasm and underwent thyroidectomy between January 2012 and December 2018. We evaluated the data about patient characteristics, preoperative ultrasound results, indications for operation and postoperative pathological valuation results and calculated ratio of Bethesda Category IV nodules and incidental malignancy rates. The malignancy rate of Bethesda Category IV nodules was 48.9 %, while the incidental malignancy rate was 30.7 %. There was no statistical difference between patients with benign and malignant pathology results in terms of gender, age, preoperative diagnosis, size of the index nodule, number and results of biopsies and the thyroidectomy performed. The most important risk factor among all parameters was hypoechogenicity of the nodule. The solid structure increases this risk. Ultrasonographic hypoechogenicity is the most important risk factor for preoperative malignancy risk assessment for Bethesda Category IV thyroid nodules. Centers should determine their malignancy rates with particular risk factors and surgical approaches in endemic regions.

Keywords: thyroidectomy, follicular neoplasm, thyroid nodules, Bethesda Category

1. Introduction

Thyroid nodules are a common clinical problem, especially in endemic areas, and constitute a significant risk for thyroid cancer. Although some authors think there is more to it than that, the incidence of thyroid nodules increases in parallel with the improvement of imaging modalities and increased follow-up frequency (1, 2). As the rate of detected thyroid nodules increases, hesitations on the clinical approach to these nodules also increase. This situation augments the importance of understanding the malignancy risk of detected nodules in the preoperative period.

The primary method used to diagnose and treat thyroid nodules is fine needle aspiration biopsy (FNABx), and it is the standard of care for evaluation (3). Although FNABx can help distinguish malignant and benign nodules, indeterminate results create clinical dilemmas, and about 30% of thyroid nodules cannot be diagnosed clearly by pathologists for various reasons (4). To standardize pathological interpretations, the National Cancer Institute introduced a system named Bethesda System for Reporting Thyroid Cytopathology (Bethesda) in 2009 (5). According to this system, thyroid FNABx results are classified into six diagnostic categories (Bethesda I-VI). Bethesda category IV is defined as follicular neoplasm or suspicion for follicular neoplasm lesions, and 22-42% of thyroid nodules are diagnosed as Bethesda category IV by FNABx (6). This category is one of the most controversial groups in the system, and the malignancy rate of Bethesda category IV is found in a wide range of 10-40% (7). The recommended treatment for Bethesda Category IV thyroid nodules is surgical excision with hemithyroidectomy (or lobectomy) or risk assessment with molecular testing before surgery. Furthermore, the American Thyroid Association (ATA) guidelines recommend surgery for Bethesda Category IV thyroid nodules (3). However, when a large number of these nodules (60-90%) are observed to be benign after surgical excision, this group of patients are considered to be at risk of unnecessary surgical morbidity. Even if the final pathology is malignant, a second surgery may be required for the opposite side. This controversial situation and uncertainty lead to the investigation of the preoperative evaluation criteria that can be used to determine the malignancy risk of these nodules or the

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medical centers own risk rates, thus enabling them to give their patients treatment under the light of these ratios (8-10).

In this study, we determined the rates of Bethesda IV thyroid nodules and calculated the malignancy rates of these nodules in a university hospital located in an endemic area for thyroid diseases. Initially, we aimed to define the predictive factors for malignancy to select patients who need surgery. Afterwards, we tried to establish a treatment approach for Bethesda Category IV thyroid nodules considering these predictive factors and incidental malignancy rates in endemic areas for thyroid diseases such as our own region.

2. Materials and Methods

Due to various indications, 1583 thyroid surgeries were performed between January 2012 and December 2018 in our institution, Rize, Turkey. We obtained Institutional Review Board approval (2021/42). We then examined the records of these patients retrospectively. We included in the study 221 patients who had preoperative FNABx for follicular neoplasm or because of suspicion for follicular neoplasm (Bethesda Category IV) and underwent thyroidectomy. These patients constituted our study group.

We obtained the data about patient characteristics, preoperative ultrasound (USG) results (including nodule size, margin status, structure, echogenicity, vascularization and microcalcification), indications for operation and postoperative pathological evaluation results from the center's digital patient records of the center. Since the region is endemic for thyroid diseases, the approach to thyroid nodules is carried out under a certain standard. Experienced radiology specialists performed all preoperative fine needle aspiration biopsies under ultrasound guidance, and a single pathologist also specialized in thyroid diseases interpreted all preoperative biopsies and postoperative pathology results.

After FNABx, we called the nodule reported as Bethesda Category IV as the index nodule. We excluded the patients who underwent biopsies for diseases other than index nodules. If the index nodule was reported malign at the final pathology report, we included it in actual malignancy rates, but in case of a malignancy other than the index nodule, we called it incidental cancer and calculated it separately.

We used the Statistical Package for Social Sciences (SPSS) version 20.0 (SPSS Inc.; Chicago, IL, USA) for statistical analysis and the Kolmogorov-Smirnov test to classify variables according to normal distribution. We then evaluated demographic, preoperative and postoperative data and pathological features after surgery through the parametric and non-parametric Mann-Whitney U test, t-test, chi-square and frequency analyses. We conducted a logistic regression analysis to correlate thyroid malignancy and USG findings in terms of risk factors and defined statistical significance as $p<0.05$.

3. Results

A total of 1583 patients underwent thyroid surgery during the study period. 13.9% (221/1583) of these patients had a preoperative Bethesda Category IV FNABx result and constituted the study group. The majority of patients were female (177/80.7% vs 44/19.9% female and male ratios, respectively), and the mean age was 50.98±11.7 (15-81) for the whole group. As well as fine needle biopsy results, the most common cause of surgical indications was multinodular goiter (83.7%), but thyroiditis, Graves' disease, toxic multinodular goiter, recurrence of multinodular goiter, solitary nodule and nodule size alone also created additional indications. The mean size of the radiologist biopsied index nodules was 20.9±12.5 mm (6-80). Most of the patients underwent total thyroidectomy (84.6%). Others underwent hemithyroidectomy (9.5%) or complementary thyroidectomy (5.9%). We determined the type of operation per the surgeon and patient's decision depending on the patient's laboratory, biopsy and imaging results.

Among the 221 patients who underwent biopsy, one hundred and eight of Bethesda IV nodules had malignancy associated with after the surgery. Thus, our study group's actual malignancy rate of Bethesda Category IV nodules was 48.9%. The remaining 113 patients had benign histopathology in Bethesda IV nodules in the final pathology, but in 68 of these patients, we detected malignancy incidentally in a different thyroid gland area. So, the incidental malignancy rate was 30.7%, while the total malignancy rate was as high as 79.6%. Table 1 represents the malignancy rates and other characteristics of index nodules. As evident, there was no statistical difference between patients who had benign or malignant pathology results in terms of gender, age, preoperative diagnosis, size of the index nodule, number of preoperative FNABx, result of FNABX and the operation performed.

We also compared preoperative ultrasound results to detect a predictive factor for malignancy in the preoperative period. Table 2 and Table 3 represent ultrasonographic risk factors differences between benign and malignancy associated with Bethesda IV nodules with odds ratios and p values. We evaluated vascularization, margins, structure, echogenicity and microcalcification. Due to the structure of a retrospective analysis, as shown in Table 2, it became clear that vascularization, margin and microcalcification data were not wholly reported in each ultrasound report. We did not evaluate these factors statistically due to the high number of unspecified data while evaluating only the structure and echogenicity for index nodules. Univariate analysis and multivariate logistic regression analysis demonstrated that the most important risk factor among these two parameters was echogenicity ($p= 0.041$). Index nodules with hypoechogenicity tended to be malign. The solid structure also increased the risk of malignancy for nodules ($p=0.004$).

Table 1. Patient characteristics, preoperative diagnosis, number of FNABx and results and operations performed for Bethesda Category IV index nodules

	Benign	Malign	P values
Gender			
-Female	91 (41.1%) [56 incidental malign]	86 (38.9%)	0.867
-Male	22 (9.9%) [12 incidental malign]	22 (9.9%)	
-Total	113 (51.1%)	108 (48.9%)	
Age (years)			
	51.11±11.06	50.84±12.54	0.934
Preoperative Diagnosis			
- MNG*	92 (41.6%)	93 (42%)	0.395
- MNG, thyroiditis	2 (0.9%)	4 (1.8%)	
- Recurrent MNG	12 (5.4%)	2 (0.9%)	
- MNG with subclinical hyperthyroidism	1 (0.4%)	2 (0.9%)	
- Toxic MNG	3 (1.3%)	1 (0.4%)	
- MNG, Graves' Disease	1 (0.4%)	1 (0.4%)	
- Solitary nodule	2 (0.9%)	5 (2.2%)	
Size of the index nodule (mm)			
	20.92±11.25	20.90±13.81	0.370
Number of FNABx performed			
- 1	73 (33%)	74 (33.4%)	0.550
- 2	38 (17.1%)	32 (14.4%)	
- 3	2 (0.9%)	2 (0.9%)	
FNABx result			
- SFN**	57 (25.7%)	53 (23.9%)	0.371
- SFN oncocytic type	26 (11.7%)	16 (7.2%)	
- FN***	23 (10.4%)	27 (12.2%)	
- FN oncocytic type	7 (3.1%)	12 (5.4%)	
Operation			
- Total thyroidectomy	91 (41.1%)	96 (43.4%)	0.063
- Lobectomy	11 (4.9%)	10 (4.5%)	
- Complementary thyroidectomy	11 (4.9%)	2 (0.9%)	

*MNG: Multinodular goiter, **SFN: Suspicious for a follicular neoplasm, ***FN: Follicular neoplasm

Table 2. Frequencies of ultrasonographic risk factors among benign and malign index nodules

	Benign Index Nodule (n=113)	Malign Index Nodule (n=108)	P values
Vascularization			
-Not increased	6	6	0.912
-Increased	41	42	
-Not specified	66	60	
Margin			
-Irregular	25	33	0.249
-Regular	11	13	
-Not specified	77	62	
Structure			
-Cystic	3	1	0.124
-Solid	62	73	
-Mixed	48	34	
Echogenicity			
-Isoechoic	14	13	0.058
-Hyperechoic	6	1	
-Mixed	58	45	
-Hypoechoic	35	49	
Microcalcification			
-Absent	7	4	0.669
-Present	53	50	
-Not specified	53	54	

Table 3. Statistical differences between ultrasonographic risk factors

Risk Factor	Odds ratio (%95 confidence interval)	P Values
Echogenicity		
- Iso/hyper/mixed	1	**0.041**
- Hypo	1.64 (0.44-6.15)	
Structure		
- Cystic/mixed	1	**0.004**
- Solid	8.4 (3.5-69.8)	

Table 4 represents the pathological differences for incidental malign nodules and index malign nodules. The most common malignancy in index nodules was oncocytic type papillary carcinoma, while oncocytic type papillary microcarcinoma for incidental nodules. The risk of follicular carcinoma was very low (0.9%). Patients with malignant index nodules were more likely to have poor prognostic markers after surgery than patients with incidental lesions.

Table 4. Differences between pathological results among incidental and index malign nodules

	Incidental malign nodule	Malignancy in Bethesda IV nodules	P
Final pathology results			
- Papillary carcinoma	-	9 (8.3%)	**<0.001**
- Papillary microcarcinoma	9 (13.2%)	4 (3.6%)	
- Follicular carcinoma	-	1 (0.9%)	
- Medullary carcinoma	-	-	
- Medullary microcarcinoma	3 (4.5%)	-	
- Oncocytic type papillary carcinoma	1 (1.5%)	61 (56.7%)	
- Oncocytic type papillary microcarcinoma	55 (80.8%)	32 (29.6%)	
- Papillary+Medullarycarcinoma	-	1 (0.9%)	
Tumor size (mm)			
	3.70 ±3.11	18.13±12.95	**<0.001**
Lymph node metastasis			
- No	27 (39.7%)	33 (30.6 %)	**0.380**
- Yes	-	4 (3.7 %)	
- Not specified	41 (60.3%)	71 (65.7 %)	
Extra capsular invasion			
- No	35 (51.5%)	13 (12%)	**<0.001**
- Yes	33 (48.5%)	95 (88%)	
Parenchymal invasion			
- No	44 (64.7%)	86 (79.6%)	**0.021**
- Yes	24 (35.3%)	22 (20.4%)	
Extra thyroidal spread			
- No	68 (100%)	102 (94.4%)	**0.071**
- Yes	-	6 (5.6%)	
Positive surgical margin			
- No	68 (100%)	97 (89.8%)	**0.010**
- Yes	-	11 (10.2%)	
Multifocality			
- No	34 (50%)	41 (37.9%)	**0.129**
- Yes	34 (50%)	67 (62.1%)	
Lymphovascular invasion			
- No	67 (98.5%)	105 (97.3%)	**0.560**
- Yes	1 (1.5%)	3 (2.7%)	

4. Discussion

In this paper, we attempted to determine the malignancy and incidental malignancy rates of patients who were operated after Bethesda Category IV thyroid nodule biopsy diagnosis and investigated the factors that could predict the risk of malignancy. We found that the cancer rates in Bethesda Category IV nodules were high in an endemic region and the risk of incidental cancer was also high. We also demonstrated the relationship of some ultrasonographic risk factors with these rates.

As Acar et al. suggested in their paper, the Bethesda system allows for more accurate predictions for suspicious and malignant lesions (11). Although this is true, the rates of malignancy of indeterminate lesions vary, and the factors that can predict the risks of malignancy of indeterminate nodules to avoid unnecessary thyroidectomies are being investigated (12, 13). This effort is also valid for patients suspected of a follicular neoplasm or follicular neoplasm, which is reflected as Bethesda Category IV in the classification system. Malignancy rates of Bethesda category IV are found in a wide range of 10-40%. This suggests that predictive factors may prevent high unnecessary thyroidectomy rates. The results of studies on predictive factors are also controversial. Lee KH et al. suggested that tumor size (>2.5cm), malignant ultrasonography diagnoses are predictive factors (14), while Lee SH et al. revealed that only high thyroglobulin levels and the presence of calcification on ultrasonography are significant predictive factors (10). Najafian A et al. added that male sex, family history of thyroid cancer and history of head and neck radiation were associated with malignancy for follicular neoplasms of the thyroid (15). We believe that all of this confusion was due to the heterogeneity of the study groups. The most compact and recent study on this subject belongs to Kuru B and Kefeli M (16). They suggested a predictive risk index and recommended surgery for patients with one or more predictive risk factors. We obtained similar results in our study; nodules with hypoechogenicity tended to be malign, and this result was significant. The nodule being also solid increased the risk of malignancy. We presented vascularity, margin and microcalcification status as frequency only due to the excess of unspecified data. We could not detect a relationship between tumor size and malignancy for Bethesda Category IV nodules.

The appropriate surgical approach for Bethesda Category IV thyroid nodules is not clear. Some centers offer total thyroidectomy to appropriate patients by taking into account the risk factors, while others recommend hemithyroidectomy (or lobectomy) to reduce surgical morbidity, especially nerve damage (17, 18). However, this leads to the risk of a second

operation in malignant patients. In this regard, we recommend that centers manage the process by considering the risk factors and determining their regional malignancy rates. In this study, the malignancy rate of index nodules was 48.9%, which constituted nearly half of the patients. This incidence increased to 79.6% by adding incidental malignant nodules, and this rate is relatively high. There may be two reasons for this result. Firstly, suspicious nodules other than index nodules were not evaluated. However, the size of the incidental cancers varied between 1-6 mm, which made radiologic evaluation difficult. Secondly, the region is endemic to thyroid diseases, and total thyroidectomy is recommended to most patients as a surgical reflex for malignancy risk, increasing the incidental malignancy ratios. However, it should be kept in mind that most of the incidental malignancies are microcarcinoma and their clinical significance is still uncertain even if half of them are multifocal, as seen in Table 4. There is still a question that needs to be answered: Does each pathology result as incidental microcarcinomas evaluated retrospectively suggest an indication for the extent of surgery?

Although there are studies showing the rates of incidental malignancy, we could not find a study comparing the incidence and index nodules (19, 20). In our study, the rate of incidental malignancy was found to be high, and a significant difference was found between the incidental malignant nodule and the malignant Bethesda IV nodule in terms of tumor diameter and capsule invasion (Table 4). It should be kept in mind that the high rate of incidental malignancy may have a survival effect and new regulations may be needed in the preoperative evaluation process. There are also limitations to this study. The most common malignancy among index nodules was papillary thyroid carcinoma. In 2016, the definition of noninvasive follicular variant papillary thyroid cancer was changed into follicular thyroid neoplasm with papillary-like nuclear features (NIFTP), which is not cancer (21). Even if we consider that the effect will be low, this will undoubtedly decrease malignancy rates. Furthermore, we did not evaluate thyroid function tests, thyroglobulin or thyroglobulin antibody levels or molecular pathological examinations. We believe that these may be important in preoperative risk assessment. Another limitation was that all five factors determined for ultrasonographic risk were not evaluated wholly on ultrasonography and the excess of lost data in each report. We conducted statistical studies with the available data only.

Our results revealed that ultrasonographic hypoechogenicity and solidity of the nodules were significant risk factors for preoperative malignancy risk assessment of Bethesda Category IV thyroid nodules. Surgeons should be careful in this respect and inform patients about the risk of malignancy and decide the extent of surgery. Tumor size did not affect malignancy in our study. The high incidence of malignancy and the high incidental malignancy rates of these patients in endemic regions may alter the surgical approach for Bethesda Category IV nodules. For this reason, we recommend that centers should determine their malignancy rates with particular risk factors and determine their surgical approaches accordingly.

Conflict of interest

We have no financial and personal relationships with persons or organizations that could have inappropriately influenced our work.

Funding

No funding was used for the study.

Acknowledgments

The authors thank Bekir Kuru from the Ondokuz Mayıs University in Samsun for advice and comments.

Authors' contributions

Concept: A.Ö., M.U., S.K., Design: A.Ö., M.U., Data Collection or Processing: S.K., M.K.C., Analysis or Interpretation: M.U., S.K., A.P., Literature Search: A.Ö., M.K.C., A.P., Writing: A.Ö., M.U.

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Journal of Experimental and Clinical Medicine
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Research Article

J Exp Clin Med
2022; 39(3): 755-760
doi: 10.52142/omujecm.39.3.31

Evaluation of factors affecting the development of re-operation due to hemorrhage after lung resection

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Received: 28.02.2022 • **Accepted/Published Online:** 24.06.2022 • **Final Version:** 30.08.2022

Abstract

In our study, preoperative factors affecting the development of re-operation after lung resection were examined.413 patients who underwent lung resection between 2018-2020 were included in our study. The preoperative data of 25 (6.1%) patients who underwent re-operation and 388 (93.9%) patients who did not undergo re-operation were compared. Preoperative hemoglobin level (p=0.009), neoadjuvant therapy (p<0.001), pneumonectomy (p<0.001), thoracotomy (p=0.005), amount of intraoperative blood loss (p<0.001), need for intraoperative blood product use (p). =0.005), intraoperative mean arterial pressure (p=0.01), pulse rate (p=0.001), postoperative hemoglobulin amount (p<0.001) were found to affect and increase the probability of re-operation. It was statistically significant that the need for postoperative blood product usage was higher (p<0.001), postoperative complications (100% vs. 22.8%, p<0.001) and mortality (0.6% vs. 4%, p=0.01) in reoperated patients. It was found that the hospitalization day in the ICU was longer in those who underwent reoperation than in those who did not (3.2 days vs. 1.5, p<0.001). Independent risk factors affecting re-operation according to multiple logistic regression analysis; neoadjuvant treatment (p<0.001), operation time (p=0.04), intraoperative pulse rate (p=0.01) and postoperative hemoglobin (p<0.001) were found. Low preoperative hemoglobin level, on the other hand, independently affected the development of re-operation at a level close to significance (p=0.06). Re-operation due to bleeding after lung resection increases the rate of cardiopulmonary complications in the postoperative period. Careful follow-up and approach of surgery and anesthesia in the intraoperative period will contribute to the decrease in the incidence of re-operation.

Keywords: lung resection, re-operation, hemorrhage, lung cancer

1. Introduction

After lung resection, complications such as bleeding, bronchopleural fistula, persistent air leak, cardiac herniation, pulmonary torsion, empyema and lung ischemia may occur. 2-3% of these complications are bleeding (1-3). Although bleeding is a rare complication, it may cause hemodynamic instability and the need for aggressive treatment after surgery (4). The possibility of mortality and morbidity increases after re-operation.

While human error and technical problems come to the fore in surgical factors that cause hemorrhages, hemorrhage foci are the most common foci of the pulmonary artery, bronchial artery, intercostal artery and veins (4). In many studies, the bleeding focus cannot be determined exactly in the development of hemorrhage (1). Uramato and Litle have shown that factors such as the presence of lung cancer and the use of neoadjuvant and antiplatelet drugs were effective (4,5).

Our aim in this study was to evaluate the factors causing bleeding in patients who were re-operated for lung cancer.

2. Material and Methods

This study evaluated the demographic data, preoperative diagnosis, surgical procedure, intraoperative bleeding, mortality and morbidity of 25 (%6.1) patients who underwent re-operation due to hemorrhage among 413 lung resection patients operated between 2018-2021. No postoperative surgery was performed in 388 (93.9%) patients. The patients were evaluated in two groups as reoperated and non-reoperated. During this period, 324 (78.5%) thoracotomy and 89 (21.5%) VATS patients who were operated consecutively were included in the study. Lung cancer was diagnosed in 300 (72.6%) patients and benign lung mass was diagnosed in 113 (27.4) patients. The Ethics Committee of the Hospital approved this study. Confirmed number/date 2022-195/10.02.2022. The study was conducted in accordance with the Helsinki declaration principles. All patients signed a written consent form.

We recorded the preoperative data of the patients and used a standard general anesthesia protocol for all patients. We preoperatively administered antibiotic prophylaxis and used

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propofol 2-3 mg/kg and fentanyl 2 µg/kg fentanyl for induction in all surgical procedures. We used 0.5 mg/kg intravenous (i.v.) rocuronium bromide as a muscle relaxant. We placed a double-lumen endobronchial tube on the right or left as appropriate, confirmed its position by fiberoptic bronchoscopy (FBS) and maintained anesthesia with 50% oxygen, 50% air, and 2% sevoflurane. We continued remifentanil iv infusion throughout the operation. We determined the surgical procedure according to current guidelines for pneumonectomy and lobectomy and performed resections via thoracotomy or video-assisted thoracoscopic surgery (VATS).

We followed the bleeding status with preoperative arterial blood gas and hemogram tests and recorded the amount of blood in the aspirator, sponge and compresses at periodic intervals. We performed intraoperative 8 g/dl hemoglobin or 24% hematocrit. We administered no other blood product (aprotinin, factor VII or others) other than ES and fresh frozen plasma (FFP). After the operation, we transferred the patients who underwent pneumonectomy and lobectomy to the surgical intensive care unit. After one day of standard follow-up, we transported the patients who did not have hemodynamic instability to the recovery room. We arranged the management of patients using anticoagulant therapy before surgery; we discontinued antiplatelets such as warfarin three days and acetylsalicylic acid and clopidogrel seven days before the procedure. For patients undergoing coronary procedures (e.g., angioplasty, stenting), we contacted their cardiologist before discontinuing antiplatelet therapy and planned their treatment according to the nature of the planned surgery (elective or emergency). We initiated enoxaparin sodium routinely at a dose of 30 to 40 mg/day subcutaneously for prophylaxis before the scheduled surgery, unless the procedure was brief or the patient was young. We started enoxaparin sodium regardless of the patient's age if lung resection was planned. Intensive care specialists, intensive care nurses, and thoracic surgery assistants routinely monitored drainage and hemodynamic parameters. We included in the revision patients who continued to drain 100 mL for 8 hours or 200 mL for 2-4 hours and did not respond to medical treatment. We decided on one of the available treatment options according to the color, density, amount, laboratory values of the postoperative drainage, and the patient's hemodynamic status. Those options were primarily medical therapy, re-operation, hemorrhage-stopping therapy, blood product replacement and fluid therapy. We decided to operate considering the patient's hemodynamic data, x-ray, and the amount of hemorrhagic drainage. Considering the patient's bleeding status and hemodynamic stability, open thoracotomy was often preferred.

We performed surgeries 4-6 weeks after neoadjuvant therapy, as neoadjuvant chemotherapy and radiotherapy would increase postoperative hemorrhage.

Pulmonary complications were collected under pneumonia, acute respiratory failure, pulmonary embolism, prolonged air leak and need for mechanical ventilation. Cardiac complications were collected under the headings of arrhythmias, hemodynamic instability, acute coronary disease, angina, heart failure (HF) and thromboembolism.

2.1. Statistical analysis

We entered patient demographics and collected data into IBM® SPSS® (the Statistical Package for the Social Sciences) Statistics version 23 and characterized variables using mean, maximum, and minimum values while using percentage values for qualitative variables. We determined whether the distribution was normal or not by the Kolmogorov-Smirnov test and reported normal distributions as mean±SD. We used the student's t-test to compare groups, the Pearson chi-square test to analyze qualitative variables, and the Fisher's exact test if the group was small. We recorded non-parametric continuous variables as median and interval distribution and compared them using the Mann-Whitney U test. We considered a value of $p<0.05$ statistically significant and conducted multiple logistic regression analyses to determine the independent risk factors affecting re-operation. The multiple logistic regression analysis determined independent risk factors using only the variables that significantly increased the probability of re-operation in the univariate analysis, $p<0.05$.

3. Results

With a mean age of 57.7 years (min=17, max=94 years), 76.8% of the patients were male, and 23.2% were female. Table 1 shows the patients' demographic, preoperative clinical and postoperative conditions. 155 (37.5%) of the patients had hypertension (HT), 82 (19.9%) diabetes mellitus (DM), 19 (4.6%) congestive heart failure (CHF), 80 (19.4%) chronic obstructive pulmonary disease (COPD), 79 (19.1%) coronary artery disease (CAD), 5 (1.2%) chronic kidney failure (CRF), 24 (5.8%) arrhythmias, 22 (5.3%) thyroid disease, and 8 (1.9%) cerebrovascular disease (CVO). Furthermore, 234 (56.7%), 113 (27.4%) and 27 (6.5%) had a history of smoking, anticoagulation and neoadjuvant therapy, respectively. No statistically significant correlation was present between the preoperative chronic diseases of the patients and the re-operation.

We used at least one blood product in 24.5% (n=101) and postoperatively in 16.9% (n=70) of the patients. While the number of patients who used at least 1 unit (U) of erythrocytes intraoperatively was 87 (21.1%), 59 (14.3%) used at least 1 U of erythrocytes postoperatively. 185 (20.6%) used at least 1 U fresh frozen plasma (FFP) intraoperatively, while the number of patients who used at least 1 U FFP postoperatively was 43 (10.4%). We used an average of 1.4 U ES and 1.0 U ES in those who underwent Thoracotomy and VATS, respectively. We used an average of 1.7 U ES in pneumonectomies and 1.2 U ES in lobectomies patients in the

intraoperative period and applied medical treatment to 100 (24.2%) postoperatively, and it was successful in 75% of these patients.

Table 1. Demographic, preoperative clinical and postoperative status of the patients

Variable	Data
Age, Year±SD	57.7±13.1
Gender, n (%)	
Male	317 (76.8%)
Female	96 (23.2%)
Diagnosis, n (%)	
Lung cancer	300 (72.6%)
Lung mass	113 (27.4%)
Disease Localization, n (%)	
Right	239 (57.9%)
Left	174 (42.1%)
Preoperative hemoglobin, mg/dl ±SD	13.1±1.6
Anjio Bypass, n (%)	40 (9.7%)
Anticoagulant, n (%)	113 (27.4%)
Neoadjuvan, n (%)	27 (6.5%)
Resection type, n (%)	
Lobectomy	297 (71.9%)
Pneumonectomy	73 (17.7%)
Wedge Rzk	43 (10.4%)
Operation type, n (%)	
Thoracotomy	324 (78.5%)
VATS	89 (21.5%)
Operation time, hour±SD	3.9±0.7
Intraoperative blood loss, ml±SD	367.6±332.9
Intraoperative blood product use, n (%)	101 (24.5%)
Intraoperative MAP, mmHg±SD	66.1±11.7
Intraoperative heart rate, n/dk±SD	67.4±11.3
Postoperative blood product use, n (%)	70 (16.9%)
Postoperative hemoglobin, mg/dl ±SD	11.6±1.7
Re-Operation, n (%)	25 (6.1%)
Postoperative complication, n (%)	116 (28.1%)
Mortality, n (%)	5 (1.2%)
ICU length of stay, days±SD	1.8±9.8

ICU: intensive care unit.MAP: mean arterial pressure

25 (6.1%) of the postoperative patients required re-operation. Preoperative hemoglobin level (p=0.009), neoadjuvant therapy (p<0.001), pneumonectomy (p<0.001), thoracotomy (p=0.005), intraoperative blood loss amount (p<0.001), intraoperative blood product use (p=0.005), intraoperative mean arterial pressure (p=0.01), pulse rate (p=0.001) and postoperative hemoglobin amount (p<0.001) increased the reoperation rate. (Table 2). It was statistically significant that the need for postoperative blood product usage (p<0.001), postoperative complications (100% vs 22.8%, p<0.001) and mortality (0.6% vs 4%, p=0.01) were higher in reoperated patients. We found that the hospitalization day in the ICU was longer in those who underwent re-operation (3.2 days vs 1.5, p<0.001).

Independent risk factors affecting re-operation according to multiple logistic regression analysis were neoadjuvant therapy (odds ratio=5.882, 95%CI: 2.344-14.727, p<0.001), operation time (per hour, odds ratio=1.662, 95%CI: 0.982-2.813, p=0.04), intraoperative pulse rate (per unit increase, odds ratio=1.039, 95%CI:1.006-1.074, p=0.01) and postoperative hemoglobin (per unit decrease, odds ratio=1.996, 95%CI:1.474-2.702, p<0.001). Preoperative hemoglobin, on the other hand, affected the development of re-operation independently at a level close to significance (p=0.06) (Table 3).

Table 2. Factors affecting re-operation and the situation in postoperative follow-up between patients who required and did not require re-operation

Variable	Non-HNP (n=388)	HNP (n=25)	p-value
Factors affecting re-operation			
Age, year±SD	57.5±12.9	59.8±16.3	0.340
Gender, n (%)			0.170
Male	295 (76.1%)	22 (88 %)	
Female	93 (23.9%)	3 (12%)	
Diagnosis, n (%)			0.591
Lung cancer	283 (73%)	17 (68%)	
Lung mass	105 (27%)	8(32%)	
Disease localization, n (%)			0.125
Rıght	243 (63%)	20 (40%)	
Left	145 (37%)	15 (60%)	
Preoperative hemoglobin, mg/dl ±SD	13.2±1.6	12.5±1.3	**0.009**
Anjio Bypass, n (%)	59 (9.3%)	4 (9.5%)	1.000
Anticoagulant, n (%)	173 (27.2%)	9 (21.4%)	0.417
Neoadjuvan, n (%)	34 (5.3%)	12 (28.6%)	**<0.001**
Resection type,n (%)			**<0.001**
Wedge Rzk+lobectomy	328 (85%)	22 (48%)	
Pneumonectomy	60 (15%)	13 (52%)	
Operation type, n (%)			**0.005**
Thoracotomy	300(77.8%)	24 (95.9%)	
VATS	88 (22.6%)	1(4.1%)	
Operation time, hour±SD	3.8±0.7	4.5±0.7	**<0.001**
Intraoperative blood loss, ml±SD	334.6±309.8	544.2±379.0	**<0.001**
Intraoperative blood product use, n (%)	90 (23.1%)	11 (44%)	**0.019**
IntraoperativeMAP, mmHg±SD	66.5±11.0	62.1±11.2	**0.012**
Intraoperative heart rate, n/min±SD	67.1±10.4	74.8±14.0	**0.001**
***The situation in postoperative follow-up between patients requiring and not requiring* re-operation**			

Postoperative blood product use, n (%)	48 (12%)	22 (88 %)	**<0.001**
Postoperative hemoglobin, mg/dl ±SD	11.7±1.7	10.1±1.4	**<0.001**
Postoperative complication, n (%)	91 (23%)	25 (100.0%)	**<0.001**
Postoperative respiratory complication	32 (8%)	8 (32%)	**<0.001**
Postoperative cardiac complication	82 (21%)	25 (100%)	**<0.001**
ICU length of stay, days±SD	1.5±7.9	3.2±2.7	**<0.001**
Mortality, n (%)	24 (0.6%)	1 (4%)	**0.01**

MAP: mean arterial pressure; VATS: Video-Assisted Thoracic Surgery; ICU: intensive care unit. Bold p-values indicate significant differences. HNP:

Postoperative complications were present in 116 (28%) patients, while 5 (1.2%) patients died postoperatively. Pulmonary complications developed in 40 (9.6%) patients, cardiac complications in 107 (25.9%), acute renal failure (ARF) in 11 (1.6%) and CVO in 2 (0.3%). The mean length of stay in the ICU was 1.6 days (min=1, max=200 days). As pulmonary complications, 20 (4.8%) patients had acute respiratory failure, 15 (3.6%) pneumonia, 2 (0.5%) air leak, 4 (1%) pulmonary edema. As cardiac complications, hemodynamic instability was observed in 52 (12.6%), arrhythmia in 48 (11.6%), angina in 6 (1.4%) and asystole in 5 (1.2%) patients.

Table 3. Evaluation of factors affecting rethoracotomy by multiple logistic regression analysis

	Odds ratio	95% CI	p-value
Preoperative hemoglobin (per unit drop)	1.292	0.987-1.690	*0.06*
Neoadjuvan (No etc Yes)	5.882	2.344-14.727	**<0.001**
Resection type (Wedge+Lob. etc Pneumonectomy)	1.374	0.812-2.322	0.235
Type of operation (Thoracotomy etc VATS)	0.340	0.074-1.681	0.185
Operation time (per hour)	1.662	0.982-2.813	**0.049**
Intraoperative blood loss (per ml)	1.001	0.998-1.009	0.804
Intraoperative blood product use (No etc Yes)	0.818	0.298-2.246	0.697
Intraoperative MAP (per mmHg)	0.997	0.961-1.035	0.892
Intraoperative heart rate (per beat)	1.039	1.006-1.074	**0.01**
Postoperative hemoglobin (per unit drop)	1.996	1.474-2.702	**<0.001**

CI; confidence interval, Bold-signed p-values show significant differences.

Table 4 shows the relationship between postoperative general and respiratory and cardiac complications, and the amount of intraoperative blood loss and intraoperative blood product use. We found that both the amount of intraoperative blood loss and the use of at least one intraoperative blood product were associated with general postoperative complications and respiratory and cardiac complications.

In the ROC analysis for re-operation, we observed a better capacity for prediction of APACI (AUC: 0.884, 95% CI: 0.842-0.927, p<0.001) and SOFA (AUC: 0.892, 95% CI: 0.837-0.948, p<0.001) scores compared to the ASA score.

Table 4. The relationship between general postoperative complication, respiratory and cardiac complications, and the amount of intraoperative blood loss and intraoperative blood product use

	Amount of intraoperative blood loss, ml±SD	**p-value**	**Intraoperative blood product use (n=101), n (%)**	**p-value**
Postoperative general complication, n (%)				
no (n=297)	320.4±325.5	**<0.001**	49 (16.4%)	**<0.001**
yes (n=116)	486.2±322.9		52 (44.8%)	
Postoperative respiratory complication n (%)				
no (n=373)				
yes (n=40)	347.4±323.3	**<0.001**	80 (21.4%)	**<0.001**
	546.5±369.9		21 (51.6%)	
Postoperative cardiac complication n (%)				
no (n=306)	329.1±330.0	**<0.001**	54 (17.6%)	**<0.001**
yes (n=107)	475.3±318.5		47 (43.9%)	

SD; standard deviation, Bold p-values indicate significant differences

4. Discussion

In this study, we examined the relationship between re-operation after lung resection and the preoperative data of the patients and determined the presence of neoadjuvant for re-operation, prolonged operation time and increased intraoperative pulse rate as independent risk factors.

Re-operation after thoracic surgery is frequently applied for postoperative bleeding (1-5). Bleeding complications after lung resection vary between 2-3%, but this rate can reach up

to 6-13.5% in completion pneumonectomies. Although the incidence of re-operation after bleeding is not common, it is 2-3% and 1% after open and VATS, respectively (4). We found the re-operation rate as 6.1% on average, consistent with the literature.

Miyazaki et al. found no correlation between intraoperative bleeding and morbidities in their study of 241 patients who underwent VATS (2). Uramoto et al. showed that there was no correlation between hemorrhage after lung cancer surgery and patients' age, gender, smoking, clinical stage, pathological stage, and histology (4). However, Nakamura et al. found a correlation between intraoperative bleeding and the operating procedure, gender, disease stage, and histologic type (6). Decaluwe et al. revealed a relationship between chemotherapy and major intraoperative complications (7). In parallel with the literature, we found neoadjuvant therapy to be among the independent risk factors for re-operation. In addition, we found no significant relationship between the chronic diseases of the patients and the re-operation.

Perioperative bleeding is lower in VATS patients than in open thoracotomy because VATS patients represent a select group with more peripheral, smaller and safer lesions for resection. Erdogu et al. observed the re-operation rate as 3.3% after thoracotomy and 0.4% after VATS (8). In the literature, postoperative hemorrhage was observed as 2-3% after thoracotomy and 1.7% after VATS. In our study, 95.2% of the patients who underwent re-operation were patients who underwent thoracotomy. We performed re-operation in 8% of those who underwent thoracotomy and 1.1% of those who underwent VATS.

Harpole et al. found the re-operation rate as 2.9% after lobectomy and 3% after pneumonectomy (9). Erdogu et al. found this rate as 11.5% in pneumonectomies and 1.7% in lobectomies (8). Our study found pneumonectomy to be among the independent risk factors for re-operation. We performed re-operation in 3.8%, 21% and 2.3% of those who underwent lobectomy, pneumonectomy, and wedges, respectively. In their study examining 27 patients who were reoperated, Sayar et al. found that pneumonectomy was the primary operation of 59.9% of the patients who underwent re-operation (1). In our study, that rate was 52%.

Factors such as technical problems (70-80%), hemodilution, hypothermia, depletion of coagulation factors (DIC) and acidosis seem to be responsible for bleeding due to surgery. Studies have shown that the possibility of postoperative complications and mortality increases in intraoperative bleedings >250 ml in patients who underwent surgery (10, 11). Yano et al. found the average intraoperative bleeding rate as 186 and 856 ml in patients who underwent lung resection and re-operation, respectively (12). In our study, the mean intraoperative blood loss was 367 ml, while it was 547 ml in those who underwent re-operation and 334 ml in those who did not. Studies have shown a relationship between intraoperative bleeding >100 ml in patients undergoing lung resection with VATS and postoperative cardiopulmonary complications, prolonged chest tube length of stay, and prolonged hospital stay (13). In their extensive retrospective study, Li et al. revealed massive bleeding in patients who underwent thoracic surgery as the leading cause of mortality (14). Nakamura et al. showed that intraoperative bleeding >381 ml in patients undergoing resection for lung cancer was an independent risk factor for patients' survival (6). The average amount of intraoperative bleeding in patients undergoing VATS resection was in the range of 100-400 ml (13). Our study revealed 259 ml of bleeding in patients who underwent VATS and 396 ml in those who underwent thoracotomy. These results are compatible with the literature.

Studies have shown that increased intraoperative heart rate (>87 beats/min) and decreased mean arterial pressure were associated with impaired myocardial function and increased incidence of mortality in patients undergoing surgery (15). Increased heart rate decreases coronary blood flow, increases myocardial oxygen requirement and causes myocardial damage. As a result of volume loss due to bleeding, decreased oxygen supply to vital organs, immune suppression, and pulmonary edema may occur due to excessive fluid overload (13). Brady et al. found a correlation between values of <55-75 mmHg for MAP and an increased incidence of postoperative acute renal failure and MI (16). Our study revealed a correlation between increased pulse rate and decreased MAP and re-operation, and postoperative cardiopulmonary complications.

A study of 3500 patients who underwent lung resection observed bleeding requiring the use of >4 U ES in 2.9% of lobectomies and 3% of pneumonectomies (5). We used in our study an average of 1.7 U ES in pneumonectomies and 1.2 U ES in lobectomies patients in the intraoperative period. Undesirable side effects may occur in patients undergoing surgery for lung cancer due to perioperative bleeding due to blood transfusion. As the amount of blood product used increases, the rate of complications such as postoperative respiratory failure, infection, and acute kidney failure increases (17-19). Thomas et al. found that the patient's age, neoadjuvant therapy, pneumonectomy and prolonged operation time were independent risk factors for blood transfusion in thoracic surgery. The same study also evinced that intraoperative >2 U ES blood transfusion increased 30-day mortality by two times (18). We found in our study a relationship between blood transfusion and postoperative respiratory and heart failure.

In this study, we examined the relationship between re-operation after lung resection and the perioperative data of the patients and determined neoadjuvant for re-operation, prolonged operation time, increased intraoperative pulse rate as independent risk factors. Rapid diagnosis and treatment of

hemorrhages that develop due to lung resection and require re-operation significantly affected mortality and morbidity. Careful observation of the markers of perioperative hemorrhage and close patient follow-up with the cooperation of clinical branches would decrease the incidence of hemorrhage-related mortality and morbidity.

This study had two limitations. Firstly, we conducted the study in a single center and retrospectively. Secondly, since our hospital is a training and research hospital with more than one clinical branch, there is a high probability of different approaches in surgery.

Acknowledgments

The Ethics Committee of the Hospital approved this study. Confirmed number/date 2022-195/10.02.2022. The study was conducted in accordance with the Helsinki declaration principles. All patients signed a written consent form.

Conflict of interest

The authors declared that there was no conflict of interest during the preparation and publication of this article.

Financial Disclosure

We used no funds for this publication.

Authors' contributions

Concept: Y.Ö., Design:Y.Ö, C.A., Data Collection or Processing:Y.Ö., C.A., Analysis or Interpretation: Y.Ö., C.A., Literature Search: Y.Ö., Writing: Y.Ö.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 761-766
doi: 10.52142/omujecm.39.3.32

Short- and mid-term clinical results of ultrasound-guided genicular nerve block in arthroplasty-related chronic postsurgical pain

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Received: 05.03.2022 • **Accepted/Published Online:** 17.05.2021 • **Final Version:** 30.08.2022

Abstract

One-third of patients experience knee arthroplasty-related chronic postsurgical pain (CPSP), and CPSP negatively affects all dimensions of quality of life. We aimed to evaluate the short- and mid-term clinical results and success of ultrasound-guided genicular nerve block in patients with knee arthroplasty-related CPSP. The secondary outcome aimed to evaluate the relationship between clinical success and the presence of neuropathic pain. This study is a retrospective chart review of patients with knee arthroplasty-related CPSP who were referred to a pain clinic and were unresponsive to conservative treatments. We obtained the clinical evaluations of the patients at one, three, and six months before and after the procedure from patient records. We evaluated pain intensity with a 10-point numerical rating scale (NRS), the effects of knee pain on function with the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scoring system, and the presence of neuropathic pain with the Douleur Neuropathic Pain 4 Questions. We accepted the clinical success of the procedure as a 50% or more reduction in pain intensity. We evaluated a total of 21 patients. The therapy was successful in 13 (61.9%), 12 (57.1%), and 7 (33.3%) patients at the first, third, and sixth-month visits, respectively. There was a statistically significant difference between the NRS and WOMAC scores. At baseline, neuropathic pain was present in 61.9% (n = 13) of the patients, and its presence was not related to clinical success at the first, third, and sixth-month visits. Ultrasound-guided genicular nerve block relieves pain and improves short- and mid-term functionality for patients with knee arthroplasty-related CPSP. The study resulted in clinical success in two-thirds of the patients in the early period and one-third in the sixth month.

Keywords: knee arthroplasty, chronic postsurgical pain, genicular nerve block, neuropathic pain

1. Introduction

Knee osteoarthritis is essential in clinical practice because of its increasing prevalence, multimorbidity, and negative impact on life (1). It is typically managed with stepwise treatment, including education, structured exercise programs, topical analgesics, specific and nonspecific nonsteroidal anti-inflammatory drugs, intra-articular steroid injections, aquatic exercises, gait aids, cognitive-behavioral therapy, and intra-articular hyaluronic acid injections (2). In patients unresponsive to conservative treatments, knee arthroplasty is the gold standard treatment (3).

The most important determinant of satisfaction in patients undergoing knee arthroplasty is pain relief and the functionality gained from pain relief (4). Persistent pain for at least three months after knee arthroplasty is defined as chronic postsurgical pain (CPSP) (5) and detected at a frequency of 16–47% (6-8). Knee arthroplasty-related CPSP can be nociceptive or neuropathic, affecting the biopsychosocial aspects of patients' quality of life because of the multifactorial etiology of knee arthroplasty-related CPSP, which is not fully understood. A multidisciplinary and comprehensive pain management approach is recommended that involves the cooperation of orthopedic surgeons, physiatrists, pain specialists, and psychiatrists (9).

No guidelines have been established for managing knee arthroplasty-related CPSP, and there is insufficient evidence about interventional treatments' clinical value and success (10). However, studies have shown that intra-articular Botulinum toxin injections (11), periarticular subcutaneous perineural injections (12), dry needling (13), and genicular nerve blocks and radiofrequency treatments (14, 15) may improve pain and functionality.

The genicular nerve consists of anastomoses of the tibial, common peroneal, saphenous, femoral, and obturator nerves, and it provides sensory innervation of the knee joint (16). To explore whether genicular nerve block application might be beneficial in knee pain (16), researchers have examined the clinical results of blockade and ablation treatments in various patient groups (14, 15, 17, 18). In this study, we retrospectively evaluated the clinical outcomes of ultrasound-guided steroid-added genicular nerve blockade and the relationship between clinical success and the presence of neuropathic pain during short- to mid-term follow-up in patients with knee arthroplasty-related CPSP.

2. Materials and Methods

2.1. Study design and setting

This study involved a single-center retrospective analysis. We

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acquired the data by screening the files of patients treated between April 1, 2019, to July 31, 2020, at our institution, after obtaining approval from the ethics committee of Necmettin Erbakan University (No. 2021/3127, approved March 2, 2021). We conducted this study per the declaration of Helsinki.

2.2. Participants

We evaluated the patients referred to the pain clinic between April 2019 and July 2020 who were unresponsive to nonsurgical treatment (physical therapy, exercise programs, and pharmacological treatments) and had pain from arthroplasty that persisted for three months. We included those who had undergone a genicular nerve block with triamcinolone and bupivacaine and excluded those who changed pharmacological therapy, exercise program, and orthotic devices two months before undergoing the procedure or at the end of the follow-up period. We also excluded those who received physical therapy or rehabilitation during the six months before the procedure, and those with a history of psychiatric disease, dementia, malignancy, inflammatory rheumatologic disorders, and neurological diseases

2.3. Variables and outcomes

We reviewed the patients' clinical follow-up files for the following: Demographic data, Douleur Neuropathic Pain 4 Questions (DN-4), pre-procedural clinical findings regarding pain duration before the procedure, and pre and post-procedural pain intensity scores (Numeric Rating Scale (NRS) and Western Ontario and MacMaster Universities osteoarthritis index (WOMAC). We analyzed pain intensity and WOMAC results at one, three, and six months after the procedure.

We obtained pain intensity data before the genicular nerve block and at one, three, and six months after the procedure. At those intervals, we asked the patients to determine the mean pain intensity from the past week using the NRS (0–10 at each visit, with 0 meaning "no pain" and 10 meaning "the most severe pain I can imagine"). Clinical success constituted a 50% or higher reduction in pain intensity (19).

The WOMAC is a valid, reliable osteoarthritis-specific questionnaire that contains 24 questions under three subheadings: pain, stiffness, and physical function. Each question is scored on a Likert scale as 0 = none, 1 = mild, 2 = moderate, 3 = severe, or 4 = very severe. The score for each section is calculated individually, and the total score ranges from 0 to 100. High scores indicate increased pain and stiffness and impaired physical function (20).

The DN-4 consists of 10 questions, seven focusing on symptoms and three determined by clinical examination. The symptom questions include the following: burning, painful cold, electric shock, tingling, pricking, numbness, and itching. The senses examined are light touch hypoesthesia, pinprick hypoesthesia, and brushing allodynia. Each question with a yes answer is given one point. The scores obtained by symptom questioning and clinical examination are added to calculate the total score, which is 10 points maximum. Patients with a score of 4 or above are considered to have neuropathic pain (21).

2.4. Intervention

The injection area was sterilized with a povidone-iodine solution. The ultrasound-guided genicular nerve block was applied as defined by Kim et al. (17). Each patient was placed in a supine position with a pillow under the popliteal fossa to avoid discomfort. A 12-MHz linear transducer (Siemens Acuson S2000, Germany), covered with a sterile disposable sheath, was first placed parallel to the lower extremity to identify the medial and lateral epicondyle of the femur and proximal aspect of the tibia. The tract of the genicular artery was identified and confirmed using color Doppler mode. The genicular nerve block target points were determined, which are usually next to the superior lateral, superior medial, and inferior medial genicular arteries. The needle was inserted into the plane of the ultrasound probe in the long-axis view. Gentle aspiration was performed, and the injectate was administered. The same procedure was performed for the three injection sites (superior lateral, superior medial, and inferior medial genicular arteries), with a total volume of 6 ml, comprising 5 ml of 0.5% bupivacaine and 1 ml of 40/mg/ml triamcinolone divided between the three injection sites for each knee. At the end of the procedure, ice was applied for 20 minutes.

2.5. Statistics

After finalizing the study, we performed a post hoc sample size analysis using the G*power software package for power analysis (version 3.1.6; Franz Faul, Kiel University, Kiel, Germany). We included 21 items in the final analysis and calculated the power as 0.69 with an effect size of 0.5 and an alpha error level of .05. We conducted data analysis using SPSS version 20.0 (IBM Corporation, Somers, NY, USA).

We used the Shapiro-Wilk and Kolmogorov-Smirnov tests to test the hypothesis of normal distribution and presented the descriptive data as frequencies (n) and percentages (%) for the categorical variables. For numerical variables, we reported whether the mean (standard deviation) and median with 25–75% percentiles were normally distributed or not. We used the Chi-square or Fisher's exact tests to compare categorical variables between independent groups and analyzed variables with non-normal distributions with Friedman's analysis. We used the Wilcoxon Rank Sum Test to compare repetitive measurements, a Bonferroni correction to avoid possible type 1 errors and Pearson's correlation coefficient or Spearman's rank correlation to assess the relationship between variables.

3. Results

We included 21 patients in the study (Fig. 1). Their average age was 64.0 (58.5–69.5), and 81.0% (n = 17) were female. The postoperative time was 18.0 (5.5–43.0) months, and the

postoperative pain duration was 7.0 (4.0–35.5) months. The postoperative times were 6 ≤ months in 6 (28.57%), 7–12 months in 3 (14.28%), 13–24 months in 4 (19.04%), and ≥ 25 months in 8 (38.09%) patients. The right side was painful in 10 (47.6%), while the left side was in 11 (52.4%). Neuropathic pain was present in 13 (61.9%).

Fig 1. Flow diagram

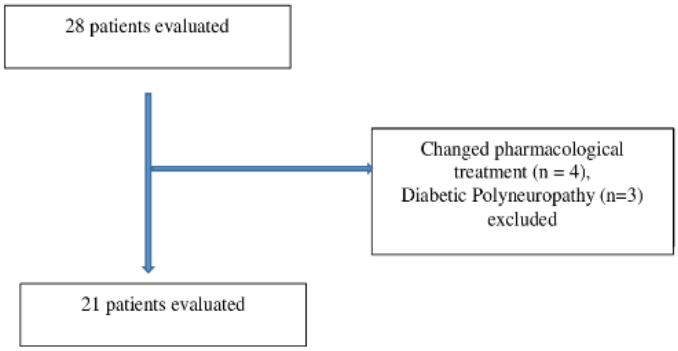

Table 1 shows the pre and post-procedural pain intensity scores (NRS). There was a statistically significant difference between pain intensity values before and after the procedure (p-value < .001). In the post hoc analysis, there was a statistically significant difference in pain intensity scores between the pre-procedure period and the first, third, and sixth-month visits (Table 2).

Table 1. Pain intensity NRS (0–10) at the follow-up period

Time	Pain intensity NRS (0-10)	
	Median (25-75% percentiles)	95%C.I.*
Baseline	7.0 (5.0-8.0)	5.77-7.33
1st month visit	3.5 (1.5-6.0)	2.37-5.03
3rd month visit	4.0 (1.5-7.0)	2.79-5.60
6th month visit	6.0 (2.0-8.0)	3.85-6.54

*95% Confidence Interval for Difference

Table 2. Pairwise comparisons of baseline and postprocedural pain intensities

Time		P*	Z score	Effect size
Baseline	**1st month**	0.000	-3.627	0.79
	3rd month	0.003	-3.232	0.70
	6th month	0.030	-2.565	0.56

* Bonferroni correction was done

Table 3 shows the pre-procedural and post-procedural WOMAC total scores. There was a statistically significant difference between pre and post-procedure total WOMAC scores (p-value < .001). In the post hoc analysis, there was a statistically significant difference in WOMAC total scores between the pre-procedure and first, third, and sixth-month visits (Table 4).

Table 3. WOMAC total scores at the follow-up period

Time	WOMAC total score	
	Mean ± standard deviation	95%C.I.*
Baseline	61.66 ± 17.09	53.88-69.44
1st month visit	44.94 ± 20.90	35.43-54.45
3rd month visit	47.52 ± 21.89	37.55-57.49
6th month visit	55.26 ± 20.47	45.94-64.57

*95% Confidence Interval for Difference

Table 4. Pairwise comparisons of baseline and postintervention WOMAC total scores

Time	P*	Z score	Effect size	
Baseline	**1st month**	0.000	-3.623	0.79
	3rd month	0.003	-3.181	0.69
	6th month	0.034	-2.121	0.46

* Bonferroni correction was done

The average percentage of pain relief after the procedure was 52.78% (14.58–73.21%) (32.29–63.65, 95% CI), 50.0 (0.0–73.21) (23.92–57.41, 95% CI), and 0.0 (0.0–55.00) (9.88–39.60, 95% CI) at the first, third, and sixth-month visits, respectively, compared with the baseline. There was a statistical difference in pain relief between the first and third, third and sixth, and first and sixth months (p = 0.027, 0.017, 0.020, respectively).

There were no statistical differences between the groups with and without neuropathic pain in terms of age, postoperative time (the time between arthroplasty surgery and steroid-added genicular nerve blockade), pain duration, baseline pain intensity, or baseline WOMAC scores (Table 5) neither in clinical success in the first, third, and sixth-month visits between the patients with and without neuropathic pain ($\chi 2 = 3.590$, p = .085; $\chi 2 = 4.863$, p = .067; $\chi 2 = 1.615$, p = .346, respectively). We also evaluated the complications. No patients developed infection, weakness, or neuralgia.

Table 5. Preoperative variables comparisons of patients with neuropathic pain and without neuropathic pain

Variables	Median (25-75%)		P
	Without neuropathic pain	With neuropathic pain	
Age (years)	62.0 (58.5-69.25)	67.0 (57.5-69.5)	0.79
Baseline pain intensity (NRS)	5.0 (4.25-7.75)	7.0 (5.5-8.0)	0.08
Baselin WOMAC score	63.54 (32.55-68.75)	64.58 (53.64-75.0)	0.53
Postoperative time (months)	12.5 (6.25-46.0)	24.0 (5.-43.0)	0.88
Pain duration (months)	7.0 (4.5-37.5)	3.5 (3.0-29.5)	0.56

4. Discussion

We found that pain intensity decreased by half in the early period, and pain intensity and functionality improved at short and mid-term follow-ups with a steroid-added genicular nerve blockade. In their randomized controlled study of 28 patients with knee arthroplasty-related CPSP, Qudsi-Sinclair et al. (17) compared pulsed radiofrequency treatment for the genicular nerve to steroid-added genicular nerve blockade. They evaluated pain intensity with the NRS and functionality with the Oxford Knee Score and observed a decrease in pain intensity that started on the first day and improved functionality that started in the first month; both results continued through the third and sixth months, without any difference between the groups (17). Erdem and Sir (15) retrospectively analyzed the pain intensity and WOMAC scores of 23 patients with chronic knee pain who had undergone pulsed radiofrequency after genicular nerve blockade. They analyzed follow-up data from the third week and the third month after the procedure. As in our study, Erdem and Sir (15) found an improvement in pain intensity and functionality in the short and mid-term. Ghai et al. (22) applied a single session ultrasound-guided genicular nerve blockade with a mixture of 4.5 mL of 0.5% bupivacaine and 1.5 mL (60 mg) of methylprednisolone to patients with osteoarthritis-related chronic knee pain. Improvement in pain severity as measured by NRS and functionality as assessed by WOMAC was demonstrated at a 3-month follow-up (22). Guler et al. (23) showed that ultrasound-guided genicular nerve blockade with a total mixture of 5 ml of 2% lidocaine and 40 mg of triamcinolone, to patients with knee osteoarthritis has sustained improvement in pain, physical function, and physical capacity for up to 12 weeks. Fonkoue et al. (24) performed a single session genicular nerve blockade with a mixture of lidocaine and triamcinolone, using scopy-controlled classic and revised target techniques, to patients with chronic knee pain due to osteoarthritis. In both groups, improvement was detected at the 12th-week control compared to baseline in pain severity assessed by NRS, functionality assessed by WOMAC, and physical and mental health assessed by SF-12 (24). The common point of these studies in patients with knee osteoarthritis is that long-term efficacy was demonstrated with a single-session block with added steroids. It has been shown that steroid-added blocks inhibit pain pathways (25). In our study, midterm efficacy may be related to the addition of steroids in the nerve blockade procedure. Elsaman et al. (26) assessed the effect of genicular nerve block on the inflammatory joint disease. In patients with knee involvement due to rheumatoid arthritis, genicular nerve blockade with bupivacaine was shown to improve pain severity and functionality in a 12-week follow-up (26). Elsaman et al. (26) argued that this clinical efficacy may be related to the anti-inflammatory effects of local anesthetics (27). In our study, we evaluated the clinical outcomes of patients with knee arthroplasty-related CPSP. It has been shown that arthroplasty surgery has a neuroinflammatory effect (28). The clinical improvement in our study may be related to the suppression of neuroinflammation by the genicular nerve blockade.

Kim et al. evaluated the clinical results of ultrasound-guided steroid-added genicular nerve blockade in patients with chronic knee pain due to osteoarthritis. They found that the pain and functionality improvements observed at weeks two and four regressed to the pre-procedure baseline levels at week eight (17). Our finding that the block efficacy lasted longer than in Kim et al.'s study may be due to the development of spontaneous remission with increased follow-up time in arthroplasty-related chronic pain (29, 30).

Our study evinced that pain scores significantly decreased at the first-month visit after the procedure, and the analgesic effect gradually decreased toward the sixth month. Erdem and Sir (15) found no statistically significant change in pain relief with the progression of the follow-up period, although the improvement in functionality decreased. Qudsi-Sinclair et al. (14) reported that the analgesic efficacy started at the month-one visit in the genicular block group remained stable during the sixth-month follow-up period. The stability of pain palliation these studies reported can be explained by the higher drug doses and volumes than ours or by the combined radiofrequency therapy. In addition, our findings supported Qudsi-Sinclair et al.'s (14) recommendation to perform strict pain follow-up after the blockade, as it may require procedure repetition in the first six months.

In our study, approximately three-fifths of the patients had neuropathic pain. This prevalence rate was higher than reported in the literature (31, 32). Comparing the groups with and without neuropathic pain, we found no difference between the groups regarding the clinical success of the genicular nerve blockade. In the literature, studies have shown that peripheral nerve blockades can be beneficial for patients with neuropathic pain of various etiologies (33, 34). Consistent with our findings, Eker et al. (34) concluded from their double-blind controlled study on patients with neuropathic pain that prolonged efficacy could be achieved using peripheral nerve blockades with added steroids.

The main limitations of the study were its retrospective design and limited sample size. In addition, the absence of control groups and the limited follow-up data make the findings difficult to generalize.

In conclusion, ultrasound-guided steroid-added genicular nerve blockade is a safe treatment option that reduces pain intensity by half in the early period and relieves pain in the short to mid-term. Its clinical success is not associated with the presence of neuropathic pain in patients with arthroplasty-related CPSP. Future long-term follow-up studies should investigate the need for blockade repetition and the addition of genicular nerve blockade to the management algorithm for knee arthroplasty-related CPSP. Also, prospectively designed

studies could evaluate the strength of our findings by using more extensive patient participation and control groups.

Conflict of interest

None.

Funding

No funding was used for the study.

Acknowledgments

Materials in this manuscript were previously presented as an oral presentation at the Sixteenth National Algology Congress.

Authors' contributions

Concept: S.B, M.Z.G., Design: S.B, M.Z.G., Data Collection or Processing: S.B, M.Z.G., Analysis or Interpretation: S.B, M.Z.G., Literature Search: S.B, M.Z.G., Writing: S.B, M.Z.G.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 767-769
doi: 10.52142/omujecm.39.3.33

The practice of COVID-19 vaccination in patients bleeding disorders

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Received: 15.03.2022 • **Accepted/Published Online:** 05.06.2022 • **Final Version:** 30.08.2022

Abstract

Subcutaneous injections are recommended instead of intramuscular for patients with bleeding disorders to avoid bleeding complications. This study aimed to determine whether the intramuscular administration of COVID-19 vaccines would increase bleeding-associated complications in patients with bleeding disorders followed in our clinic. We collected the data of 47 patients with bleeding disorders older than 18 years followed at the hematology outpatient clinic and screened between March 15, 2020, and December 31, 2021. We obtained the data from the hospital's electronic information system, including age, gender, type of bleeding disorder, factor levels, and whether they received prophylaxis. We interviewed the participants about the type of vaccine they received and the duration of compression they applied to the injection site, whether they received factor replacement before and after the injection, and whether there were any complications following the injection. We included in the study thirty-nine male patients vaccinated against COVID-19. The mean age of the patients was 39.05 (18–73 years). Factor VIII deficiency constituted 79.4%, XI 10.3%, and other bleeding disorders 10.3 % of the cases. The patients with bleeding disorders had a mean factor level of 2.02 (0–9). Twenty-nine (74.4%) patients were on regular factor prophylaxis for their bleeding disorder. In terms of the compression duration to the injection site following injection, 6 (15.4%) patients had applied compression for 10 min, 4 (10.3%) for 5–10 min, and 29 (73.4%) for less than 5 min. Two (5.1%) patients developed ecchymosis after the first vaccine dose on the injection arm. The results of this study demonstrate that the rate of bleeding complications remains low in patients with bleeding disorders if they receive intramuscular vaccination after necessary precautions.

Keywords: COVID-19, vaccination, bleeding disorders, bleeding complication

1. Introduction

Vaccination against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in Turkey began on January 13, 2021, after the coronavirus disease 2019 (COVID-19)-inactivated vaccine CoronaVac (Sinovac, Beijing, China) was approved for use (1, 2). The widespread release of this vaccine and others from different manufacturers worldwide has resulted in the administration of more than 10.5 billion doses (3). So far, 61.4% of the population in Turkey has been fully vaccinated, and 5.86% are partially vaccinated (1). Subcutaneous injections are recommended instead of intramuscular for patients with bleeding disorders to avoid bleeding complications (4). However, the subcutaneous administration of vaccines elicits lower seroconversion than intramuscular administration, leading to a faster decline of the antibody response (5, 6). This study aimed to determine whether the intramuscular administration of COVID-19 vaccines would increase bleeding-associated complications in patients with bleeding disorders followed in our clinic.

2. Materials and Methods

We collected the data of 47 patients with bleeding disorders older than 18 years followed at the hematology outpatient clinic of the Ondokuz Mayıs University (OMU), Turkey, and screened between March 15, 2020, and December 31, 2021. We included in the study 39 patients vaccinated against COVID-19 and excluded Eight patients as they were unvaccinated. We obtained the data from the hospital's electronic information system, including age, gender, type of bleeding disorder, factor levels, and whether they received prophylaxis. We interviewed the participants about the type of vaccine they received, and the duration they applied compression to the injection site, whether they received factor replacement before and after the injection and whether there were any complications following the injection, they had visited the outpatient clinic, or communicated via phone using

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their registered phone numbers in the electronic information system of the hospital. The Local Ethics Committee accepted the study with OMU KAEK 2022/67 reference number.

2.1. Statistical Analysis

After being encoded, we transferred the study data to a computer and analyzed them with SPSS software (Version 20 for Windows, SPSS Inc, Chicago, IL, USA). We expressed continuous variables by median (min.-max.) and frequency data by percentage (%) in data evaluation and used The Chi-square test to assess the correlation between bleeding and other parameters. We considered P <0.05 statistically significant.

3. Results

Table 1 shows the characteristics of 39 adult bleeding disorders patients. All the participants were male with a mean age of 39.05 (18–73 years) and a mean factor level of 2.0 (0–9). Thirty-one had hemophilia A (79.5 %), four had hemophilia B (10.3 %), and four had other bleeding disorders (10.3%). Based on their factor levels, 19 (61.2%) of the patients with factor VIII level disorder were classified as severe (<1%), 8 (25.8 %) as moderate (1%–5%), and 4(13%) as mild (>5%); while 1 (25%) of the patients with factor IX level disorder were classified as severe, 2 (50%) as moderate, and 1 (25%) as mild. Two of the patients with Von Willebrand disease were classified as Type 1.

Table 1. Clinical characteristics of patients with a bleeding disorder after COVID-19 vaccine and vaccine doses

	N (%)
Bleeding Disorder Type	
- Hemophilia A	31 (79.5 %)
- Hemophilia B	4 (10.3 %)
- Von Willebrand's disease	2 (5.1 %)
- Factor 13 deficiency	1 (2.6 %)
- Afibrinogenemia	1 (2.6%)
Hemophilia A severity	
- <1%	19 (61.2%)
- 1-5 %	8 (25.2%)
- > 5%	4 (13%)
Hemophilia B severity	
- <1%	1 (25%)
- 1-5 %	1 (25 %)
- >5 %	2 (50%)
Factor prophylaxis	
	29 (74.4)
Vaccine doses	
- 1st vaccination	39 (100%)
- 2nd vaccination	37 (95%)
- 3rd vaccination	23 (59%)
- 4th vaccination	7 (18%)

A total of 106 doses of the COVID-19 vaccine had been administered, with 29 (74%) of the patients receiving the Pfizer–BioNTech vaccine, two patients (5%) the Sinovac vaccine, and eight patients (21%) both vaccines. Out of the 39 patients, 95% had been fully vaccinated (two doses), 59% had received the first booster dose, and 18% the second.

Twenty-nine (74.4%) patients were on regular factor prophylaxis for their bleeding disorder. Only the patient with factor XIII deficiency was on regular two units of fresh frozen plasma per month, while seventeen (43.6%) patients received pre-vaccination counseling from a hematologist. In terms of the compression period following injection, six (15.4%), four (10.3%), and 29 (73.4%) patients had applied compression to the injection site for 10 min, 5–10 min, and for less than 5 min, respectively. 16 (41%) patients received prophylactic factor replacement before injection; three (7.7%) required post-vaccination factor prophylaxis. None of the patients had injection site uncontrollable bleeding. Only two (5.1%) developed ecchymosis on the injection arm after vaccination; the complication developed following the first vaccine dose. Both received prophylaxis and factor replacement before subsequent vaccine doses.

Eight patients were unvaccinated; two had chosen not to be vaccinated, four were not vaccinated due to the potential side effects of their disease, and two remained unvaccinated because they were afraid.

4. Discussion

This study examined the experiences of patients with bleeding disorders after receiving COVID-19 vaccination. We found a low rate of bleeding-related complications associated with the intramuscular administration of COVID-19 vaccines in patients with bleeding disorders.

The World Federation of Hemophilia has provided several recommendations for the vaccination of patients with bleeding disorders, such as the subcutaneous administration of the vaccine to prevent bleeding, using the smallest gauge needle (25–27 gauge), and applying a cold compress to the injection site for 5 minutes before and pressure 10 minutes after vaccination respectively (7). Following the global introduction of COVID-19 vaccination programs, hematology experts announced that patients with hemophilia with a factor level above 10% (factor VIII or IX) do not require hemostatic treatment before the vaccination to minimize vaccine-related hemorrhage. They also recommended that patients with factor levels below 10% or other rare bleeding disorders consult follow-up centers to discuss the required hemostatic precautions before the vaccination (8,9).

A study regarding other intramuscular vaccines in pediatric patients found a bleeding rate of <1% at the injection site (10); however, it provided no information concerning whether the patients had applied compress for 10 min following the vaccination to the injection site. In our patients, the post-vaccination bleeding rate (5.1%) at the injection site was higher than the general population (2.4%) (11). The high bleeding rates observed in our patients could result from most not applying compression to the injection site for 10 min after the vaccination. However, our study's bleeding rate was lower than the research conducted with adult patients with bleeding disorders vaccinated against COVID-19 (8%) (12). This difference might stem from the fact that many of our patients had received regular factor replacement.

Overall, 17% of the patients screened during the study were unvaccinated. The percentage of unvaccinated individuals for COVID-19 among the patients we followed up with was twice as high as that of the unvaccinated individuals within the general population of Turkey. Kocher F et al. found a higher proportion of bleeding disorders in patients vaccinated against COVID-19 compared to the general population (13). The most common reasons why our patients did not get vaccinated could be the side effects of the vaccines and misinformation on social media (14). Patients with bleeding disorders have high bleeding stress factors. Hence, the high proportion of unvaccinated individuals among our patients may be attributed to their fear of increased bleeding or vaccine-related complications.

The limitations of this study included not being able to directly observe whether the patients developed bleeding complications following each dose of vaccination, the retrospective evaluation of the data, and the small number of patients due to the inclusion of a single center.

Hence, the results of this study demonstrate that the rate of bleeding complications remains low in patients with bleeding disorders if they receive intramuscular vaccination after necessary precautions. Post-vaccination bleeding complications could be prevented if patients seek counseling from specific bleeding disorders specialists. The strength of this study is that it could serve as guidance for specialists and patients with bleeding disorders interested in the intramuscular administration of COVID-19 vaccines. Larger-scale, randomized, and prospective studies examining patients with bleeding disorders are required.

Conflict of interest

We declare no financial or other relationships leading to a conflict of interest. All authors read and approved the manuscript.

Funding

None to declare.

Acknowledgments

None to declare.

Authors' contributions

Concept: M.H.A, E.K., Design: M.H.A, Data Collection or Processing: M.H.A, Analysis or Interpretation: M.H.A, Literature Search: M.H.A., E.K, Writing: M.H.A., E.K.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 770-775
doi: 10.52142/omujecm.39.3.34

The effects of body weight and pneumoperitoneum on pleth variability index and total haemoglobin in patients undergoing laparoscopic cholecystectomy: A prospective observational study

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Received: 04.04.2022 • **Accepted/Published Online:** 30.06.2022 • **Final Version:** 30.08.2022

Abstract

It is not clear if there is an inaccurate effect of obesity and pneumoperitoneum on non-invasive monitoring parameters. The aim of this study is to determine the effects of CO_2 pneumoperitoneum and desufflation on non-invasive monitoring variables between obese and non-obese patients undergoing laparoscopic surgery. Sixty patients were included in the study that underwent laparoscopic cholecystectomy between February 2019 and November 2019. After induction of anaesthesia, systolic and diastolic blood pressures, heart rate, duration of operation and pneumoperitoneum, pleth variability index (PVI) and total hemoglobin (SpHb) data were recorded. The patients were divided into two groups. There was no significant difference between the groups in terms of chronic diseases and duration of operation and pneumoperitoneum. No significant difference was found between the groups in PVI and SpHb values. In comparison of PVI measurement values with T0, a significant difference was found in T3, T4 and T5 in Group 2 ($p = 0.010$, 0.012 and 0.041, respectively). In comparison of SpHb measurement values with T0, a significant difference was found in T1, T2 and T3 in Group 2 ($p = 0.008$, 0.010 and 0.037, respectively). In comparison with T5, a significant difference was found in T1, T2 and T3 in Group 2 ($p = 0.023$, 0.005 and 0.006, respectively). In obese people, pneumoperitoneum can lead to errors in PVI and SpHb monitoring. Although these two parameters are very valuable in intraoperative follow-up, they should be used carefully during laparoscopic procedures in obese patients. If these parameters (PVI and SpHb) are considered to be used in bariatric surgeries, this information should be considered.

Keywords: Laparoscopic cholecystectomy, pneumoperitoneum, pleth variability index, total hemoglobin

1. Introduction

With the development of mini-invasive technique, laparoscopic procedures form the basis of general surgery. After the introduction of laparoscopy in surgery, laparoscopic cholecystectomy has been adopted as the gold standard in the surgical treatment of cholelithiasis and gallbladder diseases (1). CO_2 pneumoperitoneum method is used in exposing sufficient image and surgical field in laparoscopy. Usually, artificial pneumoperitoneum is established by CO_2 insufflation, and intra-abdominal pressure (IAP) is frequently maintained between 10 and 15 mmHg.

There are many advantages of laparoscopic cholecystectomy (such as short hospital stay, minimal postoperative pain and rapid recovery), and systemic disadvantages are associated with increased IAP. In laparoscopic cholecystectomy procedures, cardiopulmonary changes may be observed due to the pneumoperitoneum (2). Since preload is significantly affected by IAP increase, it is important to determine the effect of IAP increase on fluid response in patients undergoing laparoscopic surgery (3).

Today, hemodynamic monitoring includes static parameters such as pulmonary capillary wedge pressure and central venous pressure, as well as dynamic parameters such as pulse pressure variation and stroke volume variation (4). One of the dynamic parameters used to predict fluid response in mechanically ventilated patients is the pleth variability index (PVI) obtained by continuous and automatic calculation of pulse oximetry plethysmographic waveform variability during the respiratory cycle.

The Masimo Radical-7 (Masimo Corporation, Irvine,

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CA) device provides an easy and continuous measurement using a finger probe. This device has been validated to predict fluid sensitivity in patients who are mechanically ventilated and undergo open abdominal surgery (5, 6). A continuous, non-invasive hemoglobin level monitoring is provided simultaneously with the same probe. This has also been shown to correlate with blood haemoglobin levels (7). As this device can be used non-invasively, it has become the preferred method for hemodynamic monitoring in patient follow-up.

However, factors such as arrhythmia, hypothermia, spontaneous breathing activity, vasoactive drug use and impaired peripheral perfusion limit the availability of PVI and SpHb monitoring (8). The ability of these dynamic parameters to predict fluid response and blood loss may be affected by respiratory, hemodynamic and physical changes that cause intrathoracic pressure change (9).

There are few studies examining the effect of pneumoperitoneum and obesity on the reliability of PVI and SpHb. Both factors have previously been shown to significantly affect the cardiopulmonary physiology and function, and potentially affect the performance and reliability of these devices (10). There are studies investigating the effects of both pneumoperitoneum and obesity in gastric bypass operations, but there is no study investigating the effects of pneumoperitoneum with equal IAP in obese and non-obese patients.

The aim of this study was to determine the effects of continuous CO_2 pneumoperitoneum with a pressure of 12 mmHg and desufflation on non-invasive monitoring variables in obese and non-obese patients undergoing laparoscopic surgery.

2. Materials and methods

2.1. Subjects

This study was carried out in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Harran University (Approval Number 2019.4.20). After the approval of the hospital ethics committee, the study was registered in the Australian New Zealand Clinical Trials Registry (Trial Id: ACTRN12620000169943). Patients who underwent elective laparoscopic cholecystectomy under general anaesthesia for cholelithiasis between November 2019 and February 2019 were included in this cross sectional study. Written informed consent was obtained from all patients. Patients older than 18 years with written consent and with an American Anesthesiologists Association (ASA) physical score 1 or 2 were included in the study. Exclusion criteria were pregnancy, acute or chronic lung disease, heart failure, body mass index (BMI) below 18 and above 45, intraoperative bleeding of more than 100 mL, intraoperative inotropic support requirement and pneumoperitoneum duration > 45 min and < 20 min. All patients were fasted for at least 8 hours before surgery, and none of the patients received premedication.

2.2. Anaesthesia management and hemodynamic monitoring

Before the induction of general anaesthesia, standard anaesthetic monitoring was performed with electrocardiography (ECG), peripheral O2 saturation (SpO2) and non-invasive arterial pressure (NIBP) monitoring. The MightySat Rx Fingertip Pulse Oximeter (Masimo Corporation, Irvine, CA) probe was attached preventing it from being affected by light to the upper extremity index finger tip, where the blood pressure cuff was not attached. Induction of anaesthesia was performed by the same anaesthetist with fentanyl (2 µg/kg) and propofol (2–3 mg/kg). Rocuronium bromide 0.6 mg/kg was used as a muscle relaxant. Volume-controlled ventilation was applied to the patients after intubation. Ventilator settings were made as follows: tidal volume was 8 mL/kg according to ideal body weight, positive end-tidal pressure (PEEP) was 5 cmH2O, inspirium–expirium ratio was 1: 2, respiratory rate was 11–14 breaths/min and end-tidal carbon dioxide pressure (EtCO2) was adjusted to be 35–45 mmHg. Sevoflurane and IV remifentanil (0.25–0.5 µg/kg/min) infusion was used in the mixture of air and oxygen for maintenance of anaesthesia.

2.3. Study design

Data were collected after induction of anaesthesia in all patients. In order to minimise the effect of vasomotor tone, any stimulation to the patients was avoided 5 min before the data collection. PVI and SpHg data were recorded by the same anaesthesiologist in six pre-determined times in the end-expiratory phase.

T0 = basal value after insufflation

T1 = 5^{th} min after insufflation

T2 = 10^{th} min after insufflation

T3 = 15^{th} min after insufflation

T4 = 5 min before desufflation

T5 = 2 min after desufflation

Systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR), oxygen saturation (SpO_2) and $EtCO_2$ values were recorded in the same time frames. In addition, BMI, duration of operation and pneumoperitoneum of the patients were recorded. Crystalloid solution of 8 mL/kg was administered to patients before the induction of anaesthesia in order to prevent sudden decrease of blood pressure, thus minimising the impact of PVI and SpHb values. Crystalloid infusion continued at the rate of 4 mL/kg/h throughout the operation.

2.4. Statistical analysis

All statistical analyses were calculated by SPSS 22.0

(Statistical Packages for the Social Sciences, SPPS Inc., Chicago, IL, USA). The normal distribution was determined by Kolmogorov Smirnov test and histogram. All the parameters were not normally distributed probably because of the low group sizes. Non-parametric tests were used for calculations. The continuous variables were expressed as median (minimum–maximum). The categorical variables were expressed as n (%). The differences of continuous variables were calculated by the Mann–Whitney U test, and the Wilcoxon test was used for repeated measures. Chi-Square test was used to determine the difference between groups of categorical variables. $P < 0.05$ was considered as statistically significant. The sample size was determined by using the preliminary data of 10 patients. In power analysis, a minimum of 48 patients were shown for $\beta = 0.1$ and $\alpha = 0.05$.

3. Results

After obtaining the ethics committee approval, 74 patients were included in the study. Out of 74 patients, 60 patients were included in this study, after exclusion of 14 patients as per the exclusion criteria (Fig. 1).

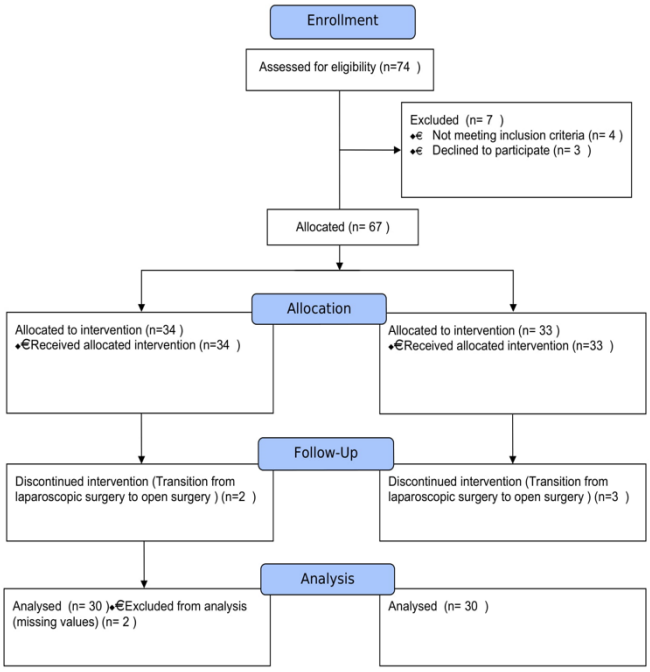

Fig.1. CONSORT (Consolidated Standards of Reporting Trials) checklist

Thirty patients with BMI < 30 were classified as Group 1, the remaining 30 patients with BMI ≥ 30 were classified as Group 2. The mean age of the patients was 38.22 ± 10.35 years, and 61.6% of the patients were women. There was no significant difference between the groups in terms of chronic diseases and duration of operation and pneumoperitoneum. Demographic data of the patients are shown in Table 1.

Table 1. Demographic data.

	Total	BMI group		p
		Group 1 BMI<30 (kg/m²)	Group 2 BMI≥30 (kg/m²)	
	median (min-max) / n(%)			
Sex (female)	18.5 (61D,6%)	18 (60%)	19 (63.3%)	0.502*
Age, years	38.22±10.35	40.05±10.23	36.40±10.52	0.235 **
BMI (kg/m²)	30.2 (19-52)	25.8 (19-29.2)	32 (30.2-52)	-
Chronic disease	10 (17.2%)	3 (10%)	7 (25%)	0.173*
Hypertension	8 (13.1%)	1 (3.3%)	7 (22.6%)	0.053*
Diabetes Mellitus	2 (3.3%)	-	2 (6.5%)	0.492*
COPD	2 (3.3%)	2 (6.7%)	-	0.238*
Operation duration	40 (28-60)	35.5 (30-60)	40 (28-60)	0.446 **
Pneumoperitoneum duration	26 (20-45)	26 (20-45)	30 (20-45)	0.253* *

BMI: Body mass index, COPD: Chronic obstructive pulmonary disease

Data on SBP, DBP and HR are shown in Figs. 2 and 3.

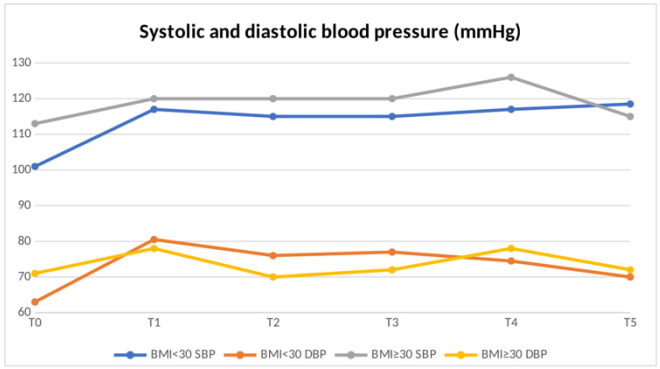

Fig.2. Systolic and diastolic blood pressure (mmHg)

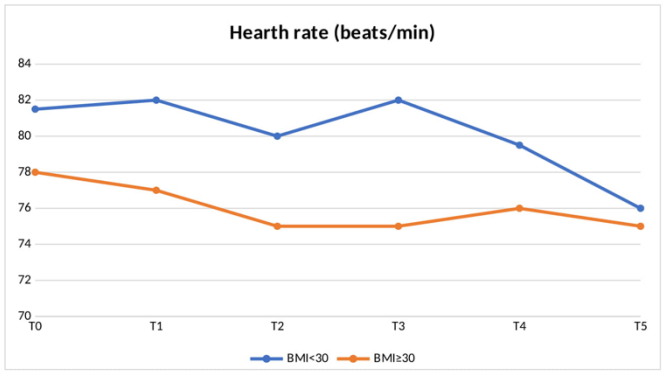

Fig. 3. Heart rate (beats/min)

From these data, it was found that only the baseline value of SBP was significantly higher in Group 2 ($p = 0.007$), but there was no significant difference between the groups in time frames and other measurements. The comparison of haemodynamic monitoring parameters between T0 and T5 is shown in Table 2.

Table 2. Hemodynamic monitoring parameters

	PVI		p*	SpHb		p*
	Group 1 BMI<30	Group 2 BMI≥30		Group 1 BMI<30	Group 2 BMI≥30	
T0	13 (6-45)	14 (8-50)	0.478	11.9 (9.8-14.5)	11.2 (9.7-14.8)	0.133
T1	15 (6-38)	15 (3-29)	0.729	11.2 (9.3-15)	10.9 (8.6-14.7)	0.222
T2	14.5 (8-32)	15 (5-25)	0.597	11 (9.5-15.5)	11.2 (8.8-14.7)	0.242
T3	12.5 (7-32)	13 (6-23)	0.465	11.1 (9.6-16)	11.3 (8.9-4.7)	0.239
T4	12 (6-33)	13 (4-29)	0.739	11.1 (9.8-16)	11.3 (9.1-14.8)	0.386
T5	12.5 (7-36)	15 (5-33)	0.845	10.9 (9.7-14.3)	11.6 (9-14.9)	0.756
p - Comparison with T0**						
T1	0.361	0.224		0.299	**0.008**	
T2	0.713	0.060		0.392	**0.010**	
T3	0.991	**0.010**		0.626	**0.037**	
T4	0.639	**0.012**		0.750	0.263	
T5	0.819	**0.041**		0.434	0.829	
p - Comparison with T5**						
T0	0.412	0.224		0.247	0.125	
T1	0.301	0.363		0.359	**0.023**	
T2	0.410	0.895		0.468	**0.005**	
T3	0.891	0.575		0.896	**0.006**	
T4	0.775	0.514		0.604	0.057	

*Mann-Whitney U test, **Wilcoxon signed rank test

PVI: Pleth Variability Index, SpHb: Total haemoglobin, T0=basal value after insufflation,T1 = 5th minute of insufflation,T2=10th minute of insufflation, T =15th minute of insufflation, T4 =5 minutes before desufflation, T5=2 minutes after desufflationers

No significant difference was found between the groups at all measurement times for PVI and SpHb values. Figs. 4 and 5 show the changes of PVI and SpHb over time. Measurements of all time periods were compared with T0 and T5 separately. In comparison of PVI measurement values with T0, a significant difference was found in T3, T4 and T5 in Group 2 (p = 0.010, 0.012 and 0.041, respectively). In comparison with T5, no significant difference was found in both groups. In comparison of SpHb measurement values with T0, a significant difference was found in T1, T2 and T3 in Group 2 (p = 0.008, 0.010 and 0.037, respectively). In comparison with T5, there was a significant difference in T1, T2 and T3 in Group 2 (p = 0.023, 0.005 and 0.006, respectively).

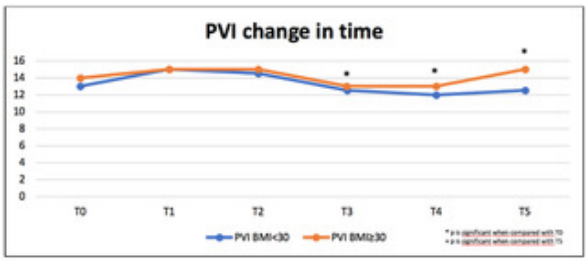

Fig.4. PVI change in time

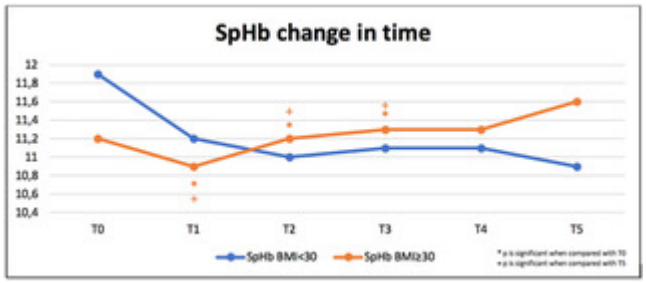

Fig.5. SpHb change in time

4. Discussion

Our study observed a false increase in PVI and a false decrease in SpHb with abdominal insufflation. Although this situation developed in all patients, it was found statistically significant only in the obese patient group (Group 2). SpHb was affected by desufflation and insufflation in obese patients.

As a result of advanced medical practices, the elderly population is increasing all over the world. Thus, the need for more surgery and the importance of haemodynamic monitoring are increase. Invasive procedures such as pulmonary artery catheters, central venous catheters and transesophageal echocardiogram require special training, increase complications, cost and application time (11, 12). Laboratory parameters such as haemoglobin and lactate and base deficit (BE) are often used in the process of intraoperative evaluation, but these measurements are not effective as they are time-consuming and expensive. Therefore, the use of non-invasive haemodynamic monitoring is increasingly important.

Estimation of blood loss and evaluation of the intravascular volume during surgery is still a major problem. Changes in intravascular volume or airway pressures can cause changes in cardiac pumping ability (13). However, as with cardiogenic shock, not all reductions in cardiac output are associated with intravascular volume. There are studies on the poor consequences of intraoperative excess fluid overload in patients. It has been reported that the application of each litre of extra fluid added to the intraoperative fluids increases the risk of postoperative symptoms and complications by 16% and 32%, respectively (14). It has been stated that patient admissions are shortened, surgical site infections are reduced and perfusion improves with sufficient but not much fluid administration (15,16). Therefore, the relevance of fluid administration has been studied for many years, and it has been reported that dynamic measurements based on cardiopulmonary interactions in mechanically ventilated patients are the best predictors of fluid response (17). Stroke volume variation, pulse pressure variation and PVI are among the most frequently used dynamic parameters in the management of perioperative fluid therapy.

PVI allows clinicians to determine fluid sensitivity in critically ill patients. SpHb allows continuous analysis of

haemoglobin concentration and helps in making decisions about blood transfusions. It is especially important for operations where there is high blood loss and time loss cannot be tolerated (18). The reliability of these parameters has been confirmed in intensive care patients and open abdominal cases in the operating room (5,19,20). However, the effect of pneumoperitoneum on these parameters in obese patients is not clear.

In fact, studies evaluating the effects of pneumoperitoneum on non-invasive haemodynamic monitoring parameters have attracted attention in recent years. Hoiseth et al. (21) showed that PVI increased during pneumoperitoneum in their studies in which they investigated the dynamic variables of fluid responsiveness in patients undergoing laparoscopic surgery. Liu et al. (3) showed that PVI increased with pneumoperitoneum and PVI value decreased to pre-pneumoperitoneum values after desufflation. However, the number of studies investigating the effects of obesity is very limited. Determination of intravascular volume is critical in obese patients. Pneumoperitoneum created for adequate visualisation of the operative area in laparoscopic surgeries results in an elevated IAP. As with non-obese patients, IAP is adjusted up to 12–15 mmHg during laparoscopy in obese patients. Normal IAP of non-obese individuals is 5 mmHg or less (22). In contrast, obese patients may have a chronically elevated IAP up to 9–10 mmHg (23). Increase in IAP increases venous stasis, reduces portal venous blood flow, increases airway pressure and impairs heart function (24). To minimise these effects, it is necessary to optimise the intravascular volume to make appropriate ventilation adjustments, to use appropriate compression devices to minimise venous stasis and to minimise the effects of increased IAP on kidney and heart function. At this point, the importance of haemodynamic monitoring is obvious for intravascular volume optimisation.

In this study, in which we examined the effects of pneumoperitoneum on non-invasive haemodynamic monitoring parameters, PVI and SpHb, we observed that PVI increased and SpHb decreased by abdominal insufflation in all patients. However, these changes were only significant in the obese patient group. DeBarros et al. (13) compared open surgery, laparoscopic obesity surgery and laparoscopic surgery in non-obese patients. In this study, they identified an incorrect increase in PVI with insufflation. This finding coincides with our study. In the same study, they observed a change on SpHb with insufflation, but this change was not statistically significant.

The difference between our study and this study may be because of inclusion of different types of laparoscopic surgeries. Since we investigated the effects of obesity and pneumoperitoneum in our study, we preferred laparoscopic cholecystectomy with minimal fluid loss and bleeding that may affect PVI and SpHb measurements and a relatively short duration for operation. We tried to provide standardisation by including the single type of surgery in our study. We also tried to minimise the effects of comorbid diseases by including only ASA 1–2 patients in our study. De barros et al. included different types of laparoscopic surgeries without ASA limitation in their studies. As a matter of fact, the average ASA level was stated as 3 in their studies. These situations may be the reason for the differences between the two studies.

Our study has some limitations. First of all, it is an observational study with limited sample size. Second, we included only ASA 1–2 patients in our study. As we evaluated the effects of obesity and pneumoperitoneum in our study, we did not investigate PVI and SpHb changes that might occur because of different comorbid diseases. More studies are needed for ASA 3–4 patients. Finally, we preferred laparoscopic cholecystectomy to standardise patient groups. The amount of intraoperative bleeding was minimal, and the need for blood transfusion did not occur in any patient. Further studies are needed to evaluate the effect of obesity and pneumoperitoneum on non-invasive monitoring in large laparoscopic operations such as large oncological resections, thoracic and colorectal surgeries, where blood loss is predicted and blood transfusion is required.

As a result, pneumoperitoneum in obese patients can lead to errors in PVI and SpHb monitoring. Although these two parameters are very valuable in intraoperative follow-up, they should be used carefully during laparoscopic procedures in obese patients. If these parameters (PVI and SpHb) are considered to be used in bariatric surgeries, which are the most common surgical procedures with the combination of obesity and pneumoperitoneum, this information should be considered. According to our knowledge, this study is the first study with standardised patient groups on the reliability of PVI and SpHb of obesity and pneumoperitoneum. In order to evaluate the usefulness of non-invasive haemodynamic monitoring in laparoscopic procedures in obese patients, further research is needed in larger laparoscopic procedures.

Conflict of interest

The authors declared no conflict of interest.

Funding

No funding was used for the study.

Acknowledgments

None to declare.

Authors' contributions

Concept: G.P., M.P., Design: G.P. ,M.P., T.G., Data Collection or Processing: M.P., T.G., M.K., Analysis or Interpretation: G.P., M.P., M.K., Literature Search: G.P., M.P., Y.G., M.K., Writing: G.P., M.P., T.G., M.K.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 776-780
doi: 10.52142/omujecm.39.3.35

Anatomical variations of cervical segment of internal carotid artery in patients with subarachnoid hemorrhage

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Received: 01.04.2021 • **Accepted/Published Online:** 14.08.2022 • **Final Version:** 30.08.2022

Abstract

The cervical segment of an internal carotid artery (cICA) usually has a straight vertical course without any branching. In the present study, variations of the cICA were evaluated based on the three-dimensional volume-rendered neurovascular images. The computed tomography angiography images of 56 patients diagnosed with subarachnoid hemorrhage (SAH) were evaluated retrospectively. Two separate researchers, blinded to each other and clinical information of the patients, evaluated the courses of cICA bilaterally. The variations were classified as tortuosity, kinking, and coiling. A total of 112 cICA segments of 56 patients were evaluated. The cICA variations were present in 21.4% of patients and 17.9% of segments. There was tortuosity in 5 (8.9%) patients, kinking in 4 (7.1%) patients, and coiling in 6 (10.7%) patients. Of 41 patients with aneurysmal SAH, there were a cICA variation in 8 (19.5%) patients. A cICA variation was detected in 4 (26.7%) of 15 patients with idiopathic SAH. The cICA variation ratio in SAH patients was concordant with studies performed in different patient groups. Although the ratio was slightly higher in idiopathic SAH patients, there was no statistical significance between the aneurysmal and idiopathic SAH groups. The coiling was more frequent in SAH patients compared to previous studies. The cICA variations tend to be bilateral in SAH patients.

Keywords: internal carotid artery, tortuosity, coiling, kinking, subarachnoid hemorrhage, dolichoarteriopathy

1. Introduction

The internal carotid arteries arise from the common carotid arteries bilaterally. The origins of the common carotid arteries are different on both sides. On the right side, the common carotid artery originates from the brachiocephalic trunk, which is the first branch of the aortic arch. On the other hand, the common carotid artery arises directly from the aortic arch on the left side. The common carotid artery is divided into the external and internal carotid arteries at the level of the superior border of the thyroid cartilage. The internal carotid artery runs vertically upward without major changes in its direction. It lies ventral to the transverse processes of the atlas, axis, and third cervical vertebrae. The internal carotid artery enters the skull via the carotid foramen of the temporal bone. For the first time, Fischer classified the segments of the internal carotid artery based on the angiographic evaluation without considering the direction of blood flow in 1938 (1). Bouthillier and several other authors have proposed different classification systems by numbering the internal carotid artery segments in the direction of blood flow (2). The portion of the internal carotid artery that extends from the carotid bifurcation to the carotid foramen is commonly accepted as the C1 or cervical segment in all proposed classification systems.

The internal carotid artery usually has a straight vertical course along the cervical segment without any branching until it reaches the carotid foramen. The anatomical variations along the cervical segment were reported in 4-66% of cases (3). Tortuosity (S or C-shaped elongation), kinking, and coiling of the artery are the anatomical variations reported previously (4). They may be congenital or acquired in etiology. In the current study, variations of the internal carotid artery along the cervical segment in SAH patients were evaluated based on the three-dimensional volume-rendered neurovascular images by describing the type of variation and reviewing the literature regarding the diagnosis, causes, symptoms, and clinical significances of the variations.

2. Materials and Methods

The study was performed after the approval of Ondokuz Mayıs University clinical research ethics committee with the number of 2014/1364. Fifty-six patients diagnosed with subarachnoid hemorrhage (SAH) were included in this study. The computed tomography angiography images of all patients were evaluated retrospectively.

Imaging data were stored in digital imaging and communications in medicine (DICOM) format and analyzed

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with Osirix 64 MD imaging software [OsiriX Foundation, Geneva, Switzerland]. Three-dimensional reconstructed images of the cervical segment of ICA (cICA) were obtained by using the 3D volume rendering function of the software.

Two separate researchers, blinded to each other and clinical information of the patients, assessed the morphology of cICA. In case of discordance, a third opinion was obtained from another researcher who was blinded to others and clinical information of the patients. Courses of the cICA were evaluated bilaterally for each patient. Regarding the classification of Weibel and Fields, S- or C-shaped elongation with medial or lateral displacement of the elongated segment was defined as tortuosity (Fig. 1). Any sharp angulation less than 90° was defined as kinking (Fig. 2), and the looped course of the artery was defined as coiling (Fig. 3) (4).

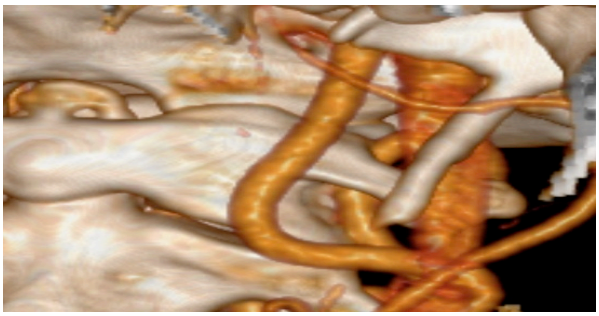

Fig. 1. Tortuosity of the left internal carotid artery cervical segment (3 dimensional reconstruction of computed tomography angiography images)

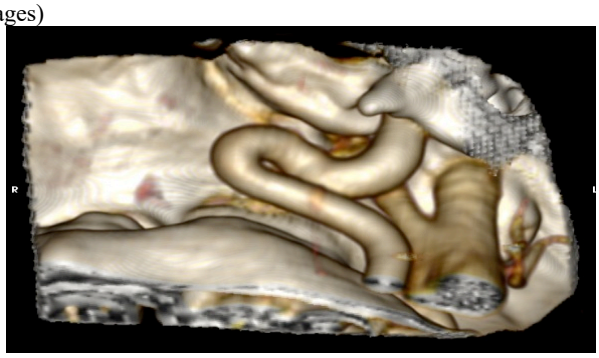

Fig. 2. Kinking of the left internal carotid artery cervical segment (3 dimensional reconstruction of computed tomography angiography images)

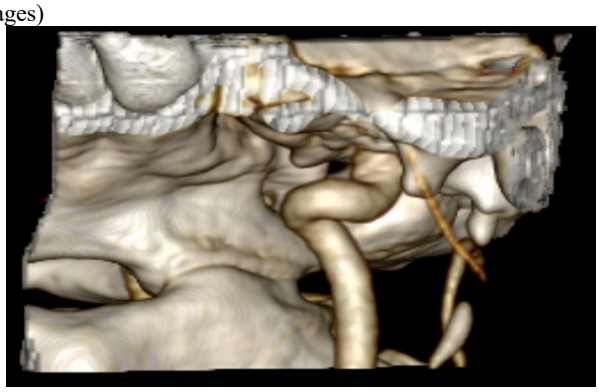

Fig. 3. Coiling of the left internal carotid artery cervical segment (3 dimensional reconstruction of computed tomography angiography images)

Statistical analyses were performed with IBM SPSS version 22.0 for windows. The cICA course variations of the aneurysmal and idiopathic SAH groups were compared by Chi-square test.

3. Results

The computed tomography angiography images of 56 SAH patients (27 male, 29 female) were evaluated. The age range of patients was 36-83 (mean, 57.96±9.61 years). Of 56 patients, the cICA segment variations were present in 12 (21.4%) patients. Male: female ratio was 1.4 (7 (58.3%) male, 5 (41.7%) female). Their mean age was 59.25±7.85. There was tortuosity in 5 (8.9%) patients, kinking in 4 (7.1%) patients, and coiling in 6 (10.7%) patients (Table 1). Variations were bilateral in 8 (66.6%) patients and unilateral in 4 (33.4%) patients. While 5 (62.5%) of 8 patients with bilateral abnormal course had symmetrical variations, the remaining 3 (37.5%) of them had different types of variations on both sides. We found coiling either on the left or right side of all patients with an asymmetrical bilateral variation. Of 4 patients with a unilateral abnormal course, variation was on the right side in 3 patients and on the left side in 1 patient.

A total of 112 cICA segments were evaluated in 56 patients. While the 92 (82.1%) cICA segments had a straight course, there were variations in 20 (17.9%) cICA segments. There was tortuosity in 8 (7.16%) segments, kinking in 6 (5.36%) segments, and coiling in 6 (5.36%) segments. Tortuosity was S-shaped in 1 segment and C-shaped in 7 segments (Table 1). Of the 20 cICA segment variations, 9 (45%) were on the right side, and 11 (55%) were on the left side. Five of the six coilings were on the left side. There was no such dominancy in either tortuosity or kinking.

Table 1. cICA variations in patients and in segments

	In patients (n=56)	In segments (n=112)
cICA variations	12 (21.4%)*	20 (17.9%)
• Tortuosity	5 (8.9%)	8 (7.16%)
• Kinking	4 (7.1%)	6 (5.36%)
• Coiling	6 (10.7%)	6 (5.36%)

*8 bilateral, cICA: cervical segment of internal carotid artery

An aneurysm was detected as a reason for SAH in 41 (73.2%) of 56 patients (aneurysmal SAH). The reason for bleeding could not be found in 15 (26.8%) patients (idiopathic SAH). Of 41 patients with aneurysmal SAH, there were cICA variations in 8 (19.5%) patients. A cICA variation was detected in 4 (26.7%) of 15 patients with idiopathic SAH (Table 2). Regarding the cICA course variations, there was no statistically significant difference between the aneurysmal and idiopathic SAH groups (p= 0.563). Interestingly, all female patients with abnormal course had an aneurysm as a reason of SAH.

Table 2. Subarachnoid hemorrhage types and the internal carotid artery cervical segment variations

SAH type	cICA variations	P value
Aneurysmal	8 (19.5%)	
Idiopathic	4 (26.7%)	>0.05

SAH: Subarachnoid hemorrhage;
cICA:cervical segment of internal carotid artery

4. Discussion

Although the cICA usually has a straight vertical course without any branching from the bifurcation of the common carotid artery to its entrance into the carotid foramen, it may have some variations in its course. These variations usually depend on the elongation of artery. Weibel and Fields classified these variations as tortuosity, kinking, and coiling (4). All of them are results of elongation of the cICA. Displacement of an elongated segment to the medial or lateral side is defined as tortuosity. It may be S- or C- shaped. Less than 90° of angulation of the vessel is defined as kinking. Coiling is the looped course of the artery.

Etiologically, variations of the cICA may be a congenital or acquired condition. Some of them are thought to be of congenital origin as they have been observed in children, and their prevalence showed no increase with age (5). Khasiyev et al. found no correlation between cICA variations and age (6). The cICA develops from the third aortic arch and dorsal aorta. Normally there is a loop between two arteries in the embryonic life stage. The cICA becomes straight with the descent of the large arteries and heart into the mediastinum. Any failure of this process may result in a loop or kink formation (7, 8). But there is no proof supporting this suggestion in the literature. On the other hand, the increased prevalence of cICA variations, especially kinking, with ageing, atherosclerosis and hypertension suggest that it is an acquired condition (9, 10). Buckling is an important factor for tortuosity. Increased lumen pressure, weakened wall and decreased axial tension cause buckling thus tortuosity formation (11). La Barbera et al. suggested that the cICA is a transitional segment between the common carotid artery (an elastic vessel) and intracranial ICA (a muscular vessel) (12). They found metaplastic changes in the tunica media of ICA, which are triggered by a stimulus such as hemodynamic forces, and they suggested that unproportional changes in vessel wall may cause buckling. Fibromuscular dysplasia also may be an etiological factor for a few patients (13).

The cICA variations are generally asymptomatic. They usually remain undiscovered until a symptom occurs due to degenerative changes such as atherosclerosis (14, 15). Riser has noticed the association between carotid kinking and cerebrovascular insufficiency for the first time (16). Although several studies suggest poor correlation between cICA variations and atheromatous plaque formation or cerebrovascular stroke, the cICA variations are thought to be an important predisposing factor for cerebrovascular insufficiency even in the absence of atherosclerosis (14, 17-19). Kinking with less than 60° of angle increases cerebral infarction risk (20).

The cICA variations presenting as a pulsatile mass may cause an abnormal sensation in the throat (21). They are also reported as a possible cause of hypoglossal nerve palsy, hemilingual spasm, and snoring (22-24).

Otolaryngologists have published many papers about the importance of cICA variations, because cICA injuries may cause fatal hemorrhages during tonsillectomy (25, 26). The cICA may be injured during the neck dissection phase of any surgical intervention performed on the neck, such as tonsillectomy, adenoidectomy, peritonsillar abscess drainage (27). The injury risk increases if the variations are not realized preoperatively or the surgeon is unaware of the probability.

Neurosurgical interventions such as upper cervical discectomy, instrumentation of odontoid fractures, and carotid endarterectomy need detailed anatomy knowledge of the cICA variations as well as the normal anatomy.

The cICA variations have a significant impact on endovascular interventions, either for imaging or treatment of cerebral vascular pathologies. Carotid stenosis is treated by carotid endarterectomy or stenting; cICA variations may complicate both. A coiling or kinking at the distal part of stenosis may cause technical failure of carotid stenting, especially during delivery of a distal protection device, which is used to prevent embolism (28-32).

The cICA variations may also complicate the endovascular interventions by preventing access to the intracranial vascular pathologies such as aneurysms and arteriovenous malformations (33).

The prevalence of cICA variations is 4-66% in literature (3). We found cICA variations in 21.4% of patients and 17.9% of cICA segments, in line with the literature. Several studies report female or male predominancy, or no sexual differences. Even though there is a tendency for a female predominance of cICA variations in literature, our male: female ratio (=1.4) is concordant with Koskas et al. (3, 6, 15, 34). Bilateral variations were reported as 24-40% in previous studies (9, 15, 35). We found that 66.6% of the cICA variations were bilateral.

There are different prevalence ratios of cICA variations in the literature regarding Weibel and Fields classification. Kinking is the most frequent, and coiling is the least frequently reported variation (3, 17). But these studies are usually performed on highly selected patients with neurologic or cardiovascular symptoms. Their result cannot be attributed to the normal population. Different prevalence rates may be explained by the non-randomized selection of the study population, different diagnostic techniques, or some nuances in the classification of cICA variations. Paulsen et al. reported

that the curved course of the cICA was more frequent than kinking and coiling in their cadaver study, which was performed on head and neck preparations randomly obtained from the anatomy department (3). Coiling was the most frequent variation (10.7 % of the patients) in our study. The coiling ratio was reported as 1.4-4% in previous studies (3, 5, 15, 17, 34-36). In the present study, the coiling ratio of 10.7% was more than two times higher than these values. The prevalence of coiling is clearly higher in SAH patients.

The relationship between some vascular pathologies and cICA course variations is a known issue, such as cICA aneurysms and dissections (37, 38). Hamada et al. reported a relationship between AVM nidus size and severity of tortuosity (39). The most important limitation of the present study is the highly selective patient population, which all have SAH. But this limitation provides us with additional information about cICA variations in patients with SAH. The cICA variation ratio in SAH patients was 21.4% in the present study, which was concordant with previous studies performed on normal or selective populations with pathologies other than SAH. This ratio was slightly lower (19.5%) in aneurysmal SAH patients and slightly higher (26.7%) in idiopathic SAH patients. There was no statistical significance when we compared the aneurysmal and idiopathic SAH groups regarding the cICA course variation. In contrast to the present study, the prevalence of convolution and looping in patients with aneurysmal SAH was higher than in idiopathic SAH, according to Nikiforov et al. (40).

The cICA variations may cause fatal vascular injury in surgical interventions performed on the neck, such as tonsillectomy, adenoidectomy, and upper cervical discectomy. Their prevalence in SAH patients is concordant with the normal population. But the coiling ratio in SAH patients reported here is one of the highest in the literature. There is no difference between aneurysmal SAH patients and idiopathic SAH patients regarding the cICA variations. A prospective randomized study performed on more patients may give more objective results.

Conflict of interest

The authors declare that there is no conflict of interest.

Funding

No funding was used for the study.

Acknowledgments

None to declare.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 781-785
doi: 10.52142/omujecm.39.3.36

Conversion from laparoscopic cholecystectomy to open surgery reasons and possible risks: A single center experience

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Received: 29.03.2022 • **Accepted/Published Online:** 30.06.2022 • **Final Version:** 30.08.2022

Abstract

Laparoscopic cholecystectomy has grown in popularity since its introduction and has become the gold standard surgical method for treating cholelithiasis. It has several advantages over open surgery, including less postoperative pain, a shorter hospital stay, a faster return to normal activities, and better cosmetic results. Converting from laparoscopic to open surgery may be necessary in some cases, either to prevent or treat difficulties. The current conversion rate from laparoscopic to open surgery is between 2 and 15%. Open surgery may be required due to advanced age, male gender, acute cholecystitis, anatomical alterations of the gallbladder or biliary system, obesity, bleeding, adhesions, and biliary tract injuries. Our study aimed to determine the risk factors that influence the conversion to open surgery. Between January 2018 and December 2021, we analyzed 921 cholecystectomy cases retrospectively at the Recep Tayyip Erdogan University Training and Research Hospital. We excluded twenty-three of these patients from the study once it was revealed that they had undergone direct open surgery or an open cholecystectomy while undergoing another operation. Twenty-eight patients had a laparoscopic cholecystectomy but had to convert to open surgery due to difficulties. We obtained the demographic and clinical information about the patients from hospital records. We analyzed whether these characteristics had a significant impact on the conversion from laparoscopic to open surgery by comparing the data of an equal number of randomly selected laparoscopic completed patients. The most common reason for conversion from laparoscopic to open cholecystectomy was adhesion due to inflammation, which accounted for 3.04 percent of conversions. In our study, cholecystitis symptoms, including multiple calculi on ultrasonography and increased wall thickness, and raised GGT and ALP levels, all affected the decision to convert to open surgery. We discovered no significant associations between gender, pancreatitis, cholangitis, stone size, and ASA score. The duration of hospitalization was considerably longer in the group that converted to open surgery. Increased rates of conversion to open surgery are associated with advanced age, obesity, previous episodes of cholecystitis, and adhesions due to previous abdominal incisions, presence of cholecystitis findings such as multiple calculi on ultrasonography, increased wall thickness and high GGT and ALP values. The most influential factor in converting to open surgery appears to have previously had cholecystitis. A preoperative patient examination can aid in predicting the risk of exposure.

Keywords: Open cholecystectomy, laparoscopic cholecystectomy, risk factors, surgery

1. Introduction

Mouret was the first to use laparoscopic surgery for cholecystectomy in 1987, and it has since become widely used for gynecological procedures (1). Its use has grown in popularity over time, and it is now the gold standard for surgical cholelithiasis treatment. In gallbladder diseases, laparoscopic surgery is most usually favored, and research shows that 85 percent of cholecystectomy cases are performed laparoscopically (1-3). Laparoscopic cholecystectomy has several advantages over open cholecystectomy, including less postoperative pain, a shorter hospital stay, a faster return to normal activities, and better cosmetic results (3, 4).

Despite its reputation as a safe surgical operation, laparoscopic cholecystectomy can cause bile duct and intestine damage and hemorrhage, which can cause severe morbidity and mortality (4). The purpose of converting to open surgery is to reduce complications. Conversion to open surgery is a method performed to ensure surgical safety, not a consequence. As a result, it is crucial to know when to convert from laparoscopic to open surgery. The rate of conversion from laparoscopic to open surgery is currently between 2% and 15% (3, 5).

The literature has shown that advanced age, male gender, acute cholecystitis, structural alterations of the gallbladder or biliary system, obesity, bleeding, adhesions, and biliary tract injuries are all efficient in converting to open surgery (6-8).

During the preoperative evaluation phase of patients

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planned for laparoscopic cholecystectomy, a good review of demographic, biochemical, and radiological results can predict the risk of conversion to open surgery. Variable risk factors among these anticipated risk parameters can aid in determining whether surgery is necessary. As a result, an appropriate operation schedule plan may be established, lowering the rate of difficulties and opening. The goal of our research was to identify the risk factors that influence the conversion to open surgery.

2. Materials and methods

2.1. Patients and data collections

Between January 2018 and December 2021, we retrospectively evaluated 921 cholecystectomy cases in the General Surgery Clinic of Recep Tayyip Erdogan University Training and Research Hospital (Ethics Committee of Recep Tayyip Erdogan University Training and Research Hospital, issued March 24, 2022, and numbered 2022/72.)

We found that open cholecystectomy was performed in 51 of these patients. In 23 of 51 patients, open cholecystectomy was started either immediately or during another procedure; we excluded these patients from the research. We assessed patients' age, gender, weight, and height, besides previous procedures, comorbidities, cholecystopathy-related laboratory test values, and ultrasonography (US) findings. Our hospital records also revealed a clinical history of acute cholecystitis, the presence of interventional procedures such as cholecystostomy or endoscopic retrograde cholangiopancreatography (ERCP), surgical findings, and the American Society of Anesthesiologists' Physical Health classification (ASA) score (9) and length of stay.

We also collected the data of 28 patients randomly selected from 921 patients who had gallbladder surgery and had it finished laparoscopically and statistically compared to see if there was a significant effect on the conversion to open surgery.

2.2. Statistical analysis

We analyzed the data with the IBM SPSS Statistics for Windows (Armonk, NY, USA, IBM Corp.) software. We presented categorical data as numbers and percentages and numerical data with mean and standard deviation values. We used the chi-square test and Fisher's Exact Test for categorical variables to determine the factors associated with conversion from laparoscopy to open cholecystectomy. We determined the distribution characteristics of continuous variables by the Shapiro Wilk and Kolmogorov-Smirnov tests and evaluated the relationships of these variables with the exposure state with the Mann Whitney U test. We accepted the statistical significance level as $p<0.05$ in all analyses.

3. Results

Between January 2018 and December 2021, we retrospectively evaluated 921 cholecystectomy cases in the General Surgery Clinic of Recep Tayyip Erdogan University Training and Research Hospital. We excluded twenty-three of these patients because they received an open cholecystectomy as part of another operation or had direct open surgery. The remaining 898 patients underwent laparoscopic cholecystectomy; however, 28 required open cholecystectomy due to various factors. In this regard, we calculated our hospital's rate of conversion to open during laparoscopic cholecystectomy as 3.04 percent.

The most prevalent reason for conversion from laparoscopic to open surgery was adhesion owing to inflammation, which accounted for 53.6 percent of the cases, followed by inability to visualize the gallbladder hilum, adhesions from prior procedures, peroperative bleeding, and further organ injury (Table 1).

Table 1. Reason for conversion to open surgery

Reason for conversion to open surgery	n	%
Adhesion due to inflammation	15	53.6
İnability to see the hilum	6	21.4
Adhesions due to previous Operations	4	14.3
Hemorrhage	2	7.1
0rgan injury	1	3.6

Patients receiving open surgery had an average age of 59, while patients undergoing laparoscopic surgery had an average age of 48, which was statistically significantly higher ($p=0.006$) (Mann Whitney U test) (Table 2).

In overweight or obese patients, the conversion rate to open surgery was 60.9 percent, whereas the rate of cases done laparoscopically was 39.1 percent, with a statistically significant difference (Mann Whitney U test, $p<0.001$). (Table 2).

Table 2. Age and BMI information of the patients

	Conversion group	Laparoscopic group	p
The average age (±SD)	59 (±14)	48 (±17)	**p<0.05**
The average BMI (kg/m^2) (±SD)	30.25 (±3.68)	25.65 (±2.28)	**p<0.001**

BMI: Body mass index, SD: Standard deviation

Males accounted for 41.1 percent (n=23) of the 56 patients enrolled in the study (28 open and 28 laparoscopic procedures completed), while females accounted for 58.9% (n=33). There was no statistically significant difference between patients who had open surgery and those who had laparoscopic surgery in terms of the male and female gender (Table 3).

Table 3. Gender and medical characteristics of patients

		Conversion group		Laparoscopic group		Total group		p
		n	%	n	%	n	%	
Gender	Male	13	56.5	10	43.5	23	41.1	**>0.05**
	Female	15	45.5	18	54.5	33	58.9	
Cholecystitis history	Yes	21	70	9	30	30	53.6	**<0.001**
	No	7	26.9	19	73.1	26	46.4	
Pancreatitis history	Yes	3	77	1	25	4	7.1	**>0.05***
	No	25	48.1	27	51.9	52	92.9	
Cholangitis history	Yes	0	0.0	2	100.0	2	3.6	**>0.05***
	No	28	51.9	26	48.1	54	96.4	
Percutaneous Cholecystostomy	Yes	2	100	0	0.0	2	3.6	**>0.05***
	No	26	48.1	28	51.9	54	96.4	
Stone size	millimetric	17	50.0	17	50.0	34	60.7	**>0.05**
	>1cm	11	50.0	11	50.0	22	39.3	
Stone number	single	3	17.6	14	82.4	17	30.4	**<0.001**
	Multiple	25	64.1	14	35.9	39	69.6	
Gallbladder wall thickness	>3 mm	19	76.0	6	24.0	25	44.6	**<0.001**
	<3 mm	9	29.0	22	71.0	31	55.4	
Priorabdominaloperation	Yes	7	58.3	5	41.7	12	21.4	**>0.05**
	No	21	47.7	23	52.3	44	78.6	

***Fisher's Exact Test is used.**

We assessed cholecystitis, pancreatitis, and cholangitis attacks for cholelithiasis consequences. In patients who had an incident of cholecystitis, we found conversion to open and laparoscopic completion as 70% and 30%, respectively, and this difference was statistically significant (Chi-square test, $p<0.001$). Furthermore, our study revealed the rate of gallbladder opening to be statistically substantially greater in patients with a gallbladder wall thickness of 3 mm or more on preoperative ultrasonography (Chi-square test, $p<0.001$) (Table 3).

This study examined whether having a cholecystectomy catheter due to pancreatitis, cholangitis, or acute cholecystitis affects the conversion to open surgery. We conducted the Fisher Exact test due to the small number of patients in this group analysed in the study and identified no statistical significance (Table 3).

While the stone size evaluation had no statistical significance, the study revealed the opening rate to be statistically high in cases with several stones (Table 3).

With a prevalence of 14.3 percent, we revealed adhesions from previous abdominal operations to be the reason for conversion to open surgery in our study. We found no statistically significant difference when comparing the incision kinds of these surgeries (Table 3).

The patients' ASA scores were largely ASA 1 and 2, and there was no statistically significant difference between the groups in terms of ASA scores ($p>0.05$) (Table 4).

We used the Mann Whitney U test to evaluate the high levels of Gamma Glutamyl Transferase (GGT), Alkaline Phosphatase (ALP), and direct bilirubin, which are among the biochemical tests performed in the preoperative period. We found all three parameters to be statistically significant in open surgery patients. ($p<0.001$) (Table 4).

Table 4. ASA score, hospitalization time (day) and biochemical parameters

	Conversion group	Laparoscopic group	p
ASA score (±SD)	3 (±1)	2 (±1)	**>0.05**
ALP (±SD)	55.8 (±25.2)	72.43 (±16.7)	**<0.001**
GGT (±SD)	59.53(±49.6)	30.3(±23.7)	**<0.001**
Direct bilirubin	5.6(±7.62)	0.2(±0.2)	**<0.001**
Hospitalization time(day)	7(±4.9)	1.8(±0.8)	**<0.001**

SD: Standard deviation
ALP: alkaline phosphatase, GGT: Gamma-glutamyl transferase

The open group's average hospital stay was 7.0 days, while the laparoscopic group's average was 1.75 days, significantly longer in the open group. (Mann-Whitney U test, $p<0.001$) (Table 4).

4. Discussion

It is impossible to determine when a laparoscopic cholecystectomy will be converted to open surgery. Although numerous risk analysis scales for conversion to open surgery have been advocated in the literature, no evaluation approach has been widely employed (2, 6).

Sutcliffe et al. (2) developed a risk score system (CLOC score) for laparoscopic to open cholecystectomy conversion, which uses the patient's age, gender, surgical indication, ASA score, gallbladder wall thickness, and common bile duct diameter to create the grading system. This system considered patients with a total score of 6 or less as low-risk candidates for conversion from laparoscopic to open surgery, whereas it considered those with a score of 6 or more as high-risk candidates. (2). The conversion rate from laparoscopic cholecystectomy to open surgery has been reported to range between 1.3 percent and 24 percent in the literature, with inflammation and fibrosis in the Callot triangle being the most common reason for conversion to open surgery (3, 6, 10).

In the UK database, Sutcliffe et al. (2) discovered a rate of conversion to open surgery of 3.4 percent in 8.820 patients across 11 investigations. The most prevalent reasons for conversion to open surgery, according to this study, were difficulty in getting crucial safe vision as well as intraoperative complications such as intestine perforation, hemorrhage, or biliary tract injury (2). Acehan et al. reported a 3.7 percent conversion rate to open surgery in 2.373 patients. Their study identified male gender, advanced age, diabetes, presence of supraumbilical median incision, multiple millimetric calculi on US, and cholecystitis signs such as increased wall thickness as factors influencing conversion to open surgery. They reported that the most common reason for these patients' conversion to open surgery was difficulty establishing safe vision due to fibrosis and inflammation in the Callot's triangle, which developed following acute

cholecystitis (3).

Our study found conversion to open surgery during laparoscopic cholecystectomy as 3.04 percent, consistent with the literature. Adhesions due to inflammation were the most common reason for open surgical conversion in our study, accounting for 53.6 percent. Other causes included an inability to visualize the gallbladder's hilum, adhesions from a previous operation, hemorrhage, and secondary organ injury. These findings are consistent with previous studies. (3,10, 11).

Although the male gender has been identified as a risk factor for conversion to open surgery (12, 13), our analysis observed no statistically significant differences in terms of gender.

Many studies have found that advanced age increases the likelihood of converting from laparoscopic to open surgery (14). The mean age in our study was 59 in the open group and 48 in the laparoscopic group, with the open group having a considerably higher mean age. Elderly patients are likely to have had more attacks with a more severe course and a higher number of comorbidities (15).

Obesity was a risk factor for conversion from laparoscopic to open surgery in various studies (5, 12); many studies have identified it as a risk factor (15, 16). Beksaç et al., on the other hand, found obesity not to be a risk factor for conversion to open cholecystectomy (6). Nevertheless, the conversion rate to overweight or obese patients was statistically significant in our study. According to the literature, the presence of pericholecystic fluid or a gallbladder wall thickness of more than 3 mm, which is accepted as an indicator of cholecystitis in preoperative ultrasonography and in patients who had had a cholecystitis attack, is a risk factor for conversion to open surgery, (15-18). Gallbladder wall thickening and fibrosis due to recurrent inflammatory attacks cause difficult dissection and therefore an increase in conversion to open surgery. In our research, individuals with cholecystitis attacks and patients with greater preoperative gallbladder wall thickness had high conversion rates to open surgery.

Fibrosis may develop in the surgical field in cases of pancreatitis and cholangitis caused by cholelithiasis, and this can be a factor determining the conversion to open surgery (19). However, there was no statistically significant difference between these conditions in our investigation. While there was no statistically significant difference in the appraisal of stone size, we found the rate of conversion to open surgery in instances with several stones statistically high.

According to studies, previous abdominal surgeries are not a barrier to laparoscopic surgery, but they are a risk factor for conversion to open surgery (3,5,6). It appeared to be a conversion factor to open surgery due to previous surgeries in our study, but we observed no statistically significant difference assessing it in terms of incision kinds. There was no significant relationship between incision styles and conversion to open surgery in Bourgouin's study (20).

Various studies have looked into whether the ASA score used to assess preoperative anesthetic risk impacts the conversion to open surgery. Our investigation identified no significant influence on the ASA score in the conversion to open surgery, as reported by Bourgouin et al. (20) and Rosen et al. (21). Among the biochemical tests performed in the preoperative period, high levels of GGT and ALP may indicate the need for open surgery (20,22). Bourgouin et al. discovered a link between GGT increase and the likelihood of undergoing open surgery (20). In patients with increased GGT and ALP, Licciardello et al. discovered a significant rate of conversion to open surgery (22). Beksac's study determined high ALP levels as a risk factor while establishing no significant relationship between ALT, AST, GGT, and T. Bilirubin values and conversion to open surgery (6). Our research discovered a relationship between high GGT, ALP, and direct bilirubin levels and the need for open surgery.

In treating cholelithiasis, laparoscopic surgery has established itself as the gold standard. Even at the best centers, conversion from laparoscopic surgery to open surgery can be inevitable despite technical breakthroughs and the development of surgical procedures and skills. Conversion to open surgery is a surgical procedure done to prevent or eliminate a potential problem.

It is challenging to anticipate which patients may require open surgery. Advanced age, obesity, past bouts of cholecystitis, adhesions related to previous abdominal incisions, presence of cholecystitis symptoms such as multiple calculi on ultrasonography and increased wall thickness, and high GGT and ALP values all influenced conversion to openness in our study. We revealed previous cholecystitis attacks as the most critical factor.

Modifiable factors can be determined in the preoperative phase by establishing the factors increasing the likelihood of conversion to open surgery. Changeable risk factors among these projected risk factors can help determine when surgery should be performed. As a result, a proper operation scheduling plan may be devised, and the rate of problems and openings can be reduced.

More experienced surgeons and centers can be referred for risk patients after identifying patients with a high risk of conversion to open surgery. Due to its many advantages, laparoscopic cholecystectomy, a gold standard approach, should be considered the first choice for individuals who are considered too risky for open surgery.

Conflict of interest
The authors declared no conflict of interest.

Funding
No funding was used for the study.

Acknowledgments

We performed this research with the Recep Tayyip Erdoğan University, Faculty of Medicine, Department of General Surgery, Rize, Turkey.

Authors' contributions

Data curation (equal), formal analysis (equal), investigation (equal), software (equal), writing-review and editing (equal), supervision (equal), validation (equal): A.Ö., conceptualization (equal), methodology (equal), project administration (equal), writing-original draft (equal), writing-review and editing (equal), visualization (equal), validation (equal): A.K., conceptualization (equal), methodology (equal), project administration (equal), writing-original draft (equal), writing-review and editing (equal), visualization (equal), validation (equal): A.P.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 786-792
doi: 10.52142/omujecm.39.3.37

The effect of listening to the music of the patient's own or others' choice during cesarean sections on pain, and its contribution to anesthesia technicians

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Received: 05.04.2022 • **Accepted/Published Online:** 01.07.2022 • **Final Version:** 30.08.2022

Abstract

To investigate the effect of listening to the music of the patient's own or others' choice during the cesarean section on the intraoperative pain of the expectant mother and its contribution to the anesthesia technicians' practice. This study is a single-centre, controlled and randomized trial involving 92 patients. During cesarean section, pregnant women in the intervention group listened to music of their own or our choice with headphones. We placed headphones on the patients in the control group but played no music. We performed cesarean sections under regional anesthesia. We collected a variety of subjective (visual analog and numerical scale for pain) and objective parameters (heart rate, blood pressure and amount of medication leftover from postoperative PCA) during the incision and suturing of the skin. We found no differences between the groups in terms of systolic-diastolic blood pressure and heart rate. However, we observed that the VAS scores in the group listening to music in the Acemashiran maqam (a melody type in Turkish classical music) were lower than the control group ($p=0.02$). On the other hand, there was no difference between the VAS scores of patients listening to the music of their own choice and that of the group listening to music in the Acemashiran maqam or from the control group ($p>0.05$). At the end of the study, 87.5% of the patients liked the music preferences, while 93.8% desired to listen to music during the next cesarean section. Listening to music reduces patients' pain during cesarean section. Comparisons with the control group showed that the effect of music in the Acemashiran maqam on pain was even more pronounced than that of the patient's own choice.

Keywords: Cesarean section, pain, music therapy, visual analog pain scale

1. Introduction

The overall cesarean section rate worldwide is 53% and increasing even more due to the perceptions and wishes of pregnant women about childbirth, the detection of risky pregnancies through intensified antenatal follow-ups, and the need for patients who have had a cesarean section before to have a cesarean section again (1). Cesarean section can be performed under general or regional anesthesia. Regional anesthesia has become more preferred with its both intraoperative and postoperative comfort (2).

Despite the increased comfort provided by regional anesthesia, patients' concerns about the unborn baby and surgical intervention cause pain during and after cesarean section (3). Many pharmacological procedures effective on pain are performed as a routine clinical practice (4). However, the search for effective non-pharmacological pain treatment has been continuing for a long time to counter the side effects of pharmacological procedures on the mother and the baby to be breastfed after delivery (5-8).

As a non-pharmacological treatment, music has an effect on pain as known since ancient times. Today, music is utilized professionally in health services. There are many studies about the effect of listening to music on reducing acute and chronic pain (6, 9-11).

Studies conducted with functional MRI and EEG to explain the effect of music on pain suggest that sounds have a general modulation effect on pain and specifically reduce pain unpleasantness induced by a positive emotional impact (12). Moreover, listening to music has been associated with dopamine release (which has a role in central analgesia) from the caudate and nucleus accumbens (13). Music modulates pain responses in the brain, brain stem, and spinal cord (14). All this evidence suggests that music-induced analgesia can be regarded as a central type of analgesia (9).

In both western and eastern cultures, music has been used for therapeutic purposes for centuries (15). However, music has a special place in treatment in Turkish culture (16).

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Because of the close relation of music with culture, listening to the music of the patient's own culture is considered to be more effective on pain (17). Similarly, it is possible to suggest that the music of the patient's own choice would be more effective on pain (10). It has also been argued that classical music with its rhythm and harmony could be more effective on pain and anxiety, even if there are no cultural affinities or personal preferences (18). Among the maqams (melody types) in classical Turkish music, especially Acemashiran maqam has been preferred because of its effect on pain. Various studies have shown that the Acemashiran maqam facilitates delivery and reduces pain (19).

It is pretty difficult to evaluate the results of effective procedures on pain. It would not be adequate to use subjective data to evaluate pain, a subjective phenomenon. Therefore, studies have involved the evaluation of both subjective and objective pain indicators.

The primary endpoint of our study was the evaluation of the effect of music on pain in patients having a cesarean section. The secondary endpoint of our study was the investigation of the effectiveness of the Acemashiran maqam on pain.

2. Materials and methods

2.1. Patient and data collections

After the approval of the local ethics committee (Lokman Hekim University non-interventional clinical research ethics committee, date: 25/12/2019, decision no: 2019/80), the study enrolled patients, who were followed up at Etlik Lokman Hekim Hospital between 01/2020 and 12/2020, and for whom cesarean section was planned due to various reasons during their antenatal follow-up. We did not regard patients' cesarean section indication as an inclusion criterion. However, we excluded patients who had maternal-fetal risk detected during pregnancy follow-up, cardiovascular disease, anxiety disorder, hearing impairment, and those not suitable for spinal anesthesia, considering that these conditions could affect the results. We explained the purpose of the study and the entire procedure to be performed within the scope of the study to all patients and obtained written consent from them.

We performed randomization according to the patients' arrival order at the polyclinic. We divided patients in the music group into two groups: Patients who would listen to classical Turkish music in the Acemashiran maqam (G1), and the music of their own choice (G2), according to the order of their arrival at the polyclinic. We asked the patients in group 2 to create a music archive on their mobile phones with 15 tracks they liked before they came for the cesarean section. We included 32 patients who arrived later as the control group (G3) in the study.

There were no patients on routine drug therapy before surgery in any group. After taking aseptic precautions, we stained and covered the patients while they were sitting or lying on their left side. We located the L3-L4 or L4-L5 disk space, inserted a 27G Whitacre spinal needle into the subarachnoid space, and confirmed its position by observing the free flow of clear cerebrospinal fluid. We administered 2.8-3 mL of 0.5% hyperbaric bupivacaine using a nasal oxygen cannula at 2-3 L/min, with the patient in the supine position. At this stage, we placed double-sided headphones covering the entire ear to prevent the operating room sounds from interfering with the operation. Patients in the group listening to classical Turkish music in the Acemashiran maqam used headphones connected to an MP3 player, while those in the group listening to the music of their own choice used headphones connected to their mobile phones. We only removed the headphones at the moment of delivery so that the mother could hear the baby's voice.

After the mother's contact with her baby, we took the baby out of the operating room, placed the headphones again and played the music until completing the suturing and dressing processes. We recorded blood pressure and heart rate parameters immediately after completing the preoperative preparations and incision after regional anesthesia (T1). We applied the numerical analog scale (NAS) to patients orally and asked them to describe the pain sensation assigning a score from 0 to 10. However, since the patient might have difficulty assessing the pain because of the excitement of having given childbirth and the stress caused by the intervention, an anesthesia technician evaluated her facial expression simultaneously, scoring the patient's pain sensation based on the Wong-Baker face scale (VAS). After suturing the skin incision (T2), we immediately evaluated and recorded the patient's blood pressure, heart rate, and NAS and VAS scores. After the operation, we asked the patients in the G1 and G2 groups questions about their musical experience before leaving the operating room. We also asked anesthesia technicians questions about the benefit of listening to music during a cesarean section in terms of anesthesia procedures. We administered IV PCA (500 mg tramadol hydrochloride in 100 cc) adjusted at a constant infusion rate of 3 ml per hour to reduce postoperative pain. With the button attached to the PCA, the patients could take an extra dose of 3 ml every half hour if feeling excessive pain. At the 24th postoperative hour, we terminated PCA and checked the remaining fluid.

2.2. Statistical analysis

We statistically analysed the results using the IBM SPSS 27.0 (SPSS Inc., Chicago, IL, USA) package program and evaluated the conformity to normal distribution with the Kolmogorov-Smirnov Test. We gave numerical variables showing normal distribution as mean +/- standard deviation, the numerical variables not showing a normal distribution as a median (minimum-maximum) value, and the categorical variables as frequencies (percentages). We used the Wilcoxon Test to compare the differences between the T1 and T2 values

of NAS and VAS data not showing a normal distribution. We used the Kruskal Wallis analysis of variance to compare the groups while analysing comparisons with the post hoc test for Kruskal-Wallis analysis. We conducted mixed ANOVA to analyse the differences between the groups in terms of T1 and T2 values regarding systolic and diastolic blood pressure and heart rate measurements that showed normal distribution. In cases where the interaction was not significant, we evaluated the comparison of repeated measurements for each group with the Dependent Samples T-Test. Using Spearman's correlation coefficient, we analysed the relationship between VAS-NAS scores and between systolic blood pressure values and VAS and NAS scores. We used the Pearson Chi-Square Test and Fisher's Exact Test to compare the groups' categorical variables and considered $p<0.05$ sufficient for statistical significance.

3. Results

We excluded 12 patients as two from G1 and one from G2 encountered a system failure of their audio player; one patient from each group wanted the earphones removed; and one patient from G1, two from G2, and four from G3 were operated under general anaesthesia. The study enrolled 92 patients, including 32 from G1, 32 from G2 and 28 from G3.

Table 1 shows the patients' demographic data, including their age and educational background, with no statistically significant differences.

Table 1. Comparison of G1, G2 and G3 in terms of age and educational status

	G1	G2	G3	p
Age	32.22±5.21	31.53±5.32	31.82±4.53	0.862
Educational Status				-
Primary school	1 (3.1%)	1 (3.1%)	1 (3.1%)	
Middle school	3 (9.4%)	4 (12.5%)	2 (7.1%)	
High school	15 (46.9%)	10 (31.2%)	12 (42.9%)	
University	13 (40.6%)	17 (53.1%)	13 (46.4%)	

G1: who would listen to classical Turkish music in the Acemashiran maqam, G2: who would listen to the music of their own choice, G3: control group

We found no differences between G1, G2 and G3 regarding the remaining amounts of PCA ($\chi2 = 0.434$, $p>0.05$).

Table 2 shows the comparison between the three groups in terms of T1-VAS and T2-VAS values. We found a difference between G1, G2 and G3 in T1-VAS values (p=0.003). In post-hoc comparisons, there was a difference between G1 and G3 (p=0.002). The T1-VAS values of patients in G3 were significantly higher than those in G1. There was no difference between G1 and G2 or G2 and G3 (p=0.218 and p=0.313, respectively), while there was a difference between G1, G2 and G3 regarding T2-VAS values (p=0.011). Post-hoc comparisons revealed a difference between G1 and G3 (p=0.011). T2-VAS values in G3 were significantly higher than in G1. We found no difference between G1 and G2 or G2 and G3 (p>0.999, p=0.095, respectively). There was a statistically significant difference between the T1-VAS and T2-VAS measurement values of G1 and G3 patients (p=0.025, p=0.010, respectively), while none between these two values in G2 patients (p=0.611).

Table 2. Comparison of G1, G2 and G3 in terms of T1-VAS and T2-VAS scores

	G1	G2	G3	
Time	**Mean±Sd Median (Min-Max)**	**Mean±Sd Median (Min-Max)**	**Mean±Sd Median (Min-Max)**	**p**
T1	0.0±0.0 0 (0-0)	0.41±1.24 0 (0-5)	0.32±0.48 0 (0-1)	**0.003**
T2	0.31±0.74 0 (0-2)	0.47±1.02 0 (0-5)	0.79±0.92 1 (0-4)	**0.011**
p	**0.025**	0.611	**0.010**	

G1: who listened to classical Turkish music in the Acemashiran maqam, G2: who listened to the music of their own choice, G3: control group. T1: incision start time T2: skin suturation time VAS: visual analog scale

Table 3 shows the comparison between the groups in terms of T1-NAS and T2-NAS values. There was a difference between G1, G2, and G3 regarding T1-NAS values (p<0.001), while post-hoc comparisons evinced a difference between G1 and G3 (p<0.001). The T1-NAS values of patients in G3 were significantly higher than those in G1. We found no difference between G1 and G2 or G2 and G3 (p=0.299 and p=0.053, respectively). There was no difference between G1, G2 and G3 in terms of T2-NAS values (p=0.092). We found a statistically significant difference between G1, G2 and G3 regarding measurement values of T1-NAS and T2-NAS (p=0.018, p=0.003 and p=0.006, respectively).

Table 3. Comparison of G1, G2 and G3 in terms of T1-NAS and T2-NAS scores

	G1	G2	G3	
Time	**Mean±Sd Median (Min-Max)**	**Mean±Sd Median (Min-Max)**	**Mean±Sd Median (Min-Max)**	**p**
T1	0.0±0.0 0 (0-0)	0.22±0.61 0 (0-3)	0.46±0.64 0 (0-2)	**<0.001**
T2	0.88±1.91 0 (0-8)	0.78±1.10 0 (0-4)	1.11±1.47 1 (0-7)	0.092
p	**0.018**	**0.003**	**0.006**	

G1: who listened to classical Turkish music in the Acemashiran maqam, G2: who listened to music of their own choice, G3: control group. T1: incision start time T2: skin suturation time NAS: numerical analog scale

The study revealed statistically significant correlations between T1-VAS and T1-NAS scores and between T2-VAS and T2-NAS scores (r=0.751, p<0.001; r=0.832 and p<0.001, respectively). In order to ascertain which of the VAS and NAS scores best represent the pain, we evaluated the correlation between these values and systolic blood pressure (Table 4). We found no correlation between T1-systolic blood pressure values and T1-VAS or T1 NAS scores or between T2-systolic blood pressure values and T2-VAS or T2-NAS scores (P=0.767, p=0.701, p=0.146 and p=0.444, respectively).

Table 4. Correlation between systolic blood pressure and NAS and VAS values in all patients

	VAS (T1 and T2)		NAS (T1 and T2)	
	r	p	r	p
T1-Systolic Blood Pressure	0.031	0.767	0.041	0.701
T2-Systolic Blood Pressure	0.153	0.146	0.081	0.444

T1: incision start time T2: skin suturation time VAS: visual analog scale NAS: numerical analog scale

Table 5 shows the comparison of patients in all three groups in terms of T1 and T2 systolic blood pressure, diastolic blood pressure, and heart rate values. We found a difference between systolic blood pressure, diastolic blood pressure and heart rate values in T1 and T2 values (p<0.001) but no difference between the groups (G: p=0.741, G: p=0.438 and G: p=0.379, respectively). The interaction was insignificant as well (GxT; p=0.672, GxT; p=0.516 and GxT; p=0.842, respectively).

Table 5. Comparison of G1, G2 and G3 in terms of systolic blood pressure, diastolic blood pressure and heart rate values in T1 and T2

	Time	G1	G2	G3	
		Mean±Sd	Mean±Sd	Mean±Sd	
Systolic Blood pressure	T1	131.34±14.39	129.94±16.44	131.11±13.53	T: p<0.001 G: p=0.741
	T2	119.81±15.64	117.22±16.49	115.71±11.96	GxT; p=0.672
Diastolic Blood pressure	T1	82.25±9.29	78.53±10.30	79.96±9.04	T: p<0.001 G: p=0.438
	T2	71.12±10.96	70.37±10.72	72.89±10.39	GxT; p=0.516
Heart rate	T1	94.91±16.39	100.16±17.82	98.54±13.53	T: p<0.001 G: p=0.379
	T2	86.09±14.42	89.19±10.76	88.21±10.96	GxT: p=0.842

G1: who listened to classical Turkish music in the Acemashiran maqam, G2: who listened to music of their own choice, G3: control group. T1: incision start time T2: skin suturation time

Since the music groups had no effects on the changes in systolic blood pressure, diastolic blood pressure and heart rate measurements that occurred over time, we analysed measurements of T1 and T2 systolic blood pressure, T1 and T2 diastolic blood pressure, and T1 and T2 heart rate in the music groups separately with the dependent samples t-test (Table 6). We found a statistically significant difference between the measurement results of T1 and T2 systolic blood pressure of all three groups (G1 mean difference: 11.53, 95% CI (5.70-17.36), p<0.001; G2 mean difference: 12.71, 95% CI (5.54-19.89), p=0.001; and G3 mean difference: 15.39, 95% CI (9.93-20.84), p<0.001). The three groups' systolic blood pressure measurement results at T2 time were statistically significantly lower than at T1 time. We found a statistically significant difference between all three groups' measurement results of T1 and T2 diastolic blood pressures (G1 mean difference: 10.56, 95% CI (5.54-15.58), p<0.001; G2 mean difference: 8.15, 95% CI (4.00-12.30), p<0.001; and G3 mean difference: 7.07, 95% CI (3.07-11.07), p=0.001).

Table 6. Comparison of the groups in terms of systolic blood pressure, diastolic blood pressure and heart rate values in T1 and T2

	Time	G1	G2	G3
		Mean±Sd	Mean±Sd	Mean±Sd
Systolic Blood pressure	T1	131.34±14.39	129.94±16.44	131.11±13.53
	T2	119.81±15.64	117.22±16.49	115.71±11.96
	p	**<0.001**	**0.001**	**<0,001**
Diastolic Blood	T1	82.25±9.29	78.53±10.30	79.96±9.04
	T2	71.12±10.96	70.37±10.72	72.89±10.39
	p	**<0.001**	**<0.001**	**0.001**
Heart rate	T1	94.91±16.39	100.16±17.82	98.54±13.53
	T2	86.09±14.42	89.19±10.76	88.21±10.96
	p	**0.007**	**<0.001**	**<0.001**

G1: who listened to classical Turkish music in the Acemashiran maqam, G2: who listened to music of their own choice, G3: control group. T1: incision start time T2: skin suturation time

Table 7. Comparison of the responses of patients in both music groups about their experiences of listening to music during caesarean section

	G1		G2		p
	n	%	n	%	
The effect of music on pain during cesarean section					
Yes	28	87.5	21	65.6	**0.039**
No	4	12.5	11	34.4	
The effect of music on stress during cesarean section					
Yes	30	93.8	24	75	0.039
No	2	6.2	8	25	
Desire to listen to music during the next cesarean section					
Yes	30	93.8	24	75	**0.039**
No	2	6.2	8	25	
Liking classical Turkish music in the Acemashiran maqam in music selection					
Yes	28	87.5			
No	4	12.5			

G1: who listened to classical Turkish music in the Acemashiran maqam, G2: who listened to music of their own choice, G3: control group.

The three groups' diastolic blood pressure measurement results at T2 time were statistically significantly lower than at T1 time. We found a statistically significant difference between all three groups' measurement results of T1 and T2 heart rates (G1 mean difference: 8.81, 95% CI (2.56-15.06), p=0.007; G2 mean difference: 10.96, 95% CI (5.46-16.47), p<0.001; and G3 mean difference: 10.32, 95% CI (5.85-14.78), p=0.001). The three groups' measurement results of heart rate at T2 time were statistically significantly lower than at T1 time.

Compared to the patients in G2, those in G1 were more willing to listen to music again during the next caesarean section and believed to a greater extent that music affected pain and anxiety (p=0.039). 87.5% of the patients who listened to classical Turkish music in the Acemashiran maqam liked the music selection. Table 7 shows the comparison of patients' responses in both music groups.

Although the effect of listening to music during a caesarean section on patient's compliance and agitation and its usefulness rate in terms of the practice of anaesthesia technicians were higher in G1 than in G2, we found that the difference was not statistically significant. Table 8 shows a comparison of the responses of the anaesthesia technician.

4. Discussion

Cesarean delivery has been increasingly common in recent years (1). Despite the availability of pharmacological methods that can be used in the postoperative period, physicians search for non-pharmacological pain relief methods (5-7, 17). Music is one of the most preferred non-pharmacological methods used for pain relief because it is easily accessible, has no side effects, and does not require additional cost and effort during its application (8). Due to the close relationship of music with culture, the music types preferred by researchers in their studies differ from each other (6, 10, 17, 20, 21). In our study that conducted to evaluate the effect of the classical Turkish music in the Acemashiran maqam on pain, we ascertained with VAS scores that this maqam (melody type) was effective on pain during cesarean sections (p=0.002).

The number of studies in the literature conducted using various types of music and musical instruments increase each passing day (8). In 2018, A study evaluating differences between meditation music, binaural rhythm, and the control group revealed that the VAS values of the two groups listening to music were lower than that of the control group during the evaluations made at the 6^{th} and 24^{th} hours (p<0.05) (2). Furthermore, In a study conducted by Farzaneh in 2019, patients who delivered by cesarean section were divided into 3 groups (patients wearing silent headphones, patients wearing headphones to listen to music (musical group) and patients not wearing headphones (control group)). Pain intensity was measured and the VAS scores were significantly lower in the music group than in the control group (p<0.001). A study conducted on three groups consisting of patients wearing silent headphones, patients wearing headphones to listen to music (music group), and patients not wearing any headphones (control group) evinced that the VAS scores were significantly lower in the music group than in the control group (p<0.001).(6) However, In another study conducted by Diri et al. in 2019 to evaluate the effect of music therapy on perceived anxiety and pain during outpatient urodynamic study (UDS) found no differences between the music and control groups in VAS values (p=0.76) another study conducted for urodynamic evaluation found no difference between the music and the control groups in terms of VAS values (p=0.76) (21).

A 2006 study conducted using VAS scores to assess the tolerance to pressure pain classified participants into three groups: Listening to white noise, relaxation music, and music of their own choice. The above study found that the participants' VAS scores listening to the music of their own choice were significantly lower than those listening to white noise and relaxation music (p<0.001) (10). In 2020, 150 patients undergoing cesarean delivery listened to the music of their own choice or Mozart's music, starting from the preoperative period until the end of the first postoperative hour, and they were then compared in terms of postoperative pain with the patients in the control group, who did not listen to any music. The study mentioned above found no difference between the group who listened to the music of their own choice and the control group in terms of postoperative pain (p=0.10), while patients who listened to Mozart's music had a lower pain rate than the control group (p=0.03) (22). Consistent with the abovementioned study, we found VAS values of G1 lower than that of G3 (p=0.011). However, we observed no differences between G1and G2 or between G2 and G3 in terms of VAS scores (p>0.999 and p=0.095, respectively).

Although it is possible to evaluate pain with VAS scores, subjective data interpretation would not be sufficient. Therefore, studies conducted to evaluate pain used objective criteria. A meta-analysis conducted in 2020, including 55 studies and 4,968 patients, concluded that listening to perioperative music reduces the need for postoperative opioids (p<0.001) (23). A controlled study conducted in 2010 providing postoperative music to patients undergoing cesarean delivery found significantly lower VAS scores in the group listening to music, while the need for a postoperative opioid was also significantly reduced (p<0.05) (24). However, we did not observe in our study any positive effect of listening to music on the need for postoperative opioids ($\chi 2$ = 0.434, p>0.05).

Changes in the cardiovascular system are often used to evaluate the patient's pain level. In a randomized controlled trial conducted in 2018 to investigate the effect of listening to music on pain and stress during cesarean sections, systolic blood pressure and heart rate during skin incision were significantly lower in the study group than in the control group

($p<0.05$); while there were no significant differences in diastolic blood pressure ($p=0.197$). The study mentioned above found no difference between the groups in terms of suturing (25). Another study investigating the effect of listening to music on anxiety and vital signs in patients who had multiple cesarean sections observed a decrease between the heart rates recorded at the beginning and the end of the operation in the study group, while there was no such change in the control group ($p<0.05$) (26). In a 2004 study, A study, evaluating the effect of listening to music before and after gynecological operations on the postoperative pain of patients performed there was no difference between blood pressure values in the music group and the control group ($p>0.05$), while observing a significant difference in heart rates ($p=0.02$) (27). A 2012 study comparing the preoperative stress and vital signs of patients listening to music in the waiting room before cesarean section with the control group, observed significant decreases in systolic ($p<0.05$) and diastolic ($p<0.01$) blood pressures in patients listening to music compared to the control group, but no significant change in heart rates ($p>0.05$) (28). Our study evinced that the music groups had no effects on the changes that occurred over time in the results of systolic and diastolic blood pressures and heart rate measurements ($p>0.05$).

Even studies showing that music does not affect pain and anxiety, patients found their experience of listening to music during the procedure favorable and were more willing to listen to music again in the next operation than the control group ($p = 0.005$) (21). In a study conducted with patients who underwent colonoscopy, the desire to listen to music again during the potential repetition of the operation was 63.2% (6). In another study, A meta-analysis to evaluate the effect of perioperative music on medication requirement, length of stay, and cost 95.5% of the participants stated that they wanted to listen to music again during the next cesarean section, while 89.7% said that music made the situation more enjoyable, and 73.4% that music calmed them (23). Our study revealed that 87.5% of the patients liked the music selection, while 93.8% in G1 and 75% in G2 desired to listen to music again during the next cesarean section ($p=0.039$). A higher rate of patients in G1 stated that music affected pain and stress during cesarean section than in G2 ($p<0.05$).

In addition to the burden and stress they create for the patient, surgical procedures are also a troublesome process for the operating team. The patients' awareness about what is being done during the procedures performed under regional anesthesia and their reactions to actions complicate the practice of anesthesia technicians, who are already busy providing medical treatment and monitoring the patient's vital signs. Various studies suggest that listening to music during surgical procedures eases the job of anesthesia technicians (29, 30). We observed in our study that listening to music during cesarean sections had a beneficial effect on anesthesia technicians' practices by improving patients' compliance and reducing their operation-induced agitation. In this respect, we found no statistically significant difference between both musical groups ($p=0.098$).

As a non-pharmacological, easily accessible, and cost-free method, music can be safely used to relieve pain during cesarean sections. Individual and cultural differences make it impossible to standardize which musical style is more effective in achieving the best results. Classical Turkish music in the Acemashiran maqam is one of the types of music that can be played for patients during cesarean sections.

Conflict of interest

The authors declared no conflict of interest.

Funding

No funding was used for the study.

Acknowledgments

The authors wish to thank Mr. Turgay Pişkin, who contributed to the study by recording his ney (a wind instrument made from a yellow cane with nodes) and bendir (a frame drum) performances in the Acemashiran maqam.

Authors' contributions

Concept: E.T., Design: E.T., Data Collection or Processing: E.T., Analysis or Interpretation: E.T., Literature Search: E.T., Writing: E.T.,

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 793-797
doi: 10.52142/omujecm.39.3.38

The effects of intermittent fasting on nighttime blood pressure levels

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Received: 21.04.2021 • **Accepted/Published Online:** 04.07.2021 • **Final Version:** 30.08.2022

Abstract

Hypertension, a preventable disease with a high prevalence, is frequently seen in obese patients with cardiovascular risk factors. Our study aimed to investigate the effect of an intermittent fasting regimen on nighttime blood pressure levels. Patients who applied to Karabuk University Faculty of Medicine Training and Research Hospital Cardiology and Family Medicine Polyclinic between January and March 2021 and were followed up for 24 hours on an outpatient basis were included in this self-controlled interventional study. Patients underwent a second 24-hour Holter blood pressure follow-up after one month of diet, and the results were compared. The study was conducted with 30 subjects who adhered to a one-month intermittent fasting regimen. 63.3% of the patients were male, and the mean age was 54.60 ± 9.71 years. One month later, 13 patients whose nighttime blood pressure improved were assigned to Group 1 and 17 patients to Group 2. There was no important variance between the groups in terms of gender, age, height, waist circumference and BMI (p=0.609, p=0.105 and p=0.087, in order of). Mean VA and BSA were considerably lower in Group 2 (p=0.027 and p=0.045, in order of). HT duration was longer in Group 1 (p=0.001). There was no important variance between the groups in terms of smoking, alcohol use, average daily sleep time, salt intake awareness and exercise habits. The rate of snacking habits in Group 2 was statistically considerably higher (p=0.033). Intermittent fasting may be beneficial in preventing nocturnal hypertension.

Keywords: hypertension, intermittent fasting, nighttime blood pressure, humans

1. Introduction

Hypertension (HT), characterized by increased intra-arterial blood pressure and caused by acquired, metabolic and genetic factors, is a condition with high mortality and morbidity, accounting for at least 45% of heart disease-related deaths and 51% of coronary heart disease-related deaths (1). Increasing risk factors such as population aging worldwide, unhealthy and sedentary lifestyles, and unbalanced diets increase the prevalence of HT (2). The incidence of HT is 25–30% in developed countries, compared with 30–35% in our country (3). While the rate of HT control with treatment is 25% in developed countries, it is only 10% in developing countries (4). Treatment of high blood pressure, regular blood pressure measurements, control of other diseases accompanying HT, a healthy and balanced diet, and stress reduction are important for good management of HT (5). The parasympathetic system is activated by nocturnal circadian rhythm, and systolic blood pressure is expected to decline by ≥10% during night rest in healthy individuals. In normotensive and hypertensive individuals, the nighttime average blood pressure decreases by ≥10% of the daytime average blood pressure, which is called the dipper pattern. On the other hand, the non-dipper pattern refers to a blood pressure, which is characterized by a nighttime dip, which is <10% lower than the daytime average blood pressure.

Intermittent fasting, i.e., periods of voluntary abstention from eating and drinking, has been practiced by people for health and religious reasons for thousands of years (6). Intermittent fasting is a dietary intervention similar to calorie restriction. It focuses on the timing of meals. It has been established that intermittent fasting regulates circadian rhythm by reducing oxidative stress and reduces cardiovascular risk and that good glucose control is achieved in individuals who are on an intermittent fasting regimen, and calorie restriction improves insulin sensitivity and blood pressure (6, 7).

In this context, our study aimed to explore the effect of prolonged nighttime fasting (the intermittent fasting regimen) on nighttime blood pressure levels in patients with high nighttime blood pressure.

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2. Materials and Methods

This self-controlled interventional study included patients who presented to the Cardiology and Family Medicine outpatient clinics in Karabuk University Faculty of Medicine Training and Research Hospital between January and March 2021 and underwent 24-hour ambulatory blood pressure monitoring within the indications. Individuals diagnosed with DM, working in shifts, pregnant, rejecting the second 24-hour Holter monitoring, and with nighttime systolic and diastolic blood pressure levels of <120/70 mmHg were excluded. A 24-hour blood pressure monitoring record was taken at admission from the patients who met the study criteria and agreed to participate in the study. Patients with a nighttime systolic blood pressure of ≥120 mm Hg and/or a nighttime diastolic blood pressure ≥70 mm Hg were considered to have nocturnal hypertension. Before the study, medical history was taken from the patients, and they were asked whether they had concomitant hypertension (HT), hyperlipidemia (HPL), coronary artery disease (CAD), cerebrovascular disease (CVD), psychiatric disorders, previous stent insertion, smoking and drinking habits. Height and body weight (BW) were measured. Body mass index (BMI) was calculated by the formula: body weight/height squared (kg/m^2), and body surface area (BSA) was calculated by the DuBois formula [$0.007184\times(W^{0.425}\times H^{0.725})$].

These patients were instructed not to eat solid foods after 18.00 for one month and not to smoke, and not to drink tea or coffee for 3 hours before bedtime. One month later, patients were asked whether they adhered to their diet, and a second 24-hour Holter monitoring of blood pressure was administered. Those with a nighttime systolic-diastolic blood pressure of≤120-70 mmHg after the intermittent fasting regimen were named “Group 1” (the group with improvement), and those without such decrease were named “Group 2” (the group without improvement).

The study data were analyzed using SPSS 22 software package. According to the results of the data analysis, pairwise comparisons were performed using the t-test for the normally distributed variables and the Mann-Whitney U test for the non-normally distributed variables. Categorical data were compared using the Chi-square analysis. The statistical significance level was set at $p<0.05$ for all analyses.

The study was approved by the Non-Interventional Clinical Researches Ethics Committee of Karabük University (Decision No: 2021/14 January 2021).

3. Results

Among a total of 129 patients who presented to the outpatient clinics during the study period and wore a Holter monitor, 24 were excluded due to being diagnosed with DM, 2 due to working in shifts, and 1 due to pregnancy. 33 patients refused the second 24-hour Holter monitorization despite the indication, and 39 patients had nighttime systolic and diastolic blood pressure levels of <120/70 mm Hg. The study was conducted on 30 individuals who adhered to a one-month intermittent fasting regimen and met the study criteria. Of the study patients, 36.7% were women, and 63.3% were men; the mean age was 54.60 ± 9.71 years (56.64±8.77 years in women, 53.42±10.25 years in men). After one month, 13 patients with improved nighttime blood pressure levels were assigned to Group 1 and 17 patients without any change to Group 2. There was no variance in total systolic, daytime systolic and daytime diastolic blood pressure levels between the two groups (p=0.176, p=0.307, and p=0.083, in order of) (Table 1).

Table 1. Comparison of changes in baseline and post-fasting blood pressure parameters between the groups

Mean Blood Pressure Variances	Group 1(n=13) Mean±SD	Group 2 (n=17) Mean±SD	*p*
Total Systolic	-5.23±10.21	-.53±8.33	0.176
Total Diastolic	-4.54±7.21	0.47±4.90	**0.031**
Daytime systolic	-4.92±11.03	-1.06±9.32	0.307
Daytime diastolic	-4.00±7.57	0.24±5.36	0.083
Nighttime diastolic	-14.15±9.44	-1.71±8.63	**0.001**
Nighttime Diastolic	-14.92±6.81	0.76±5.13	**0.0001**

The rate of men was 42.1% in Group 1, and 57.9% in Group 2, and the rate of women was 45.5% in Group 1 and 54.5% in Group 2. There was no important gender variance between the groups (p=1). The mean age was 56.00 ± 9.96 years in Group 1 and 53.53 ± 9.68 years in Group 2, and the variance was statistically unimportant (p=0.499).

The anthropometric measurements at the beginning of the study revealed no important variance in height, waist circumference, and BMI between the two groups (p=0.609, p=0.105, and p=0.087, in order of), while BW and BSA were considerably different between the groups (p=0.027 and p=0.045, in order of). The mean BW and BSA were considerably lower in Group 2 than in Group 1 (Table 2).

Table 2. Comparison of baseline anthropometric measurements between the groups

Anthropometric Measurements	Group 1 (n=13)	Group 2 (n=17)	Total (n=30)	*p**
Height (cm)	168.15 ±11.33	166.29 ±8.40	167.10 ±9.64	0.609
Waist Circumference (cm)	101.31 ±9.49	95.18 ±10.23	97.83 ±10.23	0.105
BMI (kg/m^2)	29.81 ±4.17	26.99 ±4.39	28.21 ±4.46	0.087
BW (kg)	84.23 ±14.14	74.12 ±9.60	78.50 ±12.63	**0.027**
BSA	1.98 ±0.21	1.85 ±0.13	1.90 ±0.18	**0.045**

*T-Test

According to the findings, 16.7% of the study group patients group had CAD, 13.3% had HPL, 6.7% had a stent,

3.3% had a history of CVE, and 13.3% had a psychiatric disorder. There was no important variance in the presence of HT, presence of HPL, presence of CAD, and presence of CVE between the groups (p=0.443, p=0.113, p=1, p=1). The duration of hypertension was considerably different between the groups (p=0.001). The duration of HT (11.23 ± 4.19 years) was longer in Group 1 than in Group 2 (5.12 ± 3.16 years). The mean time to diagnosis of hypertension was considerably longer in patients without improvement in nighttime blood pressure. Of the patients, 23.3% had smoking, and 3.3% had drinking habits. There was no important variance in smoking and alcohol use between the groups (p=0.613, p=0.443).

The mean sleep duration per day was 7.62 ± 1.21 hours in Group 1 and 7.65 ± 0.97 hours in Group 2, with no important variance between the groups (p=0.867). Concerning the eating and lifestyle habits of the participants, 56.7% had snacking habits, 36.7% had salt awareness, and 43.3% had exercising habits. There was no important variance in salt awareness and exercising habits between the groups (p=0.165 and p=0.454, respectively). The rate of snacking habits was statistically considerably higher in Group 2 than in Group 1 (p=0.033) (Table 3).

Table 3. Comparison of eating and lifestyle habits between the groups

		Group 1		Group 2		Total		p
		n	%	n	%	n	%	
Snacking Habits	No	9	69.2	4	30.8	13	100.0	**0.033**
	Yes	4	23.5	13	76.5	17	100.0	
	Total	13	43.3	17	56.7	30	100.0	
Salt awareness	No	7	36.8	12	63.2	19	100.0	0.454
	Yes	6	54.5	5	45.5	11	100.0	
	Total	13	43.3	17	56.7	30	100.0	
Exercising Habits	No	5	29.4	12	70.6	17	100.0	0.165
	Yes	8	61.5	5	38.5	13	100.0	
	Total	13	43.3	17	56.7	30	100.0	

4. Discussion

The rate of nighttime blood pressure reduction by intermittent fasting was 43.3% in the patients included in our study. There was no variance in gender, age, comorbidities, smoking, alcohol consumption, BMI, waist circumference, sleep duration per day, salt awareness, and exercising habits between patients with and without reduced nighttime blood pressure. Baseline BW and BSA were higher, the duration of HT was longer, and the snacking habit was less in the group with improved blood pressure than in those without any improvement.

Animal studies have reported that intermittent fasting effectively lowers blood pressure (6). The study by Toledo et al. (8) divided 1422 individuals into four groups by administering 5, 10, 15, and 20-day intermittent fasting regimens for one year and reported that the mean SBP decreased from 131.6 ± 0.7 mmHg to 120.7 ± 0.4 mmHg and DBP from 83.7 ± 0.'4' mmHg to 77.9 ± 0.3 mmHg, while the SBP and DBP decreases were higher in the group treated with a longer intermittent fasting regimen. In a similar study by Erdem et al. (9) with 60 patients, a significant decrease was established in 24-hour ambulatory blood pressure measurements and office blood pressure measurements in all patients after 30 days of intermittent fasting. Li et al.'s (10) study with 32 type 2 DM patients reported a significant reduction in systolic/diastolic blood pressure measurements in all study patients after one week of intermittent fasting. In our study, nighttime blood pressure parameters were improved in 43.3% of the patients treated with intermittent fasting. Variances between study results may be due to different study groups and study periods.

Stote et al. (11) observed a reduction in systolic and diastolic blood pressure in all patients without gender variance after intermittent fasting in 15 cases (10 women and 5 men) with a mean age of 45 years. Toledo et al. (8) also established no gender variance in blood pressure reductions. In our study, the rate of improvement in blood pressure was 45.1% in women and 42.1% in men, and the variance was statistically unimportant.

Studies in various populations demonstrated an almost linear relationship between BMI and systolic and diastolic blood pressure. A BMI of <25 kg/m2 was found to be effective in the primary prevention of hypertension, and blood pressure was reduced with weight loss in most subjects (12). Intermittent fasting studies have shown that besides energy restriction, increased ghrelin and adiponectin levels, decreased leptin and insulin levels, and increased insulin sensitivity also cause weight loss and thus a decrease in BMI (13). It has been observed that current weight and the type and duration of intermittent fasting cause different rates of weight loss. A weight loss of 2.5–9.9% has been observed in almost all intermittent fasting studies (14,15). The study conducted by Toledo et al. (8) in 2019 determined that the waist circumference measurements of the participants decreased by 4.6 ± 0.1 cm in the 5-day intermittent fasting group and 8.8 ± 0.8 cm in the 20-day intermittent fasting group. Weight loss, in turn, was 3.2 ± 0.0 kg in the 5-day intermittent fasting group and 8.6 ± 0.3 kg in the 20-day intermittent fasting group. Weight loss and waist circumference reduction were considerably higher in men than in women. The authors found the patients with the highest body mass index to have the most weight loss. The study by Heilbronn et al. (14) reported a weight loss of 2.5% with 22-day intermittent fasting in 16 healthy individuals with normal body mass index, while Eshghinia and Mohammadzadeh (16) observed a weight loss of 7.1% in overweight or obese women with 6 intermittent fasting for 6 weeks. Our study administered intermittent fasting for one month and did not evaluate changes in weight, waist circumference, and body mass index. However, when the available measurements of the patients were compared, the

mean body weight was found to be considerably lower in the group without improvement in nighttime blood pressure parameters than in the group with improvement. The small size of our study group was the most significant factor for our adequate analysis and precise results.

The mechanism of blood pressure reduction in intermittent fasting was suggested to be connected to the augmented activation of the parasympathetic system due to the brain-derived neurotrophic factor (BDNF)-induced increase in NE excretion through the kidneys, increased renal sodium excretion, and increased insulin sensitivity (8,17). BDNF is mainly produced in response to the activation of glutamatergic receptors. Intermittent fasting is also a stimulant. The effect of the factor on heart rate and blood pressure has been proven in mice studies at George Washington University (18). A study by Sutton et al. with 130 prediabetes men established a mean decrease of 11 ± 4 mmHg in systolic blood pressure and 10±4 mmHg in diastolic blood pressure in patients after 18 hours/day fasting for 5 weeks (19). A prospective study conducted with 82 people during the month of Ramadan, in turn, reported no critical reduction in diastolic blood pressure but a decrease of 3 mmHg in systolic blood pressure (20).

Our study is important because it is one of the very few studies examining the effect of intermittent fasting on the course of blood pressure and the predictive factors of improvement in nighttime blood pressure parameters. However, it is limited by the single-center design, the reduced number of patients presenting to the hospital and the outpatient clinic during the COVID-19 pandemic, and the resistance of patients with an indication for a second 24-hour Holter monitoring of blood pressure against study participation due to concerns about the risk of COVID-19 transmission. In addition, biochemical parameters and anthropometric changes such as body weight, height, and BMI of the patients were not recorded one month later. Also, the lack of reporting energy intake and expenditure may affect the outcomes of intermittent fasting studies. There is a need for further multicenter studies on this subject in larger groups, administering a longer intermittent fasting regimen.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Funding

No funding was used for the study.

Acknowledgments

None to declare.

Authors' contributions

Concept: A.H.Y.Y., A.A., B.K., D.A., Design: A.H.Y.Y., A.A., P.K., H.İ., Data Collection or Processing: A.H.Y.Y., A.A., P.K., H.İ., B.K., Analysis or Interpretation: A.H.Y.Y., A.A., D.A., P.K., Literature Search: A.H.Y.Y., A.A., B.K., H.İ., Writing: A.H.Y.Y., A.A., P.K., B.K.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 798-802
doi: 10.52142/omujecm.39.3.39

Fasting plasma c-peptide level predicts the response of glucagon-like peptide-1 agonist (exenatide) add on to metformin monotherapy in obese type 2 diabetics

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Received: 17.04.2022 • **Accepted/Published Online:** 20.07.2022 • **Final Version:** 30.08.2022

Abstract

We aimed to evaluate the ability of serum C-peptide levels to predict glycemic control in obese type 2 diabetes (T2DM) patients in which a glucagon-like peptide-1 receptor agonist (GLP1-RA), an exenatide, was added to metformin monotherapy. This was a retrospective study, in which we enrolled 44 consecutives obese, type 2 diabetic patients receiving metformin monotherapy and have inadequate glycemic control (HbA1c >7% and <10%). Twice daily GLP1-RA (10 mcg exenatide injection) was added to the treatment. Weight, height and body mass index (BMI), in addition to fasting plasma glucose, hemoglobin A1c (HbA1c), and C-peptide levels measured baseline and at the sixth months post treatment. Regardless of the initial HbA1c level, treatment success was considered a HbA1c level below 7%. Predictors of successful glycemic control were assessed by a regression analysis. The fasting plasma C-peptide level was used as a marker of β cell function. After adding the GLP-1 RA, fasting glucose, C-peptide, BMI, and body weight decreased significantly ($p < 0.01$ for all). 27 (61.4%) patients were achieved treatment success, who have HbA1c level < 7% at the sixth month. The baseline C-peptide level was correlated with the HbA1c level 6 months post-treatment (r: 0.4, p: 0.01). Multivariate logistic regression analysis showed that the baseline fasting plasma C-peptide level was an independent predictor of successful glycemic control [exp.B: 6.6 (1.63-26-9) p: 0.008]. In a receiver operating characteristics (ROC) curve analysis, a baseline plasma C-peptide level of 2.56 ng/mL was the best cut-off value. Initial fasting plasma C-peptide levels can predict the treatment response of the GLP1-RA (exenatide 10 mcg, twice daily) add on to metformin monotherapy in obese type 2 diabetics.

Keywords: C-peptide, type 2 diabetes, GLP-1 RA, exenatide

1. Introduction

The prevalence of T2DM continues to increase worldwide mainly due to obesity, which is one of the biggest health problems today. Unless there is a contraindication to its use, metformin is recommended as the first-line drug in pharmacological treatment of T2DM. Due to the progressive nature of T2DM, many patients require treatment with additional anti-diabetic drugs including injectables and insulin at some point in their disease course (1). There is no consensus on which drug should be added to metformin monotherapy.

All drugs in diabetes treatment are aimed reducing blood glucose levels. Some drugs, such as thiazolidinediones, sulfonylureas, and insulin have side effects of weight gain and hypoglycemia (2). When considering potential drug candidates to be added to metformin monotherapy, potential adverse reactions that could further complicate T2DM treatment must be taken in to account.

Glucagon-like peptide-1 (GLP-1) receptor agonists (GLP-1 RAs) are effective glucose-lowering drugs chosen for second or third line therapy in the treatment of type 2 diabetes and prescribed in combination with metformin, other oral antidiabetic drugs and insulin (1–3). These drugs are also associated with weight loss and have a low risk of hypoglycemia in comparison to other therapies (4).

Exenatide was approved in 2005 as the first GLP-1 RA drug for the treatment of T2DM (5). Exenatide is available as a short-acting formulation for administration twice daily (BID) before meals. Exenatide BID is effective therapy for glycemic control in patients with T2DM, both as monotherapy and as a component of combination therapy (5).

The glycemic response to treatment with GLP-1 RAs is

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highly variable. In the treatment of T2DM with GLP-1 RA drugs, some patients have a very effective treatment response while others may have an inadequate decrease in HbA1c (3, 6, 7). Since C-Peptide is secreted equal amounts with insulin from the pancreas, it is used to evaluate the endogenous insulin reserve in patients using insulin therapy (8, 9). Clinical markers of low β-cell function are also associated with reduced glycemic response to GLP-1 RAs therapy (10).

In the present study, we aimed to investigate the ability of serum fasting C-peptide levels predict to treatment response in obese type 2 diabetic patients in which a glucagon-like peptide-1 agonist (exenatide) was added to metformin monotherapy.

2. Materials and methods

2.1. Study subjects and design

This was a retrospective study in which 283 consecutive obese type 2 diabetic patients aged between 30 and 70 years were screened for inclusion in the study in an outpatient obesity clinic. All the included patients were receiving metformin monotherapy and they have inadequate glycemic control (HbA1c >7% and <10%). Patients who had a history of type 1 diabetes mellitus, malignancies, chronic kidney disease, chronic liver disease, pancreatitis, previous bariatric surgery were excluded from the study in addition to patients using glucocorticoids or insulin. Of these 283 patients, 44 patients were included in the present study and treated with the exenatide GLP-1 RA (injection). There were no other treatment changes during treatment changes during a 6 month follow up. The study was performed in accordance with the Declaration of Helsinki 2000. The study protocol was approved by ethnics committee of University Hospital (2020/0632) Informed consent was not required because of the retrospective nature of our study.

2.2. Data collection

The patient history, physical examination findings and anthropometric measurements (body weight and height) were obtained from the patient's charts. Other data including, weight measurements, concomitant medications and laboratory results were obtained from standard forms used in the obesity clinic. Regardless of the initial HbA1c level, treatment success was considered a HbA1c value below 7% at the sixth month. Weight was measured by Tanita (Name of Manufacturer, City, and Country) using bioelectric impedance analysis method. Blood tests were performed after 12 hours overnight fasting. Plasma C-peptide was measured using radioimmunoassay method (Immunotech). The HbA1c level was measured by high performance liquid chromatography. Fasting blood glucose was determined using the hexokinase method. At every visit, the patients were advised of the importance of diet and exercise. The body mass index (BMI) was calculated as body weight in kilograms divided by height in meters squared (kg/m2). A BMI value larger than 30 kg/m2 was considered as obesity. Weight measurements and laboratory tests were repeated after 3 and 6 months.

2.3. Statistical analysis

Statistical analyses were carried out using SPSS, version 21.0. Normality of the data distribution was evaluated by the Kolmogorov-Smirnov test. Non-normally distributed variables were analysed by Wilcoxon's-signed-ranks test for repeated measurements. The McNemar test was used for comparisons of repeated categorical measurements. Data were expressed as mean ± standard deviation (SD) for continuous variables and frequency (percentage) for categorical variables. To determine the correlation between C-peptide and HbA1c levels Pearson's correlation coefficient was used. Predictors of treatment success 6 month post-treatment we were analysed by multivariate regression analysis. A receiver operating characteristics (ROC) curve was used to determine the appropriate cut-off value for the prediction of treatment success. The sensitivity and specificity of the best cut-off value were calculated by the areas under the curve (AUC).

3. Results

In total 44 obese T2DM patients (33 females and 11 males) were included in the study. The mean age was 51.4±8.9 years at the time of the first visit. Table 1 shows that the addition of the GLP-1 RA (exenatide 10 mcg twice daily) to metformin monotherapy resulted in statistically significant decrease in fasting blood glucose and serum C-peptide levels and HbA1c, BMI and body weight (all p<0.01).

Table 1. Average measurements and laboratory values of the patients at baseline and 6 months post-treatment

Parameter	Baseline	6 month	P value
Fasting glucose (mg/dL)	166.0±54.9	148.5±62.4	<0.001
C-peptide (ng/mL)	3.67±1.43	3.31±1.24	0.002
HbA1c (%)	7.82±1.43	6.85±1.45	<0.001
BMI (kg/m^2)	43.05±6.33	40.39±6.28	<0.001
Body weight (kg)	111.64±16.66	104.70±16.24	<0.001

BMI: body mass index, HbA1c: Hemoglobin A1c; Data are given as mean ± standard deviation

The 6-month HbA1c value of 27 (61.4%) of 44 patients who had a baseline value 7%. The frequency of HbA1c in target range was statistically increased at the 6-month follow up as compared to the baseline (p<0.001). Baseline fasting C-peptide level was correlated with HbA1c level at the 6-month follow up (r: 0.4, p: 0.01). Parameters, which could be a predictor for treatment success, were evaluated with simultaneous multiple regression analysis in Table 2. Multivariate logistic regression analysis showed that baseline fasting plasma C-peptide level was an independent predictor of successful glycaemic control [exp.B: 6.6 (1.63-26-9) p: 0.008].

Table 2. Multivariate logistic regression analysis of independent parameters of treatment success (HbA1c <7%) at sixth month

	Exp (B)	95%CI	P value
Age (years)	1.0	(0.91-1.14)	0.718
Fasting glucose (mg/dl)	1.0	(0.97-1.02)	0.908
Fasting C-peptide (ng/ml)	**6.6**	**(1.63-26.9)**	**0.008**
HbA1c (%)[1]	3.2	(0.88-11.5)	0.077
BMI (kg/m^2)[1]	1.0	(0.79-1.07)	0.326

[1]BMI: body mass index, HbA1c: hemoglobin A1c; Data are given as mean ± standard deviation

In the ROC curve analysis, 2.56 ng/mL was the best cut-off value of initial fasting plasma C-peptide for indicating the specificity (100%) and sensitivity (63.1%) of the treatment success (HbA1c<7% at 6-month) (Fig**.** 1 **and Table 3**).

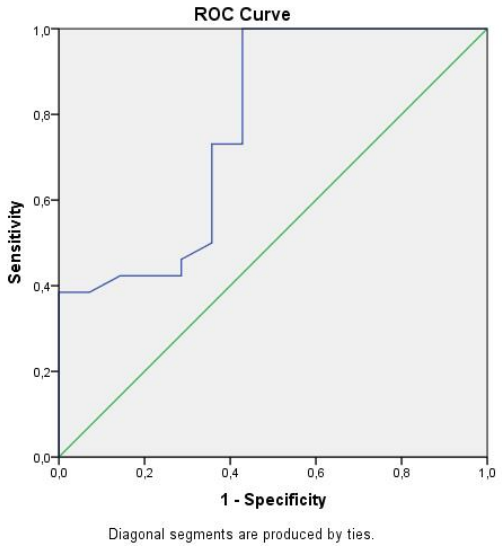

Fig.1. ROC curve showing the serum c-peptide value specificity and sensitivity for the treatment success (HbA1c<7% at the sixth month).

Table 3. Area under the curve; Test Result Valuable: Fasting c-peptide

Area	Std. Error	Asymptotic Sig.	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.775	0.083	**0.005**	0.613	0.937

4. Discussion

The goal of this study was to determine whether the C-peptide level was a predictor of treatment success after the addition of GLP-1 RA to treatment regimen in obese type 2 diabetic patients receiving metformin monotherapy. After adding GLP-1 RA, fasting glucose, C-peptide, BMI and weight decreased. The baseline fasting plasma C-peptide level was correlated with the HbA1C level at the 6-month follow-up and the baseline fasting plasma C-peptide level was an independent predictor of glycemic control.

T2DM is associated with a progressive loss of ß-cell mass and function, which result in progressive increase in blood glucose values. Successful treatment requires understanding the disease pathogenesis. GLP-1 concentrations are reduced in patients with type 2 diabetes mellitus. GLP-1 increases insulin secretion from ß-cells, decreases glucagon release from pancreatic α-cells, promotes satiety in the brain, and slows gastric emptying.

In the literature, there are several studies reporting the safety and efficacy of exenatide. Exenatide significantly reduced HbA1c in patients with diabetes not adequately controlled by maximally effective doses of metformin (11–14). DeFronzo et al., Apovian et al. and Derosa et al. showed that exenatide 10 µg BID add on to metformin reduced HbA1c of 0.8%, 0.9% and 1.2% respectively (11,15,16). Results of our study, that showed 1.0% reduction of HbA1c at sixth month, are in concordance these results reported.

In the study of DeFronzo et al., 40% (n:41) of subjects in the 10µg exenatide arm reached an HbA1c ≤7%. In that group, change in body weight was -2.8±0.5 kg from baseline weight 101±2 kg (11). Our data demonstrated that when exenatide at dose of 10 µg twice daily is added to metformin monotherapy for 24 weeks in a group of obese type 2 diabetic patients with less than-optimal glycemic control (baseline HbA1c 7.8%), there was an overall improvement in glycaemia (end of study HbA1c 6.8%), with 61.4% of patients able to reach an HbA1c treatment goal of below 7%. In our study, as patients of the study group (baseline body weight: 111.64±16.66 kg and BMI: 43.05±6.33 kg/m2) lost 6.94 kg (6.3%) on average, it is likely that the improvement in glycemic control resulted from both the treatment and the weight loss, because a 5–10% body weight reduction in overweight/obese type 2 diabetics improve blood glucose measurements (6). Similar with our results, Appovian et al. reported that exenatide BID also showed reductions in weight (–7.3 kg) in a 24-week study in 194 overweight or obese patients with T2DM (baseline body weight: 91.4 kg and BMI: 32.9 kg/m2) on stable metformin therapy. In that study, 65% of participants on metformin plus exenatide BID achieved HbA1c goal ≤6.5% at endpoint (15).

C-peptide is a biomarker for testing and also provides support for the classification of diabetes subtypes, and assists in the staging of T2DM and its clinical management (9,17). In insulin-treated T2DM, clinical markers of low β-cell function are associated with reduced glycemic response to GLP-1RA therapy (18). C-peptide and islet autoantibodies represent potential biomarkers for the stratification of GLP-1RA therapy in insulin-treated diabetes. However, higher C-peptide levels do seem to predict response to GLP-1 agonists (10).

On the other hand, there is limited and variable evidence

to support the use of C-peptide to predict treatment response to non-insulin treatments for T2DM (10, 19). High fasting C-peptide is associated with response to the thiazolidinediones, rosiglitazone and pioglitazone, which is in keeping with their action of reducing insulin resistance (20, 21). In two cohort studies of mixed DPP4 inhibitor use has shown that initial higher fasting C-peptide predicts reduction of HbA1c (8, 22). Better insulin response to glucose is seen in those patients taking Liraglutide with higher GST c-peptide levels (23). The clinical relevance of this finding is confirmed by studies showing that fasting c-peptide predict reduction of HbA1c following initiation (10, 19).

Iwao et al. reported outcomes in 39 type 2 diabetes patients with successful glucose control and 30 type 2 diabetes with unsuccessful glucose control who were converted from the insulin therapy to monotherapy with GLP-1 RA liraglutide. Urinary, fasting and postprandial C-peptide levels were measured to assess the β-cell function (24). Unlike our study, the fasting plasma C-peptide level was not predictive of treatment success in their study. The reason for this discordance might be the differences in the BMI values between the two study cohorts (43.05±6.33kg/m2 vs. 25.4±4.2 kg/m2) and difference in the baseline C-peptide levels. Additionally, the initial fasting C-peptide values of our study, the group with treatment success and the group without treatment success, are also divergent (3.67±1.43 ng/mL, 1.8±0.9 ng/mL, 0.8±0.5 ng/mL; respectively). Furthermore, the mean BMI of the patients in the treatment success group in the study by Iwao et al. (14) was significantly higher than that of the unsuccessful treatment groups. Similar to our study, they used C-peptide levels to assess ß-cell reserves in their study. The GLP-1 RA liraglutide treatment provided weight loss and helped meet treatment targets in patients with adequate ß-cell reserve (24).

The limitations of our study are that being retrospective, have the limited number of patients, and the study population consists mostly of class 2-3 obese T2DM patients.

In conclusion, initial fasting plasma C-peptide level can predict the treatment success of the GLP1-RA (exenatide 10 mcg, twice daily) add on to metformin monotherapy in type 2 diabetics. In the future, prospective studies with larger patient populations should attempt to further elucidate the effect of fasting plasma C-peptide levels on the treatment response in T2DM.

Conflict of interest

The authors declared no conflict of interest.

Funding

No funding was used for the study.

Acknowledgments

None to declare.

Authors' contributions

Concept: K.G., Design: M.S., Data Collection or Processing: S.Ö., Analysis or Interpretation: B.C., M.S., Literature Search: B.C., Writing: M.S., K.G.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 803-808
doi: 10.52142/omujecm.39.3.40

How does visual praxis based occupational therapy program effect motor skills in children with hyperactivity and attention disorder? Single blind randomized study design

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Received: 20.04.2022 • **Accepted/Published Online:** 01.07.2022 • **Final Version:** 30.08.2022

Abstract

Attention Deficit and Hyperactivity Disorder (ADHD) is a neurodevelopmental disorder characterized with problems such as; atypical attention skills increased impulsive behaviors at least two of the child's areas of daily living. In studies investigating developmental aspect of the said problems of children with ADHD, it was stated that the children usually do not demonstrate normal development regarding the motor proficiency and may fall behind their peers in exhibiting normal motor performance. These problems in motor skills are said to cause a hindered development in complex skills which may result in; distorted body image, increased anxiety and poor social communication skills. This study's aim was to investigate effects of a Visual-Praxis-based Occupational Therapy program (VPOT) on motor proficiency of children with ADHD. The 58 children who were included to the study were assigned numbers from 1 to 58 and randomized into two groups (the intervention group and the control group). Both groups were subjected to the Bruninks-Oserestry Test of Motor Proficiency 2–Brief Form (BOT2-BF). After the initial assessment, the participants in the intervention group were admitted into the VPOT program for a duration of 8 weeks with twice-weekly sessions of 45 minutes. End of the program, BOT2-BF was applied once more. The participants mean age was 8.44±1.39 years. There were no differences between the intervention and the control group in terms of age. When the BOT2-BF results for the intervention group were analyzed, it was found that there were significant differences between pre- and post-intervention scores of all sub-scores and the total score. Due to approaching the motor development aspect of ADHD within the context of motor proficiency and visual perception, this study can be considered to be one of the innovative and pioneer studies in the field and is positively unique regarding the usage of the intervention approach that was used.

Keywords: Attention Deficit and Hyperactivity Disorder, occupational therapy, Motor Skills, visual perceptions

1. Introduction

Attention Deficit and Hyperactivity Disorder (ADHD) is a neurodevelopmental disorder characterized by problems such as; atypical attention skills, increased impulsive behaviors, or physical activity affecting at least two of the areas of daily living over a period of one year, usually before the age of seven (1). It was detected that these symptoms start during early childhood and their effects continue throughout adolescence or adulthood. In addition to that, research in this area shows that these symptoms may create performance problems that may influence the social, academic, and other activities of daily living directly (2). In studies investigating the developmental aspect of the said problems of children with ADHD, it was stated that the children usually do not demonstrate normal development regarding motor proficiency and may fall behind their peers in exhibiting normal motor performance (3–6). These problems in motor skills are said to cause a hindered development in complex skills, which may result in; distorted body image, increased anxiety, and poor social communication skills (4, 7, 8). Due to the problematic areas being complex and having a high variety, a need for the treatment plans having dynamic properties within a high variety has arisen (9, 10).

It is possible to classify the approaches that offer solutions to the problems of children with ADHD into two categories; medical and rehabilitative methods. In medical treatment methods, medication is the primary treatment modality. It was emphasized that medical treatment cannot solve all problems on its own, and especially in problems regarding normal skill development and its effects on

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behaviors, supporting the process with rehabilitation-based approaches is needed (11, 12).

In studies focusing on rehabilitation-based approaches for children with ADHD, it was seen that the rehabilitation applications focused on a wide variety of developmental areas and included different health professionals. However, it was realized that most of the rehabilitation programs in the field focused on the behavioral aspect and more specific and individual problems, such as bursts of rage and infringement of interpersonal boundaries, rather than motor, sensory or cognitive skills (6, 13, 14). A very limited number of studies focused on motor and sensory skills. In studies focused on providing solutions for the sensory problems experienced by children with ADHD, the behavioral outcomes after sensory-based approaches or brain stimulations were investigated (16). On the other hand, the studies on motor skill problems usually focused on single motor skill or investigated motor outcomes as secondary outcomes of other rehabilitation approaches. It was stated in previous research that the inability to demonstrate normal motor development was on the basis of other problems experienced by children with ADHD, and the importance of conducting comprehensive intervention studies within this area of focus was emphasized (17).

In the light of this information, this aim was to investigate the effects of a Visual-Praxis-based Occupational Therapy program (VPOT) on the motor proficiency of children with ADHD.

2. Materials and methods

2.1. Ethical statement

This study was designed as a single-blind, randomized, controlled trial of the Visual-Praxis-based Occupational Therapy program (VPOT) on motor skills in children with SLD. The protocol used in this study was approved by the Clinical Ethics Boards and Commissions (ID: 2022-060), and written informed consent was obtained from every child and his/her legal guardian.

2.2. Participants

Fifty-eight children who applied to a Research and Training Outpatient Pediatric and Adolescent Mental Health Clinic were admitted to the study. The inclusion criteria were; (1) Having an ADHD diagnosis given by a pediatric psychiatrist, according to the DSM-V criteria, (2) Continuing to primary school education, and (3) Being between 7 and 10 years of age. The exclusion criteria were; (1) Having another diagnosis for a neurodevelopmental disease other than ADHD, (2) Continuing to occupational therapy, physiotherapy, or any other rehabilitation program, and (3) Being a professional athlete or sportsperson.

2.3. Procedure

The 58 children who were included to the study were assigned numbers from 1 to 58 and randomized into two groups (the intervention group and the control group) using a computer-based random number selector between 1 and 58. After the randomization, both groups were subjected to the Bruininks-Oserestry Test of Motor Proficiency 2 – Short Form (BOT2-BF) by the third author. After the initial assessment, the participants in the intervention group were admitted into the VPOT program, which was carried out by the first author for a duration of 8 weeks with twice-weekly sessions of 45 minutes. After the training program finished, the BOT2-BF was applied once more by the second author (Fig. 1).

2.4. Measurements

Sociodemographic Information Form: This form was prepared by the authors and included questions about the participants' age, gender, dominant side, and current education status.

Bruininks-Oserestry Test of Motor Proficiency 2 – Short Form (BOT2-BF): The BOT2-BF is a tool to assess the motor proficiencies of children between 4.5 and 14.5 years of age. The BOT2-BF has 8 subtests and a total of 46 items and enables assessing gross and fine motor skills alongside motor proficiency. While Dr. Robert H. Bruininks was developing the BOT2-BF, he started his work from the basis of the Oseretsky Motor Proficiency Test. While both tests are similar, the Bruininks-Oseretsky Test of Motor Proficiency had significant positive differences in its contents construction, and technical quality. 'Therefore, the Bruininks-Oseretsky Test of Motor Proficiency became a significantly important tool for therapists and researchers to assess children's motor skills, prepare prepare intervention programs, and detect current or possible motor development problems. The Bruininks-Oseretsky Test of Motor Proficiency was updated in 2005, and the Bruininks-Oseretsky Test of Motor Proficiency 2 (BOT2) was developed. The BOT2 is applicable for children between 4 and 21 years of age and takes between 45 and 60 minutes to complete, and the maximum possible score is 243. BOT2 also has a short form, namely the Bruininks-Oseretsky Test of Motor Proficiency 2 – Short Form (BOT2-SF), which was updated in 2010 to include 12 items rather than 14. The BOT2-BF takes between 15 and 20 minutes to complete, and the maximum possible score is 72. The validity and reliability of the Turkish version of BOT2-BF were previously shown to be satisfactory in children with SLD by Köse et al. in 2018 (Cronbach's $\alpha = 0.78$).

2.5. Intervention program

The Visual-Praxis-based Occupational Therapy program (VPOT): The VPOT was applied for a duration of 8 weeks with twice-weekly sessions of 45 minutes. The VPOT consists of 6 tasks in total; 4 stations and 2 activities. In each session of the VPOT, all 6 tasks were done in "activity-station-station-station-station-activity "sequence, without any breaks. Before starting the sessions, each task's rules

and contents were explained to the child in detail and an easy-to-understand manner (18).

2.6. Statistical analysis

All analyses were performed using SPSS v.26. In order to establish the homogeneity of the groups, both ages and genders of the participants were compared between groups at the baseline of the study. This comparison was made using the Chi-Square test for gender and Mann-Whitney U Test for ages due to the variable age not having a normal distribution. The participants' BOT-2BF mean scores were reported for all sub-tests and the total score. BOT2-BF results before and after the intervention program was compared in both of the groups separately. In order to see the differences in groups, the effect size for each sub-score and the total score were calculated with the post-intervention assessment results, using Cohen's d score.

3. Results

After obtaining the ethics committee approval, 74 patients were included in the study. Out of 74 patients, 60 patients were included in this study, after exclusion of 14 patients as per the exclusion criteria (Fig. 1).

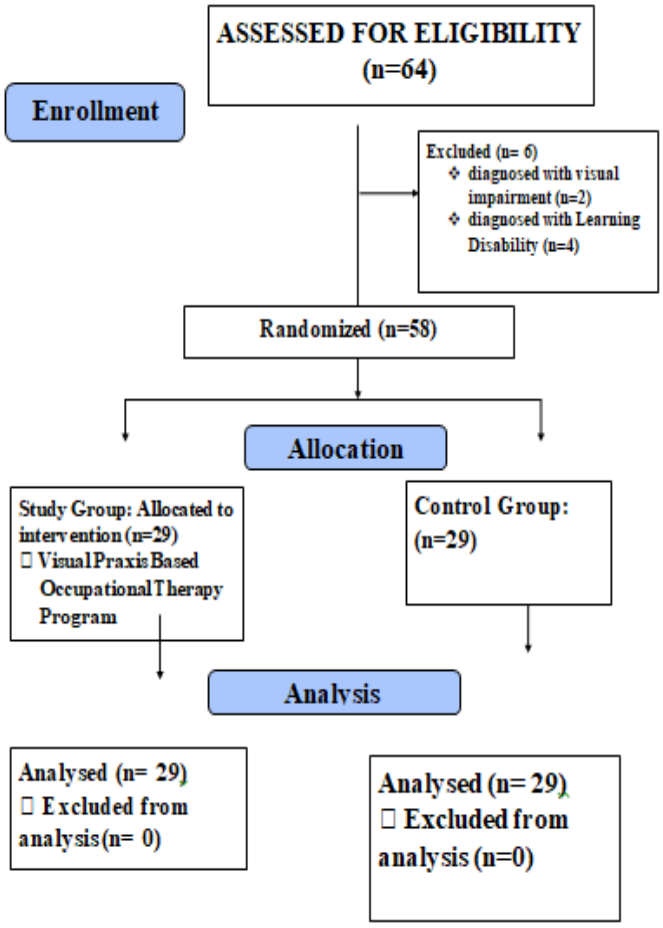

Fig. 1. Consort Diagram

A total of 58 individuals participated in this study. The participants were randomly assigned to one of the two groups. There were 29 participants in each group, and there were no drop-outs. The participants' mean age was 8.44±1.39 years, and there were 41 male participants (70.7%). There were no differences between the intervention and the control group regarding age (Table 1) and gender (Table 2).

Table 1. Baseline comparison of age between groups

	Intervention Group (X±SD)	Control Group (X±SD)	p
Age	8.72±1.60	8.96±1.17	0.318

Table 2. Baseline comparison of genders between groups

		Intervention Group	Control Group	Total	Chi-Square
Gender	Male	19	22	41	0.565
		65.5%	75.9%	70.7%	
	Female	10	7	17	
		34.5%	24.1%	29.3%	

When the BOT2-BF results for the intervention group were analysed, it was found that there were significant differences between pre- and post-intervention scores of all sub-scores and the total score. On the other hand, there were no significant differences between the pre- and post-intervention scores in none of the sub-scores or the total score of BOT2-BF in the control group. Additionally, the effect sizes which were calculated using the Cohen's d score – were between 0.54 and 1.16, which showed a medium to large effect size between the groups (Table 3) (19).

4. Discussion

In this study investigating the effects of the VPOT on the motor proficiency skills of children with ADHD, it was found that the VPOT was effective in improving all of the motor skills parameters (fine motor, gross motor, balance, coordination, etc.). Due to approaching the motor development aspect of ADHD within the context of motor proficiency and visual perception, this study can be considered to be one of the innovative and pioneer studies in the field and is positively unique regarding the usage of the intervention approach that was used.

According to the results of the study investigating the effects of a physical exercise-based rehabilitation program on the motor skills of children with ADHD, Pan et al. stated that significant improvements were achieved in locomotor skills and hand coordination while other motor proficiency skills did not improve (19). Vysniauske et al., based on the results of a meta-analysis investigating the effects of physical

activity-based rehabilitation applications on the motor skills of children with ADHD uncovered that the studies including children with ADHD were focused on a single skill and usually were found to be effective in improving a specific motor proficiency skill (endurance, hand-eye coordination, fine motor skills), however, were not effective in improving many motor skill parameters simultaneously (20). While the results of our study are in accordance with the literature in that they show the effectiveness of an intervention approach in improving motor skills of children with ADHD, they also are positively distinct in showing the effectiveness of the intervention program in improving all parameters of motor skills. We think that this difference was caused by the VPOT not focusing on a single aspect of motor skills and/or not focusing on a specific motor skill in every session, but rather utilizing a multi-skill training in each of the sessions.

Table 3. Comparison of BOT2-BF scores between pre- and post-intervention assessment in the Intervention and the Control groups

	Intervention Group					Control Group					
	Pre-Intervention		Post-Intervention		p	Pre-Intervention		Post-Intervention		p	Effect Size (Cohen's d)
BOT2-BF Score	Mean	Std. Dev.	Mean	Std. Dev.		Mean	Std. Dev.	Mean	Std. Dev.		
Fine Motor Precision	3.07	0.96	3.93	0.80	**0.001***	3.45	1.02	3.49	0.99	0.33	0.58
Fine Motor Integration	5.00	2.20	5.93	2.19	**0.001***	5.93	2.45	5.82	2.16	0.58	0.54
Manuel Dexterity	2.00	0.85	2.90	0.56	**0.001***	2.38	0.68	2.32	0.73	0.87	0.74
Bilateral Coordination	4.00	1.65	5.34	1.56	**0.001***	3.45	1.12	3.48	1.02	0.59	1.16
Balance	2.48	1.18	3.00	0.96	**0.001***	2.07	0.84	2.03	0.82	0.33	0.95
Running Speed and Agility	3.28	1.56	3.90	1.45	**0.001***	3.24	1.62	3.28	1.65	0.33	0.59
Upper Limb Coordination	4.38	2.18	6.69	2.71	**0.001***	5.17	2.70	5.10	2.64	0.54	0.57
Strength	2.52	1.12	3.34	1.34	**0.01***	2.52	1.43	2.57	1.35	0.79	0.59
Total	27.31	7.35	5.45	6.39	**0.001***	28.24	7.51	27.90	7.13	0.16	0.97

* $p<0.05$

In their study analyzing the motor skill problems of Iranian children with ADHD between 6 and 11 years of age, Lavasani et al. found that the most frequently experienced problems were; manual dexterity, balance, and bilateral coordination (21). In a study investigating the effectiveness of a neurofeedback-based rehabilitation program on the motor skills of children with ADHD between 5 and 12 years of age, Jahani et al. said that neurofeedback was an effective intervention method for increasing manual dexterity in the participants with a medium effect size. Sharon et al., on the other hand, found that an interactive metronome-based rehabilitation program was effective in improving the motor skills in children with ADHD and stated that the intervention method yielded significant improvements in bilateral coordination, and upper-extremity reaction time, and manual dexterity with a large effect size (22). When the results of our study were analyzed, it was seen that there were parallels between our results and various different applications in the literature regarding the intervention approach significantly improving the manual dexterity skills. However, while many other studies reported a large effect size regarding the improvements in manual dexterity, there was only a medium effect size in our study. We think that this situation arose from the difference between the tests used in order to assess manual dexterity skills.

In the results of their review study analyzing the motor control problems experienced by children with ADHD, Kaised et al. stated that multi-layered studies were needed to improve balance due to balance being a highly complex concept, and the balance and coordination problems of the children with ADHD were influencing their motor control skills. In addition to this, they have emphasized the need for increasing the number of high-quality studies in this field (23). Bucci et al. reported that the visual attention-based applications for children with ADHD improved accurate and correct eye movements that, in turn, influenced the dynamic balance positively and therefore were effective methods that can be used in improving balance skills (24). In a study investigating the effects of an active vestibular stimulation-based rehabilitation program on the motor skills of children with ADHD between 7 and 12 years of age, Moghadam et al. showed that the method they applied was very effective in improving visual-motor integration and balance skills (25). The VPOT training program that we used in our study had similar effects to other intervention approaches in the literature regarding improving the balance skills of children with ADHD. In addition to the similarities to other intervention methods described in the literature, the VPOT yielded a large effect size regarding the improvements in the balance skills. This may have been caused by the VPOT's structure, which involves the simultaneous usage of visual, motor, and sensory skills, being in correspondence with the complex nature of balance skills, possibly yielding a heightened effect.

As a result of their study investigating the effects of physical activity on the symptoms shown by children with ADHD throughout the prognosis of the condition, Hoza et al. found that even a basic level of physical activity was effective in improving motor coordination (26). Meßler et al. similarly found that the High-Intensity Interval Training program was effective in improving bilateral coordination in children with ADHD between 8 and 13 years of age (27). The results of our study indicated that the VPOT was an effective treatment method for improving bilateral coordination. Especially due to lowered bilateral coordination being one of the most frequently reported problems in children with ADHD and the previous studies reporting the most rapid improvements in bilateral coordination, we think that our results are in accordance with the literature.

According to its prognosis, ADHD is considered to be a neurodevelopmental disorder usually characterized by bursts of anger and not being able to conclude an ongoing task. It was reported that the inability to follow a typical motor development process was underlying these behavioral problems. In our study, the VPOT training program was used with the aim of improving motor skills, and it was found to be effective. However, the behavioral effects of the said improvements were not assessed. When the skill and behavior-based dynamic structure of ADHD are considered, this can be interpreted as a limitation of our study.

In conclusion, the VPOT, which was developed considering the typical development of visual perception and praxis skills, was found to be effective in improving all of the parameters of motor proficiency skills (fine motor, bilateral integration, balance, etc.). It is stated that the problematic developmental areas experienced by children with ADHD have a high variation. When the developmental processes are taken into consideration, it can be understood that problems in motor skills affect the children's activities of daily living. In recent studies regarding this subject, the importance of comprehensive studies from this perspective was repeatedly emphasized. The VPOT training, which was developed by the researchers, is one of the first intervention approaches involving visual perception, motor, sensory and cognitive processes. In addition to that, we think that the usage of the VPOT in this study may provide a positive contribution to the literature towards a conceptual shift in developing new intervention approaches and may act as an incentive for future studies willing to integrate multiple developmental areas and conduct more comprehensive studies.

Conflict of interest

The authors declared no conflict of interest.

Funding

No funding was used for the study.

Acknowledgments

None to declare.

Authors' contributions

B. K. and K.K. carried out the literature survey, applied for ethical approval, and contributed to the development of the data collection methods and analysis plans. B. K. applied the visual-based occupational therapy program. O.K. undertook the data collection process, which is the repeated evaluation process. E.T. and S.Ş. contributed to the analysis process, interpreted the data, and contributed to the development of the text. All the authors reviewed and edited the text and eventually approved the final version.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 809-813
doi: 10.52142/omujecm.39.3.41

The pivotal role of the number of transferred embryos in oocyte donation cycles: A retrospective cohort study

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Received: 29.04.2022 • **Accepted/Published Online:** 20.07.2022 • **Final Version:** 30.08.2022

Abstract

This research aimed to show the role of the number of transferred embryos on pregnancy outcomes of the oocyte donation cycles (ODC). This retrospective cohort study included 122 ODCs performed at a private in vitro fertilization (IVF) center between 2020 June - 2021 January. Cases with severe male infertility, tuboperitoneal, and endometrial factors were not included in the study. The median (interquartile range) recipient age was 43 (30–54) years. ODC results revealed that 10.7% of the cases were negative, 4.9% were biochemical pregnancies, and 84.4% were clinical pregnancies. Pregnancy outcomes were checked; miscarriage, preterm, and term delivery rates were 5.7%, 3.9%, and 90.4%, respectively. The rate of recipients for the younger than 40 years was 32%, between the 40–44 years was 27%, and between 45–54 years was 41% respectively. Statistically significant difference was not observed between age groups in terms of endometrial thickness ($p = 0.059$), number of transferred embryos ($p = 0.857$), number of ODC attempt ($p = 0.666$), live birth rate ($p = 0.1$), and other pregnancy outcomes ($p > 0.05$, for all). A total of 96 (78.7%) embryo transfers (ET) resulted in a live birth. In 8.2% (n=10) of cases, single embryo transfer (SET) and in 91.8% (n=112) of cases, double embryo transfer (DET) was performed. The number of embryos transferred was statistically significantly higher among cases that resulted in live births compared to cases without live births ($p = 0.002$). Significant difference was not found in terms of the recipient age ($p = 0.392$), male age ($p = 0.108$), endometrial thickness ($p = 0.478$), and the number of attempt ($p = 0.777$) between cases resulted in live birth or not. The only parameter that affects the live birth rates in ODC is the number of transferred embryos.

Keywords: Oocyte donation cycle, live birth rate, pregnancy outcome, number of transferred embryos

1. Introduction

Since the first successful IVF pregnancy was succeeded via donor oocytes in the 1980s, oocyte donation cycles (ODC) have been increasingly preferred among women who cannot conceive with their oocyte or when it is inconvenient to become pregnant with their oocyte (1). Although ODC is routinely used, it is also discussed at ethical tables, in religious settings, and in medical debates. Couples may apply to ODC for reasons such as decreased ovarian reserve due to advanced age or premature ovarian failure, hypergonadotropic hypogonadism, severe genetic diseases, and recurrent pregnancy losses (1,2). Donor age had to be used to determine the appropriate number of embryos to be transferred in ODCs (3). Moreover, a single high-quality blastocyst transfer is recommended to minimize multiple pregnancies and related complications in ODCs (3). Although using healthy and high-quality oocytes as much as possible is principal in ODC, some characteristics of the recipient woman and her husband affect the probability of successful pregnancy results. Many parameters such as the sperm quality used in fertilization, age of the recipient mother, body mass index (BMI), genetic characteristics, endometrial thickness, and pattern were associated with clinical outcomes in ODC (4-7). Single embryo transfer (SET) is recommended to avoid complications related to multiple pregnancies and to increase the live birth rate. However, specialists generally choose to transfer double embryos when they have a suspicion about the quality of embryos. Still, conventional morphological grading, which determines the embryo quality has limitations for predicting the live birth rates (8). In this research, it is expected to underline the effects of the number of transferred embryos predicting live birth rates in fresh ODC of couples with no

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embryos to be frozen.

2. Materials and methods

This retrospective cohort study included 122 ODCs conducted at a private IVF center in Cyprus. Data has collected between June 2020 and January 2021. Egg donors were 22 to 30 years old, and recipients were 30 to 54 years old. All patients were informed and signed a written consent form to share the results of treatment and to use data for research purposes in the academic area. The local Institutional Review Board (2020-10/1) has approved to do research for this study. Each patient was analyzed only once in this study.

2.1. Patients

Inclusion criteria

- Fresh donor oocytes were used in all ODC
- Couples without severe male factor or endometrial factor
- Donors aged between 22-30 years
- Recipient age <55 years
- Couples with no embryos to be frozen

Exclusion criteria

- Recipients with known endocrinologic problems and other chronic illnesses
- Donors with known genetic problems, endocrinologic problems, and other chronic illnesses
- Multiple pregnancies

2.2. Procedure

In cases where a decision was made for an ODC, 2 mg estradiol (E2) hemihydrate (17beta-estradiol) treatment was started orally three times a day and was continued at the same dose for at least 7 days. The dose was increased (2× 2 per oral per day) in cases of thin endometrium (< 7mm) or serum E2 did not reach 300 pg/ml. Simultaneously with ovulation trigger in donor women, intramuscular progesterone injection 1x100mg per day was started in the recipient women. Estradiol and progesterone were administered together after embryo transfer. These medications had been used until a βhCG test. Controlled ovarian stimulation for egg donors was done by recombinant follicle-stimulating hormone (r-FSH; Gonal-F®, Serono, Geneva, Switzerland) and suppression for LH surge was done by a gonadotropin-releasing hormone (GnRH) antagonist, cetrorelix acetate (Cetrotide®, Merck KGaA, Serono, Geneva, Switzerland). Final follicular maturation has been completed by analog trigger; Leuprolide acetate (Lupron; TAP Pharmaceuticals, North Chicago, IL, USA). Lastly, ovum pick-up is performed after 35-36 hours with transvaginal ultrasound. The fertilization process is done with Intracytoplasmic sperm injection (ICSI) for all patients. In our policy, on the fifth day of embryos 1 or 2 good quality embryo transfer (ET) was performed in all ODCs.

2.3. Variables

Variables examined in this study are as follows:

- Recipient's and husband's age,
- The thickness of the endometrium,
- Number of previous ODC attempts
- The number of transferred embryos
- Pregnancy outcomes

2.4. Assessment of pregnancy outcomes

Beta-hCH levels are checked in blood after 12 days, followed by embryo transfer (ET). In case of a positive result, an ultrasound scan is performed after the first beta-hCG test. With an ultrasound scan, foetal viability is checked. International Committee for Monitoring Assisted Reproductive Technology (ICMART) (9) criteria are used for pregnancy results. These are: Biochemical pregnancy has accepted as positive test result which is beta hCG in serum or urine but not seen in ultrasound scan, clinical (ongoing) pregnancy was accepted by the existence of heartbeat of one foetus or more at 7th weeks of gestational age and liveborn of a baby more than 24th weeks was accepted as live birth (LB).

2.5. Statistical analysis

Statistical Package for the Social Sciences v21 (SPSS Inc., Chicago, IL, USA) is used to analyse the data. The normality test is checked by Shapiro-Wilk analyses. Statistical data are shown by mean ± standard deviation or median (interquartile range; IQR). Both parametric and non-parametric tests were used in the situation of the test results. Categorical variables were analysed by the Chi-square tests. Normally distributed variables were analysed with independent samples t-test. The Mann-Whitney U test and Kruskal Wallis test are used for not normally distributed variables. Two-tailed p-values of <0.05 were accepted as statistically significant.

3. Results

The median (IQR) recipient age was 43 (30–54) years. Procedure results revealed that 10.7% was negative, 4.9% was biochemical pregnancy, and 84.4% was clinical pregnancy. Of the clinical pregnancies, miscarriage was observed in 5.7%, preterm delivery in 3.9%, and term delivery in 90.4% of the ODCs. The age of recipients who were younger than 40 years covers 32% of the total participants, for between 40–44 years, 27% and between 45–54 years were 41%. In terms of endometrial thickness ($p = 0.059$), number of transferred embryos ($p = 0.857$), number of OD attempt ($p = 0.666$), live birth rate ($p = 0.1$, Fig. 1), and other pregnancy outcomes ($p > 0.05$, for all) statistically significant difference was not found out by between age groups (Table 1).

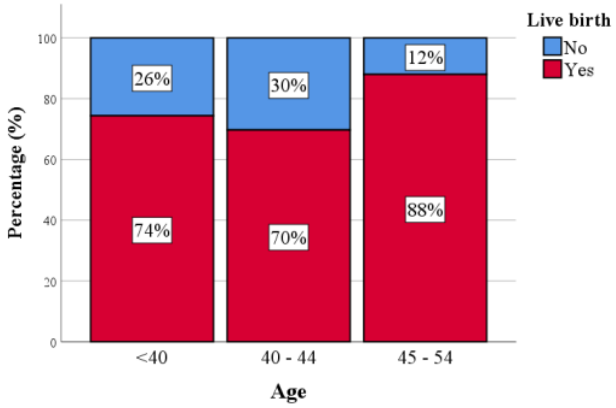

Fig.1. Live birth rates with regard to age groups

Table 1. Summary of clinical characteristics with regard to the age group

	Recipient age (years)				*p-value*
	<40 (n = 39)	**40 – 45 (n = 33)**	**46 – 54 (n = 50)**	**Total (n = 122)**	
Recipient age (years)	34 (30 - 36)	43 (42 - 43)	47 (46 - 50)	43 (37 - 46)	<0.001
Male age (years)	34 (31 - 37)	44 (41 - 49)	46 (41 - 48)	42 (36 - 47)	<0.001
Endometrial thickness (mm)	9.1 (8.7 -11.2)	9.2 (8.4 - 10)	8.7 (8.1 - 9.8)	9.1 (8.3 - 10)	0.059
Embryos transferred (no)					
1	4 (10.3%)	3 (9.1%)	3 (6%)	10 (8.2%)	0.857
2	35 (89.7%)	30 (90.9%)	47 (94%)	112 (91.8%)	
OD attempt (no)					
3	32 (82.1%)	27 (81.8%)	42 (84%)	101 (82.8%)	0.666
2	5 (12.8%)	6 (18.2%)	7 (14%)	18 (14.8%)	
1	2 (5.1%)	0 (0%)	1 (2%)	3 (2.5%)	
Result					
Biochemical pregnancy	3 (7.7%)	2 (6.1%)	1 (2%)	6 (4.9%)	0.439
Clinical pregnancy	31 (79.5%)	27 (81.8%)	45 (90%)	103(84.4%)	0.354
Miscarriage	2 (5.1%)	4 (12.1%)	1 (2%)	7 (5.7%)	0.149
Live birth	29 (74.4%)	23 (69.7%)	44 (88%)	96 (78.7%)	0.100

Data are given as median (1st quartile-3rd quartile) for continuous variables according to normality of distribution and as frequency (percentage) for categorical variables, OD: oocyte donation

A total of 96 (78.7%) ODCs resulted in a live birth. In 8.2% (n=10) of cases SET and in 91.8% (n=112) of cases DET were performed. The number of embryos transferred was statistically significantly higher among cases with live births compared to cases without live births (p=0.002). No significant difference was found in; the age of the recipient (p = 0.392), male age (p=0.108), endometrial thickness (p = 0.478), and number of attempts (p=0.777) between cases with and without live birth (Table 2).

Table 2. Summary of characteristics with regard to the live birth

Recipient age (year)	**Live birth**		*p-value*
	No (n = 26)	**Yes (n = 96)**	
	42 (44 - 37)	44 (47 - 36)	0.392
Male age (year)	44 ± 6.75	41.45±7.17	0.108
Endometrial thickness (mm)	9.2(10 - 8.6)	9.05 (10.1 - 8.2)	0.478
Embryos transferred (no)			
1	6 (23.1%)	4 (4.2%)	0.002
2	20(76.9%)	92 (95.8%)	
Oocyte donation attempt (no)			
3	22(84.6%)	79(82.3%)	0.777
2	3 (11.5%)	15 (15.6%)	
1	1 (3.8%)	2 (2.1%)	

Data are given as mean ± standard deviation or median (1st quartile-3rd quartile) for continuous variables according to normality of distribution and as frequency (percentage) for categorical variables

4. Discussion

OD had a limited area of use in its first application; however, it has become increasingly common today (1). The decreasing ovarian reserve in women of advanced age may cause infertility. Remarkably, advanced-aged couples who decide to have children pretend to use ODC. This study researched the effects of the number of transferred embryos on expecting live birth rates within fresh ODCs. Recipients' age and other parameters were not affecting the pregnancy outcome. Remarkably, results showed that the live birth rate was higher in women with double embryo transfer compared to single embryo transfer. ODC is generally preferred by relatively advanced-age women. However, advancing age is known to be associated with negative obstetric outcomes in women. Decreased successful pregnancy outcomes in advanced age are suggested to be caused by decreased blood flow to the endometrium, thus limiting the access to various hormones (10). Similarly, different studies showed that the age of recipients over 45 years negatively affects pregnancy outcomes (11-13). Contrary to these studies, many stated that the age of recipients in OD does not affect pregnancy outcomes, which is similar to this study (5, 14-17). Consistent with these studies, no significant difference was found in terms of pregnancy outcomes by the different age groups which are examined in this study. In addition, the husband's age differed between recipient age groups in our results. Previous studies showed that male age in OD does not affect pregnancy outcome (18). Therefore, this difference was assumed not to affect the results. An increased number

of embryos transferred in ICSI cycles showed and suggested to increase the probability of implantation and indirectly the possibility of live birth; however, current guidelines recommend SET in OD cycles (3). Multiple pregnancies are not very suitable due to various undesirable consequences and parents' unwillingness. More than one embryo transfer in IVF treatment increases the risk of multiple pregnancies (5, 19). Increased multiple pregnancy frequency with the increasing number of embryo transfers was shown in OD cycles (14, 20). In some studies, no significant difference was shown between DET and SET in terms of pregnancy frequency, but a significant difference was found in terms of multiple pregnancies (21). Among the parameters examined in our study, only the number of transferred embryos was related to the live birth rate. When the number of transferred embryos is compared, DET had significantly higher live birth rates than SET. In different studies, parameters affecting pregnancy outcomes in OD cycles were examined. In a study examining the parameters used to predict egg-sharing donation outcomes, Braga et al. reported that pregnancy outcome was associated with oocyte quality and successful implantation, regardless of the age of donors and recipients (5). In ICSI cycles, the day of the embryo at transfer time is one of the most important reasons referring to the success of blastocyst transfer. Our study shows that DET results have higher LBR compared to SET in ODCs. The reason why DET is recommended may be due to the moderate embryo quality. DET increases fertility per ODC; cumulating single embryo transfer in a fresh or frozen cycle after SET can significantly balance the difference in efficacy while reducing the risks of having multiple pregnancies; at least in women with high-quality embryos (22). On the other hand, concerns about the acceptability of the multiple pregnancy and related complications can be the cost of this decision. In other studies, the authors noted that the recipient's BMI and the husband's sperm quality affect pregnancy outcomes (6, 7, 23). These parameters were not examined in our study. The distribution of these parameters at different levels among the groups may have caused our results to be unconsciously misinterpreted. The most important limitations of this study are retrospective data collected from a single center. Many parameters of the donor, recipients, and husbands that may affect clinical pregnancy in OD treatment were not examined. The fact that these parameters are confounding factors and their heterogeneous distribution among the groups caused us to take a biased interpretation. In addition, age variation of the husband among different recipient age groups may affect the results.

This study exposed that the recipient's age did not significantly affect pregnancy outcomes in ODC. The only parameter affecting the live birth rate in ODC was the number of transferred embryos. Because of this, DET should be preferred to increase live birth rates in ODC cycles if the quality of embryos is not reassuring. Nevertheless, this research only included blastocyst transfer, qualities of the embryos were not included in the study, and the sample size of the population was small. Consequently, it is recommended that the impact of DET and SET with different embryo qualities at blastocyst stages needed to be examined in future studies.

Conflict of interest

The authors declared no conflict of interest.

Funding

No funding was used for the study.

Acknowledgments

None to declare.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 814-816
doi: 10.52142/omujecm.39.3.42

Surgical treatment of basket impaction, a rare complication of ERCP. Single centre experience

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Received: 25.04.2022 • **Accepted/Published Online:** 06.06.2022 • **Final Version:** 30.08.2022

Abstract

Basket impaction is a rare complication of Endoscopic Retrograde Cholangiopancreatography (ERCP). This complication is usually treated non-operatively. Surgery is required when non-operative methods fail. In this study, we retrospectively evaluated the results of five patients who underwent surgery for basket impaction between January 2017 and December 2021. The median mean age was 50 (21-82) years. Basket impaction was in the common bile duct in three patients and the pancreatic duct in two. There was no operative complication. Wound infection developed in one patient and evisceration in one in the postoperative period. The length of hospital stay was 10 (7-12) days. The overall morbidity rate was 33.3%. There was no postoperative mortality. Basket impaction can usually be treated with non-operative interventions. Surgical intervention should be preferred last.

Keywords: biliary stone, pancreatic stone, basket, impaction, surgery, endoscopic retrograde cholangiopancreatography

1. Introduction

Endoscopic Retrograde Cholangiography (ERCP) is an invasive method used to treat bile duct and pancreatic duct stones (1, 2). The overall complication rate after ERCP has been reported as 5-10% (3).

Basket impaction/wire fracture is a rare complication usually treated with non-operative methods. Surgery is the only treatment option when endoscopic treatment fails (4). This study aimed to present the results of patients who had failed endoscopic interventions and underwent surgery due to basket impaction.

2. Materials and Methods

The local ethics committee approved this study (OMU KAEK 2022/233). We obtained informed consent from all patients. We included in the study five patients who underwent surgery with the diagnosis of basket impaction between January 2017 and December 2021 and performed surgery in all patients when non-operative methods failed.

We retrospectively analyzed clinico-demographic characteristics, concomitant diseases, ERCP indications, previous ERCP attempts, stone size, basket type, basket impaction location, surgical type treatment, postoperative complications, mortality, and length of hospital stay from patient medical records.

2.1. Surgical technique and postoperative care

We conducted antibiotic prophylaxis with 3rd generation cephalosporin 30 minutes before incision. Three hepatopancreaticobiliary surgeons performed two different surgical methods according to the localization of basket impaction.

If the impacted basket is in the common bile duct, we fully achieved duodenal mobilization by performing the Kocher maneuver for a tension-free anastomosis and applied 2 cm longitudinal choledochotomy to the common bile duct and then extracted the basket with the stone. We irrigated the bile duct with saline and checked common main duct for stone clearance and the passage from the choledochus to the duodenum via a bougie. We performed cholecystectomy routinely if not done previously and choledochodoudenostomy anastomosis with continuous PDS (Polydioxanone) monofilament suture 5.0 for single-layer anastomosis. We placed an abdominal drain under the choledochodoudenostomy routinely.

If the impacted basket is in the main pancreas duct, we performed duodenotomy from the second part of the duodenum and then removed the stone and basket. We repaired the

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duodenum over a double layer. We placed an abdominal drain under the duodenum.

Oral intake started on the third postoperative day. When the amount was below 100 cc/day, we removed the drain and then discharged the patients. If T-tube was applied, we removed it on the 14th postoperative day after control cholangiography.

3. Results

The median mean age was 50 (21-82) years. Three of the patients were women. The clinico-demographics and surgical characteristics of the patients are shown in table 1. We performed ERCP in three due to bile duct stones and two due to pancreatic duct Stones (Fig. 1). ERCP features are shown in table 2. There were no intraoperative complications. Postoperative wound infection developed in one patient and evisceration in one. The length of hospital stay was 10 (7-12) days. The overall morbidity rate was 40%. There was no postoperative mortality.

Table 1. Characteristics of the patients

Age	Sex	Stone localization	Surgery	Complication	Length of stay	Comorbidity
59	F	Common bile duct	Stone and basket extraction+ Choledochoduodenostomy+Cholecystectomy	None	11	Hypertension
59	M	Pancreas	Duodenotomy and basket extraction from the pancreatic duct	Wound infection	8	Diabetes mellitus
82	F	Common bile duct	Stone and basket extraction+ Choledochoduodenostomy	Evisceration	10	Hypertension+ coronary artery disease
21	F	Pancreas	Duodenotomy and basket extraction from the pancreatic duct	None	7	None
50	M	Common bile duct	Common bile duct exploration+T tube+ Cholecystectomy	None	12	None

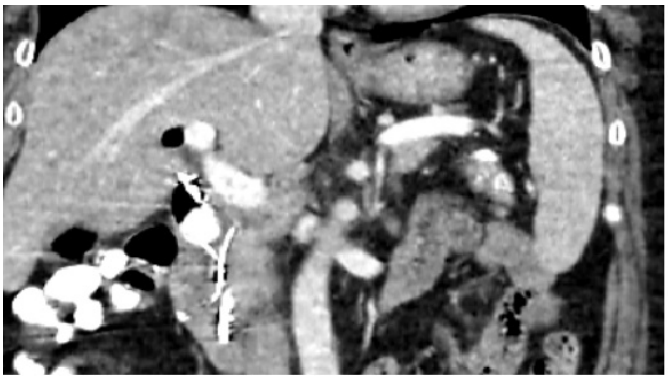

Fig. 1. Computed tomography image of the impacted basket in the common bile duct

Table 2. ERCP features

	Indication	Number of ERCPs	Duct Diameter	Basket type	Stone diameter
1	Common bile duct Stone	3	20mm	Dormia	20mm
2	Pancreatic Stone	5	7mm	Dormia	6mm
3	Common bile duct Stone	4	20mm	Dormia	20mm
4	Pancreatic Stone	2	6mm	Dormia	5mm
5	Common bile duct Stone	2	10mm	Dormia	20mm

4. Discussion

ERCP is the primary choice for the treatment of common bile duct stones. The success rate of ERCP is 85-95% (5). The European Society for Gastrointestinal Endoscopy (ESGE) recommends sphincterotomy and balloon dilation for the first treatment of common bile duct stones. ESGE recommends mechanical lithotripsy (ML) as a secondary treatment (6). ML is recommended if the stone size exceeds the CBD diameter after balloon dilation. The success rate of ML was reported as 79-96% and the complication rate as 3.5%. These complications are trapped/broken baskets, wire fracture, and broken handle perforation/duct injury (7-12).

Dormia basket entrapment/fracture is a rare complication and usually a problem of high-volume centers (4). The basket impaction/wire fracture frequency has been reported as 0.8-5.9% (13-15). If the biliary stone is over 20 mm, it is a significant risk factor for basket impaction (16). This problem is usually resolved with non-operative methods. Non-operative methods are extracorporeal shockwave, intracorporeal electrohydraulic lithotripsy, and laser lithotripsy catching the basket with a second basket. If non-operative interventions are failed, surgical intervention is required (17-19).

Pancreatic duct stones usually develop in the background of chronic pancreatitis. Removal of pancreatic duct stones is recommended because of the potential to cause persistent duct obstruction and recurrent pancreatitis. Endoscopic removal of pancreatic stones includes pancreatic duct sphincterotomy, reduction of stone with balloon or basket, ML, and pancreatic stent placement (20). Pancreatic stones are less common than biliary tract stones. However, it has been reported that the complication rate in pancreatic stones is three times higher than in the common bile ducts. Several rescue methods are available in basket impaction, including open surgery, salvage lithotripters, sphincterotomy lengthening, stent placement, EHL (electrohydraulic), and ESWL (extracorporeal shockwave lithotripsy). In the past, open surgery was the most commonly used salvage option (21).

ERCP is at the forefront in treating both common bile duct stones and pancreatic duct stones. Basket impaction can

usually be treated with non-operative interventions. Surgical intervention should be preferred last.

Conflict of interest

None to declare.

Funding

None to declare.

Acknowledgments

None to declare.

Authors' contributions

Concept: K.K., İ.G., Design: K.K., Data Collection or Processing: K.K., Analysis or Interpretation: K.K., İ.G., Literature Search: K.K., Writing: K.K., İ.G.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 817-821
doi: 10.52142/omujecm.39.3.43

Maternal-fetal characteristics, etiological factors and perinatal outcome in pregnancies with polyhydramnios

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Received: 11.05.2022 • **Accepted/Published Online:** 06.06.2022 • **Final Version:** 30.08.2022

Abstract

This study aimed to evaluate maternal and fetal characteristics, etiological factors and perinatal outcome in pregnancies with polyhydramnios. A total of 330 pregnancies diagnosed with polyhydramnios were included in this retrospective study conducted a tertiary center. Data on maternal age, obstetric characteristics, etiology of polyhydramnios, detailed prenatal ultrasonography findings on fetal anomaly, cesarean delivery and the adverse pregnancy outcomes were recorded. The polyhydramnios was idiopathic in 47.0% of cases, while the maternal diabetes (29.4%) and fetal conditions (23.6%) were responsible for the etiology in the remaining cases. The most common anomalies identified on prenatal ultrasonography were central nervous system anomalies (29.5%), followed by the gastrointestinal system anomalies (11.5%). Cesarean delivery occurred in 38.2% of pregnancies, while adverse pregnancy outcomes were noted in 64.5% of pregnancies and including delivery of a neonate with congenital anomaly (36.6%), macrosomia (21.1%), preterm delivery (18.3%), perinatal mortality (13.1%) and pregnancy termination (10.8%). In conclusion, our findings revealed the polyhydramnios to be idiopathic approximately in half of cases, and to be due to either maternal diabetes or fetal pathology (central nervous system and gastrointestinal anomalies) in the other half. Given the occurrence of adverse pregnancy outcomes in most of pregnancies with polyhydramnios, our findings emphasize the vital role of intensive monitoring of the maternal-fetal condition in pregnancies with polyhydramnios. Meticulous diagnostic approach seems crucial for timely recognition of fetal anomalies via detailed imaging studies as well as the early recognition and strict control of gestational diabetes via close follow up, given the likelihood of erroneously diagnosed idiopathic polyhydramnios to challenge the implementation of proper management and appropriate counselling of patients.

Keywords: polyhydramnios, etiology, amniotic fluid index, pregnancy outcome

1. Introduction

Polyhydramnios, a pathologic excess of amniotic fluid index (AFI) in pregnancy, refers to a high-risk obstetric condition with an incidence of 0.9-3.9% and increased risk of adverse pregnancy outcomes (1-3). Its association with increased perinatal morbidity and mortality depending on the extent of excess AFI and the underlying cause, necessitates antepartum evaluation and fetal surveillance to identify the underlying cause, direct care, and time the delivery (2, 4-7). Although the most of cases are idiopathic, polyhydramnios can also result from fetal (i.e., structural anomalies, aneuploidy) or maternal conditions (i.e., diabetes, infections) (2, 5, 7-9).

This study aimed to evaluate maternal and fetal characteristics, etiological factors and perinatal outcome in pregnancies with polyhydramnios.

2. Materials and Methods

2.1. Study population

A total of 330 pregnancies diagnosed with polyhydramnios were included in this retrospective study conducted at University of Health Sciences, Başaksehir City Hospital between May 2020 and December 2021. Pregnancies beyond the 20th week of gestation were included and those with polyhydramnios due to fetal conditions were further assessed via detailed prenatal ultrasonography for potential structural fetal anomalies.

This study was conducted in accordance with the ethical principles stated in the "Declaration of Helsinki" and approved by the institutional ethics committee (date of approval: 24.11.2021, protocol no: KAEK/2021.11.257).

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2.2. Assessments

Data on maternal age, obstetric characteristics, etiology of polyhydramnios (idiopathic, maternal diabetes, fetal conditions), detailed prenatal ultrasonography findings on fetal anomaly, cesarean delivery (rates and indications), and the adverse pregnancy outcome including preterm delivery (<37th gestational week), macrosomia (>4000 g birthweight), delivery of a neonate with congenital anomaly, perinatal mortality and pregnancy termination were recorded.

Polyhydramnios was diagnosed by two-dimensional ultrasound findings including the amniotic fluid index (AFI) > 24 cm or the single deepest pocket (SDP) > 8 cm, in addition to a 75 g oral glucose tolerance test (OGTT). A 75 g OGTT was implemented in routine pregnancy care between 24 and 28 weeks of gestation, as well as in women with polyhydramnios and a missing OGTT before 28 weeks of gestation, while after 28. gestational week the glycemic status was monitored via glycated hemoglobin (HbA1c) and blood glucose testing.

Fetal well-being after the diagnosis of polyhydramnios was monitored once or twice weekly, based on gestational week and presence of concomitant maternal risk factors.

2.3. Prenatal ultrasonography examination

The prenatal ultrasonography assessment was performed using the ARIETTA 850 (Hitachi Healthcare, C1-5 convex array probes, 1–5 MHz, Japan) and included fetal weight estimation, polyhydramnios-related amniotic fluid measurements and the detailed assessment of the fetal anatomy in cases with suspected fetal pathology.

2.4. Statistical analysis

Descriptive statistics were reported including frequencies and percentages for categorical variables.

3. Results

3.1. Maternal and obstetric characteristics and polyhydramnios etiology

Mean maternal age was 32 years (range, 22 to 47 years). Overall, 50% of women were multiparous and 32.4% were grand-multiparous (Table 1).

Table 1. Maternal and obstetric characteristics and polyhydramnios etiology

Maternal age (year), mean (min-max)	32 (22-47)
Parity, n (%)	
Nulliparous	58 (17.6)
Multiparous	165 (50.0)
Grand-multiparous	107 (32.4)
Polyhydramnios etiology, n (%)	
Idiopathic	155 (47.0)
Maternal diabetes	97 (29.4)
- Type 1 diabetes	15 (4.5)
- Type 2 diabetes	19 (5.8)
- Gestational diabetes on insulin	26 (7.9)
- Gestational diabetes on diet	37 (11.2)
Fetal conditions	78 (23.6)

The polyhydramnios was idiopathic in 47.0% of cases, while the maternal diabetes (29.4%) and fetal conditions (23.6%) were responsible for the etiology in the remaining cases of polyhydramnios (Table 1).

3.2. Prenatal ultrasonography findings on fetal conditions (n=78)

The most common anomalies identified on prenatal ultrasonography were central nervous system (CNS) anomalies (29.5%), followed by the gastrointestinal system anomalies (11.5%) (Table 2).

Table 2. Detailed prenatal ultrasonography findings for fetal conditions related to polyhydramnios (n=78)

	Fetal conditions (n=78)
Prenatal ultrasonography findings, n (%)	
Central nervous system anomalies	23 (29.5)
- Anencephaly	4 (5.1)
- Hydrocephaly	4 (5.1)
- Spina bifida	10 (12.8)
- Encephalocele	3 (3.9)
- Corpus callosum agenesis	2 (2.6)
Gastrointestinal system anomalies	9 (11.5)
- Esophageal atresia	5 (6.4)
- Duodenal atresia	3 (3.9)
- Imperforate anus	1 (1.3)
Genitourinary anomaly	7 (9.0)
Skeletal dysplasia	6 (7.7)
Non-immune hydrops fetalis	5 (6.4)
Diaphragmatic hernia	5 (6.4)
Cardiac anomalies	5 (6.4)
Pulmonary system anomalies	5 (6.4)
- Congenital pulmonary airway malformation	3 (3.9)
- Bronchopulmonary sequestration	2 (2.6)
Chromosomal anomaly	5 (6.4)
- Trisomy 18	4 (5.1)
- Trisomy 21	1 (1.3)
Fetal akinesia syndrome	3 (3.9)
Immune hydrops fetalis	2 (2.6)
Cystic hygroma	2 (2.6)
Facial defect (cleft lip-palate)	1 (1.3)

3.3. Cesarean delivery rate and adverse pregnancy outcomes

Cesarean delivery occurred in 126 (38.2%) of 330 pregnancies with polyhydramnios, due to previous cesarean history in most cases (42.9%), followed by fetal distress (19.8%), labor dystocia (14.3%) and suspected fetal macrosomia (11.9%) (Table 3).

Adverse pregnancy outcomes were noted in 213 (64.5%) of 330 pregnancies with polyhydramnios, and included delivery of a neonate with congenital anomaly (36.6%), macrosomia (21.1%), preterm delivery (18.3%), perinatal mortality (13.1%) and pregnancy termination (10.8%) (Table 3).

Table 3. Cesarean section delivery and adverse pregnancy outcomes (n=330)

Cesarean delivery, n (%)	126 (38.2)
Cesarean indications, n (%)	
- Previous cesarean history	54 (42.9)
- Fetal distress	25 (19.8)
- Labor dystocia	18 (14.3)
- Suspected fetal macrosomia	15 (11.9)
- Fetal malpresentation	8 (6.3)
- Placenta previa	4 (3.2)
- Umbilical cord prolapse	2 (1.6)
Adverse pregnancy outcome, n (%)	213 (64.5)
- Delivery of a neonate with congenital anomaly	78 (36.6)
- Macrosomia (>4000 g birthweight)	45 (21.1)
- Preterm delivery (<37th gestational week)	39 (18.3)
- Perinatal mortality	28 (13.1)
- Pregnancy termination due to concomitant multiple fetal anomaly	23 (10.8)

4. Discussion

Our findings revealed that almost half of pregnancies with polyhydramnios were idiopathic, while maternal diabetes and fetal anomalies (CNS and gastrointestinal anomalies) were responsible for ~30% and 20% of cases, respectively. Overall, 38.2% of women had cesarean delivery, while adverse pregnancy outcome was noted in 64.5% of cases, including congenital anomaly (36.6%), macrosomia (21.1%), preterm delivery (18.3%), perinatal mortality (13.1%) and pregnancy termination (10.8%).

Our findings support the predominance of idiopathic polyhydramnios with no evidence for maternal or fetal pathology in pregnancies with polyhydramnios (1, 8, 10-12), whereas indicate a lower rate of idiopathic polyhydramnios (~50% vs. ~70%) and higher rates of polyhydramnios due to maternal diabetes (~30% vs. ~20-25%) and fetal pathology (~20% vs. ≤11%) compared to most of the previous studies (1,4,10-13).

Nonetheless, there also some studies reporting similarly high rates of fetal anomaly (~30%) regardless of the degree of AFI excess, suggesting the consideration of the likelihood of fetal anomaly in all cases of polyhydramnios, even in those with only mildly elevated amniotic fluid volumes (1, 8, 14).

Especially, almost 10% of cases with idiopathic polyhydramnios were reported to be accompanied by a fetal anomaly (mainly the gastrointestinal atresia) that was only found after birth, while none of the antenatal characteristics (i.e., amniotic fluid volume, estimated fetal weight or gestational and maternal age at the time of diagnosis) was found helpful in detecting these anomalies before birth (13, 14). Also, in a study on the outcomes of children from pregnancies complicated with polyhydramnios without fetal anomalies, the authors emphasized the likelihood of polyhydramnios to be associated with increased rate of fetal malformations, genetic syndromes, neurologic disorders, and developmental delay, which may be diagnosed only after birth (15). Moreover, use of 75 g OGTT with the fixed cut offs is considered not appropriate to identify gestational diabetes in some cases, and gestational diabetes with increased birthweight is considered likely even with blood glucose levels were below the cut-offs (14, 16).

Hence, our findings emphasize the likelihood of a certain proportion of cases with apparently idiopathic polyhydramnios to actually be related to gestational diabetes or fetal anomaly (14), which seems to emphasize the contributory role of differences in accurate prenatal diagnosis rates in the discordance noted between studies on polyhydramnios etiology.

Our findings support the consideration of gastrointestinal tract anomalies, CNS anomalies, musculoskeletal anomalies, airway malformation and congenital diaphragmatic hernia amongst the most common fetal congenital anomalies associated with polyhydramnios (1, 11, 14, 17, 18). In contrast to other studies reporting the cardiac anomalies as the most frequently diagnosed anomaly in fetuses of mother with polyhydramnios (5, 19, 20). Our findings revealed a lower rate of cardiac anomalies and no cases of congenital infection as a cause of polyhydramnios. Likewise, some polyhydramnios studies did not find any case of cardiac malformation (11), and also indicated a rare frequency of congenital infection as a cause of polyhydramnios (11, 12).

The type of CNS anomalies diagnosed on ultrasonography in the current study including spina bifida in 12.8% of cases, followed by anencephaly and hydrocephaly (each in 5.1%) is in agreement with consideration of polyhydramnios as a risk factor for neural tube defects such as spina bifida, anencephaly and encephalocele (21, 22).

In the current study, maternal diabetes was responsible for ~30% of polyhydramnios cases, and the gestational diabetes rather than pre-gestational diabetes was the responsible factor (19.1% vs. 10.3%) along with presence of fetal macrosomia in 21.1% of deliveries. Especially ~25% of all pregnancies with polyhydramnios are considered to be due to gestational diabetes, and about 8-20% of all pregnancies with gestational diabetes are complicated by polyhydramnios (13, 14). Hence, our findings seem to emphasize the role of obligatory OGTT between 24 and 28 weeks of gestation, as well as the monitoring the glycemic status via HbA1c and blood glucose testing after 28 weeks of gestational age. Nonetheless, a tendency for macrosomic fetuses and increased likelihood of adverse pregnancy outcomes has also been noted in pregnancies with gestational diabetes and polyhydramnios vs. those without polyhydramnios, even with strict metabolic control after diagnosis (5, 14, 23, 24). Indeed, a 11-fold increased risk for macrosomia was reported with polyhydramnios but only in the concomitant presence of accelerated fetal growth (AFG) (9). The pregnancies with normal OGTT that develop polyhydramnios and AFG are considered to be at higher risk for maternal and neonatal complications, while isolated polyhydramnios without AFG is considered to increase the risk for delivery complications but

not the neonatal morbidity (9).

Our findings are consistent with previous reports indicated that fetal structural anomalies can be found in 8-45 % of pregnancies with polyhydramnios, whereas the fetal aneuploidies, including trisomy 13, 18, and 21 are observed in only 0.4-10% (4, 5, 10, 11, 20, 23-27). Given that chromosomal anomaly was evident in 6.4% of our cases, which is close to upper limit of the reported range, our findings may emphasize the utility of routine karyotyping in ultrasonographically isolated polyhydramnios (28). In fact, in a meta-analysis of 20 studies in 1729 pregnancies with idiopathic polyhydramnios on the risk of chromosomal aberrations, the authors reported the rate of chromosomal aberrations to range between 0 and 13.8% along with a relative risk of 3.1 for chromosomal aberration in women at high risk for aneuploidy (29). However, given the lack of studies on the relative risk for chromosomal abnormalities in low-risk women with idiopathic polyhydramnios, the authors concluded that the suboptimal quality of the evidence precludes from drawing any solid conclusions on routine karyotype testing in idiopathic polyhydramnios cases, especially in women at low risk for chromosomal aberrations (29).

Regarding the mode of delivery, cesarean delivery was noted in 38.2% of pregnancies in our study (due to fetal distress in 19.8% and fetal macrosomia in 11.9%), supporting previously reported high rates of elective cesarean sections in polyhydramnios cases due to fetal anomaly (22.9 %) and maternal diabetes (21.2 %) (10). In addition, in a retrospective matched case control study with 588 singleton pregnancies, the rate of cesarean delivery was reported to be significantly higher among women with vs. without polyhydramnios (31.3% vs. 18.7%) (30), while the multivariate logistic regression analyses also revealed polyhydramnios to be an independent risk factor for delivery by a caesarean (OR, 2.0 to 21.02) (30, 31). Also, in a study on the outcomes of children from pregnancies complicated with polyhydramnios but a normal detailed ultrasound examination during pregnancy, polyhydramnios was reported to be associated with increase in the risk of elective cesarean delivery due to suspected macrosomia (15).

Preterm delivery (18.3%), delivery with a congenital anomaly (36.6%), perinatal mortality (13.1%) and pregnancy termination (10.8%) rates in the present study, supports the consideration of fetuses with polyhydramnios and congenital anomalies to have a higher risk of perinatal complications with particular increase in the risk of preterm delivery (10, 14, 32). In addition, the ongoing risk of intrauterine fetal demise was also reported to in pregnancies affected by polyhydramnios at every gestational age (7-fold by 37 weeks, and 11-fold by 40 weeks) compared with unaffected pregnancies (33). Likewise, in a study with 50 pregnant women with polyhydramnios vs. 80 pregnant women with normal amniotic fluid, the authors also noted significantly higher occurrences of fetal anomaly, cesarean section, preterm birth, fetal distress and fetal macrosomia in patients with vs. without polyhydramnios (34). Hence, our findings support the crucial role of monitoring of the maternal-fetal condition in pregnancies with polyhydramnios given the association of prenatal diagnosis of polyhydramnios with a higher occurrence of adverse perinatal outcomes (14, 33, 34).

Certain limitations to this study should be considered. First, potential lack of generalizability is an important limitation due to single-center study design with relatively small sample size. Second, lack of data on severity and gestational age at the time of polyhydramnios diagnosis is another limitation which otherwise would extend the knowledge achieved.

In conclusion, our findings revealed the polyhydramnios to be idiopathic in half of cases, and to be due to either maternal diabetes or fetal pathology in the other half. Given the occurrence of adverse pregnancy outcomes in a considerable portion of pregnancies with polyhydramnios, our findings emphasize the vital role of intensive monitoring of the maternal-fetal condition in pregnancies with polyhydramnios. Meticulous diagnostic approach seems crucial for timely recognition of fetal anomalies via detailed imaging studies, given the likelihood of erroneously diagnosed idiopathic polyhydramnios to challenge the implementation of proper management and appropriate counselling of patients.

Conflict of interest

The authors declare that they have no conflict of interest.

Funding

None to declare.

Acknowledgments

None to declare.

Authors' contributions

Concept: Ö.Ö., G.B., U.Ç., Design: Ö.Ö., G.B., U.Ç., Data Collection or Processing: Ö.Ö., G.B., K.B., Analysis or Interpretation: Ö.Ö., U.Ç., Literature Search: Ö.Ö., U.Ç., Writing: Ö.Ö., G.B., U.Ç.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 822-821
doi: 10.52142/omujecm.39.3.44

Periaortic adipose tissue index: A new approach to the relationship between coronary stenosis severity/lesion complexity and periaortic adipose tissue

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Received: 11.05.2022 • **Accepted/Published Online:** 20.07.2022 • **Final Version:** 30.08.2022

Abstract

Periaortic adipose tissue (PAT) is associated with atherosclerosis. The severity of coronary stenosis with PAT has not been evaluated with conventional coronary angiography (CAG). The aim of the study is to determine the relationship between PAT and coronary stenosis severity/ complexity, and to evaluate it with the periaortic adipose tissue index (PATI), a new index derived from PAT. Patients who underwent CAG and thoracic computed tomography (CT) between January 2017 and January 2022 were included in the study. PAT volume was calculated by evaluating CT images, and PATI was calculated by dividing the PAT volume by the circumference of the descending aorta. Patients were divided into two groups according to the presence of ≥50% stenosis on CAG. The correlation of PAT and PATI with the SYNTAX score was evaluated. In our study, 263 patients [mean age 64.5(54/72), male 164 (62.4%)] were evaluated. Severe coronary artery disease (CAD) was observed in 181 patients (68.8%). PAT volume and PATI were significantly higher in patients with severe stenosis ($p<0.001$, for both). When PAT and PATI were evaluated alongside CAD risk factors, an independent association between PATI and severe CAD was discovered (β:0.581, p:0.97, β:0.968, p:0.006, respectively). No correlation was found between SYNTAX score and PAT and PATI (r:-0.026, p:0.73, r:-0.019, p:0.19, respectively). In our study, PAT and PATI were higher in patients with severe coronary stenosis, and there was an independent relationship between PATI and severe stenosis. We found no relationship between PAT and PATI and the SYNTAX score.

Keywords: Coronary artery disease, periaortic adipose tissue, syntax score, coronary angiography, computed tomography

1. Introduction

Coronary artery disease (CAD) is a common cause of mortality and morbidity. Although developing diagnosis and treatment methods reduce deaths due to CAD, the number of patients followed as a result of CAD is increasing (1). Many studies have been conducted to identify and treat atherosclerosis risk factors and tests predicting CAD are very beneficial. Although various invasive and non-invasive tests are used, conventional coronary angiography (CAG) is the gold standard method for diagnosis.

Visceral adipose tissue is found in many parts of the body and is named according to the area where it is located; it is especially prevalent around vessel walls (2). It is known that adipose tissue functions as endocrine organs and produces mediators (2-4) and these also cause obesity-related atherosclerosis and vascular damage (3). It is also known that increased visceral adipose tissue is associated with metabolic syndrome parameters (5). Epicardial adipose tissue (EAT) is the visceral adipose tissue type that has been most reported on (6-8). Increased EAT volume and thickness are known to be associated with CAD, cardiovascular events and arrhythmias (6-9). Although we have less research on periaortic adipose tissue (PAT), it is associated with coronary and peripheral vascular disease (10, 11). In addition, it is known that an increase in aortic diameter is associated with an increase in PAT volume (12, 13). In the Framingham Heart Study, PAT was shown to be associated with thoracic and abdominal aortic diameter and was an independent risk factor (12). The relationship between PAT and coronary lesion comes from computed tomography (CT) studies and most of these evaluated subclinical atherosclerosis (2, 10). Although studies have found that PAT is associated with coronary stenosis and calcification, studies supporting these findings are rare (2, 10). To the best of our knowledge, there exists no study evaluating coronary atherosclerosis with

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CAG and investigating its relationship with PAT. In addition, there is no regulation to minimize the effect of aortic diameter when evaluating PAT related conditions.

The aim of the study was to demonstrate the relationship between PAT volume and coronary stenosis severity/complexity with CAG. In addition, we tested this relationship with the periaortic adipose tissue index (PATI), which we defined according to the aortic diameter.

2. Materials and methods

2.1. Study population

Patients who underwent CAG between January 2017 and January 2022 at Baskent University Alanya Application and Research Center were evaluated. Patients over 18 years of age who underwent thorax CT within 6 months (before or after) the CAG date, were included in the study. Patients with previous percutaneous coronary intervention and/or cardiac surgery (such as coronary artery bypass surgery, valve surgery, and aortic surgery), known moderate and/or severe heart valve disease, heart failure, chronic kidney failure, chronic obstructive pulmonary disease (COPD), were excluded from the study. This study was approved by Baskent University Institutional Review Board (Project no: KA 22/212) and supported by Baskent University Research Fund.

Hospitalization diagnoses were evaluated during the CAG period, and the patients were divided into two groups according to the presence of acute coronary syndrome (ACS) in this period (14-16). The patients were divided into two groups as; 1- Severe CAD, 2-Normal coronary/noncritical CAD based on the presence of ≥50% of coronary lesion severity. In addition, the complexity of coronary lesions was evaluated with the SYNTAX score by consensus by two cardiologists.

Demographic characteristics and laboratory results during the CAG period were obtained using the hospital database. The Chronic Kidney Disease Epidemiology Collaboration formula was used to calculate the glomerular filtration rate (GFR) (17).

2.2. Measurement of periaortic and epicardial adipose tissue volumes

Chest CT images with and without contrast were used in the retrospective evaluation. Chest CT scans were performed with a two-slice multi-detector CT scanner (Somatom Spirit, Siemens). Chest CT protocol with tube voltage of 130 kVp and tube current of 160 mA were used for non-overlapping images with a thickness of 5 mm. The diameter of the descending thoracic aorta was measured from the outer edge of the aortic wall at its widest point, in the anteroposterior plane. All image analyses (GE, Advantage, AW) were performed on the workstation with the semi-automatic method that requires manual tracking of the borders. A CT attenuation range width of -195 to -45 Hounsfield units was used to determine adipose tissue. The volume of adipose tissue was automatically calculated in the areas monitored for PAT and EAT via the integrated software. The PAT was anatomically defined, similar to the Framingham heart study, as the region anteriorly between the area immediately surrounding the thoracic aorta (defined by a horizontal line drawn from the oesophagus connected to the left costovertebral joint) and posteriorly, between the right lateral edge of the vertebral body and the anterior edge of the vertebral body (Fig. 1) (12). The EAT volume was calculated by manually scanning the area surrounding the adipose tissue between the visceral layer of the pericardium and the heart surface (Fig. 1). PATI was calculated by dividing the PAT volume by the aortic circumference in cm.

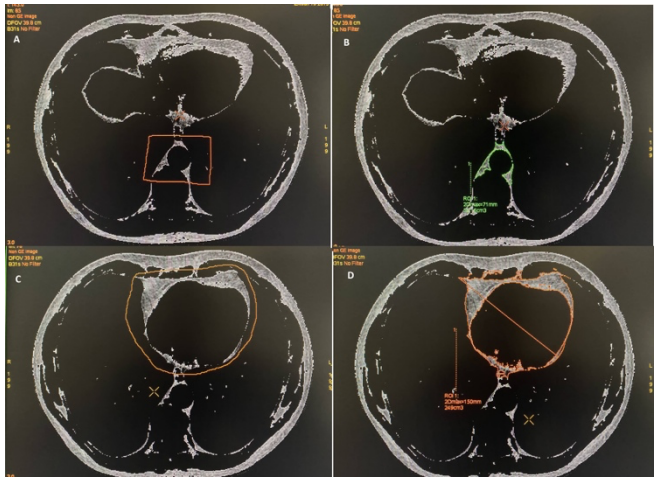

Fig.1. Evaluation of periaortic and epicardial adipose tissue volume on CT image. A-B; Appearance and evaluation of periaortic adipose tissue in computed tomography, C-D; Appearance and evaluation of epicardial adipose tissue in computed tomography

2.5. Statistical analysis

Statistical analysis was performed with the SPSS 25.0 statistical analysis software. The normality of the distribution of continuous variables was examined using the Kolmogorow-Smirnow test. Normally distributed continuous variables were expressed as mean and standard deviation, while non-normally distributed ones were expressed as medians and quartiles. Categorical variables were expressed as numbers and percentages. Normally distributed continuous variables between the groups were compared with the Student's T test, non-normally distributed continuous variables were compared with the Mann-Whitney U test, and categorical variables were compared with the Chi-Square test. The correlation of adipose tissue measurements with the SYNTAX score was analysed by Pearson correlation analysis. Relationships between coronary artery disease, adipose tissue, and other possible variables, were examined via directed acyclic graphs (DAG). DAG was plotted and analysed with the Dagitty v3.0 software (18). The relationship between variables detected as a result of the DAG analysis and fat measurements with the

presence of severe CAD, was analysed separately using the conditional forward method in binary regression analysis. The power, sensitivity and specificity values of the fat measurements in terms of detecting the presence of severe CAD were made via ROC curve analysis. All analyses were two-way and statistical significance was accepted as p<0.05.

3. Results

In our study, 263 patients [mean age 64.5(54/72), male 164 (62.4%)] were evaluated. Severe CAD was seen in 181 (68.8%) patients, and male gender was more common in patients with severe lesions (p=0.001). In addition, the number of patients who underwent CAG with the diagnosis of acute coronary syndrome was higher (p<0.001). Angiotensin converting enzyme inhibitor (ACEi)/angiotensin receptor blocker (ARB) use and statin use were higher (p=0.033, p=0.015, respectively). The group with severe CAD had higher glucose levels at admission and high-density lipoprotein cholesterol (HDL-C) levels (p=0.002, p=0.005, respectively). When visceral fat tissue was evaluated between groups, EAT, PAT, and PAT-derived PATI were found to be higher in those with severe CAD (p=0.014, p<0.001, p<0.001, respectively). There was no difference between the groups in other demographic data (p>0.05 for all) (Table 1).

When the relationship between SYNTAX score and EAT, PAT, PATI was examined in the correlation analysis, no significant correlation was found between them (r:-0.026, p= 0.73, r:-0.026, p= 0.73, r:-0.019, p=0.19, respectively) (Fig. 2).

ROC curve analysis was applied to evaluate the relationship between severe CAD and EAT, PAT, PATI. A significant correlation was found between EAT, PAT, PATI and severe CAD (AUC: 0.595, 95% CI: 0.522 to 0.688, p=0.014, AUC: 0.644, 95% CI: 0.573 to 0.716, p<0.001, AUC: 0.644, 95% CI: 0.574 to 0.714, p<0.001, respectively) (Fig.3 and Table 2).

Sensitivity and specificity of PAT in predicting severe CAD were 59.7% and 59.8%. While the sensitivity and specificity of PATI were 63% and 62.2%, EAT was found to be 55.2% and 54.9% (Table 3).

Factors associated with CAD and PAT were analysed in DAG analysis (Fig. 4). Age, GFR, Glucose, HDL-C, low density lipoprotein cholesterol, sex and smoking were found to be associated with severe CAD. These parameters were taken into regression analysis with PAT and PATI separately (Tables 4 and 5).

Table 1. Clinical and laboratory findings of patients with and without severe coronary stenosis ASA; acetylsalicylic acid, ACEi; angiotensin converting enzyme inhibitor, ARB; angiotensin receptor blocker, BP; blood pressure, CCB; calcium channel blocker, CRP; C-reactive protein, EAT; Epicardial adipose tissue, GFR; glomerular filtration rate, HDL-C; high-density lipoprotein cholesterol, LDL-C; low density lipoprotein cholesterol, PAT; periaortic adipose tissue; PATI; periaortic adipose tissue index

Parameter	Coronary stenosis <50%	Coronary stenosis ≥50%	Total	p value
Number	82	181	263	
Age, years	64.5(54/72)	66(56/76.5)	65 (56/75)	0.160
Male/female, N(%)	39(47.6)/43(52.4)	125(69.1)/56(30.9)	164(62.4)/ 99(37.6)	**0.001**
Systolic BP, mmHg	130(120/140)	130(120/145)	130 (120/140)	0.190
Diastolic BP, mmHg	80(70/85)	80(71/90)	80 (70/90)	0.082
Hypertension, N (%)	43(52.4)	110(60.8)	153 (58.2)	0.204
Diabetes, N (%)	23(28)	71(39.2)	94 (35.7)	0.080
Active smoking, N (%)	15(18.3)	32(17.7)	47 (17.9)	0.904
Acute coronary sydrome, N(%)	35(42.7)	149(82.3)	184 (70)	**<0.001**
Medications, N (%)				
ASA	20(24.4)	66(36.5)	86 (32.9)	0.053
ACEi/ARB	27(32.9)	85(47.6)	112 (42.6)	**0.033**
Beta blocker	22(26.8)	57(31.3)	79 (30)	0.445
CCB	17(20.7)	47(26)	64 (24.3)	0.359
Statin	10(12.2)	46(25.4)	56 (21.3)	**0.015**
Hemoglobin, g/dl	12.8±1.9	13.2±2.2	13.1±2.1	0.160
Glucose, mg/dl	112(98.5/136.5)	130.5(105/173)	121 (103/162.5)	**0.002**
GFR, ml/min/1.73 m2	77.8(45.8/92)	79(57/94)	79 (56.7/92.3)	0.396
CRP, mg/L	7.7(1.5/22.5)	5.1(1.7/26.6)	6 (1.6/24)	0.329
Total Cholesterol, mg/dl	190(175/213)	193(166/213)	192 (169/213)	0.975
HDL-C, mg/dl	44(41/52)	43(35/47)	43 (38/47)	**0.005**
LDL-C, mg/dl	115(96/136)	116(96/143)	115 (96/138)	0.527
Triglyceride, mg/dl	149(101/163)	149(118/192)	149 (115/171)	0.166
EAT volume, ml	167(120/215)	189(135/261)	181 (130/250)	**0.014**
PAT volume, ml	27(20/38)	34(25/51.5)	32 (24/46)	**<0.001**
Aortic diameter, mm	28(25/31)	29(26/31.5)	28 (26/31)	0.387
PATI, ml/cm	4.38(3,51/5.89)	5.5(4.13/7.76)	5.1 (3.9/7.16)	**<0.001**

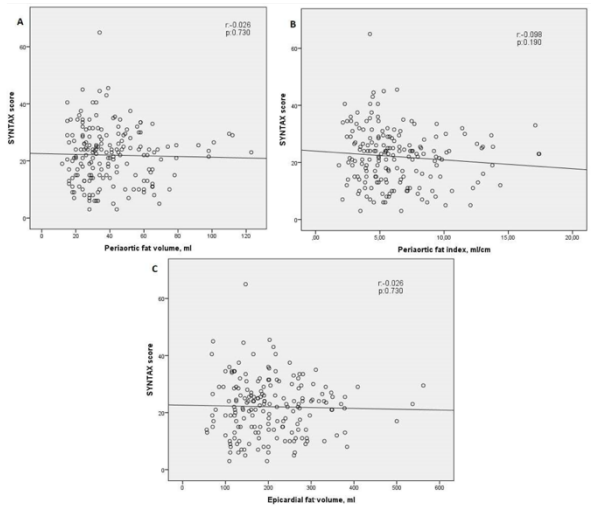

Fig.2. Correlation of Syntax score with visceral adipose tissue markers

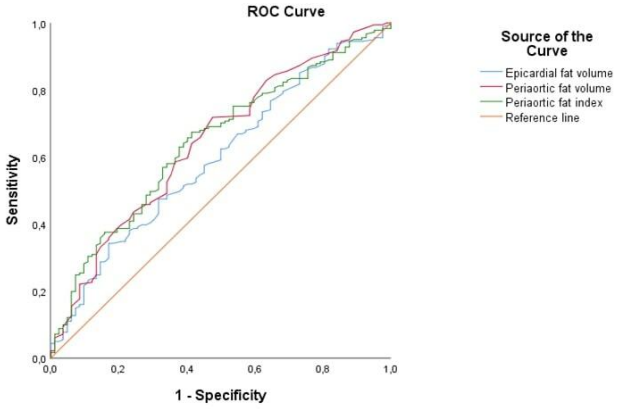

Fig.3. ROC curve analysis of visceral adipose tissue markers to predict significant coronary stenosis

Table 2. ROC curve analysis of visceral adipose tissue parameters to predict significant coronary stenosis

Variables	Area under curve	Significance	95% Confidence Interval
EAT volume, ml	0.595	0.014	0.522-0.668
PAT volume, ml	0.644	<0.001	0.573-0.716
PATI, ml/cm	0.644	<0.001	0.574-0.714

EAT; Epicardial adipose tissue, PAT; periaortic adipose tissue; PATI; periaortic adipose tissue index.

Table 3. Sensitivity and specificity of visceral adipose tissue parameters in the prediction of presence of significant coronary stenosis

Parameter	Value	Sensitivity, %	Specificity, %
EAT volume, ml	177.50	55.2	54.9
PAT volume, ml	30.50	59.7	59.8
PATI, ml/cm	4.8429	63.0	62.2

EAT; Epicardial adipose tissue, PAT; periaortic adipose tissue; PATI; periaortic adipose tissue index.

An independent relationship was found between PATI and the presence of severe CAD (β: 0.968, p=0.006) (Table 4). This association with PAT disappeared when evaluated together with other risk factors (β: 0.581, p=0.097) (Table 5). Age, GFR, HDL-C, sex, and glucose were independently associated with severe CAD in both analyses (Tables 4 and 5).

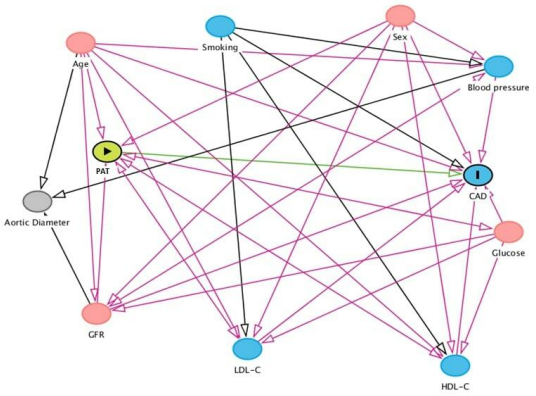

Fig. 4. Directed acyclic graph that show associations of periaortic adipose tissue, coronary artery disease and possible covariates. CAD; coronary artery disease, GFR; glomerular filtration rate, HDL-C; high density lipoprotein cholesterol, LDL-C; low density lipoprotein cholesterol, PAT; periaortic adipose tissue

Table 4. Binary logistic regression analysis of periaortic adipose tissue index and possible covariates for the association with significant coronary stenosis

Variables	B	S.E.	Sig.	Exp (B)
Age*	2.557	0.817	0.002	12.897
GFR*	0.925	0.31	0.003	2.521
HDL-C*	-1.483	0.673	0.028	0.227
Glucose*	1.464	0.492	0.003	4.322
Sex **	-0.886	0.312	0.005	0.412
PATI*	0.968	0.354	0.006	2.631

Model: Age, GFR, Glucose, HDL-C, LDL-C, PATI, sex and smoking. *Natural logarithm of non-normally distributed parameters was used. ** Male sex, GFR; glomerular filtration rate, HDL-C; high-density lipoprotein cholesterol, LDL-C; low density lipoprotein cholesterol, PATI; periaortic adipose tissue index

Table 5. Binary logistic regression analysis of periaortic adipose tissue volume and possible covariates for the association with significant coronary stenosis

Variables	B	S.E.	Sig.	Exp (B)
Age*	2.237	0.812	0.006	9.363
GFR*	0.908	0.307	0.003	2.480
HDL-C*	-1.492	0.667	0.025	0.225
Glucose*	1.460	0.489	0.003	4.307
PAT*	0.581	0.351	0.097	1.789
Sex **	-0.832	0.319	0.009	0.435

Model: Age, GFR, Glucose, HDL-C, LDL-C, PAT, sex and smoking. *Natural logarithm of non-normally distributed parameters was used. ** Male sex, GFR; glomerular filtration rate, HDL-C; high-density lipoprotein cholesterol, LDL-C; low density lipoprotein cholesterol, PAT; periaortic adipose tissue.

4. Discussion

To the best of our knowledge, this is the first study comparing PAT with stenosis severity and lesion complexity in patients undergoing CAG. PAT volume was significantly higher in patients with severe CAD, while PATI was higher and showed an independent association with severe CAD. However, we did not observe any relationship between EAT, PAT, PATI and SYNTAX score.

CAD risk factors have been evaluated numerous times before and used in risk modification, namely male gender, age, diabetes mellitus (DM), hyperlipidemia and smoking (15, 16). In our study, male gender, high glucose levels and low HDL-C were observed more frequently in the group with severe CAD. Although age and GFR were not different between the groups, regression analysis revealed an independent risk factor for severe CAD in addition to male gender, glucose and low HDL-C. Smoking was not different between the groups. Although the frequency of DM and HT was more frequent in the group with severe CAD, there was no statistical difference. No difference was observed between the groups in other cholesterol parameters except HDL-C. This may be due to the fact that we grouped them according to the severity of stenosis, instead of grouping them according to the presence of atherosclerosis. In addition, it may be due to the significantly higher use of statins in the group with severe stenosis.

As expected, the frequency of ACS was higher in the group with severe CAD in patients who underwent CAG. In the other group, in addition to coronary imaging with the diagnosis of stable angina, coronary imaging was required before valve surgery or aortic surgery. In our study, the severity of the coronary lesion and the complexity of the lesion were evaluated as a number of previous studies have shown that EAT is associated with coronary lesion severity (7, 8). However, lesion complexity differs in studies (6-8): Kaya et al. evaluated 93 patients in their study, while the EAT volume was higher in patients with severe CAD, they did not find a relationship between lesion complexity and EAT (7). On the contrary, in a different CT study, patients with familial hypercholesterolemia were evaluated and it was found that EAT volume was associated with lesion complexity (8). Similarly, Erkan et al. evaluated EAT thickness in 183 patients echocardiographically in their study (6). EAT thickness was associated with the SYNTAX score and the Gensini score, which assesses the extent of atherosclerosis (6). In our study, EAT volume was higher in patients with severe CAD, but no correlation was found between this result and the SYNTAX score.

Obesity is one of the cardiovascular risk factors like DM and is associated with increased visceral fat volume (19, 20). The relationship between the criteria used in the diagnosis of metabolic syndrome and PAT volume has been shown (5). An increase in PAT volume was observed with the presence of each criterion defining the metabolic syndrome (5). Pro-inflammatory cytokine release increases with increasing adipose tissue (3, 21, 22). An increased pro-inflammatory response causes atherosclerosis and vascular damage (3, 21, 22). Mazotta et al. evaluated the relationship of pro-atherogenic mediators with PAT. In their study, they evaluated samples taken from PAT in patients who were going to have coronary, valve or aortic surgery. They showed that pro-atherogenic mediators were higher in those with CAD (21).

PAT volume has been shown to be associated with coronary and peripheral artery disease (10, 11, 23). Increased PAT volume has been demonstrated in patients with COPD and patients undergoing peritoneal dialysis (24, 25). In addition, in a study evaluating PAT volume in ischemic stroke, higher periaortic fat attenuation was observed while there was no change in PAT volume in cardioembolic strokes (26). In peripheral arterial disease, PAT was demonstrated to be an independent risk factor in the Framingham heart study, and in this study, increased PAT volume was associated with lower ankle brachial index and claudication (11). The relationship between carotid intima-media thickness and plaque size was investigated by Yun et al. and associated with PAT volume (5).

Mamopoulos et al. showed that the assessment of periaortic adipose tissue, aortic size and calcification is reliable and comparable in advanced and undeveloped devices, regardless of imaging parameters (slice thickness or CT) (27).

Subclinical disease has generally been evaluated in studies associated with PAT and coronary atherosclerosis, as well as studies evaluating the relationship of severe CAD are rare (2, 10). Contrary to EAT and to the best of our knowledge, there are no studies evaluating lesion complexity. The relationship between PAT and atherosclerosis has been principally evaluated with CT, and one of the most important of these reports is the Framingham Heart Study (2, 10). This study shows that PAT is associated with coronary calcification and is a risk factor for cardiovascular disease (2).It should be noted that the purpose of this study was not to compare stenosis severity and lesion complexity, but to demonstrate increased atherogenic mediators in PAT in those with CAD (21). Efe et al. evaluated the relationship between PAT and severe CAD in their study and observed more severe stenosis in the group with high PAT volume. PAT volume and coronary stenosis severity were determined by CT. However, when they evaluated PAT volume with other risk factors, they did not detect an independent risk factor (10). Our study supports the CT study.

It is known that PAT volume is associated with aortic enlargement as well as with atherosclerosis (12). In the Framingham heart study, it was shown that PAT tissue is associated with thoracic and abdominal aortic diameters and

is an independent risk factor (12). This association may possibly be stronger than that of coronary atherosclerosis. As the aortic diameter increases, it is expected that the surrounding fatty tissue will increase. We aimed to eliminate the effect of aortic width in all patients by dividing the PAT by the aortic circumference, instead of just evaluating the volume. PATI represents the volume of adipose tissue per cm relative to the aortic circumference and has been associated with coronary lesion severity, and this relationship was found to be an independent risk factor when evaluated together with CAD risk factors. PATI can thus be used to balance PAT volume due to a change in aortic diameter.

We found that PAT volume is associated with severe CAD. However, we did not observe a relationship between PAT and SYNTAX score. PATI derived from PAT and calculated according to aortic circumference was both associated with severe CAD and was found to be an independent risk factor, when evaluated together with CAD risk factors.

Conflict of interest

The authors declared no conflict of interest.

Funding

This study was approved by Baskent University Institutional Review Board and Ethics Committee (project number: KA 22/212) and supported by Baskent University Research Fund.

Acknowledgments

None to declare.

Authors' contributions

Concept: E.A., S.A., Design: E.A., AC., Data Collection or Processing: E.A., I.U., Analysis or Interpretation: S.A., A.A., I.H.M., Literature Search: E.A., S.A., Writing: E.A., S.A.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 829-832
doi: 10.52142/omujecm.39.3.45

Investigation of the changes in the general characteristics of COVID19 patients with the vaccination program

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Received: 17.05.2022 • **Accepted/Published Online:** 30.05.2021 • **Final Version:** 30.08.2022

Abstract

Since the onset of the Coronavirus Disease 2019 (COVID-19) pandemic, many vaccine research studies have started. Changes in the demographic characteristics of the patients hospitalized from the emergency room to the intensive care unit due to COVID-19 has caught our attention since the vaccination program began in Turkey. The purpose of this study is to investigate whether our investigation is scientifically valid and meaningful. Thus, it will be helpful to investigate the effect of priority ordering in vaccination programs in future pandemics. Demographic characteristics and hospitalization processes of patients hospitalized in the intensive care unit before and after vaccination were compared. For comparison, Charlson Comorbidity Index (CCI) and Acute Physiologic Assessment and Chronic Health Evaluation (APACHE) scores, as well as intensive care unit duration of stay and mortality were used. While age [mean (SD); 70,8 (12,2) vs 66,2 (15,2), p=0,032] and duration of intensive care stay [day; mean (SD); 6,4 (6,3) vs 9,4 (7,4); p<0,001] increased in the post-vaccination group, a statistically significant decrease was observed in APACHE [mean (SD); 26,9 (9,2) vs 20,9 (9,0); p=0,008] and CCI scores [mean (SD); 4,3 (2,2) vs 3,6 (2,7); p<0,001]. Regulating the priorities of those to be vaccinated causes rapid changes in the patient population. For this reason, vaccination of vulnerable groups will contribute to the operation of the health system properly.

Keywords: COVID19, SARS-CoV-2, vaccination, prognostic factors

1. Introduction

Since the beginning of the coronavirus disease 2019 (COVID-19) pandemic, vaccine development studies have been initiated in almost all developed countries. Vaccines that were developed and approved for emergency use started to be applied even before a year had passed. After emergency use approval was obtained on January 13th, 2021 for COVID-19 vaccine studies in Turkey (CoronaVac), the Pfizer-BioNTech vaccine began to be used in Turkey as of March 12th, 2021.

Due to the limited number and distribution of vaccines in the early days, each country had to identify their own priority groups. The first group that received access to the vaccine in Turkey was healthcare workers considered to be at the highest risk. Then, the vaccination program was applied gradually, taking into account the people with advanced age and chronic diseases that experience a more severe course of the disease. After the vaccination program started, it was observed that the demographic characteristics of the patients hospitalized in the intensive care units changed in the clinic where we worked, and in the feedback that was received from colleagues around the world. This observation constituted the basis for this research. Whether there has been a statistically significant change in the demographic characteristics of patients who need intensive care before and after the vaccination program was examined. Thus, an attempt was made to measure the contribution of the vaccination program to the adequacy of the intensive care capacity even in the early period.

It was reported in the weekly epidemiological update of the World Health Organization that over 22 million new cases and over 59,000 new deaths occurred between January 24th and January 30th, 2022, and that there was a 9% increase in new deaths when compared to the previous week. As of January 30th, 2022, over 370 million confirmed cases and over 5.6 million deaths had been reported globally (1). The leading argument that anti-vaccinists put forward is the lack of sufficient research Therefore, it is necessary to demonstrate the value of vaccination using scientific evidence to counter anti-vaccination arguments.

2. Materials and Methods

Our hospital is 110 kilometers from the city center of İzmir and has a capacity of 300 beds, serving approximately 250,000 people. In this hospital, 24 beds, including isolated rooms, are reserved for intensive care pandemic patients. This was a retrospective, observational, pre-, and during cohort analysis. The study was approved by the Clinical Research Ethics Committee of the University of Health Sciences, İzmir Dr. Suat Seren Chest Diseases and Surgery Training and Research Hospital, dated 2021 and number 2021/48-59. The ethical guidelines of the Declaration of Helsinki were

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observed. This study covered the period of 2 months before the start of the vaccination program and 15 days after the second dose, which was thought to have established the effectiveness of the vaccine. For these dates, the data were extracted directly from a systematic query of the electronic health record as part of the Health Data & Management Solution System by examining the COVID-19 patients hospitalized in the intensive care unit day by day, and the average age, number of inpatients, average Acute Physiologic Assessment and Chronic Health Evaluation (APACHE) scores, average Charlson comorbidity index (CCI) scores, total hospital stay of the patients hospitalized on the specified day, length of stay in the intensive care unit, and intubation and discharge rates for each day were calculated. These data were recorded on the data record form separately for each day. Using this data, the pre-vaccination group (Grp 1) and post-vaccination group (Grp 2) were compared. The purpose of this study is to examine the effect of vaccination on intensive care hospitalizations and the clinical severity of patients with COVID-19.

2.1. Statistical analysis

The frequencies and percentages were given for the categorical variables, and the mean, standard deviation (SD), median, and range (minimum-maximum) values were given for the numerical variables as descriptive statistics. The categorical variables were compared using the Pearson chi-square test, while the numerical variables were compared using the Wilcoxon rank sum Mann Whitney U test between the groups. Statistical significance was assessed at $P < 0.05$ and all statistical analyses were performed using R software version 4.0.5 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

A total of 252 patients admitted to the emergency department and hospitalized in the pandemic intensive care unit were included in this study. The general data of all of the patients are shown in Table 1. When the patients were evaluated pre- and post-vaccination, the following statistically significant changes were observed: the mean age of the patients decreased, the number of hospitalization days increased, the APACHE score decreased, and the CCI score decreased (Table 2). Although the number of intubated patients and the number of patients receiving noninvasive mechanical ventilation decreased, these changes were not statistically significant, and although there was a decrease in intensive care deaths, there was no statistically significant change.

The change in the age group distribution of the patients hospitalized in the intensive care unit is highlighted in Fig. 1.

The aim of this study is not to compare the outcomes of vaccinated and unvaccinated patients, but to investigate the change in the general characteristics of patients who need intensive care with vaccination. However, when the patients were examined, 35 (14%) of 252 had one dose and 14 (5.5%) had two doses of vaccine during their admission to the intensive care unit. Moreover, 203 (80.5%) patients were not vaccinated. All of the vaccines that the patients had were CoronaVac (by Sinovac) inactivated vaccine, which was approved for emergency use in Turkey on January 13th, 2021.

Table 1. Overall patients characteristics

	Mean (SD)	Median	Range	Yes	No
Age	69,1 (13,6)	70,0	25,0-94,0		
Apache score	24,3 (9,7)	24,0	4,0-47,0		
Icu day	7,7 (6,8)	6,0	1,0-36,0		
Entubation				85 (33,7%)	167 (66,3%)
MV				85 (33,7%)	127 (50,4%)
Ex				119 (47,2)	133 (52,8%)

Table 2. Comparison of before and after vaccine periods

	Group 1	Group 2	Totally	P
Age				
Mean (SD)	70,8 (12,2)	66,2 (15,2)	69,1 (13,6)	**0,032**
Median	70,5	70,0	70,0	
Range	26,0-94,0	25,0-94,0	25,0-94,0	
Gender [N (%)]				
Man	95 (60,1%)	54 (57,4))	149 (59,1%))	0,676
Woman	63 (39,9%)	40 (42,6%)	103 (40,9%)	
Apache score				
Mean (SD)	26,9 (9,2)	20,6 (9,0)	24,3 (9,7)	**0,001**
Median	27,0	20,0	24,0	
Range	11,0-46,0	4,0-47,0	4,0-47,0	
CCI score				
Mean (SD)	4,3 (2,2)	3,6 (2,7)	4,0 (2,5)	**0,008**
Median	4,0	3,5	4,0	
Range	0,0-11,0	0,0-14,0	0,0-14,0	
ICU length of stay (day)				**0,001**
Mean (SD)	6,4 (6,3)	9,4 (7,4)	7,7 (6,8)	
Median	5,0	8,0	6,0	
Range	1,0-31,0	1,0-36,0	1,0-36,0	
Entubation [N (%)]				
Yes	57 (36,1%)	28 (29,8%)	85 (33,7%)	0,307
No	101 (63,9%)	66 (0,2%)	167 (66,3%)	
MV [N (%)]				
Yes	57 (36,1%)	28 (29,8%)	85 (33,7%)	0,214
No	73 (46,2%)	54 (57,4%)	127 (50,4%)	
Noninvaziv	28 (17,7%)	12 (12,8%)	40 15,9%)	
Ex [N (%)]				
Yes	81 (51,3%)	38 (40,4%)	119 (47,2%)	0,096
No	77 (48,7%)	56 (59,6%)	133 (52,8%)	

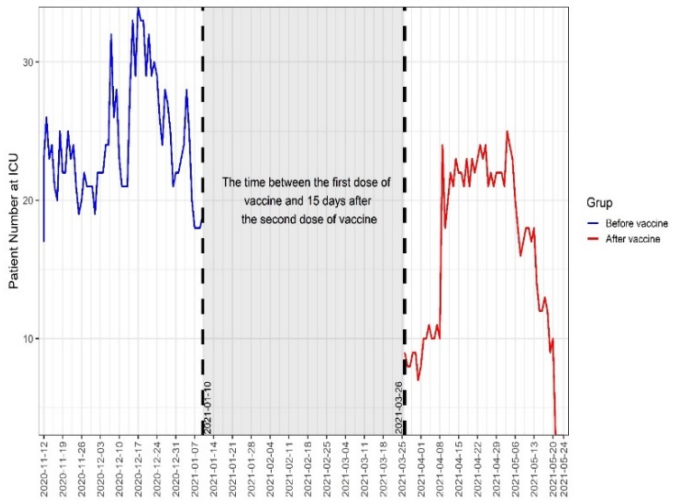

Fig. 1. Change in the number of patients hospitalized in intensive care unit over time

4. Discussion

Haas et al. (2) reported at the conclusion of a large-scale study in Israel that vaccination with two doses of BNT162b2 was quite effective in preventing symptomatic and asymptomatic SARS-CoV-2 infections, as well as COVID-19-related hospitalizations, severe illness, and death in all age groups (16 years, including adults over the age of 85).). These findings suggest that the COVID-19 vaccine may help to control the pandemic. In that study, the protection rate was 97% against symptomatic COVID-19. Polack et al. reported that the two-dose BNT162b2 regimen provided 95% protection against COVID-19 in people aged 16 and over, and the safety for a median of 2 months was similar to that of other viral vaccines (3). In a study investigating the effect of the vaccination program on reducing the burden of disease in South Korea, it was found that it reduced negative outcomes, such as intensive care unit admissions and deaths, by 45% and 43%, respectively (4). It was reported that SARS-CoV-2 vaccines administered in Brazil, by giving priority to people over the age of 75, were quite effective in reducing the number of deaths associated with COVID-19, with a protection ratio of 19.31 (95% CI: 18.20–20.48), and an attributable protection ratio of 94.8%. In the same study, it was also observed that the protection after the second dose was as high as 99.2% (5). In a Brazilian study, a rapid increase in vaccination coverage among the elderly was associated with a significant decrease in relative mortality rates when compared to younger individuals. It was stated that if mortality rates among the elderly remained proportional to those observed until the 6th week, an estimated additional 43,802 COVID-related deaths could be expected by the 19th week (6). In the study, in which the vaccination campaign in Israel was estimated to have prevented 158,665 COVID-19 infections, 24,597 hospitalizations, 17,432 critical or severe hospitalizations, and 5532 deaths (7), it was also estimated that these prevented deaths and hospitalizations were of people aged 65 years and older, and that prevented illness, hospitalization, and deaths were from the fully vaccinated group. In a study conducted in Poland, it was stated that vaccinated patients hospitalized for COVID-19 constituted a small (1.2%) group when compared to all of the hospitalized COVID-19 patients, and vaccination provided assurance in preventing severe infection and death (8).

In a multicenter study conducted in the USA, it was observed that the need for emergency room admission and hospitalization due to COVID-19 was an extremely rare incidence in fully vaccinated patients. Emergency room visit rates among fully vaccinated people were low. It was stated that if hospitalization was required, elderly patients with significant comorbidities were at high risk for serious consequences regardless of their vaccination status (9).

In a retrospective study conducted in Saudi Arabia, it was stated that most of the COVID-19 patients hospitalized to the intensive care unit were unvaccinated, and most of the partially vaccinated patients were infected before developing immunity (10).

This study aims to scientifically demonstrate the benefit of the vaccine as early as possible. Therefore, it is based on the evaluation of the entire patient group as a single patient rather than the individual conditions of the patients before and after the vaccination program. The most important limitations of the study were that it was conducted in a very early period, was single-centered, and it was designed retrospectively.

Even in the early period of vaccination, the demographic characteristics of patients in need of intensive care have changed, emphasizing the importance of vaccination in ending the pandemic. Larger multicenter, prospective, and controlled studies will be conducted in the future to confirm the effectiveness and the success of vaccination.)

Conflict of interest

The authors declared no conflict of interest.

Funding

No funding was used for the study.

Acknowledgments

None to declare.

Authors' contributions

Concept: O.O., O.H., Design: O.O., O.H., S.S., Data Collection or Processing: O.O., O.H., S.S., Ş.K.Ö., Analysis or Interpretation: O.O., S.S., Literature Search: O.O., Ş.K.Ö., Writing: O.O., O.H., S.S.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 833-837
doi: 10.52142/omujecm.39.3.46

The consequences of cesarean section and vaginal delivery on ovarian reserve in infertile women

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Received: 19.05.2022 • **Accepted/Published Online:** 24.06.2022 • **Final Version:** 30.08.2022

Abstract

According to the data of the World Health Organization (WHO), although the rate of cesarean section is increasing worldwide, one out of every five births is performed by cesarean section. Cesarean section is life saving for both mother and baby depending on the situation. However, it also brings some complications. Its effect on fertility also raises concerns. The aim of the present study was to investigate the consequences of cesarean section on ovarian reserve. Anti-Mullerian hormone, Follicle-Stimulating Hormone, Luteinizing Hormone, Estradiol, antral follicle count, ovarian volume, and ovarian elastography results of 120 patients under the age of 30 who were not obese and were compatible with the criteria for unexplained infertility of the American Society for Reproductive Medicine were analyzed in both pre-pregnancy and postpartum period. There were 57 patients with vaginal delivery and 63 patients with cesarean section. Differences between the groups according to the mode of delivery and between the pre-pregnancy and postpartum groups were investigated. There was no statistically significant difference for the vaginal birth group in terms of AFC, AMH, FSH, LH, E2, SWE and ovarian volumes between pre-pregnancy and postpartum. In the cesarean section group, AFC, AMH and ovarian volumes were found to be significantly lower in the postpartum period than before pregnancy. As a result, cesarean delivery may have negative consequences for ovarian reserve tests.

Keywords: AMH, cesarean, elastography, ovarian reserve, vaginal birth

1. Introduction

Modern cesarean section techniques started with the Sänger technique in the early 1900s, and the Pfannenstiel-Kerr technique formed the infrastructure of today's technique as a double layer closure with a lower segment transverse incision (1). Cesarean section is life saving for both mother and baby depending on the situation (2). However, it also brings some complications. Some of these are infection, fever, excessive blood loss, injury to other intra-abdominal organs, endometriosis, urinary retention, and anesthesia-related side effects (3–5). While the rate predicted by the World Health Organization for cesarean section was between 10% and 15% in the 1980s, it is over 40% in some regions today (6). Today, the increasing frequency of cesarean section raises concerns with the studies showing some negative effects on fertility (7–9). However, these studies had limitations, such as the older age of the cesarean section group, the co-morbidities of the cesarean section group, and the non-homogeneous distribution of the groups (10, 11).

The most important indicator of female fertility is the ovarian reserve, and it is directly related to the number of antral follicles in the ovary (12). Anti-Mullerian hormone (AMH), antral follicle count (AFC), Follicle-Stimulating Hormone (FSH), Luteinizing Hormone (LH), and Estradiol (E2) are tests used to evaluate ovarian reserve (13). AMH blood level is not affected by the menstrual cycle like FSH, LH, and E2 (14).

Ultrasound elastography, which has become widespread in recent years, shows the elasticity of soft tissues and is also used in the ovary's evaluation in gynecology practice (15).

The aim of this prospective study was to observe the consequences of cesarean section on ovarian reserve tests in infertile women.

2. Material and Methods

After obtaining the approval of the local ethics committee, patients who applied to the obstetrics and gynecology outpatient clinic of our tertiary hospital because of unexplained infertility were evaluated for this study. The criteria for unexplained infertility were compatible with the criteria of the American Society for Reproductive Medicine (16). Patients who gave birth before, over 30 years of age and obese (Body Mass Index ≥ 30) were not included in the study.

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AMH, FSH, LH, and E2 blood levels were analyzed on the 3rd day of the participants' menstruation. FSH, LH, and E2 tests were carried out by a chemiluminescence immunoassay method (Abbott®). Reference ranges were in follicular phase 3.03-8.8 IU/L for FSH, 1.8-11.78 IU/L for LH, and 21-251 ng/L for E2. AMH levels were determined by another chemiluminescence immunoassay method (Beckman-Coulter®), its reference ranges were 0.07 -7.35 ng/ml.

Antral follicle count was performed by Samsung HS70A ultrasound device® via 2D imaging for both ovaries transvaginally. Follicles with a diameter of 2-10 mm were antral follicles. Length (L), height (H), and width (W) measurements were made for each ovary. The volume of the ovaries was calculated using the formula L x H x W x 0.523 (Figure 1). Shear wave elastography (SWE) evaluation was also performed (Figure 2). Participants' age, height, and weight data were recorded. Body mass index (BMI) was calculated with weight (kilogram)/height(meter)². Data of 240 participants were recorded for the study. Pregnancy occurred in 182 of these participants within 1 year. 151 of 182 pregnancies resulted in live births. In the study, 31 patients who did not continue their follow-up for various reasons were dropped out. In order to ensure standardization and to comply with the recommendations of the Ministry of Health, 400 micrograms of folic acid in the first trimester of pregnancy and Decavit Pronatal® (Kocak Farma) once a day from the third month to delivery were recommended. Ultrasound measurements and hormone measurements were repeated on the 3rd day of menstruation when the 120 participants included in the study had menstruation at least 6 months after live birth.

Participants using postpartum hormonal contraceptive methods were not included in the study. The data of the participants constituted two groups who gave birth by cesarean (CS) and delivered vaginally (VB).

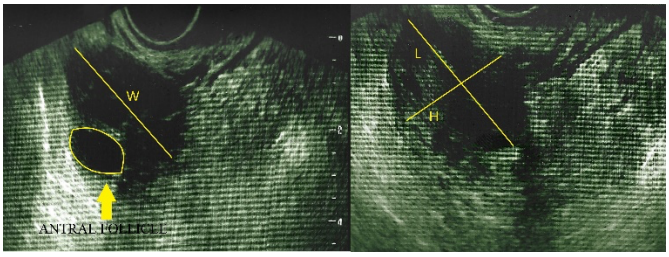

Fig. 1. Calculation of the volume of the ovaries (L x H x W x 0.523) and antral follicle

G* Power software version 3.01 (Franz Foul, Kiel, Germany) was used for sample size calculation. In a study published in 2017, it is estimated that there are approximately 1.7 million infertile women in Turkey (17). Power analysis was performed using the mean and number of study participants in the study of Gurol-Urganci et al. (10). All data obtained were analyzed with IBM SPSS Statistics for Windows, Version 23.0, and $p \leq 0.05$ was significant. Compatibility with the normal distribution was evaluated using the Kolmogorov-Smirnov test. The difference between the CS and VB groups was analyzed using the Independent-T test for normally distributed data, and the Mann-Whitney U test for non-normally distributed data. The changes in pre-pregnancy and postpartum data for VB and CS were analyzed with the paired T-test for data with normal distribution, and with the Wilcoxon Signed test for data without normal distribution. Correlation between data was analyzed by a Pearson Correlation test.

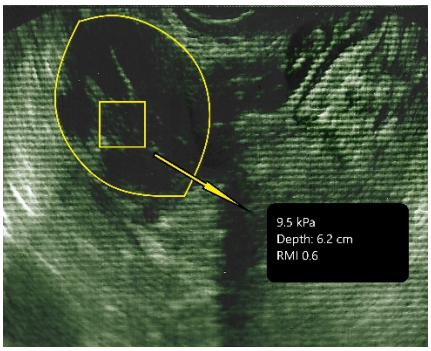

Fig. 2. Evaluation of the ovary with Shear Wave Elastography

Ethical permission for the study was obtained from the Clinical Research Ethics Committee of Kanuni Training and Research Hospital (2019/47).

3. Results

There were 120 primiparous pregnant women included in the present study. While 63 (52.5%) of the participants gave birth vaginally, 57 (47.5%) gave birth by cesarean section. The ages of the participants ranged from 19.2-28.8 years, and their BMI ranged between 19-28.9 kg/m2. There was no difference between the groups in terms of age, BMI and duration of conception (Table 1).

Table 1. Descriptive characteristics of patients according to delivery type

	Vaginal delivery (*n* = 57) (mean ± SD)	**Cesarean section (*n* = 63)** (mean ± SD)	***p***
Age (year)	24.1 ± 3.1	23.6 ± 2.7	0.423*
BMI (kg/m²)	23.4 ± 3.2	23.7 ± 3.4	0.512*
Duration of conception (month)	7.0 ± 2.7	7.3 ± 3.1	0.315**

BMI: Body Mass Index * Independent-T test ** Mann-Whitney U test

There was no significant difference for the VB group in terms of AFC, AMH, FSH, LH, E2, SWE and ovarian volumes between before pregnancy and postpartum period. In the cesarean section group, AFC, AMH and ovarian volumes were found to be significantly lower in the postpartum period than before pregnancy. In the postpartum period, a significant difference was found between the VB and CS groups in terms of AFC, AMH, ovarian volumes and SWE in the left ovary (Table 2).

According to the method of conception, patients were

classified as spontaneous, intrauterine insemination (IUI), in vitro fertilization (IVF). There was no difference between CS and VB groups in terms of conception method (Table 3).

Table 2. Laboratory results and ultrasound findings of patients according to delivery type

	Vaginal delivery (n = 57)	Cesarean section (n = 63)	p value
[b]AFC in the right ovary	7.6 ± 1.4	7.8 ± 1.2	0.808[*]
[a]AFC in the right ovary	7.4 ± 1.6	6.1 ± 1.1	**0.431**[*]
p value[i]	0.512	**0.145**	
[b]AFC in the left ovary	7.6 ± 1.5	7.3 ± 1.4	0.380[*]
[a]AFC in the left ovary	7.6 ± 1.4	6.0 ± 1.0	**0.027**[*]
p value[i]	0.716	**0.044**	
[b]AMH (ng/ml)	2.2 ± 0.7	2.3 ± 0.7	0.292[**]
[a]AMH (ng/ml)	2.2 ± 0.7	1.7 ± 0.5	**0.033**[**]
p value[ii]	0.746	**0.025**	
[b]FSH (IU/L)	6.8 ± 1.5	6.7 ± 1.8	0.452[**]
[a]FSH (IU/L)	7.0 ± 1.7	6.8 ± 1.3	0.528[**]
p value[ii]	0.253	0.439	
[b]LH (IU/L)	7.6 ± 2.8	7.3 ± 2.7	0.509[**]
[a]LH (IU/L)	6.9 ± 2.5	8.2 ± 2.6	0.054[**]
p value[ii]	0.343	0.278	
[b]E2 (ng/L)	42.7 ± 11.6	36.7 ± 14.9	0.064[**]
[a]E2 (ng/L)	37.6 ± 12.2	34.3 ± 13.9	0.302[**]
p value[ii]	0.189	0.248	
[b]SWE in the right ovary (kPa)	8.3 ± 1.9	8.2 ± 2.1	0.540[**]
[a]SWE in the right ovary (kPa)	8.4 ± 2.0	8.9 ± 1.6	0.304[**]
p value[ii]	0.618	0.057	
[b]SWE in the left ovary (kPa)	8.4 ± 2.0	9.3 ± 1.9	0.056[**]
[a]SWE in the left ovary (kPa)	8.2 ± 1.8	9.3 ± 1.9	**0.039**[**]
p value[ii]	0.056	0.612	
[b]Right ovarian volume (mm^3)	9.5 ± 1.7	9.6 ± 1.7	0.421[**]
[a]Right ovarian volume (mm^3)	9.7 ± 1.7	7.6 ± 1.2	**0.028**[**]
p value[ii]	0.415	**0.031**	
[b]Left ovarian volume (mm^3)	10.0 ± 1.9	10.0 ± 2.0	0.462[**]
[a]Left ovarian volume (mm^3)	9.7 ± 1.5	7.9 ± 1.3	**0.043**[**]
p value[ii]	0.415	**0.029**	

AFC: Antral Follicle Count, AMH: Anti-Mullerian Hormone, FSH: Follicle Stimulating Hormone, LH: Luteinizing Hormone, E2: Estradiol SWE: Shear Wave Elastography
[a]after birth; [b]before pregnancy; [*] Mann-Whitney U test; [**] Independent-T test;
[i] Wilcoxon Signed test; [ii] paired T-test

Table 3. Distribution of conception types according to delivery type

	Vaginal delivery (n = 57)	Cesarean section (n = 63)	p [*]
Spontaneous	8 (14%)	9 (14.3%)	0.359
IUI	38 (66.7%)	41 (65%)	0.432
IVF	11 (19.3%)	13 (20.7%)	0.248

IUI: Intrauterine insemination, IVF: In vitro fertilization [*] Chi-square test

4. Discussion

Although there are studies on the consequences of a mode of delivery on ovarian reserve in the literature, there is no comparative study covering the pre-pregnancy period. Ovarian reserve tests during pregnancy differ between pre-pregnancy and trimesters (18). Therefore, the current study provides new information on this aspect. Past studies either did not include a standardized group or the analysis was carried out using retrospective data. In some studies, it has been indirectly associated with some factors, such as intra-abdominal adhesions and vascular injury, that may affect ovarian reserve (10).

Today, with the change in the obstetric population, there have also been changes in obstetric practices. The average age of women at first pregnancy was increasing, and the number of women with additional diseases who achieve pregnancy with assisted reproductive techniques was also increasing (1). The incidence of cesarean is high in advanced maternal age and in pregnancies with assisted reproductive techniques (19,20). In addition, the frequency of cesarean section varied during the first birth due to legal problems (21). The thought that cesarean delivery may have a negative consequence for ovarian reserve worries both physicians and patients (7).

In the present study, AMH, AFC, FSH, LH, E2, ovarian volume, and SWE were analyzed both in the pre-pregnancy and postpartum period. There was no significant difference in ovarian reserve tests in the vaginal delivery group. However, AMH, ovarian volume and AFC were significantly adversely affected in the CS group. The present

study showed that cesarean section may adversely affect ovarian reserve tests.

With the increasing cesarean rate, the consequences of the mode of delivery on fertility is a more striking issue, and some studies have suggested that cesarean section may cause negative reproductive outcomes (7–9). However, some studies suggest that there was no such relationship (10,11, 22). These studies on the consequences of cesarean section on ovarian reserve were not in the infertile patient group, but included a patient population that could cause bias. In our study, prospective follow-up was performed in the unexplained infertile patient group and the groups were similar to each other in terms of descriptive characteristics.

AMH measurement is widely used in determining ovarian reserve, however, the lack of an international standard and being affected by many factors are its handicaps (23). One of these factors is age and with increasing age, serum AMH level decreases, especially after 35 years of age (23). In our study, there was no difference in age between the groups and the mean age was also found to be 23.9 ± 2.9 years.

Although there are studies suggesting the evaluation of ovarian reserve by looking at serum FSH, LH and E2 levels on the 3rd day of menstruation (24), the combination of AFC, AMH, FSH, LH gave more reliable results (25). Therefore, ovarian reserve tests were evaluated in combination in our study. Although ovarian volume was not a stand-alone ovarian reserve test, it might correlate with AFC (26). Ovarian volumes were also evaluated in our study, and a significant decrease was observed in postpartum ovarian volume in the CS group.

The deterioration of ovarian reserve tests with the increase in BMI should be considered (27). In the present study, patients considered obese according to the World Health Organization were not included (BMI ≥ 30), and the participants' BMI ranged from 19 to 28.9 kg/m2.

Evaluation of the ovaries using SWE has been studied in patients with polycystic ovary syndrome, and it has been shown that SWE values increase in the polycystic group. In the study in which the elasticity of the ovaries was evaluated, the SWE value was found to be approximately 8 kPa in the non-patient control group (28). In the present study, the SWE results of the ovaries were approximately 8.6 kPa, and the results of delivery and elasticity did not show a significant difference in both the VB and CS groups.

In the study of Romanski et al., it was reported that IVF cycles had no effect on the ovarian reserve (29). Pregnancy was achieved by various methods, and the methods were similar between the CS and VB groups in the current study.

Limitations

There were approximately two years between the time the patients were first evaluated and the time they were evaluated postnatally. The decrease in age-related ovarian reserve is observed especially over the age of 30 (30). Since the mean age of the patients in our study was 23.9 at the beginning, no significant decrease was observed in ovarian reserve tests with advancing age.

As a result, cesarean delivery may have negative consequences for ovarian reserve tests. There is a need for further studies investigating the consequences for long-term outcomes and clinical pregnancy rates.

Acknowledgments

The authors thank Prof Dr Sevim KOSE for their assistance in editing the study.

Conflict of interest

The authors declared none.

Authors' contributions

Concept: K.B.E., Design: K.B.E., D.K., Data Collection or Processing: K.B.E., D.K., Analysis or Interpretation: K.B.E., D.K., Literature Search: K.B.E., D.K., Writing: K.B.E., D.K.

Funding

None.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 838-841
doi: 10.52142/omujecm.39.3.47

Evaluation of the effect of vacuum assisted closure in the surgical management of Fournier's gangrene: A single center experience

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Received: 20.05.2022 • **Accepted/Published Online:** 15.06.2022 • **Final Version:** 30.08.2022

Abstract

Fournier's gangrene is a surgical emergency arising in the perineum and genital area and is characterized as necrotizing fasciitis of the perineum and genital area. It quickly spreads between the fascial planes and causes soft tissue necrosis. Diabetes mellitus is the most prevalent predisposing factor. Early diagnosis and management are critical in the progression of the disease. Surgical debridement and wide-spectrum antibiotic therapy are the first steps in treatment. Despite advancements in diagnosis and treatment procedures and changes in critical care techniques, the disease has a death rate of 16-40%. This research compares patients operated on for Fournier's gangrene between January 2016 and January 2022, including those treated with vacuum-assisted closure technique, vs those who were not. We acquired and analyzed the data on the patients' demographic and clinical features from hospital records. The study involved 16 patients, six (38%) in Group 1 and 10 (62%) in Group 2, 11 men (68 %) and five women (32%). Swelling in the wound region was the most prevalent complaint. The most common gangrene site was the perianal region, and diabetes was the most common predisposing condition. Group 2 had a significantly shorter hospital stay (p=0.02). There was no statistically significant difference in mortality or other parameters between the groups. The main advantages of VAC therapy are that it requires fewer dressings, causes less pain, and reduces the risk of contamination. The advantages of the traditional wet dressing include its ease of use and low cost and the fact that VAC therapy promotes faster wound healing and shorter hospital stays.

Keywords: Fournier's gangrene, vacuum-assisted closure, surgical treatment, diabetes mellitus

1. Introduction

Necrotizing fasciitis of the perineum and genital area is known as Fournier's gangrene (FG) (1). FG is a common surgical emergency that affects the perineum and genital area, spreads quickly between the fascial planes, and leads to soft tissue necrosis (2, 3).

In a case reported by Jean-Alfred Fournier in 1883, the disease, first identified as necrotizing fasciitis of the genital area by Bauriene in 1764, became known as Fournier's gangrene (4). Meleney was the first to use surgical debridement in FG in the 1920s (5). Surgical treatment is the most effective technique for reducing mortality today.

Every disease or condition that reduces tissue circulation and suppresses the immune system has been proposed as a risk factor. Diabetes mellitus is the most prevalent predisposing factor (6). Hypertension, coronary and peripheral artery disease, obesity, smoking and drug use, poor hygiene, alcoholism, cancer, and immunosuppression are all critical risk factors (7, 8). Although males are more commonly affected, the disease can affect both sexes and people of all ages (9). The etiology includes urogenital and anorectal infections, as well as trauma. FG is a disease with a fulminant and deadly course that is difficult to diagnose before necrosis and gangrene develop (10). Early detection and treatment are critical in the progression of the disease. Surgical debridement and wide-spectrum antibiotic therapy are the first steps in treatment (11). The continuation of treatment requires frequent and effective wound dressings and recurrent wound debridement's. Vacuum-assisted wound closure techniques (VAC) have become increasingly popular in recent years (12). Despite advancements in diagnosis and treatment procedures and changes in critical care techniques, the disease's death rate remains between 16 and 40% (13).

Our research aimed to assess the etiological characteristics, comorbidities, current treatment techniques, and factors influencing mortality in patients with FG, as well as to share the impact of VAC use on treatment outcomes.

2. Materials and Methods

This study compared patients operated on for FG between January 2016 and January 2022 and those treated with VAC with those who were not. We defined two groups of patients: Group 1 included patients operated on for FG and treated without VAC, while Group 2 included patients treated with

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VAC. We obtained and analyzed from hospital records patient demographics, leukocyte and lactate dehydrogenase (LDH) levels at admission, symptoms, affected area, etiological factors, comorbid diseases, number of debridements, diverting ostomy status, reconstruction methods, length of hospitalization, and mortality status.

2.1. Statistical analysis

We analyzed the data with IBM SPSS (Statistics for Windows. Armonk, NY, USA, IBM Corp.) software. We presented numerical data with mean and standard deviation values and categorical data with numbers and percentages. We evaluated relationships between categorical data using the chi-square test. We determined the distribution characteristics of continuous data with the Kolmogorov Smirnov test and the differences in numerical variables between the groups with the Mann Whitney U test. We accepted the significance level as $p<0.05$ in all statistical analyzes.

3. Results

This study involved 16 patients, six (38%) in Group 1 and 10 (62%) in Group 2, 11 men (68%) and five women (32%). Patients came to the emergency room with a variety of complaints. Swelling in the wound region was the most common complaint at the diagnosis. The most common gangrene site was the perianal region, and diabetes was the most common predisposing condition. We considered many factors to be associated with the etiology.

Table 1. Patient demographics, clinical features, etiological and predisposing factors

Parameters	Group 1, (n:6)	Group 2, (n:10)
Age* (years)	65.5 (±13.4)	65.4 (±12.1)
Gender, n (%)		
Male	4 (25)	7 (44)
Female	2 (12)	3 (19)
Initial symptoms, n(%)		
Fever	1 (6)	0
Pain	0	1 (6)
Swelling	2 (12)	6 (38)
Crepitus	1 (6)	0
Necrosis	1 (6)	2 (12)
Septic shock	1 (6)	1 (6)
Involved area, n(%)		
Perianal	4 (25)	4 (25)
Perineal	2 (12)	2 (12)
Genital	0	2 (12)
Predisposing factors, n(%)		
Diabetes	4 (25)	7 (44)
Hypertension	1 (6)	2 (12)
Cerebrovascular disease	0	1 (6)
Etiology, n(%)		
Obesity	1 (6)	5 (31)
Cigarette	2 (12)	3 (18)
Poor hygiene	1 (6)	0
İmmunosuppression	0	1 (6)
Anorectal diseases	2 (12)	1 (6)
Leukocyte count (u/L)*	17.8 (±2.8)	15.5 (±1.9)
LDH level (u/L)*	841 (±231)	720 (±305)

*Data is presented as mean (standard deviation), LDH: Lactate Dehydrogenase.

The primary causes were smoking, anorectal disorders, and obesity. Blood tests taken at the time of admission to the emergency department revealed elevated levels of leukocytes and LDH (Table 1).

During hospitalization, we conducted debridement on an as-needed basis in the operating room. The number of debridements was not significantly different between the two groups (p=0.646). We opened ostomies in two (33%) of Group 1 and three (30%) of Group 2 patients. The difference between the groups was not statistically significant (p=0.654). We applied various reconstructive approaches to patients whose wounds no longer required debridement. We used graft repair and secondary healing more frequently in Group 1, while primary repair was used more frequently in Group 2. Some patients in both groups died before we could complete wound repair. The difference between the groups was not statistically significant (p=0.064). Group 2 had a considerably shorter hospital stay (p=0.02). In both groups, there was no statistically significant difference in mortality (p=0.489) (Table 2).

Table 2. Treatment outcomes for Fournier's gangrene

Parameters	Group 1, (n:6)	Group 2, (n:10)	P
Number of debridement**	4 (3-6)	3 (1-6)	0.646
Ostomy, n(%)	2 (33)	3 (30)	0.654
Reconstruction methods, n(%)			
Flap	0	2 (12)	
Graft	2 (12)	0	0.064
Secondary recovery	2 (12)	1 (6)	
Primary repair	0	5 (31)	
Not implemented	2 (12)	2 (12)	
Length of hospital stay (day) **	24 (17-38)	16 (3-41)	**0.020**
Mortality, n(%)	2 (12)	2 (12)	0.489

*Data is presented as mean (standard deviation), **Data is presented as median (min-max value)

4. Discussion

FG is a serious, rare disease that can be fatal if diagnosed and treated too late. It is a fulminant disease that can affect the anal, perineal, and genital areas individually or in combination. Although FG can occur at any age, it becomes more common after the age of 50 (14). It is most commonly diagnosed between the ages of 30 and 60 (15). Some studies suggest that the prognosis worsens with age, but some show that age does not affect disease mortality (16, 17). In our study, the disease often occurred after the sixth decade, consistent with the literature. This could be related to increasing etiological factor exposure and lower immunity as people get older. Although the disease most commonly affects men, it can also affect women and children (18). The disease was more frequent in males in our sample, consistent with the literature.

FG may manifest itself as fever, chills, fatigue, and local discomfort a few days after symptoms; it may change to a

rapidly deteriorating clinical picture with severe pain, edema, erythema, post-induration necrosis, and crepitation in the later period (19). Delay in diagnosis and treatment leads to the rapid spread of the infection, especially in diabetic or severely immunosuppressed patients, and even extends to more distant organs and tissues within hours, leading to an increase in morbidity and mortality (20, 21). In our study, the most common complaint at first admission was swelling in the affected area.

Studies have suggested that the disease develops from genitourinary causes in 24%, anorectal in 24%, intra-abdominal in 10%, traumatic causes in 52%, and undetected causes in 38% (22). We observed that gangrene originating from the perianal region was more common in both groups, consistent with the literature. This could stem from the perianal region being poorly ventilated and hygienic.

Diabetes mellitus is the most common predisposing factor in Fournier's gangrene. Diabetic patients have impaired chemotaxis, phagocytosis, and cellular function. This causes an increased tendency to infections (6, 21). Our study's most common comorbid disease was diabetes, consistent with the literature. This finding implies that diabetes mellitus increased sensitivity to FG once cellular activities were damaged. Other etiological risk factors include chronic alcohol consumption, obesity, cancer, poor hygiene, low socioeconomic status, trauma, immunosuppression, paraplegia, and idiopathic causes.

A high leukocyte count at the first admission increases the risk of mortality (23). In addition, increased serum creatinine kinase and lactic acid levels, high FG severity index and APACHE II score are associated with poor prognosis (20-22). In our study, leukocyte and LDH, the infection parameters, were significantly higher at first admission, but we could not evaluate the relationship between mortality and blood values due to the insufficient number of patients.

Early diagnosis, emergency debridement, and wide-spectrum antibiotics are FG's most essential treatment components (1). All necrotic tissue is debrided, and the procedure is repeated if necessary to control the infection. If the anorectal region and sphincter are involved, or if there is fecal contamination, a colostomy may be preferred to reduce contamination (24). We applied aggressive surgical debridement and wide-spectrum antibiotic therapy to all patients. There was no significant difference between the two groups regarding the number of debridements. We applied a diverting ostomy to approximately 40% of the patients, but there was no difference between the two groups. In most of the patients who underwent ostomy, the gangrene site was the perianal region. We may have opened more diverting ostomies in these patients, as we foresaw that the area would be easily contaminated and could not be taken under control. Studies have reported that patients with an ostomy prognosis are worse (16). Our study revealed diverting ostomy in all patients with a mortal course, in line with the literature. Patients are treated with wet dressing or VAC treatments, and consecutive surgical debridements are performed. Wet dressings are an effective, safe, and inexpensive method of treatment commonly used to keep the wound clean. In recent years, VAC has been widely used in treatment as an alternative to wet dressing (25). VAC therapy also has several advantages. It allows for minimizing contamination, especially in wounds with the possibility of contamination, reducing the number of dressings compared to traditional dressings, and providing less pain to the patient. In addition, negative pressure accelerates healing with the increased blood supply in the wound and provides faster clearance of inflammatory mediators (26). Studies show that the number of dressing’s decreases and the length of stay is shortened with VAC treatment (27). Our study found that patients treated with VAC were discharged in a shorter time, in line with the literature. We believe that this is due to the accelerating effect of VAC therapy on wound healing, as mentioned above.

Early closure of the defective area is an integral part of the treatment. There are various reconstruction methods to achieve this with different functional and cosmetic results. The patient's clinical characteristics and the surgeon's preference are essential in determining the reconstruction method used (28). Our study evinced that primary repair was performed significantly more in VAC patients, but there was no statistically significant difference between the two groups. We believe that the reason behind the more frequent use of primary repair in VAC patients was faster wound healing and a faster approach to wounds.

Diabetes, female gender, presence of malignant disease, and the time from the onset of the disease to the first surgical treatment were reported as independent risk factors affecting mortality (29). Pawlowski et al. reported the mortality rate in FG to be 16-40% (30). Our total mortality rate was 25%, compatible with the literature, though with no statistical significance between the two groups.

Our study had several limitations. It was retrospective and could not be randomized. Therefore, limitations such as irregularity in some case records and the inability to access all the desired data have emerged. Thus, we could not use the Fournier Gangrene Severity Index (FGSI), which is used to determine the severity and prognosis of the disease, as we could not obtain all the necessary information.

FG is an emergency surgical condition progressing rapidly and has a high mortality rate if not treated early. Early diagnosis, aggressive surgical debridement and appropriate antibiotic therapy are essential factors in the prognosis of the disease. Reconstructive procedures can be performed successfully using various dressing, and wound closure techniques in patients followed up after early surgical debridement. The important advantages of VAC therapy are

less requirement for frequent dressings and less pain and risk of contamination. According to our results, both methods have advantages over each other. The prominent features of the classical wet dressing are that it is easily accessible and cheaper and that VAC therapy provides faster wound healing and shorter hospital stays.

Conflict of interest

The authors declared no conflict of interest.

Funding

No funding was used for the study.

Acknowledgments

None to declare.

Authors' contributions

Concept: M.U., T.A., Design: M.U., T.A., Data Collection or Processing: M.U., T.A., Analysis or Interpretation: M.U., T.A., Literature Search: M.U., T.A., Writing: M.U., T.A.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 842-847
doi: 10.52142/omujecm.39.3.48

Awareness of temporomandibular joint involvement in rheumatoid arthritis patients by physicians dealing with rheumatology

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Received: 14.06.2022 • **Accepted/Published Online:** 30.06.2022 • **Final Version:** 30.08.2022

Abstract

The aim of this study is to evaluate the attitudes and awareness of physicians dealing with rheumatology about temporomandibular joint (TMJ) involvement while evaluating rheumatoid arthritis (RA) patients with the TMJ questionnaire we created. For this purpose, an online TMJ evaluation questionnaire consisting of 10 questions was prepared and completed by the physicians via the Google Forms application. Among those who participated in the survey; the percentage of correct answers to the question of whether the TMJ joint is a synovial joint was 95.5%. While the rate of those who correctly knew that more than half of the RA patients had TMJ involvement remained at 35.5%, the rate of those who correctly identified that RA rarely affected the first TMJ was 75.5%. The rate of those who knew that TMJ involvement is frequently bilateral in RA was 62.7%. The number of participants who answered the question of what the clinical findings are in TMJ involvement correctly was over 60%. When asked about radiological findings in TMJ involvement in RA, more than 75% of the participants expected joint space narrowing, erosion, and mandibular destruction. On the other hand, the number of physicians who expected radiological findings such as ostephitis and sclerosis as radiological findings was below 40%. While the number of physicians who performed TMJ inquiries and examinations in RA patients was only 15.5%, if there was a TMJ complaint, this rate increased to 80%. The number of participants using objective tests for the evaluation of TMJ was below 30%. Also, 85.5% of the participants ignored TMJ involvement in RA. In conclusion, our study showed that physicians dealing with rheumatology have low awareness of TMJ involvement in RA.

Keywords: Temporomandibular joint, rheumatoid arthritis, awareness, survey

1. Introduction

The temporomandibular joint (TMJ) is a synovial joint formed between the mandible and the temporal bones. When the functional anatomy of the joint is examined, it has an extremely special design based on neuromuscular control and the integrity of the soft tissue elements. This joint has an important place in the human body because it takes part in vital functions such as chewing, swallowing, and speaking.

According to the National Institute of Dental and Craniofacial Research, TMJ problems have been reported as the second most common complaint after low back pain (1). They can affect up to 5-12% of the entire population, which can create enormous treatment-related costs (1).

Since Rheumatoid arthritis (RA) is an inflammatory arthritis involving synovial joints, it is not surprising that TMJ joint involvement. However, TMJ involvement is rarely the first joint involved in the course of RA (2, 3). Therefore, TMJ complaints and evaluation in RA patients are often neglected by physicians because they focus more on other joints. This neglect may cause serious damage to the joints and disability (4).

Our clinical observations are that RA patients do not have obvious TMJ complaints and unless they apply to the physician with these complaints, history asking, physical examination, and imaging studies about TMJ are insufficient by physicians.

When we went through the literature, we could not find any studies showing how much the physicians dealing with RA tended to TMJ problems. In this study, we aimed to evaluate the attitudes and awareness of physicians dealing with rheumatology about TMJ involvement while evaluating

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RA patients with the TMJ questionnaire we created.

2. Material and Methods

2.1. Participant and data collection

An online questionnaire consisting of 10 questions was prepared to evaluate the attitudes and awareness of physicians towards TMJ involvement in RA patients, and an e-mail form was prepared for physicians (rheumatology specialists and sub-branch assistants, physical medicine and rehabilitation specialists and assistants, internal medicine specialists and assistants) who are interested in rheumatology throughout Turkey. An invitation containing the subject, purpose and method of the study was sent via mail and/or online groups. In this survey, the volunteers were not asked for personal information except their age. The study questionnaire form is shown in Table 1. A 'Google Forms' application link was sent to those who accepted to participate in the study survey.

Table 1. Attitude and Awareness Questionnaire towards Temporomandibular Joint Involvement in Patients with Rheumatoid Arthritis

1. The temporomandibular joint is a synovial joint.		
a) Correct	b) No answer	c) Wrong
2. Temporomandibular joint involvement occurs in more than half of rheumatoid arthritis patients.		
a) Correct	b) No answer	c) Wrong
3. Temporomandibular joint is rarely the first joint affected in patients with rheumatoid arthritis.		
a) Correct	b) No answer	c) Wrong
4. The temporomandibular joint is often bilaterally involved in patients with rheumatoid arthritis.		
a) Correct	b) No answer	c) Wrong
5. What are the clinical findings in temporomandibular joint involvement? (More than one option can be ticked)		
a) Pain in the joint	b) Swelling	
c) Morning stiffness in the joint	d) Weakness in chewing muscles	
e) Sound coming from the joint	f) Joint movement limitation	
6. What are the radiological findings seen in temporomandibular joint involvement? (More than one option can be ticked)		
a) Decreased joint space	b) Erosion	
c) Destruction of the mandibular condyle	d) Osteophyte	e) Sclerosis
7. I do the temporomandibular joint questioning and examination in every rheumatoid arthritis patient whether there is a complaint or not.		
a) Yes	b) No	c) No answer
8. I do temporomandibular joint questioning and examination only in patients with rheumatoid arthritis who have complaints.		
a) Yes	b) No	c) No answer
9. Which examination methods do you know exactly for temporomandibular joint? (More than one option can be ticked)		
a) Evaluation of pain and tenderness by palpation		
b) Evaluation of crepitation and clicking sounds coming from the joint with palpation	c) Incision opening measurement	
d) Measurement of lateral glide distances	e) Measurement of the strength of the chewing muscles	
10. Do you think that you are more interested in other joints in rheumatoid arthritis patients and ignore the temporomandibular joint?		
a) Yes	b) No	c) No answer

While determining the sample size, 10 patients per item is regarded optimum in terms of validity in survey research (5). Given that our survey had ten items, we aimed for a sample size of at least 100. The survey was set to last for a maximum of six months. If the minimum required number of participants is not reached within 6 months, it has been decided to extend these periods by one month until the minimum required number is reached, or to wait until the end of the 6-month period even if the minimum required number is reached, and to reach the maximum number of participants.

This study was conducted between October 2021 and March 2022, following the approval of the local ethics committee (Number: E-77192459-050.99-48888, Decision number 2021/606), in accordance with the principles of the Declaration of Helsinki. The study was designed as a cross-sectional, descriptive survey study.

2.2. Statistics

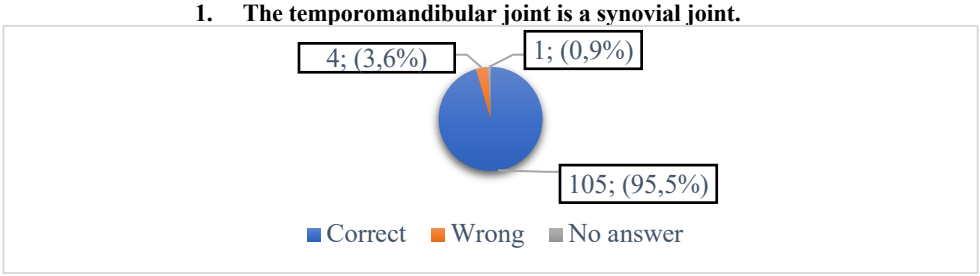
Only descriptive statistical analysis was performed on the collected data. SPSS version 22 (IBM Corp., Armonk, NY, USA) was used for this process.

3. Results

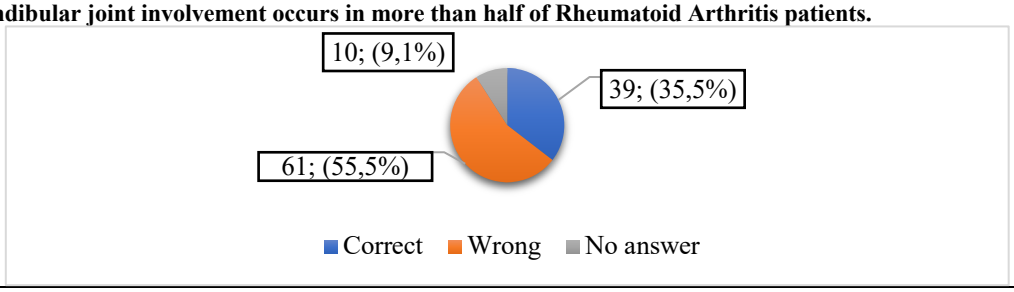
In the study, survey invitations could be sent to a total of 246 people. Within a 6-month period, 110 of them agreed to participate in the study. The mean age of the participants was 37.1± 8.6 (years). 105 (95.5%) of the participants included in the study gave the correct answer to the question whether TMJ is a synovial joint. Table 2 shows the answers given by our participants to the survey questions.

Table 2. Response results of the participants who participated in the survey study n (%)

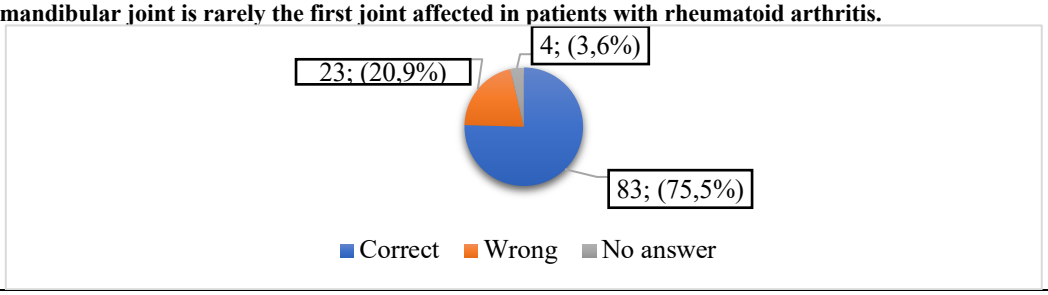
1. The temporomandibular joint is a synovial joint.



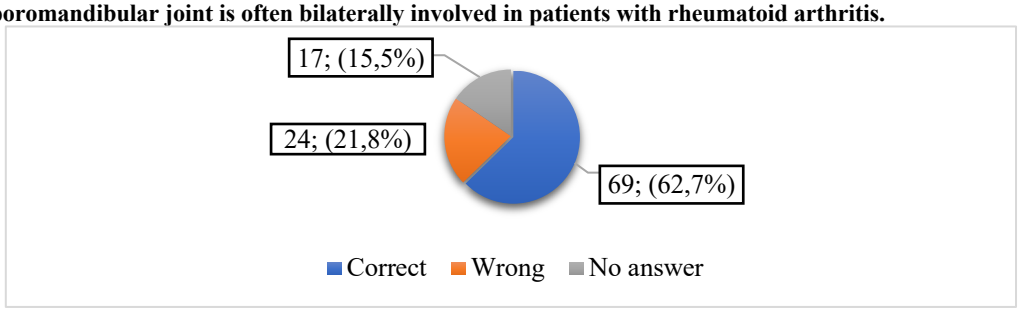
2. Temporomandibular joint involvement occurs in more than half of Rheumatoid Arthritis patients.



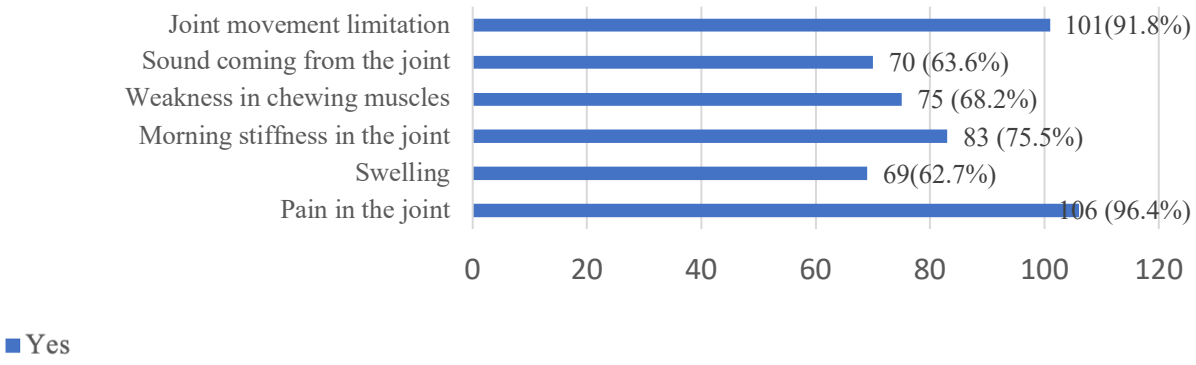
3. Temporomandibular joint is rarely the first joint affected in patients with rheumatoid arthritis.



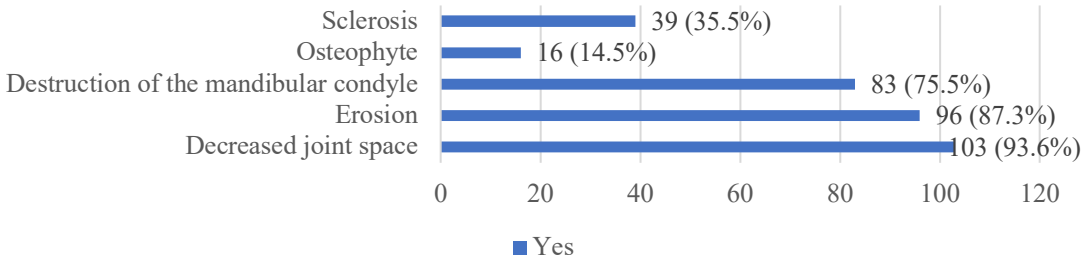
4. The temporomandibular joint is often bilaterally involved in patients with rheumatoid arthritis.



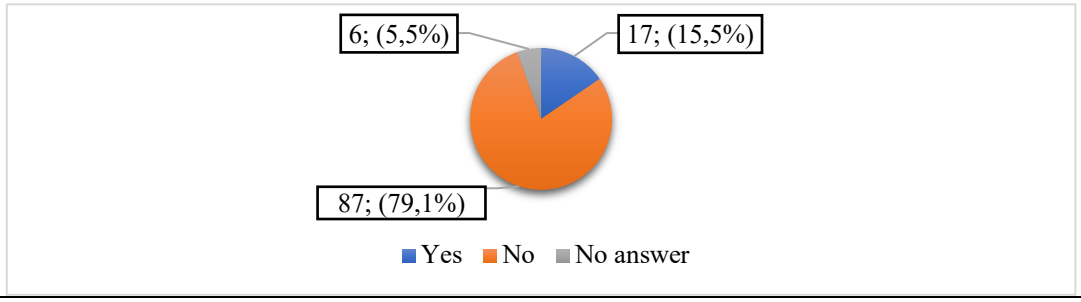
5. What are the clinical findings in temporomandibular joint involvement? (More than one option can be ticked)



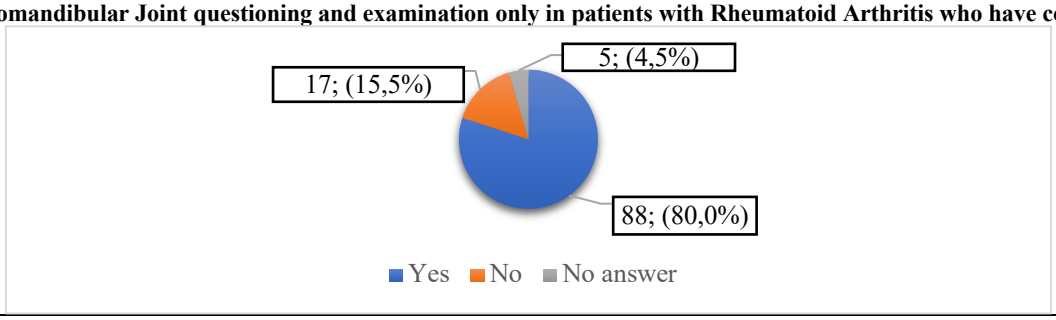
6. What are the radiological findings seen in temporomandibular joint involvement? (More than one option can be ticked)



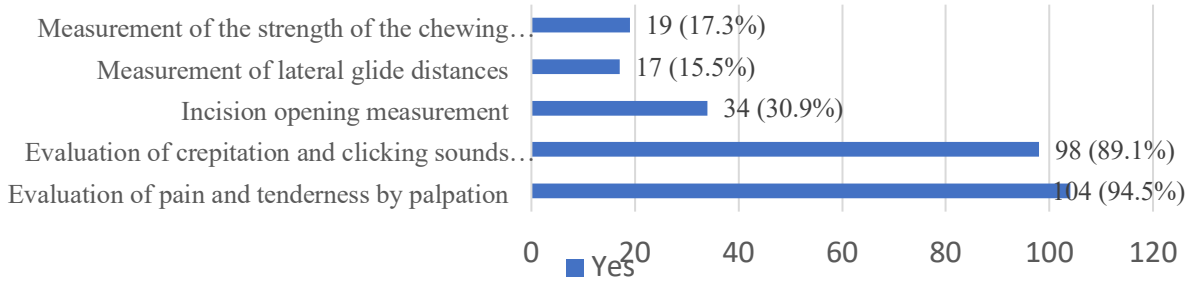
7. I do the temporomandibular joint questioning and examination in every rheumatoid arthritis patient whether there is a complaint or not.



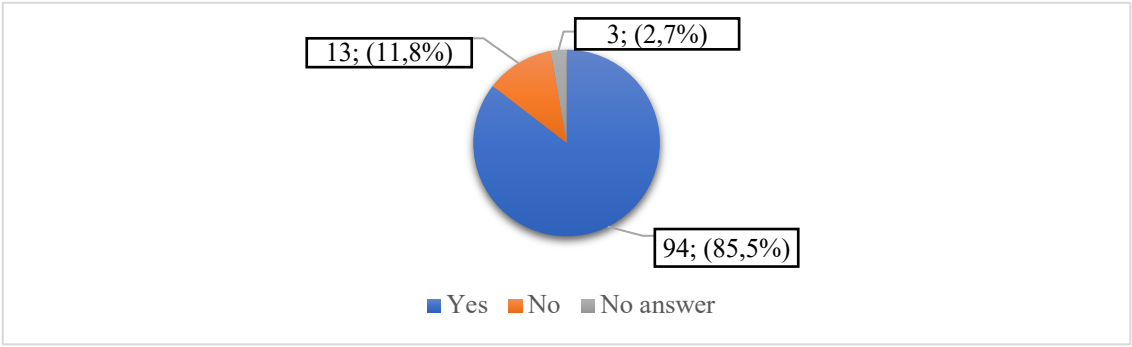
8. I do Temporomandibular Joint questioning and examination only in patients with Rheumatoid Arthritis who have complaints.



9. Which examination methods do you know exactly for Temporomandibular Joint? (More than one option can be ticked)



10. Do you think that you are more interested in other joints in rheumatoid arthritis patients and ignore the temporomandibular joint?



4. Discussion

Rheumatoid arthritis is among the most common types of inflammatory arthritis. We know very well that the disease's course with joint deformities makes people suffering from this disease worried about their future. As physicians, our duty should be to keep the quality of life of RA patients at the highest level and to prevent deformity. In this, we should know the RA, which is insidiously in front of us, and measure the disease activity correctly. TMJ is not taken into account when calculating disease activity scores such as DAS28, which we use to measure disease activity in today's outpatient practice (6). Perhaps because the disease activity of a patient with TMJ involvement but not peripheral joint involvement is seen in remission, treatment changes are not made incorrectly. When we examine the HAQ, which is also the RA health assessment score, it is very thought-provoking that while the daily loss of function caused by peripheral joint involvement is questioned in the section about eating, the inadequacies of the TMJ joint are not questioned (7). We think that physicians dealing with rheumatology should be mindful of this issue.

Temporomandibular joint: as it is known, the glenoid fossa of the temporal bone is a synovial joint consisting of 4 articulated surfaces consisting of the upper and lower surfaces of the articular disc and the mandibular condyle (8). Almost all of the physicians participating in the survey answered correctly that the TMJ joint is a synovial joint.

TMJ involvement is frequently encountered in patients with RA. In the literature, Moen et al. reported that 77% of RA patients had pain and dysfunction due to TMJ involvement (9). Again, most studies show that more than 50% of RA patients clinically show TMJ involvement (2). However, the number of participants who correctly answered the question "More than half of RA patients had TMJ involvement" in our study remained only at 35.5 percent. TMJ involvement in RA may be ignored because physicians believe it is uncommon.

According to the literature, TMJ involvement is rarely the first joint involved in the course of RA, and it is striking that it is often bilateral when TMJ involvement is present (2, 3). In our survey, more than 60% of participants correctly answered the questions 'Temporomandibular joint is seldom the first joint affected in patients with rheumatoid arthritis.' and 'Temporomandibular joint is commonly bilaterally implicated in patients with rheumatoid arthritis.'

Clinically, in TMJ pathologies due to RA, there is preauricular pain or tenderness during joint movement (10). As the causes of this pain; retrodiscal tissue compression, inflammatory changes in the joint, stretching of the joint capsule, and synovitis have been demonstrated (10). Morning stiffness, weakness in masticatory muscles, swelling in the joint and limitation of movement are also seen in TMJ joint involvement. The percentage of participants who answered these questions correctly in our study was over 60%. In other words, although our study participants ignored the TMJ involvement, they were well acquainted with the clinical findings of TMJ involvement.

The diagnosis of rheumatoid arthritis and TMJ involvement is based on the history, physical findings, and radiographic findings. Radiographic changes of TMJ include flattening of the mandibular condyle, cortical erosion, gradual reduction in joint space, deossification, condyle head deformity, and subcortical cysts (10-12). Again, in the advanced stages of the disease, a sharp and pointed condyle, osteophyte formation, lipping, shortened posterior ramus length causing premature posterior occlusion, anterior open bite and deepening of the antegonial notch can be counted among the radiographic changes (13). Since they were questioned in terms of radiological findings in TMJ involvement in RA, the majority of the participants said that they expected joint space narrowing, erosion and mandibular destruction. On the other hand, the number of physicians who expected radiological findings such as ostephitis and sclerosis as radiological findings was very low. The reason for this may be that these radiological findings (ostephitis, sclerosis) are seen in osteoarthritis joint involvement rather than inflammatory arthritis (14).

While the number of physicians who inquire about TMJ history and examine patients with RA remains at only 15%, if there is a TMJ complaint, this rate rises to 80%. From this point of view, we think that physicians ignore TMJ involvement in patients with RA who do not have TMJ complaints.

In our study, we found that the majority of the participants searched for pain and tenderness by palpating the joint, as well as looking for crepitation and clicking sound in the joint in detecting TMJ involvement. However, there are more objective tests for the evaluation of TMJ; we found that measurement of incision opening, measurement of lateral glide distance, and measurement of chewing muscle strength are not frequently known. However, objective methods may be more valuable in terms of patient follow-up.

Finally, 85% of the participants admitted that they ignored TMJ involvement in RA. We could not find any other study in the literature investigating the awareness of TMJ involvement in RA and the awareness of physical examination methods in detecting TMJ involvement. In the literature, there are only studies reporting that RA patients have low awareness of the problem related to TMJ (15).

The most important limitation of our study is that we could not reach many clinicians in our country due to the low participation in online surveys, while the strength of our study is that it is the first study investigating physicians' awareness of TMJ involvement in RA.

Our findings are significant as they reveal that physicians dealing with rheumatology ignore TMJ involvement in RA

and are not aware of the objective measurement methods that they can use when TMJ involvement occurs. We think that the examination of TMJs, which is a crucial component of our vital activities such as nutrition and speech, should be added to the standard joint examination. In addition, we think that it should be included in disease activity ratings and health assessment questionnaires.

We believe that if our study results are supported by future national and international studies, awareness of TMJ insertion will increase.

Conflict of interest

None to declare.

Acknowledgments
None to declare.

Funding
The authors received no financial support for the research and/or authorship of this article.

Authors' contributions

Concept: M.İ., İ.İ., Y.D., A.K.C., P.Ç., Design: M.İ., İ.İ., Y.D., A.K.C., P.Ç., Data Collection or Processing: M.İ., İ.İ., Y.D., A.K.C., P.Ç., Analysis or Interpretation: M.İ., İ.İ., Y.D., A.K.C., P.Ç., Literature Search: M.İ., İ.İ., Y.D., A.K.C., P.Ç., Writing: M.İ., İ.İ., Y.D., A.K.C., P.Ç.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 848-856
doi: 10.52142/omujecm.39.3.49

Role of cannabinoid receptor-2 in small intestinal fasted myoelectric activity of rats

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Received: 27.05.2022 • **Accepted/Published Online:** 13.06.2021 • **Final Version:** 30.08.2022

Abstract

Cannabinoid receptor-1 (CB_1R) and cannabinoid receptor-2 (CB_2R) have significant roles in the esophagus, small intestine, and colon motility in the postprandial period besides gastric emptying, secretion, and defecation in the gastrointestinal system. Furthermore, our previous study showed that activation of peripheral CB_1R inhibited migrating myoelectric complex (MMC), forming the source of fasted small intestinal motility. However, the role of the central/peripheral CB_2Rs on the MMC pattern is still unknown. The present study aimed to investigate the roles of peripheral and central CB_2Rs in forming and regulating small intestine MMC patterns in rats. In this study, we used 42 adult male Sprague-Dawley rats (n:7). We implanted bipolar electrodes in three different jejunum sites of rats (J1, J2, J3) to record the MMC pattern. We placed a cannula in the right lateral ventricle to perform drug intracerebroventricularly (i.c.v.) and implanted a catheter in the right jugular vein to inject the drug intravenously (i.v). After the amelioration period, we conducted experiments following an 18-hour fasting period and later took at least a one-hour baseline recording of the MMC pattern. Then, we injected JWH 133, a CB_2R agonist i.v. (1.25-10 mg/kg) or i.c.v. (2.5-20 µg/rat) and also administered AM 630, a CB_2R antagonist, i.v. (0.25-2 mg/kg) or i.c.v. (2.5-20 µg/rat). We compared the effects of JWH 133 or AM 630 on the MMC pattern to the vehicle group (10% dimethyl sulfoxide). Centrally or peripherally injected JWH 133 and AM 630 did not cause any change in the spike frequency and the number of the MMC cycle. The results of the present study propose that CB_2Rs are involved in neither endogenous formation nor exogenous regulation of the fasting myoelectric activity in healthy fasted rats.

Keywords: cannabinoids, cannabinoid receptor-2 (CB_2R), gastrointestinal motility, migrating myoelectric complex (MMC), rat

1. Introduction

Small intestinal motility mixes the chyme with digestive enzymes and bile secretion, and transports the content throughout the gut in coordination with the digestive and absorption functions. The postprandial pattern, which occurs in the small intestine following food intake, consists of segmentation and peristalsis. In the fasted state, a unique and complex pattern comes into play in the whole small intestine (1). This pattern consists of 3 phases in which the contractile activity at any point in the small intestine has shown recurrent differences. Firstly, there are not almost contractions for about 40-50 minutes (phase I), and later many irregular contractions occur for 30-40 minutes (phase II). Finally, intense contractions occur for 5-10 minutes (phase III). Then phase I begins again, and the cycle rhythmically continues approximately every 90-120 minutes until the next meal in human. The cycle starting in the most proximal part of the small intestine moves (migrates) towards the distal portion of the intestine (2). Because of its migration, this complex has been termed "the migrating motor/motility complex" by Foulk *et al.* in 1954 (3). The physiological significance of this complex is that it moves the undigested food residues, gastrointestinal (GI) secretions, and dead cells from the stomach and small intestines to the large intestine, having prevented the bacteria from transitioning from the large intestine to the small intestine. Because of these crucial functions, the migrating motor/motility complex is called the "housekeeping of the gastrointestinal tract" (4).

First, Szurszewski (1969) demonstrated that the electrical changes (slow and spike waves), which are recorded in the smooth muscle of the small intestine in dogs, forming the origin of the migrating motor/motility complex and also termed the "migrating myoelectric complex: the MMC" (5). Obviously, the control mechanism of the MMC is complex because it occurs rhythmically and regularly. Many neurohumoral factors play a role in the formation/ regulation of the MMC, such as mainly motilin, acetylcholine, somatostatin, pancreatic polypeptide, serotonin, xenin, and ghrelin (6-13). Furthermore, the enteric nervous system initiates, maintains, and coordinates the MMC while the parasympathetic/sympathetic nervous system is only involved

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in the coordination of the MMC (14-16).

The endocannabinoid system comprises endogenous ligands, synthesis/degradation enzymes, and primary receptors (cannabinoid receptor-1 and cannabinoid receptors-2). Endocannabinoid ligands, mainly anandamide and 2-AG, are produced on demand from membrane phospholipids in postsynaptic neurons. The ligands are released immediately without being stored in vesicles and then function as retrograde messengers (17, 18). Cannabinoid receptor-1 (CB_1R) is mainly expressed in the central nervous and enteric nervous systems, and one of its primary functions is modulating neurotransmitter release in several neurons (19). In contrast, cannabinoid receptor-2 (CB_2R) is mainly expressed in immune system organs, such as the thymus, and spleen and is often referred to as the peripheral cannabinoid receptor. The major receptors of endocannabinoids are seven-transmembrane G-protein-coupled receptors, leading to the suppression of adenylate cyclase activity (20, 21).

Endocannabinoid system components are present in the GI tract. Endogenous ligands or their pharmacological modulation (ligand, receptor agonists, inhibitor of catabolic enzyme) can cause inhibition in GI functions, such as gastric emptying, peristalsis, and defecation; conversely, suggesting that specific CB_1R/CB_2R blockage can lead to excitation of the functions (18, 22, 23). In the postprandial state, there is substantial evidence that CB_1R/CB_2R mediates the inhibitor effects of cannabinoids on GI motility. *In vitro* study results have shown that nonspecific CBR agonists and specific-CB_1R agonists activate CB_1R and reduce the amplitudes of smooth muscle contractions induced by electrical field stimulation in the gastric fundus, antrum, ileum, and colon preparations state (24-29). Similarly, *in vivo* studies supported that CB_1R activation has inhibited GI transit in the fed states (18, 30-32). Furthermore, particular CB_2R receptor agonists have been proven in multiple studies to reduce increased GI transit caused by various pathological conditions (e.g., inflammation, colitis, irritable bowel syndrome). Consistent with these findings, peripheral CB_1R activation has also inhibited the MMC pattern generating the origin of fasting motility (33).

However, it is unknown whether **(a)** central/peripheral CB_2Rs have a tonic role in the regulation/formation of the MMC and **(b)** whether central/peripheral CB_2R activation by exogenously administered CB_2R agonist affects the MMC pattern. For this purpose, to investigate the role of CB_2Rs in migrating myoelectric complex, we examined whether **(1)** central and peripheral administration of CB_2R antagonists and **(2)** central and peripheral administration of CB_2R agonists caused any change in the MMC pattern.

2. Material and Methods

2.1. Animals

We obtained adult male Sprague Dawley rats weighing 250 to 300 g from the Animal House of Ondokuz Mayıs University (Samsun, Turkey). We housed them in a quiet, temperature- and humidity-controlled room at 22±1°C for a 12-h alternating light-dark period, and gave food and water ad libitum. We conducted all protocols and procedures under the Guide for the Care and Use of Laboratory Animals (NIH Publication, 865–23, Bethesda, MD, USA).

2.2. Surgical procedures

We used ketamine [50 mg/kg; intraperitoneal (i.p)] and chlorpromazine (25 mg/kg; i.p) to anesthetize the male Sprague-Dawley rats and, through a midline incision, placed three bipolar stainless Ni/Cr wire electrodes (Driver-Harris, Cedex, France) into the muscular wall of the small intestine 15 (J1), 25 (J2), and 35 (J3) cm distal to the pylorus for electromyographic (EMG) recordings. We placed a catheter in the right jugular vein to reveal the peripheral effect of the substance, and a 24-gauge cannula in the right lateral ventricle (1.5 mm caudal, 2 mm lateral from Bregma; 6 mm ventral from the skull surface) to administer drug centrally. We tunneled the electrodes and cannula subcutaneously to exit the back of the animal's neck. Later, we tunneled both the EMG electrodes and the catheter subcutaneously and externalized them at the back of the animal's neck. We then fixed the EMG electrodes to the skull with dental acrylic. We housed all the male rats, one in each cage after surgery. We treated the rats with antibiotics (Ampicillin; 100 mg/kg; intramuscular) and analgesics (Metamizole Sodium; 100 mg/kg; i.p.) for the first three days following surgery (34).

We adapted the rats to the experimental conditions by putting them in Bollman cages (Bahadır Co., Turkey). We performed this putting process for 2 hours a day until the experiment day (7th post-operative day).We made the rats hungry for 18 hours without water restriction in wire-bottomed cages before experiments for EMG recording (12, 14, 33). We performed all the experiments on conscious rats in the Bollman cages. We recorded and analyzed EMG recordings amplified with a bioamplifier (ML132, ADInstruments, Australia) by the PowerLab data acquisition system (ML870/P, PowerLab 4/SP, AD Instruments, Castle Hill, NSW, Australia)

2.3. Design of electromyography studies

The experiments started with a control recording of baseline myoelectric activity with three MMC cycles propagated over all three sites (J1, J2, J3) for at least one hour. We started administering the drug at the end of the fourth MMC cycle at J1 sites. After drug application, we took recordings for a minimum of one hour. We tested each animal was tested 2-5 times with an interval of 3 days.

- In the first series of experiments, we injected a vehicle (10% DMSO) intravenously (i.v.) in a volume of 1 ml/kg (n=7).
- In the second series of experiments, we injected a vehicle (10% DMSO) intracerebroventricularly (i.c.v.) in a volume of 5 µl/rat (n=7).

- In the third series of experiments, we injected JWH 133, CB_2R agonist, at doses of 1.25-10 mg/kg intravenously (n=7).
- In the fourth series of experiments, we injected JWH 133, CB_2R agonist, at doses of 2.5-20 µg/rat intracerebroventricularly (n=7).
- In the fifth series of experiments, we administered AM 630, CB_2R antagonist, at doses of 0.25-2 mg/kg intravenously (n=7).
- In the sixth series experiment, we administered AM 630, CB_2R antagonist, at doses of 2.5-20 µg/rat intracerebroventricularly (n=7).

2.4. Drugs and chemicals

We prepared all drugs on the experimental days just before drug administration. We dissolved agonists and antagonists were in 10% dimethyl sulfoxide (Sigma, St. Louis, MO). We injected i.c.v. with a Hamilton syringe in a volume of 5 µl/rat, and administered i.v. within a volume of 1 ml/kg with an insulin syringe. We purchased from JWH 133 (CB_2R-selective agonist) and AM 630 (CB_2R-selective antagonist) from PolyPeptide (Strasbourg, France). We selected the agonist and antagonist doses investigated in our study by considering the doses of these agents that are effective on the GI system in the literature (35-41).

2.5. Data analysis

We characterized the typical feature of intestinal myoelectric activity in the interdigestive period, or phase III of the MMC, as a period of clearly distinguishable intense spike waves, propagating aborally through the all recording segment and followed by a period of quiescence, phase I of the MMC having only slow waves and identified phase II of the MMC as a period of irregular spike waves. Within 1 hour after drug administration, we calculated spike frequency and numbers of the MMC cycles in all three sites (J1, J2, and J3) and analyzed them with the LabChart 7.0 program (12, 33).

2.6. Statistics

We converted all of the obtained EMG recordings to numerical values and used GraphPad Instat (v3.06) software for statistical analysis (San Diego, CA, USA). After determining that all the data were normally distributed, we performed a one-way analysis of variance (ANOVA) and Tukey–Kramer post hoc tests for multiple comparisons between groups. We expressed all values used in the graphics as mean±standard error (SEM). We considered $p< 0.05$ was considered statistically significant for all statistical tests,

3. Results

In the interdigestive period, all animals showed a fasted motor motility with recurrent MMC cycles propagated to the distal intestinal segments. Fig. 1 shows the characteristics of the MMC pattern at all theree jejunum sites in the EMG recording.

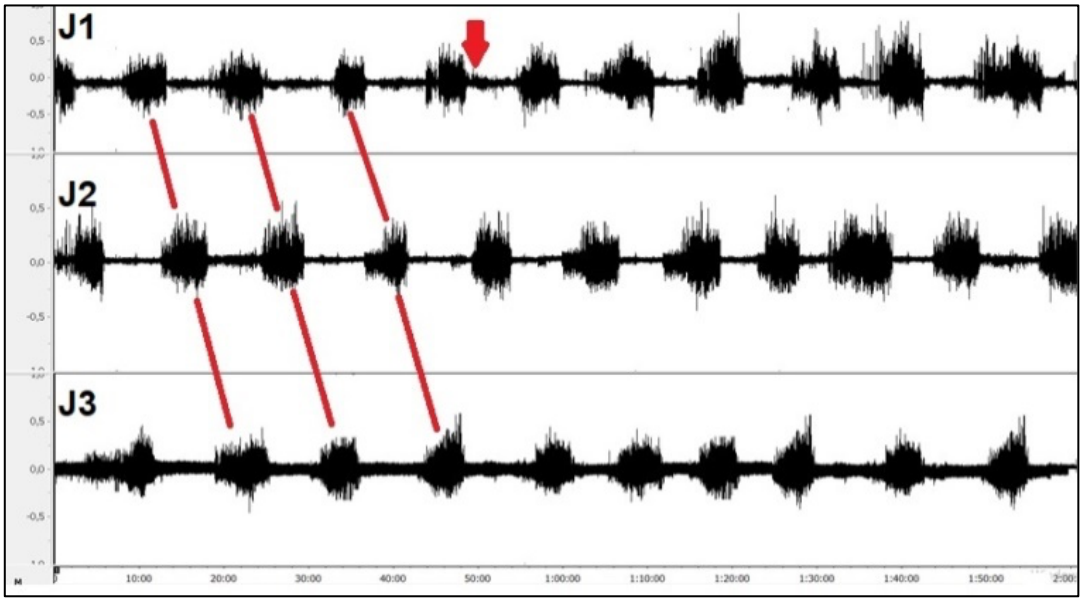

Fig. 1. In a conscious and fasted rat, three regular the MMC cycles at all three points (red lines) and drug injection at the end of the 4th the MMC cycle at J1 point (red arrow)

3.1. Effects of exogenously administered JWH 133, *CB_2R agonist,* on fasting myoelectric activity by activation of peripheral/central CB_2Rs

The effect of intravenously administered JWH 133 on the MMC pattern

Intravenous 10% DMSO administration (1 ml/kg), the solvent of JWH 133, did not change spike frequency and numbers of the MMC cycles at J1, J2, and J3 sites within one hour after administration compared to the baseline recording period. We, therefore, compared the effects of JWH 133 doses (1.25-10 mg/kg, i.v.) on the MMC pattern to the i.v. vehicle group. After i.v. injection of the CB_2R selective agonist JWH 133 at

1.25-10 mg/kg, it did not cause a statistically significant change in spike frequency at all three jejunal sites (Fig.2a). Additionally, the number of the MMC cycles remained unchanged by i.v. administered JWH 133 at jejunal recording sites (Fig. 2b).

The effect of intracerebroventricularly administered JWH 133 on the MMC pattern

After 10% DMSO injection (5 µl/rat, i.c.v.), the MMC pattern on baseline recording remained unchanged at jejunal recording sites compared to the control period. For this reason, we compared i.c.v. administrations of JWH 133 doses (2.5-20 µg/rat) on the MMC pattern according to the vehicle group. JWH 133 administered intracerebroventricularly, at doses of 2.5-20 µg/rat, did not cause a statistically significant change in spike frequency and the number of the MMC cycles in all recording sites compared to the vehicle group (Fig. 3a and b).

3.2. Tonic involvement of CB_2Rs in formation/regulation of fasting myoelectric activity

The effect of intravenously administered CB_2R antagonist AM 630 on the MMC pattern

After intravenous administration of AM 630, at doses of 0.25-2 mg/kg, it did not induce a statistically significant alteration in spike frequency at all three jejunal sites (Fig. 4a).

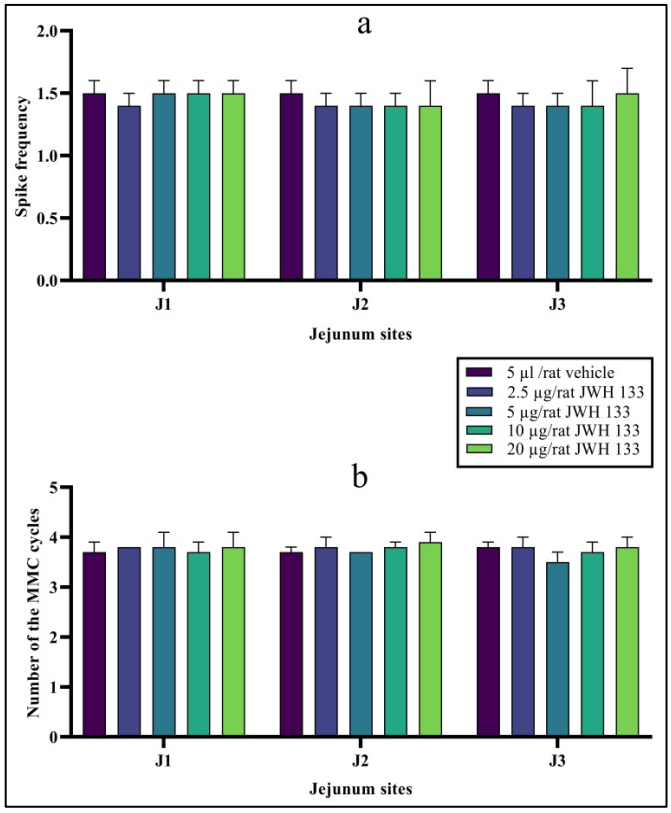

Fig. 3. Comparison of effect of different intracerebroventricular doses of CB_2R selective agonist, JWH 133, on the MMC pattern in its spike frequency **(a)** and number of the MMC cycle **(b)** (Mean±S.E.M., n=7)

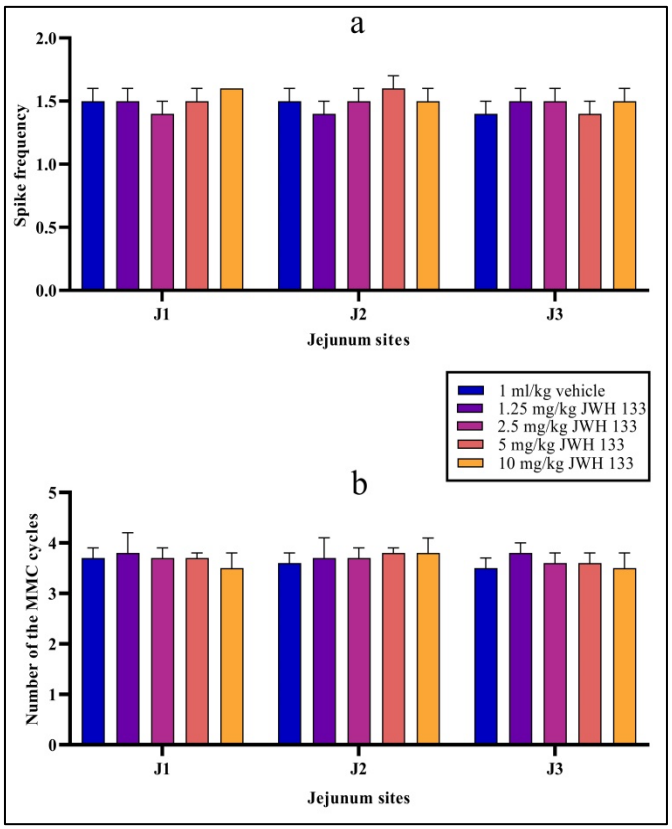

Fig. 2. Comparison of effect of different intravenous doses of CB_2R selective agonist, JWH 133, on the MMC pattern its spike frequency **(a)** and number of the MMC cycle **(b)** (Mean±S.E.M., n=7)

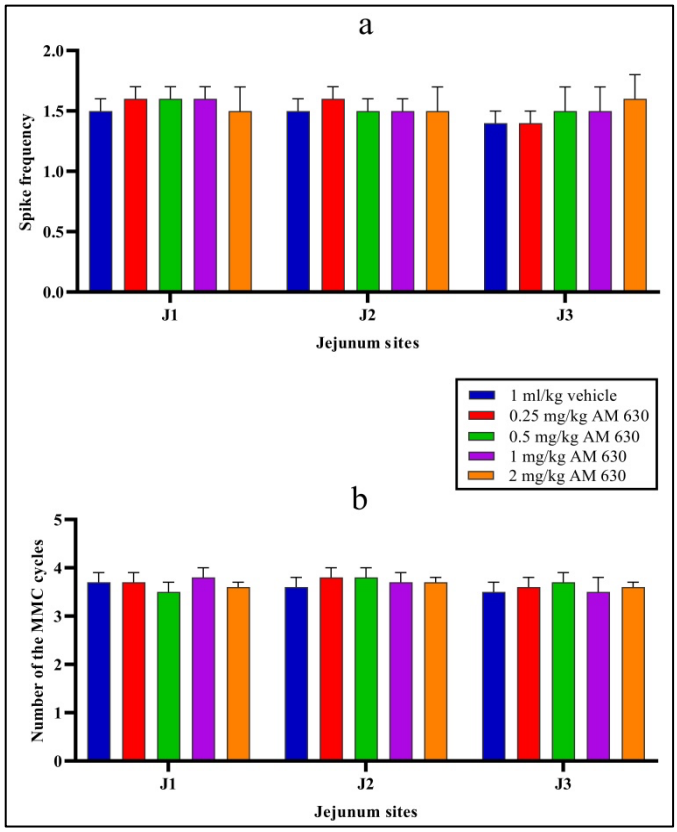

Fig. 4. Comparison of effect of different intravenous doses of CB_2R selective antagonist, AM 630, on the MMC pattern in its spike frequency (a) and number of the MMC cycle **(b)** (Mean+S.E.M., n=7)

Also, the number of the MMC cycles at J1, J2, and J3 sites did not alter by i.v. administration of AM 630 dosages compared to the vehicle group (Fig. 4b).

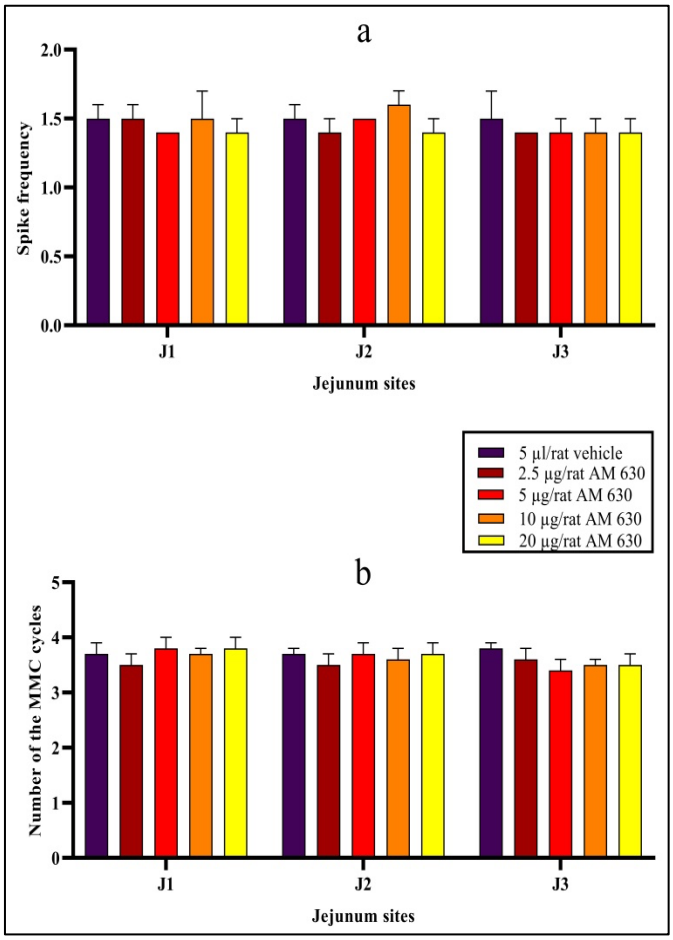

Fig. 5. Comparison of effect of different intracerebroventricular doses of CB_2R selective antagonist, AM 630, on the MMC pattern in its spike frequency **(a)** and number of the MMC cycle **(b)** (Mean+S.E.M., n=7)

The effect of intracerebroventricularly administered CB_2R antagonist AM 630 on the MMC pattern

Following approximately a one-hour baseline recording of i.v. AM 630 injection (2.5-10 µg/rat), neither the spike frequency nor the number of the MMC cycles were affected at J1, J2, and J3 sites compared to the vehicle group (Fig. 5a and b).

4. Discussion

Our study investigated the roles of CB_2Rs, which function in the gastrointestinal tract, in forming and regulating of the MMC in rats. For this purpose, we investigated the possible effect of CB_2R agonists and antagonists on the MMC pattern. Intravenously and intracerebroventricularly administrations of CB_2R agonist JWH 133 and CB_2R antagonist AM 630 were ineffective on the MMC pattern, the spike frequency and the number of the MMC cycles.

Many studies have shown the presence of endocannabinoid system components in the gastrointestinal tract. Mechoulam *et al.* (1995) isolated 2-AG in the canine intestine (42). Izzo *et al.* (2001) demonstrated the presence of anandamide in the mouse's small intestine (43). Moreover, the enzyme FAAH, responsible for breaking down anandamide, was observed in the intestines of mice and rats (44-46). CB_1R expression has been demonstrated in the enteric nervous system and epithelial cells by immunohistochemical studies, revealing that these receptors are associated with intrinsic primary afferent neurons, motor neurons, and interneurons of the GI tract (46, 47).

On the other hand, CB_2Rs are located in macrophages, plasma cells, and epithelial cells in the GI tract, suggesting that their expression increases in the inflammation process, indicating CB_2Rs function in the GI system in pathological conditions rather than physiological states (48-50).

According to the results of *in vitro* studies, endocannabinoids, phytocannabinoids, or synthetic cannabinoids bind to CB_1Rs on enteric neurons, leading to a decrease in the release of acetylcholine from neurons and, finally, inhibition of contractile responses (51-56). Similarly, *in vivo* animal studies shown that it suppresses small intestine and colon transit and defecation emerging in the fed period through the activation of CB_1Rs (56-59). Likewise, in our previous study, the CB_1R-specific agonist ACEA dose-dependently caused an inhibitory effect on the MMC pattern via the activation of peripheral CB_1Rs. Activation of peripheral CB1Rs not only exerts an inhibitory effect on postprandial motility but also has an inhibitory effect on fasting myoelectrical activity (33).

Many animal and human studies suggest that CB_1Rs act as inhibitor receptors on postprandial motility, but there is no involvement of CB_2Rs (18, 31, 32, 37, 59). However, it was unknown whether CB_2Rs participate in fasting intestinal motility activity. Therefore, we investigated here the role of CB_2Rs on the MMC. CB_2R agonist JWH 133 administered peripherally (1.25-10 mg/kg, i.v.) and centrally (2.5-20 µg/rat, i.c.v.) did not cause any change in the MMC in the present study in coherence with the studies in the literature. Furthermore, we administered CB_2R antagonist AM 630 both peripherally (0.25-2 mg/kg, i.v.) and centrally (2.5-20 µg/rat, i.c.v.); however, the antagonist did not affect the MMC pattern. Therefore, when evaluated together with the experimental results mentioned above, these findings suggest that CB_2Rs are not involved in both formation and regulation of the MMC as well as postprandial intestinal motility.

Although it was believed that CB_2Rs do not have a function in intestinal motility, the data revealed in recent years suggest that CB_2Rs have a role in intestinal motility changing in pathophysiological conditions rather than physiological states. Mathison *et al*. (2004) reported the CB_2R agonist JWH 133 decreased liposaccharide-induced intestinal transit in rats (39). Kimball *et al*. showed that JWH 133 normalized the accelerated transit in a colitis model in mice (40, 41). Lastly, Lin *et al.* (2019) observed that administering a new specific CB_2R agonist, AM 1241, returns to basal colon

motility in rats with diarrheal-irritable bowel syndrome (60). For this reason, it can considered normal that CB_2Rs did not participate in basal MMC activity in healthy fasted rats in this study.

In conclusion, the results of the present study suggest that CB_2Rs do not have tonic participation in the formation or regulation of MMC. Moreover, exogenous CB_2Rs activation does not have a role in the regulating of MMC forming source of fasting motility activity in healthy fasted rats.

Conflict of interest

None to declare.

Acknowledgments

None to declare.

Ethical Approval

The study was approved by the Ethics Committee of Ondokuz Mayıs University (date: 05.06.2020, No.2020/22).

Funding

The authors declare that this study received financial support from TUBİTAK 1002 Project (Project No. 220S126).

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 857-862
doi: 10.52142/omujecm.39.3.50

The effects of ATP sensitive potassium channel (K_{ATP}) opener and blockers on Bcl-2, Bax, and Cyt-c gene expression levels in epileptic rats

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Received: 01.07.2022 • **Accepted/Published Online:** 05.07.2022 • **Final Version:** 30.08.2022

Abstract

In this study, it was aimed to investigate the gene expression levels of Bcl-2, Bax, and cytochrome c (Cyt-c), in the cortex region of pinacidil as a K_{ATP} channel opener and glibenclamide as a blocker on penicillin model epilepsy. Male Wistar-Albino rats were used. A total of 4 main groups were formed: Control, Epilepsy, Epilepsy-opener, and Epilepsy-blocker groups, then three-time points were formed subgroups (1st day, 4th, and 8th). 48 rats were used in total. The epileptic focus was created by intracortical administration of penicillin at a dose of 500 IU/2 µl. Cortex is removed from all animals and cyt c, Bcl-2, and Bax gene expression levels were determined by qPCR. The SPSS 21 program was used for statistics. Bcl-2 and Bax gene expression levels were increased in the cortex regions of rats with epilepsy ($p<0.05$). Bcl-2, Bax gene expression levels, which increased due to epilepsy with the effect of K_{ATP} channels opened with pinacidil, returned to normal levels in the epilepsy opener group ($p<0.05$). Bcl-2 gene expression level, which was increased as a result of epilepsy due to the effect of K_{ATP} channels closed with glibenclamide, was higher than in the control and epilepsy-opener groups ($p<0.05$). Bcl-2 and Bax gene expression levels are increased in the cortex region due to epilepsy indicates that the apoptotic pathway could be activated. This study also It has been shown that the apoptotic pathway activated by epilepsy can be inactivated by pinacidil.

Keywords: epilepsy, Bcl-2, Bax, Cytochrome c, KATP channels

1. Introduction

Epilepsy is a common neurological disorder characterized by the temporary occurrence of abnormal, excessive neural activity in the brain (1). The role of neuronal death mechanisms in epilepsy has been debated for years. Neuron death from causes other than seizures generally does not involve a specific molecular mechanism and these cells cannot be rescued (2). In contrast, regulated cell death involves a cellular mechanism, and this mode of death can be manipulated by pharmacological and genetic means (3). Apoptosis is usually induced in two main ways. In the extrinsic pathway, apoptotic cell death is induced by death receptors, while in the intrinsic pathway, death signals directly or indirectly regulate mitochondria, leading to the release of cytochrome and the formation of the apoptosome complex (4).

Seizure-induced neuronal damage may involve both excitotoxic and apoptotic mechanisms. Bax/Bcl-2 expression rate increases in the hippocampus due to seizures, resulting in apoptotic cell death (5). Bcl-2 and Bcl-XL protein were increased in humans with seizure brains compared with controls (6).

The Bcl-2 gene family consists of pro-and antiapoptotic genes. This family performs its functions by forming dimers. The ratio of death antagonists (Bcl-2, Bcl-xL) to agonists (Bax) has been shown to have a critical role in determining the fate of cells (7,8). The Bcl-2 protein family determines the survival or death of a cell by controlling the release of mitochondrial apoptogenic factors, cytochrome, and apoptosis-inducing factors (AIFs), which activate activation (9,10).

There are two types of K_{ATP} channels in the cell: cytoplasmic and mitochondrial. The ones on the cytoplasmic membrane of K_{ATP} channels open outwards and are unidirectional. MitoK_{ATP} channels open into the mitochondria. During the seizure, the increased calcium in the cell passes to the mitochondria and the apoptotic pathway takes place here. Because calcium increases mitochondrial permeability, increases the release of cytochrome-c, and the pathway is stimulated (11).

Although it is not known for certain epilepsy, if the general characteristics of K_{ATP} channels are taken into account; cytoplasmic channels are opened and potassium is taken out of the cell, the cell becomes hyperpolarized (12). It is among our hypotheses that the apoptotic pathway may be inhibited by simultaneously opening the all K_{ATP} channels and allowing potassium to enter the mitochondria and that it may have a role

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in this pathway (13).

Pharmacological studies have proven that K_{ATP} channels play an important role in controlling the seizure threshold (14). It has been determined that K_{ATP} channel agonists (diazoxide, cromakalim, pinacidil, levcromakalim, nicorandil, minoxidil, etc.) reduce seizures in the experimental model of epilepsy-induced by pentylenetetrazole (PTZ) and 4-aminopyridine (4a-AP: Aminopyridine) (15). K_{ATP} ion channel antagonists (glibenclamide etc.)(16) improve epileptic seizures. In other words, these agents alter neuronal excitability by modulating K_{ATP} ion channels (16).

Although the underlying mechanisms of K_{ATP} ion channel agonist and antagonist on epileptic seizures are not fully understood, few studies have investigated the effects of K_{ATP} ion channel agonist and antagonist in the penicillin-induced epilepsy model (17,18). It was determined that the K_{ATP} channel agonist cromakalim and the antagonist 5-HD have different effects in diabetic rats in a PTZ epilepsy model (18). Bepridil and P1075, which are selective K_{ATP} agonists, reduce seizures when administered before and after seizures. HMR1098 and 5HD, which are selective K_{ATP} antagonists, have been shown to increase seizures when administered before and after seizures (19).

Penicillin parenteral treatment has resulted in generalized seizures in cats and rats (20,21). It works by inhibiting the GABAergic inhibitory system (22). One of the other convulsive drugs, pilocarpine, has a PTZ-like action.

The penicillin model of epilepsy was used for this work because we previously explored the electrophysiological effects of KATP channel openers and blockers in prior studies. On the other hand, it is a readily applicable acute model. The focus of this research was to look at how the impacts discovered in prior studies alter gene expression. We wanted to look at this impact after a single dosage of penicillin was given for 8 days.

The most affected areas of the brain in epileptic seizures are the hippocampus and the cortex. This study, it was aimed to clarify the effect of non-selective K_{ATP} channel openers and closers on Bcl-2/Bax, cytochrome c gene expression, which is effective in the apoptotic process in the penicillin model epilepsy.

2. Materials and Methods

2.1. Experimental animals

The experimental animals used were obtained from BAIBU Experimental Animals Application and Research Center. All experimental animals have been treated based on the guiding principles approved by the animal ethical committee of Bolu Abant Izzet Baysal University as well as all the treatments comply with recommendations provided on the Declaration of Helsinki (Registration number:2018/36/A2).

The animals were kept for the 12 hours in a light/dark environment with a relative humidity of 60-70% and were fed ad libitum. Wistar albino male rats aged 2-4 months were used. Four groups were formed Control, Epilepsy, Epilepsy-Opener, Epilepsy-Blocker. Subgroups of each group were formed on 1stday, 4thday, and 8thday. Total of 48 animals were used. An epileptic focus was created as a result of intra-cortical 500IU/2 µl penicillin administration. Then, on the 1st, 4th, 8thdays after the seizure, the animals were decapitated and their cortex regions were removed, and Bcl-2, Bax, cyt c m RNA expressions were determined by qPCR. SPSS 21 program was used for statistics. ANOVA was used to determine the differences between the groups, and the LSD test was used as a post hoc test to determine which group this difference was from, $p<0.05$ values were considered significant (Fig. 1).

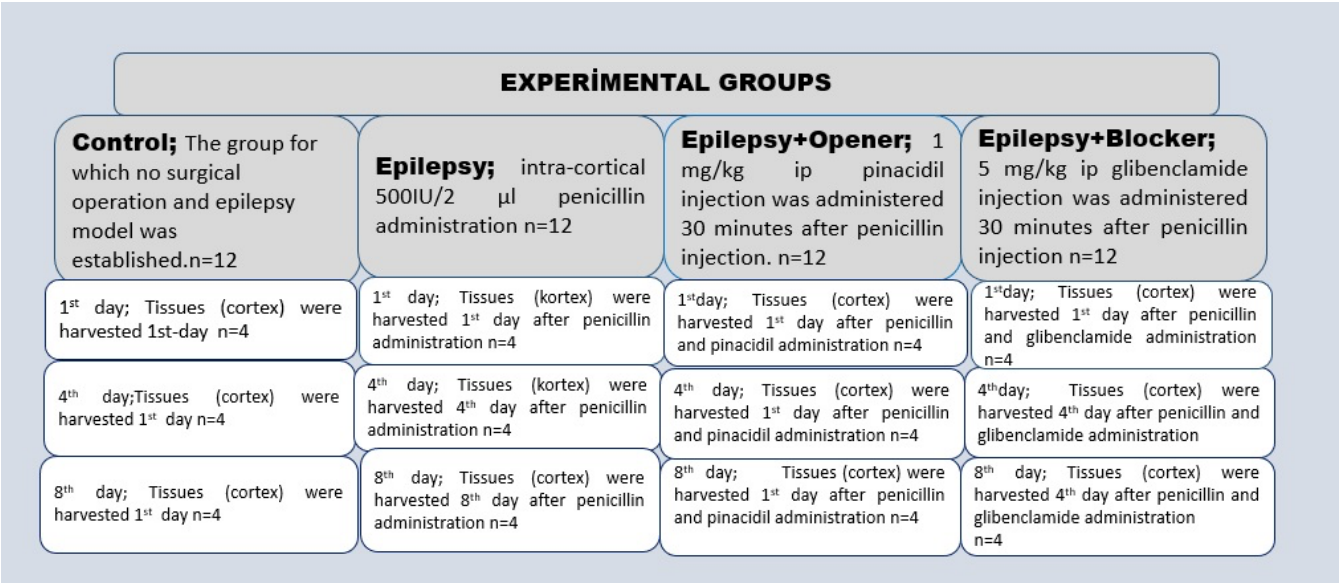

Fig. 1. Experimental groups

2.2. Surgical operation

All rats were anesthetized with 1.2 g/kg urethane intraperitoneally (i.p). and placed in a stereotaxic device. Left cerebral cortex 2mm posterior to bregma and 3 mm lateral to sagittal skull bone is removed, then dura matter is removed. To create epileptic focus 500IU with a Hamilton microinjector to a depth of 1.2 mm, 2µl of penicillin G was injected.

2.3. Drug administration

In this study, K_{ATP} channel opener Pinacidil (1mg/kg), and blocker Glibenclamide (5mg/kg) were given i.p. All drugs were applied 30 min after penicillin administration.

2.4. Q-PCR method

To detect changes in gene expression levels, total RNA was isolated, cDNA synthesis was performed, and qRT-PCR experiments were performed.

RNA isolation: For RNA isolation from tissue samples, 1 ml of Trizole solution was added to a 50 mg tissue sample and homogenized. The tubes were incubated at room temperature for 5 minutes, then 200µl chloroform was added, and manually shaken quickly for 15 seconds. The tubes were kept at room temperature for 3 minutes, centrifuged at 12,000g, and 4 °C for 15 minutes. The transparent colored upper phase was taken into a new tube and 500µl of 100% isopropanol was added. After incubation at room temperature for 10 minutes, the tubes were centrifuged for 10 minutes at 12,000g and 4 °C At this stage, the RNA in the sample formed a white precipitate at the bottom of the tube. The liquid in the tube was removed and the RNA precipitate was washed with 1ml of 75% ethanol and centrifuged at 7500g and 4 °C for 5 minutes. The resulting RNA was dissolved with 20-50µl of DEPC-ddH2O and its concentration was measured.

c DNA Synthesis: For each sample, 1µg of RNA, 2 µl of oligo dT, and DEPC-ddH2O were mixed with a final volume of 8µl and incubated for 5 minutes at 70 °C. After 10µl of 2X reaction buffer and 2µl of reverse transcriptase enzyme was added, the samples were incubated for 1 hour at 42 ˚C and 5 minutes at 80 ˚C. The cDNA samples were stored at -20˚C.

Table 1. Primer list

Primers name	Primers	Tm (°C)
Bcl-2-F	ATGGGGTGAACTGGGGGEGGATTG	66
Bcl-2-R	TTTCATATTTGTTTGGGGCAGGTC	59
Bax-F	GAGAGGATGGCTGGGGAGAC	63
Bax-R	GGTGAGCGAGGCGGTGAGGACT	68
Sitokrom C-F	TGGACAGCCCCGATTTAAGT	57
Sitokrom C-R	TCAATAGGTTTGAGGCGACAC	58
GAPDH-F	ACCACCATGGAGAAGGCTGG	61
GAPDH-R	CTCAGTGATGCCCAGGATGC	61

Quantitative Real-Time PCR (qRT-PCR): Primers that bind with high specificity to the target gene regions to be tested for RT-PCR experiments were designed (Table 1). To investigate the level of mRNA expression, 1µl of cDNA, 1µl of primer mixture (10 µM, forward+reverse), 10µl of 2X SYBR Green, and 8µl of DEPC-ddH2O were added to each qRT-PCR reaction. The following program was used for the reaction:95˚C for 5 min, [95˚C for 15 sec, 60˚C for 30 sec, 72˚C for 30 sec] x40, 72˚C for 5 min.

Analysis of the qRT-PCR results: Normalization with a housekeeping gene such as GAPDH was performed to prevent differences between samples and possible pipetting errors during the detection of gene expression levels. The analysis was performed using the ddCt method by the following equation.

ddCt = Ct (target gene) - Ct (housekeeping gene)

Target gene expression = 2^ (- ddCt)

3. Results

Bax gene expression level in the Epilepsy-8thday group; Control, Epilepsy-1stday, Epilepsy-4thdays, Epilepsy-opener-1stday, Epilepsy-opener-4thday, Epilepsy-opener-8thday, Epilepsy-blocker-1stday, Epilepsy-blocker-4thday, Epilepsy-blocker-8thday gene expression levels were statistically higher ($p<0.05$) (Fig. 2, Fig. 3). There was no statistically significant difference between the groups in cytochrome c, gene expression levels $p>0.05$ (Fig. 2).

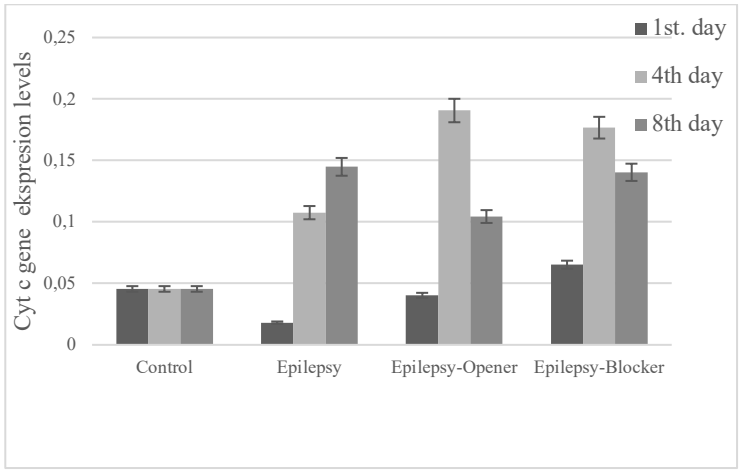

Fig. 2. Cyt C gene expression levels. There was no statistically significant difference between the groups

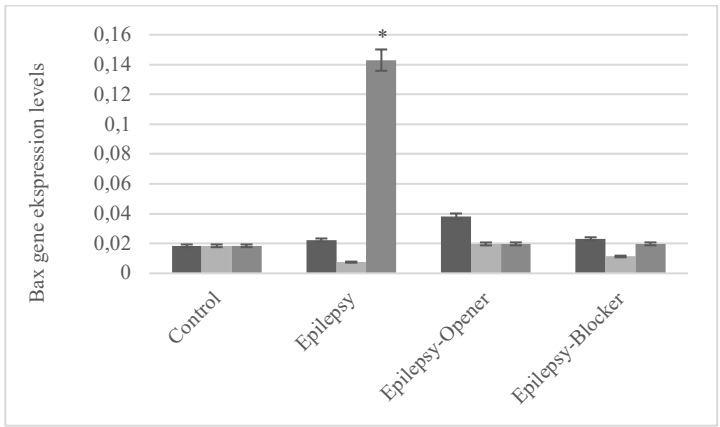

Fig. 3. Bax gene expression levels. * means significantly higher than the other groups $p<0.05$

Bcl-2 gene expression levels were statistically higher in the Epilepsy 8thDay group than in the Control, Epilepsy 4thDay group, Epilepsy-blocker 4thDay groups ($p<0.05$). Bcl-2 gene expression levels were statistically higher in the Epilepsy-Blocker 8thDay group than in the Control, Epilepsy 4thDay groups, Epilepsy-Blocker 4thDay groups ($p<0.05$) (Fig. 4).

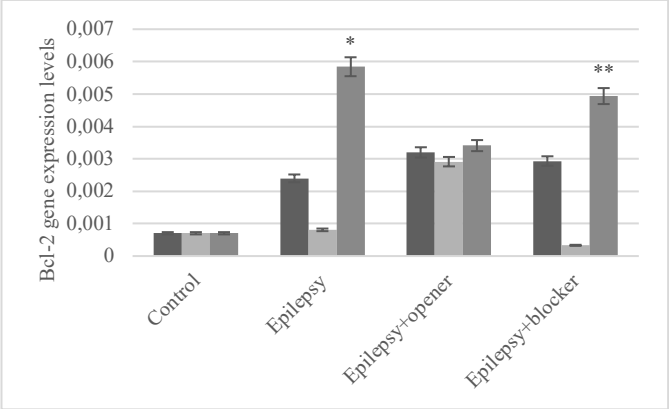

Fig. 4. Bcl-2 gene expression levels. *epilepsy-8thday group was significantly higher than the control, epilepsy-4thday, epilepsy-blocker-4thday groups $p<0.05$. ** Epilepsy-blocker-8thday group was significantly higher than the control, epilepsy-4thday, and Epilepsy-blocker 4thday groups

4. Discussion

Recurrent brief seizures lead to progressive loss of hippocampal neurons (23). It is unclear whether the role of the Bcl/Bax gene family and neuronal death is direct in the epileptic phase of seizure formation, but it occurs secondary to the effects of severe and prolonged seizures in epileptogenesis. It has been shown that the Bcl-2 gene family and apoptosis have a role in epileptogenesis (23).

Overactivation of glutamate receptors, accompanied by Ca^{+2} overload, is thought to be responsible for the death of neurons in various conditions such as stroke and epilepsy. In addition to such an excitotoxic mechanism, neurons die if deprived of important growth factors and trophic effects, which are conditions sensitive to certain oncogene products such as the Bax protein (5).

Opening and blocking of K_{ATP} channels are known to affect contractility: cell adhesion, gap, and tight junction regulation, protection against metabolic ischemia and hypoxia, cell health, and cellular adaptation to stress (24). In this study, a neuronal loss that may occur in the cortex region of the non-selective K_{ATP} opener pinacidil and its blocker glibenclamide, which is used in penicillin-induced epilepsy, and Bcl-2, Bax and cyt c gene expression levels on the 1st, 4th and 8th days after the seizure were determined. As a result, Bcl-2 and Bax gene expression levels increased in the Epilepsy 8thday group. It was shown that the expression level of Bcl-2 and Bax gene expression levels increased 8thday after seizure induction.

Mitochondria play an important role in the apoptosis of mammalian cells by releasing various apoptogenic proteins, including cytochrome c (cyt c), into the cytoplasm (9,10). The Bcl-2 protein family regulates these mitochondrial changes during apoptosis (9,10).

In this study, cytochrome c levels were determined, but no significant difference was found between the groups. According to this result, it may be that a single seizure does not cause a neuronal loss in the cortex and that the increase in gene expression of Bcl-2/Bax genes may indicate that neuron loss is controlled.

Diazoxide, a selective mitochondrial K_{ATP} channel opener, affected the increase of Bcl-2 levels in the cortex of rats exposed to cerebral ischemia-reperfusion injury. Non-selective K_{ATP} channel blockers have been shown to have the opposite effect (25). Bepridil and P1075, which are selective K_{ATP} agonists, reduce seizures when administered before and after seizures. The selective K_{ATP} antagonists HMR1098 and 5HD have been shown to increase seizures when administered before and after seizures (19). Drugs that have an impact on specific mitochondrial K_{ATP} channels or cytoplasmic channels have been investigated (19).

In this study, pinacidil showed neuronal loss caused by epilepsy by opening both channels. Glibenclamide, on the other hand, closed both channels in the opposite way to pinacidil. Bcl-2 and Bax, which were increased with epilepsy and decreased with pinacidil, were found to be the same with glibenclamide in the epilepsy group. It has been observed that the closure of K_{ATP} channels does not affect neuronal loss. Pinacidil has been shown to have a protective effect against neuronal loss caused by epilepsy.

Epileptogenesis and status epilepticus are the stages in which epilepsy becomes chronic, treatment resistance develops, spontaneous and tonic-clonic seizures occur, and the seizure threshold falls. Neuronal loss increased as a result of the high electrical activity throughout this phase. Many processes have been attributed to this process, one of which is ion channel alterations. We hypothesized that the main source of the decrease in seizure threshold and the occurrence of very severe seizures is at the cellular level, that ion channels may have an effect on the apoptotic process at the cellular level, and that we wanted to investigate the levels of Bcl-2, Bax, and cyt c gene expression. We selected K_{ATP} channels as ion channels since we previously demonstrated their impacts in electrophysiology. Many investigations have revealed K_{ATP} channels in the mitochondrial membrane, and it has been suggested that they protect the cell from apoptosis. With this study, we demonstrated that a single seizure can activate an apoptotic pathway at the cellular level, that it may play a role in epileptogenesis, that opening of K_{ATP} channels may play a role in this process, and that it should be taken seriously in the first and only seizure in the treatment process. We demonstrated that therapies that promote antiapoptotic gene expression can be recommended in this process. In prior research, we have established electrophysiologically that K_{ATP} channel openers lessen seizures. In this study, we demonstrated

the antiapoptotic effects of K_{ATP} channel openers. This study implies that the decrease in seizures caused by the impact of K_{ATP} channels could be due to a shift in antiapoptotic gene expression. The mechanism of the seizure decrease could be attributed to increased expression of antiapoptotic genes and decreased expression of the Cytochrome c gene. Many researchers have attempted to prevent and treat seizures in the chronic period. In contrast to these researchers, we wanted to look into the effects of a single seizure on gene expression during the acute phase of epilepsy. In the future, we intend to assess gene expression levels in chronic models. This study is strong in the mentioned aspects.

In these studies, the effects of KATP channel opener and blockers on penicillin model epilepsy, which is an acute model, were investigated, and bcl2, bax and cyt c gene expression levels in the cortex, the region most affected by epilepsy, were investigated.

We were unable to include procedures such as immunohistochemistry or Elisa to validate gene expression data due to a limited budget when organizing the study. We were only able to determine their gene expression due to funding constraints. As a result, we isolated RNA from all cortical tissues from rats and used Q PCR to evaluate gene expression.

In conclusion, it has been shown in this study that K_{ATP} channel openers, which have been shown to reduce seizures on epilepsy in previous studies, can reduce neuronal loss in the epilepsy-related cortex region. At the same time, it was shown in this study that opening K_{ATP} channels during seizures may be effective in reducing neuronal loss.

Conflict of interest

The authors declared no conflict of interest.

Funding

This study was financed by the Abant İzzet Baysal University Research Foundation (BAP-grant number: 2019.10.01.1408).

Acknowledgments

None to declare.

Ethical Committee Approval

All experimental animals have been treated based on the guiding principles approved by the animal ethical committee of Bolu Abant Izzet Baysal University as well as all the treatments comply with recommendations provided on the Declaration of Helsinki (Registration number:2018/36/A2).

Authors' contributions

Concept: Ü.K, H.S., Design: Ü.K, H.S., Data Collection or Processing: Ü.K, H.S., Analysis or Interpretation: Ü.K, H.S., Literature Search: Ü.K, H.S., Writing: Ü.K, H.S.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 863-867
doi: 10.52142/omujecm.39.3.51

Factors affecting women's access to antenatal care services

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Received: 30.06.2022 • **Accepted/Published Online:** 01.07.2022 • **Final Version:** 30.08.2022

Abstract

The aim of this study was to quantitatively investigate the antenatal care provided by healthcare physicians in a rural area of North East Anatolia, Turkey, and the effective factors. Patients who applied to a Second-tier health institution for delivery were asked how many times they went for a check-up during their pregnancy. In addition, the sociodemographic characteristics of the responding patients were recorded. In this study, the rate of pregnant women who received inadequate antenatal care was 41.0%. Examining the risk factors for inadequate antenatal care in pregnant women who attended fewer than four antenatal care visits revealed that the risk was 2,115 (confidence interval [CI] = 1.328–3.368) times higher for those receiving spousal education for <5. Longer years than those with ≥6 years of partner training. Pregnant women who received inadequate antenatal care in the North East Anatolia region preferred to visit Secondary and Tertiary healthcare institutions instead of visiting primary care physicians. In conclusion, it is necessary to establish a referral chain through health plans and expand Primary antenatal care services to reduce both maternal and neonatal mortality and morbidity.

Keywords: antenatal care, pregnant, infant mortality, health service delivery, health personnel

1. Introduction

In the most general sense, the prenatal care is a series of regular contact between a healthcare provider and a pregnant woman at scheduled intervals from the confirmation of pregnancy to the onset of childbirth (1) Prenatal care provides routine pregnancy examinations along with training, counseling, and treatment services (2). These services are to ensure early intervention through adequate prenatal care and to address the complications that may occur in the mother and baby during pregnancy. In addition, these services aim to decrease maternal and neonatal mortality and morbidity (3).

In Maternal and Neonatal Care Standards, the World Health Organization (WHO) recommends prenatal care for minimum four times for every pregnant woman (1,2) In Turkey, the Ministry of Health set the standards of the adequate prenatal care services pursuant to the circular issued in 2008. Accordingly, the pregnant women should receive prenatal care at least 4 times (4).

In relevant international literature, considerations related to prenatal care services are largely aligned with the Kessner Index. This index refers to the timing of the onset of prenatal care and classifies the care as weak, medium, or adequate (5). Certain Turkish studies suggested that the prenatal care should be introduced as early as in the first 3 months of pregnancy and should be continued at regular intervals by a healthcare professional. Furthermore, these studies suggested that fewer than 5 follow-up visits would be considered insufficient (6).

However, both the Kessner Index and the assessments related to prenatal care services in Turkey are incomplete when assessing their quality. However, it is imperative to note that these assessments not make a distinction in terms of providing tier-based healthcare services. The aim of this study is to investigate the numerical adequacy of antenatal care and to examine the factors that affect the provision of adequate prenatal care.

2. Material and Methods

2.1. Study area

The present study was performed in a province in the North East Anatolia region. The study region had relatively lower levels of income, with the low and very low welfare levels of 16.6% and 51.6%, respectively. Furthermore, the average durations of education for women and men were 4.4 and 5 years, respectively. In this region, agriculture and animal husbandry are the main sources of livelihood (7).

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2.2. Type of study
Hospital-based cross-sectional study

2.3. Study population
The hospital records from the year 2020 were used to determine the study population. The number of pregnant women who gave birth in 2020 was 2183. The number of births in 2021 was predicted to be identical to that in 2020; thus, the study population was considered as 2183.

2.4. Study sample
The number of people included in the sample was calculated using the following formula: n = Nt2 p q / d2 (N − 1) + t2 p q. Herein, "N" is the number of individuals in the population, "n" is the number of individuals to be included in the sample, "p" is the frequency (probability) of occurrence of the event in question, "q" is the frequency (probability) non-occurrence of the event in question, "t" is the theoretical value in the t table with a certain degree of freedom and a certain level of error, and "d" is the ±deviation from the frequency of the event.[8] Accordingly, the sample size was calculated as 327 participants based on p, q, t, and d values of 0.50, 0.50, 1.96, and 0.05, respectively.

2.5. Verbal/written consent and ethics committee approval
The required ethics committee approval was obtained from the Ethics Committee of Faculty of Medicine, Kafkas University (Approval number: 80576354-050-991/ 38; date: dated 31.03.2021). In addition, written approval of the hospital administration and written and verbal consents of the patients were obtained before collection of the study data. The study was conducted in accordance with the Declaration of Helsink Structuring the data collection form: The data collection form was prepared by the researchers upon a literature review.

2.6. The dependent variable of the study
The dependent variable was the number of prenatal care services that the pregnant women received from the First-tier healthcare institutions. In Turkey, it is legally required to receive at least 4 prenatal care services during pregnancy. Therefore, receipt of fewer than 4 prenatal care services was considered inadequate in the study (5,6).

2.7. Independent variables of the study
The sociodemographic characteristics of the pregnant women.

2.8. Preliminary application of the study
Preliminary application was performed with 5 women who gave birth but were not included in the study. Required adjustments were made to the data collection form upon the preliminary application.

2.9. Selection of the individuals included in the study and collection of data
Data were collected through in-person interviews between January and June 2021. The data were collected by an obstetrician and a gynecologist.

2.10. Statistical analysis
The Chi-squared test was used for binary analyses. The statistically significant variables as confirmed by the Chi-squared test ($p < 0.05$) were tested by the Backward Likelihood Ratio (LR) logistic regression analysis.

3. Results
In the study, 41.0% women received inadequate prenatal care. The average age of women included in the study was 28.7 ± 2.59 years. The factors for receiving an inadequate number of prenatal care services at the First-tier healthcare institutions are shown in Table 1. The Chi-squared test was conducted with the data. Upon binary comparisons, statistical differences were observed in the adequacy of received prenatal care services according to the regions where the study participants resided ($p = 0.041$), women's level of education ($p = 0.034$), the partner's level of education ($p < 0.001$), type of woman's marriage ($p = 0,021$), and number of pregnancies ($p = 0,017$). However, binary comparisons revealed that there were no significant differences in the adequacy of received prenatal care services in terms of the women's age ($p = 0.355$), the partner's age ($p = 0.147$), employment status of the women ($p = 0.684$), and employment status of the partner ($p = 0.464$), the health insurance of the women ($p = 0.074$), the total income of the household ($p = 0.784$), the family type ($p = 0.972$), the number of individuals living in the house ($p = 0.348$), official marital status ($p = 0.164$), kinship with the partner ($p = 0.544$), and the woman's ($p = 0.836$) and her partner's desire for the pregnancy ($p = 0.244$) ($p > 0.05$).

The significant parameters associated with the receipt of adequate prenatal care services upon binary analyses were subject to logistical regression analysis. Table 2 shows the logistic regression analysis results table. Notably, among the pregnant women who received inadequate prenatal care services, the number of women who received less than 5-year partner education was 2.202 (CI = 1.389–3.491) times higher than the number of women who received partner education for ≥6 years.

4. Discussion
A literature review revealed the majority of countries determined the adequate or inadequate prenatal care service according to their specific conditions, but not pursuant to the criteria set by the World Health Organization (at least 4 prenatal care services). Although Turkish studies suggested that 4 prenatal care visits were quantitatively sufficient, according to the National Institute for Health and Care Excellence (NICE) 2021 recommendations, the nulliparous and multiparous women should receive 10 and 7 prenatal controls, respectively (9). Therefore, a quantitative comparison of prenatal care between different countries constitutes a confusing predicament.

In accordance with the Turkey Demographic and Health Survey of 2018 (TNSA), nearly 9 out of every 10 pregnant

women in Turkey received prenatal care. Furthermore, 93.6% of pregnant women received prenatal care from a physician. However, the TNSA 2018 did not specify the specialties of the physicians, who administered the required care. Furthermore, the TNSA 2018 reported the rate of women who underwent an ultrasonographical examination as 98.4% (9). Given that the ultrasonographical examination is performed by obstetricians and gynecologists in practice, the statistics in the TNSA 2018 are considered to have been based on the prenatal care services provided by the Second- and Third-tier healthcare institutions.

Table 1. Risk Factors for Inadequate Prenatal Care

Independent variables		Antenatal care		Total	X^2	P
		Insufficient	Sufficient			
		number (%)*	number (%)*	number (%)**		
Where the woman lives	Rural	73 (46,8)	83 (53,2)	156 (47,7)	4,173	**0,041**
	Urban	61 (35,7)	110 (64,3)	171 (52,3)		
Woman's age	19 ≤	16 (38,1)	26 (61,9)	42 (12,8)	2,073	0,355
	20-34	97 (39,8)	147 (60,2)	244 (74,6)		
	≥35	21 (51,2)	20 (48,8)	41 (12,5)		
Age of partner (median)	32 years and older	69 (37,5)	115 (62,5)	184 (56,3)	2,105	0,147
	31 years and under	65 (45,5)	78 (54,5)	143 (43,7)		
Women's education	5 years and below	75 (46,9)	85 (53,1)	160 (48,9)	4,504	**0,034**
	6 years and above	59 (35,3)	108 (64,7)	167 (51,1)		
Partner's education	5 years and below	64 (53,3)	56 (46,7)	120 (36,7)	11,964	**0,001**
	6 years and above	70 (33,8)	137 (66,2)	207 (63,3)		
Woman's job	formal sector	18 (43,9)	23 (56,1)	41 (12,5)	0,166	0,684
	İnformal sector	116 (40,6)	170 (59,4)	286 (87,5)		
Partner's job	formal sector	57 (38,8)	90 (61,2)	147 (45,0)	0,536	0,464
	İnformal sector	77 (42,8)	103 (57,2)	180 (55,0)		
Women's health insurance	No	13 (59,1)	9 (40,9)	22 (6,7)	3,199	0,074
	Yes	121 (39,7)	184 (60,3)	305 (93,3)		
Income from home	Insufficient	86 (41,5)	121 (58,5)	207 (63,3)	0,075	0,784
	Sufficient	48 (40,0)	72 (60,0)	120 (36,7		
Family type	Wide	69 (41,1)	99 (58,9)	168 (51,4)	0,001	0,972
	Core	65 (40,9)	94 (59,1)	159 (48,6)		
Person living at home	5 and above	84 (43,1)	111 (56,9)	195 (59,6)	0,879	0,348
	4 and below	50 (37,9)	82 (62,1)	132 (40,4)		
Way of marriage	by agreement	51 (34,0)	99 (66,0)	150 (46,0)	5,315	**0,021**
	by family request	83 (46,6)	94 (53,4)	177 (54,0)		
Civil marriage	No	14 (53,8)	12 (46,2)	26 (8,0)	1,934	0,164
	Yes	120 (39,9)	181 (60,1)	301 (92,0)		
Kinship with spouse	No	33 (44,0)	42 (56,0)	75 (22,9)	0,367	0,544
	Yes	101 (40,1)	151 (59,9)	252 (77,1)		
Number of pregnancies	1-2	64 (35,2)	118 (64,8)	182 (55,7)	5,736	**0,017**
	3 and above	70 (48,3,)	75 (51,7)	145 (44,3)		
The woman's desire for pregnancy	Yes	117 (41,2)	167 (58,8)	284 (86,9)	0,043	0,836
	No	17 (39,5)	26 (60,5)	43 (13,1)		
Man's desire for pregnancy	Yes	121 (40,1)	181 (59,9)	302 (92,4)	1,360	0,244
	No	13 (52,0)	12 (48,0)	25 (7,6)		
Total		**134 (41,0)**	**193 (59,0)**	**327 (100,0)**		

*row percent **column percent

Table 2. Logistic Regression analysis results table

Independent variables		**B**	**SE.**	**Wald**	**Odds Ratio**	**%95 CI* (EK-EB değer)****
Partner training	5≤	0,789	0,235	11,262	2,202	1,389-3,491
	≥6				1 (reference)	

*Confidence Interval **Minimum-Maximum value

A pre-pandemic study conducted in Sanliurfa province reported the rate of receiving adequate prenatal care as 80.6%. In the present study, 41% of pregnant women received inadequate care. The fact that the Sanliurfa province had a higher prenatal care rate compared to the present study despite it was one of the regions with the most inadequate prenatal care rate in Turkey may be attributable to the fact that women were apprehensive of visiting the hospital during the pandemic (9,10). The pregnant women limited themselves owing to the restrictions and out of fear of being infected during pandemic. In addition, the prenatal care services may also have been disrupted by the imposed curfews.

Indeed, 1,000 women die every day in the low and middle income countries because of preventable causes related to pregnancy. Therefore, providing all the pregnant women with prenatal care services is imperative (11). The Turkish field surveys reported that the rate of women who received ≥4 prenatal care services from the First-tier healthcare institutions varied between 53.3% and 71.9%. According to the same studies, the obstetricians and gynecologists administered ≥4 prenatal care services to approximately 9 out of 10 women. (12-14). To summarize, the quantity of prenatal care services at the First-tier healthcare institutions does not comply with the prescriptions of the Ministry of Health. The likely reason for this situation is the lack of tire-based healthcare provision (referral chain).

A review of the previous studies on prenatal care in Turkey indicated that the receipt of prenatal care increased with adequate income level and social health insurance. Nevertheless, there were no significant differences in the present study. We believe that this was attributable to the fact that the healthcare services were provided free of charge to pregnant women pursuant to today's healthcare policies and that all the women had the right to receive equal prenatal care (10, 15).

The logistic regression analysis results table is shown in Table 2. The rate of pregnant women who received inadequate prenatal care services was 2.202 (CI: 1,389–3,491) times higher in women who received partner training of 5 years education than in those who received partner training of ≥6 years. The binary analyses reported by the Turkish studies suggested that prenatal care services were inadequate in terms of quantity and quality when the partner had a lower level of education (12-14). The relevant international studies also reported that the partner's education level affected the levels of prenatal care in pregnant women (11). We believe that this is because of the fact that the socioeconomic level and health literacy further increase as the Partner's level of education increases and, therefore, the partner pays more attention to pregnant women's control visits (16).

Upon admission to the obstetric ward the women were asked about whether they received prenatal care and the quality thereof. In addition, all the hospital records during pregnancy were retrospectively reviewed on the basis of the hospital system and e-Nabız system. Therefore, the probability of patients forgetting or providing incomplete information was reduced. This is also the first study to examine our region's data on this topic.

Although the study was performed in a hospital, where the most of the deliveries in the region took place, the fact that it was designed as a single-centered study and did not cover all the births in the region is a limitation of our study.

In conclusion, the pregnant women in our region received inadequate prenatal care and those, who received the prenatal care, preferred the Second- and Third-tier healthcare institutions over the First-tier physicians.

It is necessary to establish the referral chain by implementing healthcare plans and ensuring that the First-tier prenatal care services are adequately proliferated to decrease maternal and neonatal mortality.

Currently, as the effects of the COVID 19 pandemic persist, pregnant women should be given more easy access to prenatal care services and its importance should not be ignored. Otherwise, increase in preventable maternal and neonatal mortality and morbidity would be inevitable.

Conflict of interest

None to declare.

Acknowledgments

None to declare.

Authors' contributions

Concept: Ü.A.T.A., E.K., Design: Ü.A.T.A., S.K., Data Collection or Processing: Ü.A.T.A., Analysis or Interpretation: Ü.A.T.A., E.K., S.K., Literature Search: Ü.A.T.A., E.K., S.K., Writing: Ü.A.T.A., S.K., E.K.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 868-873
doi: 10.52142/omujecm.39.3.52

Investigation of the effects of pinacidil and glibenclamide administration on HCN1, KCNT1, Kir 6.1, SUR1 gene expressions in hippocampus and cortex regions in epileptic rats

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Received: 01.07.2022 • **Accepted/Published Online:** 05.07.2022 • **Final Version:** 30.08.2022

Abstract

The purpose of this study was to look into the effects of pinacidil and glibenclamide on HCN1, KCNT1, Kir 6.1, and SUR1 gene expression in epileptic rats hippocampus and cortex. Male Wistar-Albino rats were used in this study. The drugs pinacidil and glibenclamide were utilized. Control, Epilepsy, Epilepsy-O, and Epilepsy-B were the five groups formed. The epileptic focus was created by intracortical administration of penicillin at a dose of 500 IU/2 µl. Hippocampus and Cortex are removed from all animals and Kir 6.1, SUR1, HCN1, and KCNT1 gene expression levels were determined by qPCR. The SPSS 21 program was used for statistics. HCN1 gene expression level is equal in the hippocampus and cortex ($p<0.05$). KCNT1, SUR1, and KIR6.1 are more expressed in the hippocampus than in the cortex ($p<0.05$). In epilepsy groups, HCN1 gene expression level was found to be higher than KCNT1, SUR1, and KIR6.1 gene expression levels ($p<0.05$). KIR6.1, SUR1, gene expression levels decreased with the application of pinacidil and glibenclamide ($p<0.05$). SUR1 and KIR6.1 gene expression levels were considerably lower in the epilepsy pinacidil group compared to the other groups. The gene expression levels in the hippocampus were found to be considerably higher than in the cortex group, according to this study. The fact that HCN1 gene expression levels are significantly greater in both the brain and the hippocampus 24 hours following the commencement of epileptic convulsions suggests that preventive medication may be possible.

Keywords: epilepsy, Kir 6.1, SUR1, HCN1, KCNT1, gene expressions

1. Introduction

Epilepsy is a neurological disorder characterized by recurring seizures that affect more than 70 million people worldwide, making it one of the most common brain diseases (1, 2).

Despite the progress in preclinical and clinical studies, the pathogenesis of epilepsy remains unclear (3). Commonly used anti-epileptic drugs relieve symptoms rather than stop the progression of epilepsy. Existing medications restore neurotransmitter equilibrium via acting on ion channels, transporters, and receptors.

Ion channels play an important role in epilepsy pathogene sis. Understanding the function of these channels is critical for both understanding the mechanism of drug-resistant epilepsy and developing new therapeutic options. HCN1, KCNT1, and K_{ATP} channels, for instance, have been proven to be useful in epilepsy in various investigations.

HCN channels are classified into two types: voltage-gated K+ channels and cyclic nucleotide-gated (CNG) channels (4). When the expression profiles of all HCN isoforms in the brain were examined, it was discovered that HCN1/HCN2 is widely expressed in cortical regions while HCN2/HCN4 is largely expressed in subcortical areas (5).

In both humans and mice, HCN2 is the most predominant isoform in cortical and subcortical regions (6). Although the presence of HCN3 is the most controversial, it is assumed that it has a role in early development since its expression in different species has been observed (7**).** Finally, HCN4 is associated with neurons with spontaneous rhythmic activity (6). Although at least three of the four HCN isoforms are expressed in neurons (8,9) there are reports that this is primarily linked to HCN1 isoform changes (10). HCN channels have been shown to function as pacemakers, and are involved in burst action potentials due to an increase in Ca^{++} in thalamocortical neurons. On the other hand, HCN channels in dendritic synapses form a shunt mechanism that restricts temporal summation while accelerating EPSP amplitude and duration (11). HCN1 and HCN2 are two isoforms related with hereditary epilepsies in humans (12). According to studies associating HCN channel expressions with epileptogenesis, dendritic HCN1 and HCN2 channels are downregulated in the pilocarpine model, but expression increases when epilepsy occurs in the chronic term (13).

Although KCNT1 channels are extensively expressed in adult central nervous system neurons, immunological labeling tests have revealed that KCNT1 is also extensively expressed

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in embryonic hippocampus and cortical mouse neurons, indicating that cells contribute to early excitability (14).

KATP channels are found in all cell types. They are found in the cell, on both the cell membrane and the inner membrane of the mitochondria. KATP channels have an important function in cell metabolism and membrane excitability (15). In cases where metabolic activity is low in the cell, K_{ATP} channels are open and the membrane is hyperpolarized, which has a cell-protective effect on vascular cells and neurons (16,17).

KATP channels are generally composed of the following Kir and four SUR1 subunits, are found in the plasma membrane, and come in a variety of topologies with a wide range of Kir and SUR subunits (18).

The effects of KATP channel openers and blockers, as well as KCNT1 and HCN1 channels, on epilepsy have been studied extensively in earlier investigations (19–21). However, the effects of employing KATP channel openers pinacidil and blockers glibenclamide in epilepsy on gene expression levels of both KIR6.1, SUR1, KCNT1, and HCN1 channels have never been studied. Previous research has found that KATP channel openers lessen seizures in a variety of epilepsy models (22,23).

The purpose of this study was to look at the levels of gene expression for Pinacidil and glibenclamide KATP (Kir 6.1, SUR1), HCN1, and KCNT1 channels in the hippocampus and cortex of rats with penicillin-induced epilepsy.

2. Material and Methods

2.1. Experimental animals

Experimental animals to be used in the study were obtained from BAIBU Experimental Animals Application Research Center. All experimental animals were treated in accordance with the guiding principles established by the animal ethical committee of Bolu Abant Izzet Baysal University, and all treatments adhered to the recommendations included in the Helsinki Declaration (Registration number:2018/36/A2). The animals were kept in the Experimental Animals Application Research Center in a relative humidity of 60-70% in a 12 hours light and 12 hours dark environment, and fed ad libitum until the study started and during the study period. Male rats of the Wistar albino breed aged 2-4 months were used. Four groups were created: Control (C), Epilepsy, Epilepsy-O, Epilepsy-B. A total of 36 animals were used, with 9 animals in each group. Rats were anesthetized with 1.2 g/kg (IP) urethane. The epileptic focus was created by intracortical administration of penicillin at a dose of 500 IU/2 µl. Drugs were applied 30 min after penicillin administration. Hippocampus and cortex tissues were removed from animals 24 hours after penicillin administration. After that Kir6.1, SUR1, HCN1, KCNT1 gene expression levels are detected in the hippocampus and cortex.

2.2. Surgical operation

All rats were anesthetized with 1.2 g/kg urethane (IP) (Sigma-Aldrich Chemical Co., St. Louis, Missouri, USA) and placed in a stereotaxic device. Left cerebral cortex 2 mm posterior to bregma and 3 mm lateral to sagittal skull bone is removed, then dura matter is removed. To create epileptic focus 500IU with a Hamilton microinjector (701N, Hamilton Co., Reno, NV, USA) to a depth of 1.2 mm, 2 µl of penicillin G was injected.

2.3. Drug administration

In this study, K_{ATP} channel-opener Pinacidil (1 mg/kg), and K_{ATP} channel-blocker glibenclamide (5 mg/kg) were given intraperitoneally (i.p). All drugs were applied 30 min after penicillin administration.

2.4. QPCR method

To detect changes in gene expression levels, the total gene was isolated, cDNA synthesis was performed, and quantitative real-time PCR (qRT-PCR) experiments were performed.

RNA isolation: For RNA isolation from tissue samples, 1 ml of Trizole solution was added to a 50 mg tissue sample and homogenized. The tubes were incubated at room temperature for 5 minutes, then 200 µl chloroform was added, and manually shaken quickly for 15 seconds. The tubes were kept at room temperature for 3 minutes, centrifuged at 12,000 g, and 4 °C for 15 minutes. The transparent colored upper phase was taken into a new tube and 500 µl of 100% isopropanol was added. After incubation at room temperature for 10 minutes, the tubes were centrifuged for 10 minutes at 12,000 g and 4 °C At this stage, the RNA in the sample formed a white precipitate at the bottom of the tube. The liquid in the tube was removed, taking care not to touch this precipitate, and the RNA precipitate was washed with 1 ml of 75% ethanol and centrifuged at 7500 g and 4 °C for 5 minutes. The resulting RNA was dissolved with 20-50 µl of DEPC-ddH2O and its concentration was measured.

cDNA Synthesis: For each sample, 1 µg of RNA, 2 µl of oligo dT, and DEPC-ddH2O were mixed with a final volume of 8 µl and incubated for 5 minutes at 70 °C. After 10 µl of 2X reaction buffer and 2 µl of reverse transcriptase enzyme were added, the samples were incubated for 1 hour at 42 ˚C and 5 minutes at 80 ˚C. The cDNA samples were stored at -20˚C.

Quantitative Real-Time PCR (qRT-PCR): Primers that bind with high specificity to the target gene regions to be tested for RT-PCR experiments were designed. The oligo design was carried out using the Amplify program, and its properties such as melting temperatures (Tm) and primary-dimer formation were studied using the same program. To ensure that the selected primers do not bind to other unwanted regions (unspecific) in the genome, the primers were selected from the exon-intron junction regions. However, the specificity of the primers was confirmed by the in-silico PCR method using the UC Genome Browser. To investigate the level of gene expression, 1 µl of cDNA, 1 µl of primer mixture (10 µM, forward+reverse), 10 µl of 2X SYBR Green, and 8 µl of ddH2O were added to each qRT-PCR reaction. The following program was used for the reaction: 95˚C for 5 min, [95˚C for 15 sec, 60˚C for 30 sec, 72˚C for 30 sec] x40, 72˚C for 5 min

Analysis of the qRT-PCR results: Normalization with a housekeeping gene such as GAPDH was performed to prevent differences between samples and possible pipetting errors during the detection of gene expression levels. The analysis was performed using the ddCt method by the following equation (Table 1).

ddCt = Ct (target gene) - Ct (housekeeping gene)
Target gene expression = 2^(- ddCt)1

Table 1. Primers list

Primers name	Primers	Tm (°C)
HCN1-F	GGATCCCAATTTCGTGACGG	59
HCN1-R	AGAGCCGTCTGTCAACTTCA	57
KCNT1-F	ATACTTCCAGCCCAGCCTTT	57
KCNT1-R	AGATGAAGGCAGTGGAAGCT	57
SUR1-F	TGGGGAACGGGGCATCAACT	61
SUR1-R	TGGCTCTGGGGCTTTTCTC	59
KIR6.1-F	GAGTGAACTGTCGCACCAGA	59
KIR6.1-R	GGATCACCAGAACTCAGCAA	57
GAPDH-F	ACCACCATGGAGAAGGCTGG	61
GAPDH-R	CTCAGTGATGCCCAGGATGC	61

2.5. Statistical analysis

The difference between groups was analyzed with SPSS v.21 ANOVA and the Post-Hoc LSD test. A p-value of <0.05 was considered significant.

3. Results

HCN1 gene expression levels in the hippocampal area are significantly higher in the Epilepsy group than in the other groups p<0.05. The level of HCN1 gene expression in the cortex is significantly higher than in the control group p<0.05 (Fig. 1).

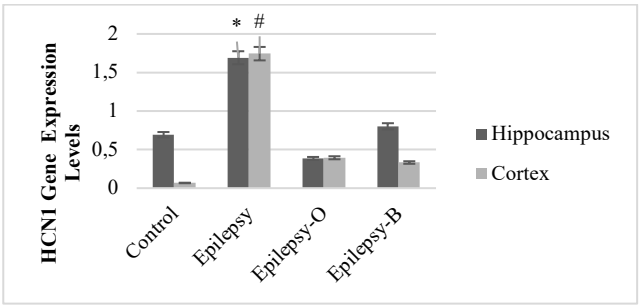

Fig. 1. HCN1 Gene expression levels in the hippocampus and cortex *p<0.05 compare with all groups in the hippocampus. # p<0.05 compared with all groups in the cortex

The control group's KCNT1 gene expression level in the hippocampal region was significantly higher than the other groups *p<0.05. The level of KCNT1 gene expression in the cortical region was significantly higher in the epilepsy group than in the other groups #p<0.05 (Fig. 2).

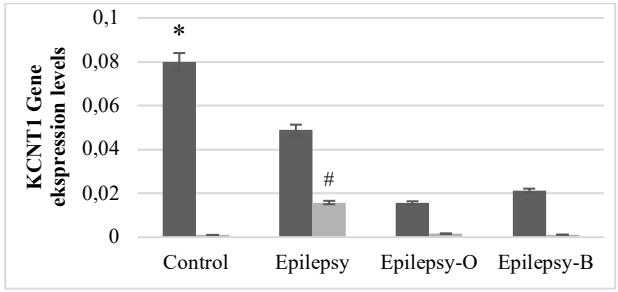

Fig. 2. KCNT1 Gene expression levels in the hippocampus and cortex *p<0.05 compare with all groups in the hippocampus. # p<0.05 compared with all groups in the cortex

The expression level of the Kir.6.1 gene expression levels in the Hippocampus region was significantly higher in the Epilepsy-B group than in the Epilepsy and Epilepsy-O groups. *p<0.05 Kir.6.1 gene expression levels in the cortical region were significantly lower in the Epilepsy group than in the Epilepsy-O and Epilepsy-B groups. #p<0.05 (Fig. 3).

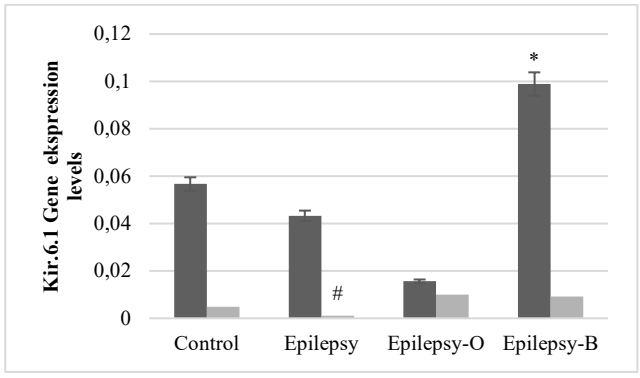

Fig. 3. Kir.6.1 Gene expression levels in the hippocampus and cortex *p<0.05 compare with all groups in the hippocampus. # p<0.05 compared with all groups in the cortex

When compared to the other groups, SUR1 gene expression was significantly lower in the Epilepsy-O group in the hippocampal region (Fig. 4).

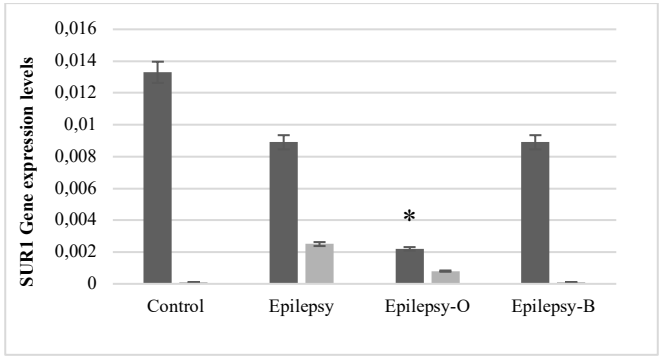

Fig. 4. SUR.1 gene expression levels in the hippocampus and cortex *p<0.05 compare with all groups in the hippocampus. # p<0.05 compared with all groups in the cortex

When gene expression levels are compared according to regions, there is no significant difference in HCN gene expression level. HCN gene expression level was expressed in both cortex and hippocampus. KCNT1, Kir.6.1, SUR1 gene expression levels are significantly higher in the Hippocampus region than in the Cortex region (Table 2).

Table 2. Gene expression levels in the hippocampus and cortex

	Region	Mean	SEM	Min	Max	F	P
HCN1	Hippocampus	0.893	0.1766	0.01	4.68	0.258	0.486
	Cortex	0.635	0.29078	0	1.08		0.450
KCNT1	Hippocampus	0.041	0.00615	0	0,13	22.513	0.001*
	Cortex	0.004	0.00296	0	0,13		0.001*
Kir.6.1	Hippocampus	0.054	0.00931	0.01	0.25	36.01	0.001*
	Cortex	0.006	0.00114	0	0,03		0.001*
SUR1	Hippocampus	0.008	0.00109	0	0.02	20.693	0.001*
	Cortex	0.0008	0.0005	0	0,02		0.001*

4. Discussion

Epilepsy is a complicated condition with numerous risk factors. In most cases, a genetic component is regarded in the absence of a recognized cause of epilepsy, and thanks to developments in genomic technology, the complex genetic structure of epilepsy, the mechanisms of its generation, and their link with mutations have begun to be proven. Comorbidities are becoming more widely acknowledged as major etiological and prognostic indicators. Anti-epileptic medications decrease seizures in more than two-thirds of persons with epilepsy but have no long-term effect on prognosis. In terms of quality of life, morbidity, and risk, epilepsy is a significant burden (2). Epilepsy is characterized by recurrent spontaneous seizures (24). It is an illness that affects a large number of people and costs a lot of money in terms of social and medical care (25). It is more common in children (26,27), and it is typically associated with a disorder that causes impairment (28,29). Recently, many studies aimed at revealing the mechanism of epilepsy have focused on ion channels and related pathways. Excessive depolarization of cells is involved in the known mechanism of epilepsy, and in this case, aiming to hyperpolarize the cells is among the strategies of anti-epileptic drugs. There are ion channels that help the hyperpolarization of the cell. In these channels, HCN1, KCNT1, and K_{ATP} channels have a role in the hyperpolarization of cells.

In neurons, HCN channels have two functions. HCN channels are partially open at rest in many cells and contribute to the correction of membrane potential. Second, HCN channels inherently provide negative feedback due to their ability to endure both membrane hyperpolarization and depolarization. HCN channels open when the membrane is hyperpolarized, and the depolarizer generates a current that flows directly into the cell. The HCN channels become inactive when the membrane is depolarized, making hyperpolarization easier. As a result, HCN channels can actively attenuate both inhibitory and excitatory inputs arriving at the cell membrane, assisting in the stabilization of the membrane potential and playing a key role in controlling the excitability and electrical response of cells (30).

In addition to the information in all of these investigations, the epilepsy group's HCN1 gene expression level was shown to be considerably greater in both the hippocampus and the cortex. HCN1 gene expression increased, demonstrating its importance in epilepsy. The KCNT1 channel subunit is engaged in gradual hyperpolarization following a single action potential or the recurrent firing of action potentials (31). It is thought that in individuals with KCNT1 mutations, increased potassium current in inhibitory interneurons generates an imbalance between neuronal excitation and inhibition, resulting in protracted hyperpolarization and seizures (32).

In either situation, it is predicted that the KATP channels will open and protect the neuron by blocking activation (33). KATP channels have been found in numerous kinds of neurons inside the central nervous system, where they may play a protective function during anoxia or hypoglycemia (33,34).

Kir6.1 was found to be only mildly expressed in the neurons of the rat central nervous system, in contrast to the widely distributed Kir6.2 component. Although positivity was relatively low in many brain areas, considerable immunoreactivity was found in the Striatum, Dorsomedial, and paraventricular hypothalamic nuclei, zona incerta, and substantia nigra pars compacta. Surprisingly, the dispersed Kir6.1-immunopositive neurons in the striatum were previously identified as tonically active large cholinergic interneurons using double-label immunofluorescence (35). It should be explained if Kir6.1 contributes to the KATP channels that protect neurons during energy depletion in all of these neurons (36), maybe synergizing with Kir6.2, or whether they perform additional functions that have not yet been determined.In the pinacidil group, HCN1 gene expression was lower than in the control and epilepsy groups. Under normal conditions, it can be thought that the role of HCN1 channels is more. HCN1 gene expression level decreased in groups using pinacidil and glibenclamide. HCN1 gene expression may be reduced by pinacidil and glibenclamide.

The cytoplasmic membrane and the inner membrane of the

mitochondria are both home to K_{ATP} channels in the cell. They work depending on ATP. Under normal conditions, the K_{ATP} channels are closed, but when the amount of intracellular ATP decreases, these channels open and allow the cell to hyperpolarize. KATP and HCN1 channels may have some sort of link. In this study, the K_{ATP} opener Pinacidil and the blocker Glibenclamide were used. The level of HCN1 gene expression was found to be low when Pinacidil and Glibenclamide were administered compared to those in the Epilepsy groups. Opening or blocking K_{ATP} channels may be effective in decreasing HCN1 gene expression levels. Under normal conditions, when the amount of ATP in the cell decreases during excessive activation in the cell, the ATP channels open and the cell becomes hyperpolarized.

The seizures during epilepsy occur due to the depolarization of cells, and seizures can affect the entire brain with the propagation of this depolarization wave. Thus, hyperpolarization of cells is a protective strategy. Ion channels located in the cell membrane contribute to hyperpolarization. Among these channels, the K_{ATP}, KCNT1, and HCN1 ion channels contribute to the hyperpolarization of the cell.

We investigated how much three of these channels contribute separately in epilepsy, how they act, do they have an effect on HCN1 and KCNT1 gene expression by opening or blocking K_{ATP} channels during an epileptic seizure. As a consequence, it's been discovered that giving pinacidil and glibenclamide to people with epilepsy lowers the amount of HCN1 gene expression.

Excessive and synchronous depolarization occurs in epilepsy, resulting in seizures. The realization of hyperpolarization in cells, on the other hand, is critical, and ion channels play the most vital role. The effects of pinacidil and glibenclamide on the gene expression levels of HCN1, KCNT1, and KATP channels in epilepsy were investigated in this study. As a result, HCN1 gene expression is highest in the hippocampus and cortex of epilepsy patients. Although the gene expression levels of KCNT1 and K_{ATP} channels were not significant during epilepsy, both K_{ATP} channel openers and blockers decreased KCNT1 and HCN1 gene expression levels. But the expression levels of Kir6.1 and Sur1 gene reduced with openers, Kir6.1 rose with blockers, while Sur1 remained unaffected.

The fact that there is a drug-resistant group in epilepsy indicates the need to conduct studies that can pave the way for developing new treatments against epileptic seizures and help to accurately understand the pathogenesis of the disease.

The fact that HCN1 gene expression levels are significantly higher in both the cortex and the hippocampus 24 hours after the onset of epileptic seizures provides promise for preventative therapy.

Conflict of interest

The authors declared no conflict of interest.

Funding

This study was financed by the Abant İzzet Baysal University Research Foundation (BAP-grant number: 2019.10.01.1408).

Acknowledgments

None to declare.

Ethical Committee Approval

All experimental animals have been treated based on the guiding principles approved by the animal ethical committee of Bolu Abant Izzet Baysal University as well as all the treatments comply with recommendations provided on the Declaration of Helsinki (Registration number:2018/36/A2).

Authors' contributions

Concept: Ü.K, H.S., Design: Ü.K, H.S., Data Collection or Processing: Ü.K, H.S., Analysis or Interpretation: Ü.K, H.S., Literature Search: Ü.K, H.S., Writing: Ü.K, H.S.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 874-878
doi: 10.52142/omujecm.39.3.53

Predictive factors and importance of Critical View of Safety in difficult elective laparoscopic cholecystectomy

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Received: 12.07.2022 • **Accepted/Published Online:** 20.07.2022 • **Final Version:** 30.08.2022

Abstract

Knowing the surgical anatomy and related variations, revealing the factors that indicate difficult cholecystectomy, knowing and applying various safe surgical cholecystectomy techniques and guidelines recommended to prevent injuries can prevent complications. This study, it is aimed to evaluate the preoperative factors that will predict difficult elective cholecystectomy. We retrospectively analyzed the data of patients treated for cholelithiasis by an experienced hepatobiliary surgeon or under his supervision between March 2018 and March 2020. Clinical, laboratory, and imaging data obtained from patients' files were evaluated. According to the Modified Nassar Scale (MNS), 140 (79.5%) patients were considered grade 1-2 (easy) and 36 (20.5%) grade 3-5 (difficult) patients within the framework of intraoperative findings. Critical View of Safety was successfully performed in 170 (96.6%) of the patients. Converting laparoscopic cholecystectomy to open was performed in two (1.1%) patients who had an MNS of 4 and 5. The most common comorbidity was hypertension.

Male gender, previous cholecystitis and ERCP, and increased gall bladder wall thickness from preoperative USG findings are independent risk factors for difficult cholecystectomy in patients scheduled for laparoscopic cholecystectomy. In addition, it should be kept in mind that LC can be difficult in patients with hypertension and coronary artery disease.

Keywords: Modified Nassar Scale, laparoscopic cholecystectomy, diagnosis, prediction, treatment

1. Introduction

Laparoscopic cholecystectomy (LC), which is the gold standard in the treatment of gallstones disease, is globally the most frequently performed surgical procedure (1,2). In line with the increasing number of laparoscopic cholecystectomies, there has been an increase in bile duct injuries. The risk of LC-related biliary injury is 0.1-1.5%. This rate is 0.1-0.2% in open cholecystectomy (3). The leading predisposing factors that cause bile duct injuries are anatomical variations, disease-related pathology, and inappropriate surgical techniques. Bile duct and vasculobiliary injuries are associated with high morbidity, mortality, and reduced quality of life. It has serious medicolegal effects as well (4,5).

Knowing the surgical anatomy and related variations, revealing the factors that indicate difficult cholecystectomy, knowing and applying various safe surgical cholecystectomy techniques and guidelines recommended to prevent injuries can prevent complications (2).

The most widely accepted and recommended method is the Critical View of Safety (CVS) technique, which was described by Strasberg in 1995 (1,3). Preoperative prediction of difficult cholecystectomy in patients who will be planned for laparoscopic cholecystectomy, informing the patient and their relatives about possible complications, and the surgeon's preparation of an operation plan in advance is vital to reduce the rate of mortality and morbidity (1,6).

The first aim of our study is to define the preoperative factors that will predict difficult elective cholecystectomy, and the second aim is to present our clinical results in cases where we applied the Critical View of Safety.

2. Materials and methods

The study was designed and carried out in accordance with the Declaration of Helsinki and was conducted with the approval of the Our Clinical Research Ethics Committee (20.01.2022 / 0013). All patients signed a written informed consent form.

The files of 176 patients who underwent laparoscopic cholecystectomy by an experienced hepatopancreaticobiliary (HPB) surgeon or under his supervision between March 2018 and March 2020 were reviewed retrospectively.

Our 1100-bed hospital with an average of 1350 cholecystectomies per year is also a regional HPB center. After examining the surgical notes, the patients were divided into two groups as easy (non-difficult) and difficult laparoscopic cholecystectomies using the Modified Nassar Scale (MNS) (3) (Table 1). Those with a MNS of 3 to 5 were considered difficult laparoscopic cholecystectomy (Group B).

In both groups, demographic data (age, gender, body mass

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ındex), ASA grade, previous acute cholecystitis, pancreatitis, and endoscopic retrograde cholangiopancreatography (ERCP) history, co-morbidities, history of anticoagulant and anti-aggregant use, previous abdominal operation, preoperative ultrasonography findings and inflammation criteria (leukocyte, neutrophil-to lymphocyte (NLR), and neutrophil-to platelet ratio) were examined. Intraoperative findings, perioperative complications, operation time, transition information, and hospital stay were noted. The morbidity and mortality that developed during the 30-day follow-up of all patients were recorded.

During the operation, all patients were given 1 g cephalosporin antibiotic prophylaxis.

Table 1. Operative difculty grading: Modifed Nassar Scale

Grade	Description
I	Gallbladder—foppy, non-adherent
	Cysticpedicle—thin and clear
	Adhesions—simple up to the neck/Hartmann'spouch
II	Gallbladder—mucocele, packed with Stones
	Cysticpedicle—fat laden
	Adhesions—simple up to the body
III	Gallbladder—deep fossa, acute cholecystitis, contracted, fbrosis, Hartmann's adherent to CBD, impaction
	Cysticpedicle—abnormal anatomy or cystic duct—short, dilated or obscured Adhesions—dense up to fundus; involving hepatic flexure or duodenum
IV	Gallbladder—completely obscured, empyema, gangrene, mass
	Cystic pedicle—impossible to clarify Adhesions—dense, fibrosis, wrapping the gallbladder, duodenum or hepatic flexure difcult to separate
V	Mirizzi Syndrome type 2 or higher, cholecysto-cutaneous, cholecysto-duodenal or cholecysto-colic fistula

2.1. Surgical procedures

Laparoscopic cholecystectomy was performed using the four-port technique. A Veress needle was inserted (in 7 patients open access was performed with the Hasson technique) and the abdominal cavity was insufflated with the maximum insufflation pressure being 12mm Hg. All patients underwent cholecystectomy. Nasogastric tubes were placed in all patients. Simultaneous umbilical hernia repair was performed in 11 patients. Hepatocystic triangle dissection was performed with CVS (7,8) method in all patients. The hepatocystic triangle was cleared of fat and fibrous tissue, the peritoneum over the infundibulum was opened from the medial and lateral aspect, and the lower 1/3 of the gallbladder was separated from the liver bed and the cystic plate was exposed. Only two tubular structures were observed to enter the gallbladder. Intraoperative cholangiography was not performed in any of the patients.

2.2. Statistical analysis

Data were evaluated in the statistical package program IBM SPSS Statistics 25.0 (IBM Corp., Armonk, New York, USA). The conformity of the data of continuous variables to the normal distribution was evaluated with the Shapiro Wilk test and Q-Q charts. Descriptive statistics were given as frequency (n), percent (%), median (M), 25th percentile (C1), and 75th percentile (C3). Mann-Whitney U test, Fisher exact test, and Fisher Freeman Halton test were used for comparisons between groups. Risk factors (multivariate logistic regression) for difficult cholecystectomy were investigated by logistic regression analysis. $p<0.05$ was considered statistically significant.

Table 2. Univariate analysis for risk factors of difficult and easy cholecystectomy group

	Modified Nassar Scale		
Variable	1-2 n=140, n (%) **Group A**	3-5 n=36, n (%) **Group B**	P
Age (years)			0,750
>50	62 (44.29%)	10 (27.78%)	
<=50	78 (55.71%)	26 (72.22%)	
Gender			**0.017**
Female	106 (75.71%)	20 (55.56%)	
Male	34 (24.29%)	16 (44.44%)	
Body mass index			0.946
<20	3 (3.06%)	1 (4%)	
20-24	22 (22.45%)	6 (24%)	
25-30	48 (48.98%)	11 (44%)	
>30	25 (25.51%)	7 (28%)	
Previous acute cholecystitis			**<0.001**
No	131 (93.57%)	20 (55.56%)	
Yes	9 (6.43%)	16 (44.44%)	
Previous ERCP			**<0.001**
No	134 (95.71%)	26 (72.22%)	
Yes	6 (4.29%)	10 (27.78%)	
Previous acute pancreatitis			0.212
No	128 (91.43%)	30 (83.33%)	
Yes	12 (8.57%)	6 (16.67%)	
Diabetes mellitus			0.092
No	115 (82.14%)	25 (69.44%)	
Yes	25 (17.86%)	11 (30.56%)	
Hypertension			**0.017**
No	89 (63.57%)	15 (41.67%)	
Yes	51 (36.43%)	21 (58.33%)	
Coronary artery disease			**0.029**
No	129 (92.14%)	28 (77.78%)	
Yes	11 (7.86%)	8 (22.22%)	
Pulmonary pathology			0.186
No	138 (98.57%)	34 (94.44%)	
Yes	2 (1.43%)	2 (5.56%)	
Chronic kidney disease			0.499
No	138 (98.57%)	35 (97.22%)	
Yes	2 (1.43%)	1 (2.78%)	
Used anticoagulants			0.271
No	137 (97.86%)	34 (94.44%)	
Yes	3 (2.14%)	2 (5.56%)	

Used antiaggregant			0.231
No	126 (90.65%)	30 (83.33%)	
Yes	13 (9.35%)	6 (16.67%)	
ASA score			1.000
1-2	130 (92.86%)	34 (94.44%)	
3-4	10 (7.14%)	2 (5.56%)	
Previous abdominal operation			**0.036**
No	96 (68.57%)	31 (86.11%)	
Yes	44 (31.43%)	5 (13.89%)	
Drain			**0.006**
No	32 (22.86%)	1 (2.78%)	
Yes	108 (77.14%)	35 (97.22%)	
Hepatosteatoz			0.710
Grade 1≤	68 (68.69%)	16 (72.73%)	
Grade>1	31 (31.31%)	6 (27.27%)	
Gallbladder wall thickness			**<0.001**
<4mm	131 (93.57%)	21 (58.33%)	
≥ 4mm	9 (6.43%)	15 (41.67%)	
Common bile duct diameter			**0.007**
<6 mm	129 (92.14%)	27 (75%)	
>6mm	11 (7.86%)	9 (25%)	
Contracted gallbladder			**0.049**
No	134 (95.71%)	31 (86.11%)	
Yes	6 (4.29%)	5 (13.89%)	
White blood cell count	7.32 (6.11;8.72)	7.43 (6.1;8.87)	0.885
C-Reactive Protein	0.14 (0.05;0.40)	0.27 (0.03;0.9)	0.278
Neutrophil-to-lymphocyte ratio	1.88 (1.47;2.37)	1.87 (1.62;2.3)	0.549
Platelet-to-lymphocyte ratio	122.3 (92;157)	125 (94.1;175)	0.520
Postoperative pathology			**<0.001**
Others	2 (1.43%)	10 (27.78%)	
Chronic cholecystitis	138 (98.57%)	26 (72.22%)	
Operation time	65 (54;80)	86.50 (72;107)	**<0.001**
Lenght of hospital stay	1 (1;1)	2 (1;3)	**<0.001**

3. Results

Of 176 patients who underwent elective laparoscopic cholecystectomy, 126 (71.59%) were female and 50 (28.41%) were male, with a mean age of 52.9 (19-84) years. 174 (98.86%) patients were operated on with the diagnosis of symptomatic gallstone disease and 2 (1.14%) patients with a preliminary diagnosis of gallbladder polyps. There was a history of cholecystitis in 25 (14.2%) patients, pancreatitis in 18 (10.2%) patients, and ERCP in 16 (9.1%) patients. The most common comorbidity was hypertension (HT) (Table 2). Of the patients, 19 (10.7%) were receiving anti-aggregant and 5 (2.8%) anticoagulant treatment,and 49 had a history of previous surgery (Table 2). The rate of previous surgery was significantly higher in Group A patients (p=0.036). Converting laparoscopic cholecystectomy to open was performed in two (1.1%) patients who had a MNS of 4 and 5. In our study, no intraoperative bile duct injury and/or major artery injury occurred. Pathology results were consistent with 164 (93.2%) chronic cholecystitis. 6 (3.4%) ulceroflegmanous cholecystitis, 3 (1.7%) chronic cholecystitis with acute attack, 2 (1.1%) biliary polyps, 1 (0.6%) xanthogranulomatous cholecystitis (Table 2).

Complications developed in 3 patients during the postoperative period. Two patients had superficial surgical site infection in the subxiphoid incision. One patient was reoperated for postoperative bleeding. In the operation, hematoma drainage was performed and no active bleeding focus was observed. This patient had a history of heart valve replacement and aortic aneurysm stenting and anticoagulant use. No mortality was observed in our series.

According to the MNS, 140 (79.5%) patients were considered grade 1-2 (non-difficult) and 36 (20.5%) grade 3-5 (difficult) patients within the framework of intraoperative findings. CVS was successfully performed in 170 (96.6%) of the patients.

In the univariant analyzes, a statistically significant difference was found between difficult and easy cholecystectomy group gender, previous cholecystitis and ERCP history, HT, atherosclerotic coronary heart disease, previous operation history, the wall thickness of the bladder, the diameter of the common bile duct, and contracted gall bladder variables on ultrasound (Table 2).

There was a statistically significant difference between the difficult and easy cholecystectomy group and the mean operative time and hospital stay, drain use, and pathology results (Table 2).

In the multivariate analysis, ERCP, HT, and a wall thickness greater than 4 mm were seen as independent risk factors for difficult cholecystectomy. When the pathology is considered as a variable, this is seen as a protective factor. The presence of ERCP increases the probability of difficult cholecystectomy 20,947 times, the wall thickness of the bladder > 4mm increases 6.482 times, and the presence of HT increases 3.206 times (Table 3).

4. Discussion

To date, different parameters and criteria have been used to define difficult laparoscopic cholecystectomy. Initially, the conversion from laparoscopy to open and long operation times were defined as difficult laparoscopic cholecystectomy criteria. With the scoring systems developed in recent years, the LC difficulty level has been standardized (1,9). The MNS, which we used in our study, is an easily applicable intraoperative difficulty score, and it was defined by Nassar et al. in 1996 and its reliability has been demonstrated in studies (10-12).

Despite advances in LC techniques, biliary tract injuries continue to be an important problem today. In a recent study, Strasberg described a safe 3-step cholecystectomy method to prevent bile duct injuries (5). Here CVS is a threshold point. Today, the CVS technique is accepted as the most effective

method for reducing morbidity and mortality associated with laparoscopic cholecystectomy. The European Association of Endoscopic Surgery and the Society of American Gastrointestinal and Endoscopic Surgeons recommend the CVS as the most effective approach to preventing bile duct injury (13-15). In the study of Sgaramella et al., it was shown that CVS plays a protective role in the prevention of intraoperative complications in multivariate analysis (2). Kaya et al.applied the CVS method in their 120 LC series, which. none of the patients had biliary tract injury (14). Similarly, in their systematic review on the prevention of biliary tract injuries in laparoscopic cholecystectomy, Graaf et al found the incidence of biliary tract injury to be 0.03%, the median success rate 95.8%, and the median coverage rate 0.95% in CVS (13). In our study, our CVS success rate was 96.6% and our conversion rate to open was 1.1%, while no biliary tract injury was observed in any patient.

In some studies, advancing patient age has been defined as a predictor for difficult cholecystectomy, as it may cause an increase in the number of cholecystitis attacks (1,11). In our study, as the patient's age was taken as a cut-off 50, it was not found to be a risk factor for difficult elective LC.

It has been suggested that the reason why difficult cholecystectomy is more common in men is the fact that men can tolerate the symptoms caused by gallstones more than women, and that, as a result, they apply to a physician late with a more advanced disease picture (1,6,11). Similarly, in our study, the male gender was found to be a risk factor for difficult elective LC.

Contrary to studies in the literature reporting that obesity is a risk factor for difficult cholecystectomy, Sgaramella et al. reported that obesity reduces complications such as biliary tract injury and bleeding during LC (2). In our study, no statistically significant difference was found between body mass ındex and difficult elective cholecystectomy.

Recurrent cholecystitis attacks prevent dissection with increased inflammation in the hepatocystic triangle and cause difficult cholecystectomies. Studies are showing the effect of pancreatitis attacks and ERCP on the difficulty of cholecystectomy with a similar mechanism (6,9,16). A stronger association was found in a recent study modifying Randhawa's model of prior biliary inflammation and procedure (1). In our study, history of acute cholecystitis and ERCP were defined as significant predictors for difficult elective LC. However, no statistically significant correlation was found with pancreatitis.

In our study, the presence of hypertension and coronary artery disease were found to be independent risk factors for difficult elective LC. Although leukocyte activation and infiltration have been found in many tissues in cases of arterial hypertension, the mechanisms causing this remain unclear (17). In the study of Sgaramella et al., the presence of more than one co-morbid disease was found to be associated with poor prognosis and intraoperative complications (2). It should be kept in mind that cholecystectomy may be difficult in patients with hypertension and coronary artery disease who are scheduled for LC.

ASA grade is a globally recognized grade. It is well validated as a marker of patients preoperative health status. Contradictory studies are reporting the effect of ASA grade on difficult LC (6,11). In this study, no statistically significant relationship was detected.

A good preoperative ultrasonography is very valuable in predicting difficult elective LC. In particular, gallbladder wall thickness > 4 mm, signs of the contracted bladder, and bile duct diameter greater than 6 mm have been described in the literature as important predictive factors in difficult LC and the need for conversion to open surgery (1,6). In our study, all three findings were found to be significant risk factors in the univariant analysis.

There is literature studying NLR as a predictive factor in predicting complicated and uncomplicated cholecystitis. In these studies, NLR was found to be associated with the severity of acute cholecystitis (18-19). In our study, we studied NLR as a predictive factor in predicting difficult cholecystectomy in patients who will undergo elective LC. As a result, no significant difference was found between difficult and easy elective LC.

Studies are reporting that acute inflammation increases the difficulty of cholecystectomy and surgical complications (1,6). According to the final pathology results in our study, 99% were patients who had acute-chronic cholecystitis. However, cholecystitis other than chronic cholecystitis was observed significantly more frequently in Group B patients. When the pathology is considered as a variable, this is seen as a protective factor.

In the study of Sgaramella et al., the conversion rate (CR) averaged 4.9% (2). In the study of Bhandari et al., the average CR was found to be 8.9% (6). We think that our rate of conversion to open surgery is lower than the literature since all surgeries were performed by a single surgeon who was experienced in hepatobiliary surgery and performs advanced laparoscopic surgery. Especially in patients with risk factors for difficult cholecystectomy, surgery should be planned by making necessary preliminary preparations.

With the development of laparoscopic surgery, an acceleration in the time to return to work after cholecystectomies, a lower need for analgesics in the postoperative period, and a shorter hospital stay have been reported (2). In our study, the duration of operation and hospital stay were found to be longer in group B patients in accordance with the literature.

Many factors have been defined in studies to predict difficult LC preoperatively. Male gender, previous

cholecystitis and ERCP, and increased gall bladder wall thickness from preoperative ultrasonography findings are independent risk factors for difficult cholecystectomy in patients scheduled for elective LC. In addition, it should be kept in mind that LC can be difficult in patients with hypertension and coronary artery disease, except for biliary inflammation and interventions. Knowing these risk factors can assist surgeons in selecting suitable patients for elective laparoscopic cholecystectomy, preoperative preparation, and postoperative patient follow-up.

Conflict of interest

The authors declared no conflict of interest.

Funding

No funding was used for the study.

Acknowledgments

None to declare.

Authors' contributions

Concept: H.K., A.A., Design: H.K., A.A., Data Collection or Processing: H.K., A.A., Analysis or Interpretation: H.K., A.A., Literature Search: H.K., A.A., Writing: H.K., A.A.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(3): 879-883
doi: 10.52142/omujecm.39.3.54

Histopathological effects of nimodipine and pentoxifylline on the vessel wall in end-to-end anastomoses in rat carotid arteries

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Received: 16.07.2022 • **Accepted/Published Online:** 30.07.2022 • **Final Version:** 30.08.2022

Abstract
When reperfusion following ischemia occurs, oxygen returns to the ischemic tissue, increasing free oxygen radicals and inducing paradox secondary damage. Before infarction, revascularization may influence the morbidity rate. Successful revascularization is not always achieved due to stenosis incidence, proliferation of smooth muscle cells, and intimal hyperplasia. This study compares the effects of nimodipine that prevents vasospasm and pentoxifylline, which stimulates growth factors and reduces collagen synthesis on intimal hyperplasia. Eighteen randomly selected Sprague-Dawley rats were divided into three groups: Group 1, the control group; Group 2, intraperitoneally administered nimodipine; Group 3, orally administered pentoxifylline. Their right-sided carotid arteries were used for anastomosis and the left-sided ones for the control. After a 7-day treatment, both the right and left carotid arteries were removed. In the biopsy, the lumen's area and diameter, thickness of tunica media thickness, thrombus, edema, intimal hyperplasia, vessel wall injury, and inflammation were analyzed. Pentoxifylline was effective in preventing intimal hyperplasia and tunica intima was similar to that in untreated carotid arteries. However, nimodipine inhibited intimal hyperplasia, but it was not as effective as pentoxifylline. The effects of pentoxifylline after anastomosis should be further assessed in vasoprotective treatment taking into account its efficacy against intimal hyperplasia

Keywords: nimodipine, pentoxifylline, end-to-end anastomoses, carotid arteries, cerebral ischemia

1. Introduction

Cerebral ischemia occurs when the required oxygenation for cerebral tissues cannot be maintained as the arterial blood flow is reduced (1). When reperfusion occurs following ischemia, the oxygen reaches the ischemic tissues, causing an increase in free oxygen radicals and inducing paradox secondary damage (1, 2). Thus, performing revascularization before infarction may affect the morbidity rate.

Owing to the occurrence of stenosis, proliferation of smooth muscle cells, and intimal hyperplasia, revascularization attempts are always not successful. These complications, following vascular reconstructive interventions, result in higher mortality and morbidity rates. Neointimal hyperplasia, resulting from smooth muscle cell migration, proliferation, and extracellular matrix accumulation, plays a crucial role in late narrowing or restenosis (3, 4).

Nimodipine is a dihydropyridine calcium channel blocker, which is mainly effective against central nervous system disorders and particularly useful in preventing and treating arterial vasospasm resulting from subarachnoid hemorrhage (5). Pharmacological agents inhibiting calcium entry into the cell or intracellular calcium-dependent events may delay the proliferation and migration of smooth muscle cells, thus reducing neointimal thickening after arterial injuries (6). Pentoxifylline is a xanthine derivative, phosphodiesterase inhibitor elevating cAMP (cyclic adenosine monophosphate) levels by inhibiting phosphodiesterase. Increased cAMP levels obstruct the growth of vascular smooth muscle cells. Therefore, pentoxifylline stimulates several growth factors in vascular smooth muscle cells and reduces collagen synthesis and the rate of neointimal hyperplasia occurring after vascular injury (7).

In this study, we compared and analyzed the effects of both drugs, nimodipine and pentoxifylline, on intimal hyperplasia. For this purpose, we conducted common carotid artery anastomosis in rats, administered drugs postoperatively, performed and histopathological evaluation.

2. Material and Methods

This experimental study was carried out after obtaining approval from the Dicle University Medical Faculty Animal Experiments Local Ethics Committee, approval number 9 dated 20/01/2016.

The study design consisted of two steps: first, common carotid arterial anastomosis; then, drug administration in rats.

In this study, eighteen randomly selected male and female Sprague-Dawley rats were used. The right carotid artery was used for anastomosis and the left as control. After one week of

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adaptation, the rats were randomly divided into three equal groups: Group 1 rats were the control group; Group 2 were intraperitoneally given a single dose of 10 mg/kg nimodipine (8) daily for 7 days; Group 3 were orally given a single dose of 100 mg/kg pentoxifylline (Trental® 400 mg; Sanofi Aventis, Turkey) (9) daily for 7 days.

The average time to develop pseudointima in the anastomotic line is 7 days (10); therefore, the effects of the anastomosis were evaluated at a limited treatment period of 7 days, when intimal hyperplasia is most prominent and fibrin and thrombus wastes are disposed of (10).

After a 7-day treatment, 1 cm segments of the right and left carotid arteries were removed and dispatched for further pathological examinations. The obtained segments were stained in formol solution, followed by hematoxylin-eosin, periodic acid–Schiff (PAS), and Masson's trichrome stains. The same pathologist conducted the pathological and staining examinations, blindly. Inflammation, vessel wall damage, intimal hyperplasia, lumen's area and diameter, thickness of tunica media, thrombus, and edema were analyzed.

2.1. Surgical technique

The rats were fasted for 4 h prior to the surgery. On the day of the experiment, rats were anesthetized with 80 mg/kg ketamine hydrochloride and 10 mg/kg xylazine hydrochloride (intraperitoneally) and were fixed in the supine position (11).

After sterilization, a horizontal right-sided neck incision was performed. Fatty tissues were raised toward the cranial as a pedicle flap. The sternocleidomastoid muscle was removed laterally with a retractor and the neurovascular bundle near the paratracheal muscle was reached (Figure 1A). To avoid respiratory distress, it is necessary not to pull the muscles too hard during the operation. A clamp was placed prior to the dissection.

The carotid artery was transected 1 cm proximal to the bifurcation with microscissors and washed with Ringer's lactate solution using a silicone tube (Figure 1B). The adventitia layer was peeled off using microforceps. Sutures were passed through the media layer with 10-0 prolene suture. First, 0- and 180-degree sutures were made (Figure 1C) and then the anterior and posterior surface sutures. Subsequently, suturing was completed with 8 sutures (Figure 1D). Then, the clamp was removed and pressure was applied with a moist tampon for 5 min. When the bleeding stopped completely, the layers were closed anatomically.

2.2. Preparation of pathology specimen

Following the treatment, the procedure was carried out again under anesthesia. To reveal the anastomosis line, 1 cm segments of the anastomosis and common carotid arteries were removed. Moreover, the left common carotid artery sample without anastomosis was removed, including the area 1–1.5 cm proximal to the bifurcation. Then, the removed vascular segments were sent to the pathology laboratory, preserved in formol solution, for analysis.

To evaluate the effects of the surgical procedure, the control group received anastomosis surgery but were not given any medication. For assessing the drug effects, a sample from the left carotid artery of the rat was taken as control.

2.3. Statistical analysis

Bivariate correlation (Pearson's R and Spearman's tests) was utilized for estimating the correlation between the datasets, while Chi-square test was used for categorical data. Mann–Whitney *U* test was employed for analyzing independent data not considered within the normal distribution. Statistical Package for the Social Sciences (SPSS) for Windows (version 20.0) was used for analysis and $p < 0.05$ was considered statistically significant.

3. Results

The lumen's diameter and area, thickness of tunica media, edema, inflammation, damage to the vessel wall, thrombus, and intimal hyperplasia were compared between the groups.

In the control group (Group 1), the lumen diameter and area were significantly decreased when comparing the right and left carotid arteries ($p = 0.023$; $p = 0.012$, resp.). In Group 2, the decrease in the lumen diameter was less than that in Group 1 but insignificant ($p = 0.079$). In Group 3, the decrease in the lumen diameter was less than that in Group 1, but statistically significant ($p = 0.026$). No difference was observed between Groups 2 and 3 ($p = 0.826$) (Table 1).

The lumen area in Groups 2 and 3 was bigger than that in Group 1; the difference was statistically significant ($p = 0.002$, $p = 0.005$); however, there was no difference between Groups 2 and 3 ($p = 0.918$) (Table 1). The increase in media thickness was significant for Group 1 and for the right and left carotid arteries was statistically significant ($p = 0.003$). However, it was less for both Groups 2 and 3. Although the thickness of tunica media was increased in all groups, when compared it was found to be insignificant ($p > 0.05$) (Table 1).

Edema and inflammation in Groups 2 and 3 were less than those in the control group; however, the difference was statistically insignificant ($p = 0.220$; $p = 0.220$, resp.). No difference regarding thrombus was observed in all groups ($p = 1.000$) (Table 2). When the right and left carotid arteries were compared in Groups 1 and 2, vascular wall damage was found to be statistically significant ($p = 0.014$; $p = 0.045$, resp.). Damage to the vessel wall was not observed in Group 3; however, the difference was insignificant when compared with that of Groups 1 and 2 ($p = 0.070$; $p = 0.220$, resp.) (Table 2). The occurrence of intimal hyperplasia was less frequent in Group 3 and comparing the right and left carotid arteries, the difference was not statistically significant ($p = 0.296$). The treatment in Group 2 was more effective than that in Group 1, and the results when comparing Groups 2 and 3 with Group 1 were statistically significant ($p = 0.035$; $p = 0.009$, resp.) (Table 2) (Fig. 2).

Table 1. Comparison of lumen diameter, lumen area, and tunica media thickness between groups

Group / Parameter	Control group (n = 6)		*p*	Nimodipin group (n = 6)		*p*	Pentoxifylline group (n = 6)		*p*	*p* *
	Right	Left		Right	Left		Right	Left		
Lumen diameter (µm)	383.6	501.2	*p=0.023*	484.8	547.9	p=0.119	518.6	568.4	p=0.396	cxn=0.079 *cxp=0.026* nxp=0.826
Lumen area ($µm^2$)	122433	200422	*p=0.012*	233516	253612	p=0.203	222961	254509	p=0.468	*cxn=0.002* *cxp=0.005* nxp=0.918
Tunica media thickness ($µm^2$)	65.03	52.54	*p=0.003*	63.05	56.60	p=0.143	57.20	50.20	p=0.204	cxn=0.831 cxp=0.196 nxp=0.449

* *p* values for the right-side analysis; cxn = control group versus nimodipine group, cxp = control group versus pentoxifylline group, nxp = nimodipine group versus pentoxifylline group.

Table 2. Comparison of edema, vessel wall injury, intimal hyperplasia, and inflammation between groups

Group / Parameter		Control group (n = 6)		*p*	Nimodipin group (n = 6)		*p*	Pentoxifylline group (n = 6)		*p*	*p* *
		Right	Left		Right	Left		Right	Left		
Edema (+)		3	0	*p=0.045*	1	0	p=0.296	1	0	p=0.296	cxn=0.220 cxp=0.220 nxp=1.000
Vessel wall injury (+)		4	0	*p=0.014*	3	0	*p=0.045*	1	0	p=0.296	cxn=0.550 cxp=0.070 nxp=0.220
Intimal hyperplasia	+	2	0	*p=0.002*	4	0	*p=0.014*	1	0	p=0.296	*cxn=0.035* *cxp=0.009* nxp=0.078
	++	4	0		0	0		0	0		
Inflammation		2	0	p=0.121	0	0	p=1.000	0	0	p=1.000	cxn=0.120 cxp=0.120 nxp=1.000

* *p* values for the right-side analysis; cxn = control group versus nimodipine group, cxp = control group versus pentoxifylline group, nxp = nimodipine group versus pentoxifylline group

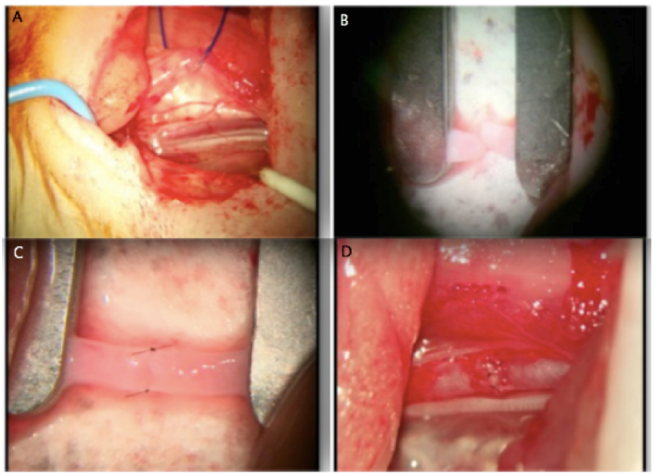

Fig. 1. (A) The carotid and vagus nerve after the sternocleidomastoid muscle is removed medially (black arrow); (D) removal of fixation sutures, completion of the anastomosis, and control of vessel patency

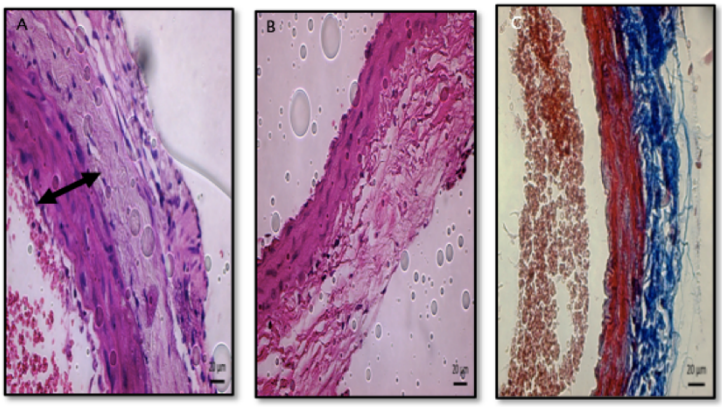

Fig. 2. (A) In the control group, tapering of the tunica intima, tunica media moderate edema, and thickening (black arrow) intimal hyperplasia are observed (hematoxylin & eosin dye, 40×). (B) In the nimodipine group, intimal hyperplasia is observed (hematoxylin & eosin dye, 40×). (C) In the pentoxifylline group, intimal hyperplasia is observed (Masson's dye, 40×)

4. Discussion

Several previous researchers reported the effects of nimodipine and pentoxifylline; however, studies investigating the effects of these drugs on anastomosis are limited.

Pharmacological agents inhibiting the calcium entry into the cells or intracellular calcium-dependent events may delay the proliferation and migration of smooth muscle cells following arterial injuries. Based on this fact, using calcium channel blockers to inhibit neointimal thickening in arterial injuries is recommended (6, 12).

Kadioglu et al. reported that nimodipine diminished endothelial dysfunction, accelerated and increased proliferation, and increased hyperplasia in the smooth muscle layer; however, this effect was reduced with prolonged treatment duration (13).

Hammerman et al. performed a study on rat intestine, demonstrating that pentoxifylline acts as an antioxidant by inhibiting the xanthine oxidase enzyme, thereby playing a role in preventing ischemia-reperfusion injury (14).

Busk et al. found that after balloon angioplasty in rabbit iliac arteries, subcutaneously administered pentoxifylline decreased the neoadventural hyperplasia at the end of 28 days and reduced cytokine and collagen accumulation in the vessel wall in the study group compared to those in the control group. Neointimal hyperplasia in the study group was less than that in the control group (15).

Chen et al. demonstrated that pentoxifylline inhibits platelet-induced growth factor after vessel damage and decreases collagen synthesis stimulated by TGF-β (transforming growth factor beta) in vascular smooth muscle cells; consequently, the vessel diameter in the pentoxifylline group was greater than that in the control group, following vessel damage (7).

Takahashi et al. revealed the proliferation of smooth muscle cells and intimal migration were induced by two growth factors in the media layer, the basic fibroblast growth factor and platelet-derived growth factor. The basic fibroblast growth factor is secreted from damaged smooth muscle and endothelial cells and regulates smooth muscle cell proliferation, whereas the platelet-derived growth factor is released by platelets and vascular cells and provides profiling and migration of smooth muscle cells (16).

In Ustunsoy et al.'s study, pentoxifylline decreased the blood viscosity by enhancing erythrocyte flexibility and preventing platelet aggregation. Accordingly, they stated that pentoxifylline led to increased and improved capillary blood flow and tissue oxygenation (107).

Taking into account the findings of these previous studies, we compared the inhibitory effects of nimodipine and pentoxifylline on intimal hyperplasia and restenosis. In this study, the vasoprotective effects were assessed by comparing the lumen diameter, lumen area, tunica media thickness, edema, vessel wall damage, thrombus, intimal hyperplasia, and inflammation parameters.

After anastomosis, the luminal diameter and area decreased in all groups compared to that those in other carotid arteries; however, Group 3 had the least degree of reduction. Although the reduction by nimodipine was not as effective as that by pentoxifylline in Group 2, it had a positive impact on the luminal diameter and area.

Upon evaluation, in terms of intimal hyperplasia, pentoxifylline effectively inhibited intimal hyperplasia, and tunica intima was similar to that in untreated carotid arteries. Moreover, nimodipine was successful in preventing intimal hyperplasia, but to not the same extent of pentoxifylline. The effects of pentoxifylline after anastomosis should be further evaluated in vasoprotective treatment protocols considering its effectiveness against intimal hyperplasia.

Conflict of interest

The authors declared no conflict of interest.

Funding

No funding was used for the study.

Acknowledgments

The publication of this article was partly supported by the Turkish Neurosurgical Society.

Authors' contributions

Concept: A.A., P.A.O., Design: A.A., P.A.O., Data Collection or Processing: A.A., P.A.O., Analysis or Interpretation: A.A., P.A.O., Literature Search: A.A., P.A.O., Writing: A.A., P.A.O.

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Journal of Experimental and Clinical Medicine
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Review Article

J Exp Clin Med
2022; 39(3): 884-892
doi: 10.52142/omujecm.39.3.55

Mammalian cell lines used in bioprocessing

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Received: 21.04.2022 • **Accepted/Published Online:** 05.06.2022 • **Final Version:** 30.08.2022

Abstract

A various number of expressions and host systems are used in biologics manufacturing. The most commonly preferred systems are based on bacteria, yeast, mammalian cells, insect cells, and transgenic animals. A wide range of molecules, including insulin, mAbs, vaccines, and recombinant proteins, are produced using different host systems. Because of several reasons impacting the product quality and yield, mammalian cells are utilized. Moreover, mammalian cells are generally used in virus-based vaccine manufacturing. Chinese Hamster Ovary (CHO) is the most widely used cell line for high yield stable recombinant protein production, while Human Embryonic Kidney (HEK) is favoured for transient transfection low yield protein manufacturing, viral-based vaccine and gene and cell therapy-related vector production. Other mammalian cell lines such as NSO, Sp2.0, Vero, MRC-5 and PerC.6 are also used in both recombinant protein and virus productions. Multiple modifications are carried out on industrial cell lines to make them more suitable for high yield and high-quality protein production. Thanks to these alterations, high productivity and quality levels are achieved in the biotechnology industry.

Keywords: bioprocessing, industrial cell lines, mammalian cell lines, biologics manufacturing

1. General introduction and expression systems used in bioprocessing

Biologics are gaining importance daily; even though biologics are expected to share %32 of sales, they are estimated to contribute to %50 of the Top 100 product sales by 2024 in pharma (1). Rapidly increased market demand for biologics improved expression systems and bioprocessing (2). Advancements in bioprocessing caused a remarkable increase in the yield of products, which eventually led to effective therapies against various complex and rare diseases (3). Several expression systems are used to manufacture recombinant therapeutics, including bacteria, yeast, insect cells, transgenic animals, and mammalian cells (4-7). On the other hand, gene and cell therapies are carried out mainly by using mammalian host cells (3).

Each expression system has advantages and disadvantages used for specific needs. For instance, bacteria offer large-scale manufacturing of recombinant proteins in a short process due to their fast growth rate with a doubling time of 20 minutes for *E.coli*. Simple culture conditions and low costs are required in these systems. Disadvantages of bacterial systems can be associated with accumulation of proteins in inclusion bodies, protease contamination from host proteins leading to degradation of the expressed protein, endotoxin accumulation and lack of proper post-translational modifications (8, 9). Despite these disadvantages, several molecules such as single-chain variable fragments (ScFvs), antibody fragments (Fabs), single-domain antibody (sdAb), insulin and its analogs, meningococcal vaccines, hormone analogs, pegfilgrastim and enzymes are manufactured by using *E.coli* (10).

Although bacterial expression systems are considered one of the oldest in biotech history, other expression platforms have been developed because of the drawbacks mentioned above.

Yeasts are commonly utilized as host expression systems due to their fast growth rate, similar but less than bacteria, predisposition to genetic manipulations, existing genetic information, low-cost fermentation characteristics, and capability of providing proper post-translational modifications. *Saccharomyces cerevisiae* and *Pichia pastoris* are the most widely used yeast expression systems for biologics manufacturing (11-13). Various genes associated with secretion and Golgi trafficking of proteins have been modified

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in *S.cerevisiae* to increase the productivity up to a gram per liter level (14). However, improper glycosylation, like hypermannosylation, which consequently causes faster blood clearance, is also present. This problem has been solved by removing the enzyme specific for mannosylation, called the mannosyltransferase gene (15).

Table 1. Main expression platforms used in biologics manufacturing and several examples of products manufactured by mammalian cell lines

Product	Producer	Molecule Type	Host cell
Perjeta (Pertuzumab)	Roche	Monoclonal antibody (mAb)	CHO
Aimovig (erenumab)	Amgen	mAb	CHO
Fasenra (benralizumab)	AstraZeneca	mAb	CHO
Taltz (ixekizumab)	Eli Lilly	mAb	CHO
Lifmior (Etanercept)	Pfizer	Fusion recombinant protein	CHO
Truxima (rituximab)	Celltrion	mAb	CHO
Retacrit (epoetin alfa-epbx)	Eprex and Erypo	Recombinant protein	CHO
Shingrix (zoster vaccine)	GlaxoSmithKline	Recombinant Vaccine	CHO
Zessly (infliximab)	Sandoz	mAb	CHO
Herzuma (trastuzumab)	Celltrion	mAb	CHO
Adcetris (Brentuximab-Vedotin)	Takeda	Antibody-drug conjugate	CHO
Mylotarg (Gemtuzumab ozogamicin)	Pfizer	Antibody-drug conjugate	CHO
Kadycla (Trastuzumab emtansine)	Genentech (Roche)	Antibody-drug conjugate	CHO
Vihuma (simoctocog alfa)	Octapharma	Recombinant protein	HEK
Luxturna (voretigene neparvovec-rzyl), nonreplicating adeno-associated virus expressing human *RPE65* gene	Spark Therapeutics	Gene therapy	HEK
Covid-19 vaccine	Astra Zeneca	Adenoviral vaccine	HEK
Lartruvo (olaratumab)	Eli Lilly	mAb	NS0
Mylotarg (gemtuzumab ozogamicin)	Pfizer	Antibody-drug conjugate	NS0
Inflectra (infliximab-dyyb)	Hospira	mAb	Sp2/0
Erbitux (cetuximab)	Merck	mAb	Sp2/0
Rekovelle (follitropin delta)	Ferring	Recombinant protein	PER.C6
Covid-19 vaccine	Johnson and Johnson	Adenoviral vaccine	PERC.6
Gardasil (HPV vaccine)	Merck	Recombinant VLP vaccine	*S.cerevisiae*
Engerix B (HBV Vaccine)	GSK	Recombinant VLP vaccine	*S.cerevisiae*
Recombivax HB (HBV Vaccine)	Merck	Recombinant VLP vaccine	*S.cerevisiae*
Novolin R (Insulin human)	Novo Nordisk	Recombinant Insulin	*S.cerevisiae*
Lantus (Insulin Glargine)	Sanofi	Recombinant Insulin derivative	*E.coli*
Humalog (Insulin Lispro)	Eli Lilly	Recombinant Insulin derivative	*E.coli*
Covid-19 vaccine	Biontech/Pfizer	mRNA vaccine	*E.coli*
Covid-19 vaccine	Moderna	mRNA vaccine	*E.coli*
Covid-19 vaccine	Sinovac	Inactivated vaccine	Vero
Imlygic (talimogene laherparepvec), an engineered herpes simplex virus type 1 expressing GM-CSF	Amgen	Gene therapy	Vero
Cervarix (HPV Vaccine)	GSK	Recombinant VLP vaccine	Hi-5 cell line (Baculovirus)

Moreover, besides removing the hypermannosylation gene (OCH1), glycosyltransferase and glycosidase genes are also transferred to manufacture the expected glycoprotein in another platform (16). The second most striking yeast expression system is based on *Pichia pastoris*, which can secrete adequately folded and active proteins, sustaining lower protein glycosylation and reaching high cell densities (17, 18). Since N-linked glycosylations are different in higher eukaryotes, some yeasts have been genetically modified to carry out human-like N-linked glycosylation (19). One of the most significant drawbacks of the *P.pastoris* expression system is the degradation or truncation of the protein of interest, which leads to low yield and loss of functional activity. Several methods have been developed to eliminate this problem, such as adding casamino acids, yeast-based peptone, protease inhibitors, creation of protease deficient strains, reduction of temperature and pH, and usage of different carbon sources (20). Considering all these advantages and disadvantages, it can be inferred that the yeast expression systems can be characterized by medium overall cost, good folding and glycosylation, medium manufacturing time, easy handling, medium to fast growth rate, easy genomic modifications, and low-cost contamination risk (21).

Furthermore, insect expression platforms can be used for multiple recombinant products like HPV vaccine. Insect cells are grown up to the required viable cell number and then transduced with a recombinant baculovirus, including the gene of interest (22, 23). Due to the lack of glycosyltransferase

enzymes, the N-linked glycosylation pattern in the insect system is not desirable. This problem can be overcome by introducing glycosylation related genes (24). The most widely used cell line in the baculovirus expression system is Sf9 cells. Besides Sf9, S2, Sf21, Tn 368 and Hi-5 cells are used to produce recombinant proteins (25, 26). Insect expression systems are associated with proper protein folding, slow growth rate, high productivity, medium cost and manufacturing time, and very low risk of contamination (27).

In addition, transgenic animals are used as host expression systems as well. These animals include a gene that codes a protein integrated into their chromosome and can pass to their offspring, rendering them transferring the transgene to generations (28). The most common ways of acquiring recombinant proteins in transgenic animals are from their milk and eggs (29). Although they usually provide suitable post-translational modifications (PTMs), there are some concerns that the producers face, such as the presence of zoonotic pathogens and ethical questions about producing transgenic animals (30).

Transgenic animals are characterized by high product yield and quality, low scale-up capacity, a very high risk of contamination, and high overall cost.

Table 1 shows the list of biological products with different host and expression platforms.

2. Why are mammalian cell lines preferred in bioprocessing, and how are they processed?

Mammalian cell-based expression systems are the most dominant platform for recombinant protein and viral-based vector and vaccine production purposes. They can produce complex molecules such as mAbs, and several therapeutic proteins with high molecular weight. Compared to bacteria and yeast systems, introducing a gene of interest into the host is time-consuming and labour intensive. Also, the selection procedure while creating stable cell lines is much longer than the microbial systems.

The crucial phases during cell-line development are associated with selecting the most suitable cell line, transfection method, and the most appropriate expression vector (31). In order to select the single-cell clones to ensure monoclonality, several high throughput screening devices have currently been used. These devices can be classified as FACS (fluorescence-activated cell sorting), colony picker and single-cell printer instruments. It is also crucial to picture single-cell clones to prove monoclonality, which is extremely important in ensuring the consistent manufacturing of biological molecules (32). Several instruments are used for this purpose, some of which have cell printing ability.

When selecting the best clones after the screening, the primary factors are proteins with high expression levels, suitable post-translational modifications, stable production patterns, and critical quality attributes. Other specifications, which are taken into account during the selection processes, are viable cell density, cell viability, and easiness to scale up both in fermenter and downstream processing. So, after determining the compatibility with industrial standards and meeting the quality criteria, the best clones are selected and used for further activities (33). Generally, the cell line development procedure is followed by process optimization and, subsequently, scale-up studies. Process optimization is usually performed at lab scale, and just after scaling up to large scales, clinical and commercial batches are carried out.

Monoclonal antibodies are the most commonly used biological therapeutics on the market. Most of the mAbs present on the market are IgG class-based, consisting of two heavy and light chains connected by disulfide bridges (34). Besides disulfide bonds, glycosylation is a common and crucial post-translational modification to ensure the molecule's activity. It should be emphasized that the post-translational modifications are present in mammalian cell lines, but glycan types may differ from human-based glycosylations. As expected, human cell lines such as HEK293, HKB11, PER.C6, HeLa and CAP cells are better for getting human-like glycans (35). IgG1 molecules include a single N-linked glycan at the Asn^{297} position in both heavy chains. While synthesizing N-glycans, multiple sugar structures can be added to form different glycosylation patterns such as G0, G1, G1F, G2S, G1FS, G2FS, G2FS2, G0-GlcNAc, etc.

Glycosylation has a critical role in complement-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC) by modifying the binding to the Fcy receptor. Both ADCC and CDC are involved in eliminating cancer cells, and particular glycans are necessary to provide therapeutic efficacy by increasing the specific interaction with various FcyR receptors and complement factor receptor C1q (36). For instance, G1F glycosylation has significantly increased the binding property of the mAb molecule to the C1q receptor, which eventually increased the complement system activity (37). Moreover, modifications associated with afucosylation have induced IgGs interaction with different FcyR receptors like FcyR IIa R131, FcyR IIa, FcyR IIb, FcyR IIIa, FcyR IIIa V158, FcyR IIIa F158 or FcyR IIIb. This strong interaction is known to boost ADCC activity (38, 39). Therefore, elevating the activity of mAbs by glycosylation modification is of great importance in the functional activity of biological molecules.

These desired glycans can be acquired either by using human cell lines such as HEK293, Per.C6, HeLa or by carrying out glycosylation engineering on most commonly used mammalian cell lines like CHO (3, 40). However, handling these human cell lines on large scales for commercial manufacturing is still in the improvement phases; hence most people in the biotech industry still prefer glycosylation modified CHO cell lines (41, 42). Besides genetic manipulations to reach the critical quality attributes in

glycosylation, media-feed screening and clone selection procedures are essential to acquire the optimal glycosylation pattern (43).

Furthermore, mammalian cell lines are preferred for manufacturing molecules requiring relatively high doses, such as monoclonal antibodies. Monoclonal antibodies (mAbs) are generally administered at 1-10mg/kg doses on average, while vaccines are usually applied with much lower doses, like the Hepatitis B vaccine at 20µg/ml (44, 45). Therefore, almost a thousand times more product is required for mAb production. Because of high yield requirements, mammalian cell lines are commonly used to manufacture these therapeutic molecules. So, stably transfected CHO cells are generally utilized, and more than 3g/L titers are found favorable in the industry; also, up to 9-10 g/L yields at maximum are acquired in fed-batch cultures (46). On the other hand, up to 12-fold more yield is expected in perfusion cultures, which renders mammalian cells suitable hosts for monoclonal antibody productions (47). However, several modifications are carried out in host cells to achieve high titers in these expression platforms, which are described in detail in section 3.

Mammalian cell lines are often used to manufacture viral vectors and vaccines (48). The advent of mammalian cell cultures allowed us in vitro production of viruses and opened the path for developing a various number of vaccines (49). The most common viral vaccination forms are attenuated and inactivated vaccine forms based on mammalian cell culture passages (50). Several vaccines, such as measles, poliovirus, rubella, rabies, hepatitis A, adenoviral- based covid-19 vaccines, were developed using cell culture techniques.

On the other hand, viral vectors, which are utilized in gene therapies, viral vector vaccines and cell therapy transductions, were also developed in animal cell culture. Recent developments in mammalian cell culture technologies enabled people to improve the yields of viral vectors and allowed scale-up in suspension cultures (51). Gene therapy is a very promising technology, which will be strikingly remarkable in the near future, along with cell-based therapies. They have the potential to correct the inherited defective genes causing several disorders (52). However, highly scalable and productive gene therapy processes must achieve clinical and commercial success. Typically, adenoviruses, adeno-associated viruses, retroviruses, lentiviruses, and herpes viruses are used for gene therapy approaches and cell therapy transductions (53). Stable producer or packaging cell lines are required for viral vector formation. Generally, Human Embryonic Kidney (HEK) cells are used for this purpose (54). For instance, for AAV formation, crucial genes are required for the assembly and infectivity of the virus. These crucial genes are separated into different plasmids containing E2A, E4 and VA. The transfer plasmid, Rep/Cap, and the helper plasmid are transfected into HEK293 cells, which contain the adenovirus gene E1, to create fully infectious AAV particles. So, the E1 gene, one of the most important genes of AAV formation, is provided with HEK packaging cell lines (55). Similar features can be considered for lentiviruses and retroviruses as well.

Moreover, viral vector-based vaccines are manufactured with the help of several mammalian cell lines such as HEK, Vero, HER.96 etc. Usually, vector-based vaccines cannot replicate themselves but can deliver antigens to the immune cells in the form of nucleic acid, DNA or RNA (56). HEK cell line is commonly used to propagate adenoviral vector-based vaccines, which are replication-defective and lack E1 genes (E1A and B) (57). These viruses are the most frequently used vaccine vectors. They are easily propagated in HEK cells or Per.C6 cells with high viral titers. Eventually, transgenes are expressed, activating the immune system (58). Rather than adenoviruses, poxviruses, herpes simplex viruses, cytomegaloviruses, alphaviruses, and adeno-associated viruses are considered viral vector-based vaccine options (48).

Also, in cell therapy applications, T cells are transduced with lentiviruses, propagated by mammalian cells. The most common cell line used for lentiviral production is HEK. The viral vectors for approved cell therapies are manufactured in the HEK cell line (59, 60).

3. Genetic modifications to create industrial mammalian cell lines

Primary human cell lines were isolated from the patients for a very long time ago. Animal cells such as CHO and Sp2.0 were also isolated from their primary sources long before. However, these cell lines cannot carry out industrial production of biological molecules because several requirements are present to meet the critical quality attributes (CQA) of the respective molecule. Thus, many genetic modifications were applied to make the cells more convenient for the biopharmaceutical industry (61). Classical genetic modification techniques are applied to increase cell lines' efficiency in manufacturing.

Besides these techniques, the latest progressions in gene editing nuclease enzymes such as CRISPR/Cas systems, zinc finger nucleases (ZFNs), and transcription activator-like effector nucleases (TALENs) render cell modifications cheaper and more applicable (62, 63). Several cases showed the successful utilization of these enzymes (64, 65).

The modifications applied to increase the efficiency of host cells are related to cell growth, maintaining cell viability, preventing cell death, promoting post-translational modifications, and enabling viral infection. These alterations are generally implemented by regulating apoptosis, performing metabolic engineering, growing cells at lower temperatures, carrying out chaperone and glyco-engineering, and modifying cell receptors to facilitate viral entry (66). Table 2 shows the genes modified during the host cell improvement process.

Table 2. Crucial genes modified to increase the yield of industrial cell lines

Pathway		Gene	Mechanism
Anti-apoptosis		mcl-1	overexpression
		30kc-6	overexpression
		bcl-2	overexpression
		bcl-w	overexpression
		bcl-xl	overexpression
		Aven	overexpression
		e1b-19k	overexpression
		Xıap	overexpression
		Crma	overexpression
Suppression of pro-apoptosis		Bax	inhibition
		Bok	inhibition
		Bak	inhibition
		Caspases	inhibition
Regulating cell cycle progression		p21	overexpression
		p27	overexpression
		CDKs	inhibition
		mTOR	overexpression
Chaperone engineering		Protein disulfide isomerase (PID)	overexpression
		BIP	inhibition
		XBP1	overexpression
		ERp57	overexpression
		C1GALT1C1	overexpression
		GRP94	overexpression
Metabolic engineering	Ammonia reduction	ornithine transcarbamylase	overexpression
		carbamoyl phosphate synthetase I	overexpression
	Lactate reduction	pyruvate carboxylase	overexpression
		lactate dehydrogenase	inhibition
		pyruvate dehydrogenase kinase (PDKs)	inhibition
Engineering cells for hypothermic growth		cold-inducible RNA binding protein (CIRP)	overexpression
Ribozyme engineering		mir-557	overexpression
		mir-1287	overexpression
		mir-30	overexpression
		mir-17	overexpression
		cgr-mir-7	overexpression
		mir-1b	overexpression
		mir-92a	overexpression

First of all, several metabolic engineering approaches have been applied to increase the yield of host cell lines. Accumulation of metabolic byproducts like ammonia and lactate is very frequent in mammalian cell cultures. These byproducts may negatively influence cell viability and product-specific yield. In order to get rid of these adverse effects of metabolites, several alterations have been implemented. For example, ammonia formation is prevented by overexpressing the glutamine-synthetase (GS) gene in CHO cells, and these cells are grown in a glutamine-free environment. This alteration provides a healthier environment for the cell and increases the viability and yield of CHO cells. Another advantage of this system is enabling people to grow the cells in a glutamine-free medium, reducing the manufacturing cost (67). Besides ammonia, lactate is a detrimental molecule for mammalian cells used in biologics production, so it is also essential to inhibit lactate production and accumulation, achieved by modifying lactate dehydrogenase pyruvate carboxylase, and pyruvate dehydrogenase kinase expressing genes (68).

Also, prevention of apoptosis is crucial in maintaining cell viability and eventually leads to elevation of productivity and extension of lifespan in antibody-expressing cells (69, 70). This is achieved by overexpressing cell survival and anti-apoptotic genes and downregulation of apoptosis-inducing genes (71). So, overexpression of anti-apoptotic survival genes like Bcl-2, Bcl-xl, Bcl-w, Mcl-1, Xiap, etc. and suppression of apoptotic genes such as Bax, Bok, Bak, Caspases (Caspase-3, 6, 7, 9), etc. increase the therapeutic protein concentration (72).

Additionally, inhibition of cell cycle progression and apoptosis is significant in elevating cell viability, viable cell density, and molecule-specific productivity (73). The cell cycle process can be disrupted by inhibiting cyclin-dependent kinases or activating CDK inhibitors. Cell cycle arrest in CHO cells caused a two to three-fold increase in productivity (74).

Generally, cell cultures are kept at 36.5 to 37 degrees Celsius. Decreasing the temperature of mammalian cell culture to 30-33 degrees Celsius has also improved biologics' productivity. These changes lower the growth of cells and extend the viability, significantly increasing the product's yield

(75). For instance, in the case of hypothermic growth, cold-related stress genes are induced and start to be expressed. Thanks to the increasing resistance to stress conditions, the cells produce more protein of interest (76).

Proper protein folding and post-translational modifications are crucial in sustaining the quality of molecules of interest and helping to meet the CQAs of the product. Enzymes related to folding proteins, such as chaperones and foldases, have crucial roles in maintaining the proper 3-dimensional structure of therapeutic molecules (77). Keeping proper disulfide bonding is also critical to maintaining therapeutic proteins' activity and stability. Overexpression of disulfide formation-related enzymes like protein disulfide isomerase induces disulfide bonding, which eventually leads to increased expression of mAbs (78).

Glycosylations are considered the most effective post-translational modifications of recombinant proteins in CHO cells. These structures have been shown to have a significant role in elevating the molecule's activity (79). Monoclonal antibody molecules used in therapy are generally based on IgG1 structures, containing N-linked glycosylation at Asn^{297} residue of heavy chains. Multiple glycans can be added to create various glycoforms such as G_0, G_1, G_0F, G_1S, G_2S, etc. (80). Antibody-dependent cell cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) are the two main mechanisms by which mAbs demonstrate their biological activity. Any modifications or changes in the glycan moieties of mAb molecules may influence these activity mechanisms. High fucosylation levels and relatively low levels of bisecting-N- acetylglucosamine (GlcNAc) are associated with monoclonal antibodies manufactured in CHO cells (81). Therefore, to increase ADCC activity, some modifications to the N-acetylglucosaminyltransferase III (GnTIII) gene were carried out for overexpression. Also, ADCC is induced by eliminating fucosylation on mAbs, which was achieved by knocking out the fucosylation related enzyme fucosyltransferase (FUT 8) in CHO cells (82).

In addition, facilitating the entry of viruses into host cell lines is also a very crucial point to be focused on. As stated above, several mammalian cells have been used for viral-based vaccine manufacturing so far. Inducing the entry of viruses into the host cells significantly increases the yield and decreases the cost of vaccine production (83). It was observed that eliminating several genes, alone or in combination, enhanced viral yields more than 20 to 50-fold in poliovirus production (84). Other cellular modifications such as adding adenovirus-related pIX gene and overexpression of tethering protein to prevent the release of endogenous retroviruses can increase the productivity of viral vaccine productions (85).

4. Conclusion: Modified mammalian cells offer new opportunities for biologics manufacturers

Several hosts and expression platforms are utilized in biologics manufacturing. Bacteria, yeasts, insects and mammalian cells are the most frequently used host systems. Different kinds of molecules like wild type insulins and their derivatives, mAbs, recombinant and traditional vaccines, and therapeutic proteins are manufactured using various host systems. For instance, some bacterial vaccines and insulins are produced with the help of bacterial expression systems, while virus-like particle (VLP) and recombinant vaccines are manufactured in yeast systems.

On the other hand, mammalian cells are commonly used to produce monoclonal antibodies, recombinant therapeutic proteins, gene therapies, viral-based inactivated and attenuated vaccines, vector-based vaccines, and vector productions for cell therapy applications. Several mammalian cell lines such as CHO, HEK, NSO, Sp2.0, Vero, MRC-5, and PerC.6 are involved in biologics manufacturing processes.

Many improvements are implemented to make these cell lines more convenient for high yield and quality protein productions. These modifications can be classified as metabolic engineering of cells, glyco and chaperone engineering of therapeutic molecules, regulation of apoptosis pathway, making improvements to keep the cells viable, and applying temperature shift to grow the cells at lower temperatures. For this purpose, several modifications were done at the genome level, and these specific changes eventually led to more productive manufacturing (86).

Moreover, the discovery of recent gene-editing tools concerning ZFNs, TALENs and CRISPR/Cas9 made the host cell modifications easier and more applicable (87). CRISPR stands out for host cell engineering for biologics manufacturing within these systems. However, it should be noted that patent restrictions should be overcome while creating an industrial cell line by using this system (88). It is crucial to target the open chromatin sites to express the gene of interest effectively. Thanks to the CRISPR system, it is possible to target the genome in desired regions that facilitates the required modifications to increase productivity. Also, open chromatin (euchromatin) region targeting mechanisms are widely used in cell line engineering approaches to boost the yield and increase stability. These mechanisms can be classified as Scaffold/matrix attached regions (S/MAR) and Ubiquitous Chromatin Opening Element (UCOE) (89, 90).

Thus, advances in cell line optimization techniques at different levels enable host cells to be used in the industrial production of biopharmaceuticals.

Conflict of interest
None to declare.

Funding
None to declare.

Acknowledgments
None to declare.

Authors' contributions
Concept: N.E., Design: N.E., Data Collection or Processing:

N.E., H.T., Analysis or Interpretation: N.E., H.T., Literature Search: N.E., H.T., Writing: N.E., H.T.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Review Article

J Exp Clin Med
2022; 39(3): 893-900
doi: 10.52142/omujecm.39.3.56

Structure and functions of spexin as a new neuroendocrine signal

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Received: 26.05.2022• **Accepted/Published Online:** • **Final Version:** 30.08.2022

Abstract

Spexin (SPX) is a recently discovered endogenous peptide consisting of 14 amino acids. It was found that SPX, kisspeptin (KISS), and galanin (GAL) peptides belong to the same gene family and are also endogenous ligands of GAL2 and GAL3 receptors. The amino acid sequence of the SPX peptide is relatively conserved in vertebrates and invertebrates. The mRNA and protein of SPX are highly expressed both in peripheral organs and in the peripheral/central nervous system of mammals, birds, and fishes. Many biological roles of SPX has been found in non-mammal/mammals, including food intake, energy metabolism, reproduction, nociception, gastrointestinal motility, stress, and endocrine functions. This review collectively mentions the peptide structure of SPX, its receptors and distribution in tissues, and the biological activities of SPX on various organs.

Keywords: galanin-2 receptor, galanin-3 receptor, Neuropeptide, NPQ, spexin

1. Introduction

Spexin (SPX) is an endogenous peptide discovered through the Hidden Markov Model in 2007 (1). The C12orf39 gene, located on chromosome 12 in the human genome, encodes preprospexin (2). After preprospexin has undergone through a series of protein synthesis processes “mature SPX” is formed, which is an effective form for cellular physiological processes. The amino acid sequence of the SPX peptide has been evolutionarily conserved in all vertebrates and invertebrates (1, 3-5). Therefore, it is claimed that SPX is essential for survival and may exist in various tissues/organs as it carries out many body functions. In fact, SPX mRNA/protein commonly presents in the main systems of the body, such as cardiovascular, skeletal, digestive, urinary, reproductive, endocrine, and central nervous systems. The widespread synthesis of SPX has pointed out that it regulates many physiological functions in the body. For instance, SPX affects food intake (6-10, 25) glucose/fat metabolism (11-15) gastrointestinal motility (1, 16), pain perception (5, 17, 18), endocrine (10, 12, 19-21), reproductive (7, 10, 20, 22-24) and cardiovascular functions (17). Additionally, the important roles of SPX have been revealed in pathological conditions such as obesity, anorexia nervosa, diabetes, anxiety, and depression (26-31). SPX exerts the above-mentioned effects by binding to galanin-2 (GAL2) and galanin-3 (GAL3) receptors (5, 14, 16, 32). Studies on SPX have increased significantly in recent years. This review discusses the molecular structure and roles of SPX in physiological and pathological conditions in both mammalian and non-mammalian species.

2. Spexin peptide structure

Spexin gene (called C12orf39 gene) located in chromosome 12 in the human genome encodes a preprospexin peptide of 116 amino acid residues (2). The preprospexin peptide contains both a hydrophobic signal peptide (SP) and two dibasic prohormone cleavage/amidation sites (RR/KR & GRR) (Fig. 1) (4). A small amino acid region among dibasic cleavage sites forms the 14 amino acids of spexin, also called neuropeptide Q (NPQ). C12orf39 gene consists of 6 exons and 5 introns in humans. The 1st and 2nd exons have encoded the signal peptide while the 3rd and 4th exons encode the active peptide (1). The 14 amino acids sequence of SPX is highly conserved with only minor changes in humans and other species. In cats, dogs, and pandas, for example, serine amino acid is replaced with alanine in the 6th position (Fig. 2).

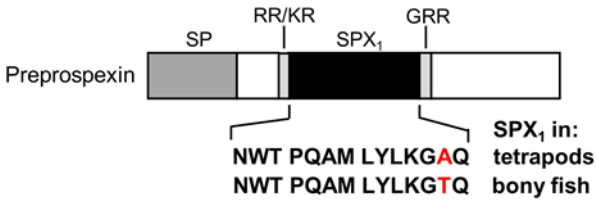

Fig. 1. Organization of SPX1 (SPX) coding sequence in tetrapods and fish models, SP: signal peptide; N: asparagine; W: tryptophan; T: threonine; P: proline; Q: glutamine; A: alanine; M: methionine; L: leucine; Y: valine; K: lysine; G: glycine; SP: signal peptide; RR/KR and GRR: cleavage sites. Figure is taken from Ma et al. (4).

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Wong et al. introduced the three-dimensional structure of SPX in goldfish for the first time. In amino acids sequence of SPX, while the first 4 amino acids (Asn1-Pro4) form a structure at the amino terminus (N-terminal), from the 5th to 14th amino acids (Gln5-Gln14) constitute an α-helix structure that extends to the carboxyl group [C-termius (COOH)]. This study also revealed that the Lys11 position of the SPX sequence is hydrophobic, and this region plays a key role in the activation of its receptor (6).

In 2014, Kim et al. firstly investigated the evolutionary mechanisms of SPX, and they revealed that SPX is phylogenetically a member of the GAL/KISS peptide family, but SPX is closer to the GAL family than the KISS family. Moreover, they discovered another form of SPX, called SPX2 (33). Accordingly, the first discovered SPX is now termed SPX1. Unlike SPX1, SPX2 is not found in mammals, but has been detected in many species such as chickens, fish, birds and frogs (Fig. 3). SPX2 is encoded by a different gene and differs from SPX1 in terms of the prohormone cleavage/amidation sites, amino acid sequence, and species in which it is located (Figs. 2 and 3). Nonetheless, the amino acid sequences of SPX1/2 are highly conserved, suggesting that SPX has performed essential functions for survival (4, 33).

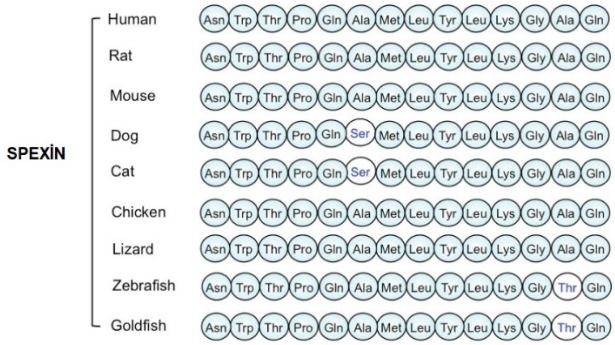

Fig. 2. The amino acid sequence of SPX in various species. The figure modified from Lv et al. (32).

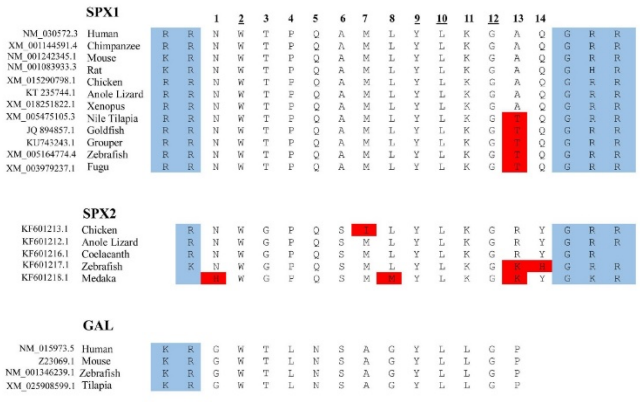

Fig. 3. Comparison of the protein sequence of SPX and GAL in diverse species. The prohormone cleavage/amidation sites is in the red box and the amino acid replacementis is in the red box. Figure taken from Reference 38.

3. Receptor of spexins

Molecular studies have shown that SPX peptide is somewhat similar to GAL peptide. The amino acids at positions 2, 3, 9, 10, and 12 (Trp2, Thr3, Tyr9, Leu10, Gln12) in the amino acid sequence of SPX1 are the same as at the corresponding position in the GAL (Fig. 3). Because amino acids at positions 2, 3 and 9 in the GAL sequence (corresponding to amino acid Trp2, Thr3, Tyr9) are the main criteria for binding to and activation of GAL receptors it has been suggested that SPX can also bind and activate galanin receptors (33). Three types of GAL receptors have been identified in mammals: GAL1 (1a and 1b), GAL2 (2a and 2b), and GAL3 receptors. GAL receptors are G-protein coupled; Gq/11 coupled receptors activate the intracellular signaling pathway by activating the phospholipase C/protein kinase C pathway. On the other hand, Gi/o coupled receptors show inhibitory effects on target cells by suppressing the adenylate cyclase/protein kinase A pathway (34). While GAL1 and GAL3 receptors have generally mediated inhibitory effects through Gi/o protein coupled receptors; activation of GAL2 receptors causes both inhibitory effects through Gi/o and excitatory effects via Gq/11. The ligand-receptor interaction study demonstrated that the SPX (SPX1 and SPX2) can activate GAL2 and GAL3 receptors but not GAL1 receptors (33, 35, 36, 37). It is concluded that SPX is the endogenous ligand for GAL2 and GAL3 receptors, and this conclusion is also consistent with the knowledge that SPX is from the galanin/kisspeptin gene family.

4. Distribution of spexin in tissues/organs

After discovering SPX, a comprehensive analysis of its localization in various species (such as the rat, mouse, human, and fish) has been carried out. While SPX1 was found in both mammalian and non-mammalian vertebrates, SPX2 was only described in non-mammalian vertebrates.

4.1. Distribution in mammalian vertebrates

SPX was first discovered in the submucosal layer of the rat esophagus and stomach (1). Sonmez et al. have shown that SPX is found in neurons secreting the CRH in mesopontine tegmentum including neurons secreting tyrosine hydroxylase and tryptophan hydroxylase in PAG and the Barrington nucleus in rats (3). Additionally, it was demonstrated in the locus cerelous and laterodorsal tegmental nucleus. Studies using immunohistochemical staining methods revealed that SPX is present in ependymal cells of the choroid plexus, superior cervical neurons, trigeminal ganglia, retinal photoreceptors, cerebellar purkinje cells, paraventricular and supraoptic nuclei in the hypothalamus (39). Moreover, SPX immunoreactivity was found in rat skin (epidermis and adipocyte), gastrointestinal system (esophagus, stomach, small/large intestine, liver, exocrine part of pancreas), endocrine tissues (adrenal cortex and medulla, thyroid and parathyroid glands, endocrine part of pancreas), the respiratory system (alveolar epithelium and bronchi), muscle tissue (heart, skeleton and smooth muscle), the genital system

(ovary and testis) and the placenta in rat, fish, human (19, 39-42). Besides, it has been demonstrated in trophoblastic cells of the human placenta (2) and type I glomic cells in carotid body (43).

4.2. Distribution in non-mammalian vertebrates

Both SPX1 and SPX2 have been shown in many living non-mammalian genomes such as chicken, lizard, frog, and fish. However, there are very few studies on the distribution and functions of SPX2 (33, 44). Tian et al. (2020) have demonstrated that spexin mRNA is widely expressed in the gastrointestinal tract, liver, and hypothalamus in Siberian sturgeon (Acipenser baeri) which is a type of experimental fish (40). Also, it was indicated that SPX1 is found in the hypothalamus, telencephalon, optic tectum, pituitary gland, cerebellum, brainstem, ventromedial thalamic nuclei and medial longitudinal fasciculi in goldfish. In a study conducted on zebrafish through the in-situ hybridization method, the localization of SPX1 and SPX2 in the brain was determined in detail (44). According to this study, while SPX1 positive neurons are highly found in the middle and hindbrain, SPX2 has been found densely in the preoptic area of hypothalamus. SPX1-positive neurons in the hindbrain project to the spinal cord and these neurons are GABAergic inhibitory neurons, and it has also been demonstrated that these neurons connect with neurons in the spinal cord where GAL2b receptors are located. Additionally, SPX1 neurons have been detected in the dorsal habenula in which GAL2a and GAL2b receptors are located, and these neurons have projected to the interpeduncular nucleus from the dorsal habenula (44).

5. Effects of spexin

The widespread distribution of SPX in both central/peripheral tissues in many living species suggests that it plays a very important role in many physiological and pathological functions. In studies conducted consistent with the suggestion, SPX has been found to be involved in the physiology/pathophysiology of reproductive, gastrointestinal, cardiovascular and endocrine systems including especially in food intake and, energy metabolism (carbohydrate/fat). Below, these effects are comprehensively discussed under the main topic.

5.1. Food intake

Studies have shown that SPX suppresses food intake and nutritional behaviors (6, 7, 8, 9, 10, 25). As it is known, the level of circulating insulin increases following food intake. A study revealed that increased insulin following food intake mediates rises of SPX expression in both brain and the liver of fish (45). Therefore, it is thought that there is a link between food intake and SPX expression through insulin. Furthermore, in studies conducted in fish, it was observed that SPX expression increases in telencephelon, hypothalamus, and optic tectum after food intake (6, 8). However, there are also studies claiming the opposite of these findings (7, 9, 10).

Ma et al. (2017) highlighted the mechanism of SPX's inhibitory effect on food intake in their study on fish. Following food intake, the level of glucose in circulation rises and glucose increases insulin secretion in the liver (concurrently in the pancreas); then, insulin causes SPX upregulation at the hepatic level through insulin receptors (small amounts of IGF1R), which is coupled to MKK3/6/P38 MAPK and PI3K/Akt pathway, acting in an autocrine and paracrine manner. With the hepatic exit of SPX, SPX levels in the blood increase and the peripheral SPX reaches the central nervous system by passing through the blood-brain barrier. Meanwhile, insulin released by the liver (together with secretion from the pancreas) can increase insulin level in circulation and has a central effect of upregulating SPX in brain areas involved in control of food intake via InsR. Later, the combined effect of both central expression and peripheral secretion of SPX can regulate the central orexigenic and anorexigenic signals pathway. Eventually, SPX suppresses food intake either by suppressing orexigenic factors [neuropeptide Y (NPY), agouti-Related Peptide (AgRP), and apelin] or by increasing anorexigenic factors [proopiomelanocortin (POMC), cocaine-and amphetamine-regulated transcript (CART), melanin-concentrating hormone (MCH), kholecystokinin (CCK), nucleobindin-2 (NUCB-2), and peptide YY (PYY)] in the nuclei in the hypothalamus (Fig. 4) (4, 6, 8). Besides, in a recent study in mice, it was revealed that spexin suppresses food intake via GAL3 receptors (40).

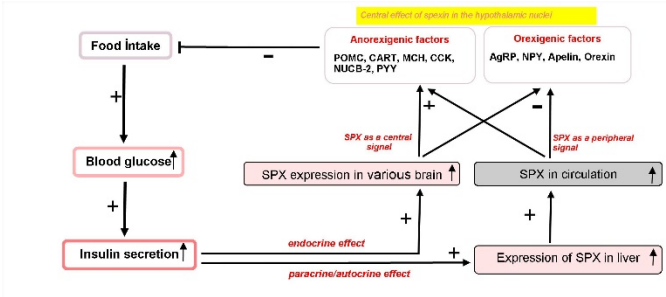

Fig. 4. Functional role of SPX on food intake in fish models (e.g., goldfish, ya fish, grouper, and zebrafish) (refer to the text for details). SPX: Spexin; POMC: Proopiomelanocortin; CART: cocaine-and amphetamine-regulated transcript; MCH: Melanin-concentrating hormone; CCK: Cholecystokinin; NUCB-2: Nucleobindin-2; PYY: Peptide YY; AgRP: Agouti-Related Peptide; NPY: Neuropeptide Y. Figure is rearranged from Ma et a.l (4).

5.2. Energy metabolism

SPX plays an important role in lipid metabolism associated with body weight control. It is well known that the long-chain fatty acid uptake and storage into adipocytes are significant factors in the control of body weight (46). Waleswski et al. (2014) found that SPX treatment decreases the uptake of long-chain fatty acids into adipocytes in mice (47). Similary, SPX inhibits the uptake of fatty acids into hepatocytes, resulting in a reduction in hepatic lipid content (48). Kolodziejski et al. demonstrated that SPX decreases adipogenesis and lipogenesis but increases lipolysis in murine and human (49). In the same study, they also found that it

suppresses glucose uptake into visceral adipose tissue. Additionally, it has been shown that SPX leads to lipid oxidation and increases locomotor activity (47). Moreover, SPX levels were also compared in obese and non-obese adults with clinical human studies. It has been shown that the C12orf39 gene was 14.9-fold lower in omental and subcutaneous adipose tissue of obese individuals compared to individuals with normal body weight (47). Also, circulating SPX levels and SPX expression in adipocytes are low in obese patients (50). As it is known, obese patients have high leptin levels. A negative correlation was revealed between leptin and SPX levels in obese individuals (13, 51). These two peptides may have an antagonist role in the regulation of body weight, hunger/satiety, and energy metabolism. Additionally, Kumar et al. (2018) showed that circulating SPX levels dropped after Roux-en-Y gastric bypass surgery in youth with severe obesity, and the low SPX levels negatively correlated with insulin resistance (HOMA-IR) and body mass index (52). Therefore, it is suggested that SPX can be a biomarker especially in adult and childhood obesity (47, 50). SPX can also be an important marker in diseases associated with energy metabolism. It has been reported that SPX may potentially have beneficial effects in various metabolic diseases. Bitarafan et al. found that SPX decreases hepatic lipids, serum alanine aminotransferase (ALT) and aspartate aminotransferase in mice with hepatic steatosis/nonalcoholic fatty liver disease (HS/NAFLD), which are markers of HS/NAFLD (53). Another clinical study results showed that serum SPX levels were significantly lower in the metabolic syndrome (MetS) group than the healthy individuals in women (54).

5.3. Reproduction

Spexin has been shown to inhibit the reproductive axis in different fish species in vitro and in vivo (20). Liu et al. (2013) firstly demonstrated that intraperitoneal injection of SPX suppresses LH release in goldfish. Also, it was found that the expression of SPX is lower during the breeding season than during non-breeding seasons (20) and in the breeding season; SPX injection has not affected LH expression in pituitary in the orange spotted grouper (7). In this study, it was also shown that expression of spexin in the hypothalamic nuclei is modulated by gonadal hormones such as estrogens (10, 20). For example, estrogen treatment causes downregulation of SPX expression (20). Additionally, while SPX injection induces expression of gonadotropin-inhibitory hormone (GnIH) and gonadotropin-releasing hormone-3 (GnRH3) (9). SPX suppresses the growth hormone expression in orange-spotted grouper and half-smooth tongue sole (7, 9). However, in a study with grouper fishes, SPX treatment does not affect mRNA expression of LH and FSH in the pituitary (7). Contrary to this data, there are studies showing that SPX can inhibit LH and FSH secretion (9, 20, 22). In another study, the effect of SPX on gamete maturation and puberty onset was investigated, and it was reported that SPX knockout zebrafish has fertility without abnormality in the timing of pubertal onset or gamete maturation in the testes and ovary (55). This data demonstrates that SPX is not essential for fish reproduction. Finally, it has recently been revealed that there was no in vivo evidence of the role of SPX in modulation of the ovine gonadotropic axis. These data show that the effect of SPX on the reproductive axis may be type-specific, and further investigations are needed to find out whether it is effective in various types of reproduction.

5.4. Nociception

Early immunohistochemical investigations indicated that spexin mRNA/protein is found in the brainstem, periaqueductal gray, brain cortex, and trigeminal ganglia, which is well known to be associated with nociceptive processes (3, 39). So, it has been thought SPX can play a role in nociceptive pain transmission/modulation. Indeed, intracerebroventricular injection of SPX caused antinociception in mice tail withdrawal test (17). Also, Pirzeh et al. (2014) show that pain sensitivity was decreased in the formalin test in female rats administrated SPX into intrahippocampal CA3 region (56). Similarly, hippocampal CA3 injection of SPX caused a reduction in pain sensitivity in the same pain test in ovariectomized rats. Furthermore, co-injection of SPX and progesterone caused a higher antinociceptive effect than progesterone administered alone (18). In another study to elucidate its possible mechanism in SPX antinociception, Lv et al. (2019) found that central spexin showed an antinociceptive effect in both tonic pain (formalin test) and visceral pain tests (writhing test) at supraspinal level. Additionally, findings of molecular analysis indicated that after central SPX is injected in mice formalin pain test, GAL3 receptors are activated and then GAL3 receptors cause to up-regulate dynorphin and k-opioid receptor gene/protein. As for the writhing test, SPX administrated in the lateral ventricle stimulates GAL2 receptors and then activates POMC/mu-opioid receptor gene/protein (5). All results have shown the antinociceptive effect of the central SPX, but the effect of spexin is still unclear at the peripheral level.

5.5. Digestive system

Mirabeau et al. (2007) firstly showed that SPX mRNA is located in the submucosal layer of the esophagus and the stomach fundus in mice by using in situ hybridization (1). Additionally, they demonstrated SPX induces contraction in rat stomach fundus smooth muscle in a dose-dependent manner in vitro, providing evidence of the first known physiological effect for SPX (1). According to another in vitro study, spexin also stimulates intestinal and colonic contraction via GAL-2 receptors in mice. Besides, intraperitoneal injection of SPX enhances all of the bowel transit via GAL2 receptor by activating L-type voltage-gated calcium channels in fed mice, but not GAL3 receptors (16). Nevertheless, it is not known whether centrally administered SPX has an effect on gastrointestinal motility and therefore,

further studies are needed to show the effect of spexin on intestine motility mediated by central nervous system. The effect of SPX on bile acid synthesis has also been investigated. It was found that both acute and chronic peripheral SPX treatment reduce the levels of total bile acid in circulating and liver, and also decrease hepatic cholesterol 7α-hydroxylase 1 (CYP7A1) mRNA level through GAL2 and GAL3 receptors (14). Furthermore, it was revealed that SPX plasma levels are reduced in patients with constipation. Finally, the mRNA levels of SPX were reported to be reduced in rat jejunum and ileum after hunger stress in rat (16).

5.6. Stress

The presence of SPX was shown in the Barrington nuclei by using in-situ hybridization, indicating that it can play a role in stress response. In fact, Sonmez et al. (2009) showed that SPX collocates with CRF (corticotropin-releasing factor) in the Barrington nuclei (3). Moreover, Zhuang et al. (2020) showed in their detailed study in rats exposed to chronic stress that SPX mRNA level was decreased, while CRF mRNA level was raised in hippocampus (57). Additionally, it was indicated that hippocampal CRF administration reduces expression of SPX mRNA in hippocampus, hypothalamus, pituitary in mice, and this inhibitory effect is mediated by the CRF receptors.

5.7. Endocrine effects

SPX has been demonstrated in many endocrine tissues, including the hypothalamus, thyroid, adrenal gland, testicles, ovaries, pancreas, and adipose tissue. Rucinski et al. (2010) found that incubation with SPX enhanced aldosterone secretion in zona glomerulosa cells and induced corticosterone secretion in adrenocortical cells in rat in vitro (19) showing that SPX plays a role in the control of adrenocortical endocrine functions. Additionally, SPX has an inhibitory effect on LH hormone release (20). It was revealed that administration with 17β-estradiol reduced expression of the SPX in the hypothalamic nuclei of spotted scat (10). While intraperitoneal treatment with SPX increases GniH, GNrH expression in the hypothalamus, it suppresses GH, FSH expression. These findings suggest that SPX has an endocrine effect on reproduction; however, all studies have been conducted on fish models (9). Furthermore, treatment with spexin indicated increases in the viability and proliferation of pancreatic islets cells in vitro (12). Spexin decreases insulin gene expression as well as insulin promoter factor 1, a transcription factor, but not expression of insulin receptor. The same study revealed that SPX reduces glucose-stimulated insulin secretion in isolated pancreatic islets (12). In another study in obese rats, it was shown that while SPX treatment decreases levels of serum ghrelin, leptin, corticosteron, it increases serum T3 and glucagon levels (21). These studies suggest that SPX may be an endocrine factor.

5.8. Other effects

It has been suggested SPX has cardiovascular and renal roles. In rats, the central injections of SPX lead to an enhancement in mean blood pressure and a reduction in renal excretion and heart rate (17). However, whereas peripherally administered SPX causes a sharp pressor and bradycardia, SPX does not alter renal urine output rate. The reason for the opposite result may be that peripherally administered SPX is rapidly metabolized. Additionally, Porzionato et al. (2012) found that there was SPX expression in both human and rat carotid body, and SPX mRNA levels were increased by hypoxia exposure for 2 weeks in neonatal rats, suggesting that SPX expression in the carotid body may be related to sensing O2/CO2 levels (43). Moreover, it was shown that SPX improves mitochondrial dysfunction and the imbalance in energy homeostasis of cardiomyocytes due to exposure to hypoxia (58).

In conclusion, SPX is a 14-amino-acid endogenous peptide that is well-conserved. To date, SPX1 and SPX2 are the two forms of SPX discovered. SPX1 was identified in both mammalian and non-mammalian cells, but SPX2 was exclusively discovered in non-mammalian cells. Studies have shown that SPX performs its functions through GAL2 and GAL3 receptors. The biological function of SPX includes feeding behavior, reproduction, nociception, glucose/lipid metabolism, gastrointestinal motility, and stress. Also, SPX plays a role in pathological processes such as obesity, anorexia nervosa, diabetes, anxiety, and depression. Therefore, its possible potential role in therapeutic targets will be beneficial for the development of new technology for the cure of various disorders. Furthermore, the functional relevance of SPX2, which is found in non-mammalian vertebrates, should be investigated further.

Conflict of interest

None to declare.

Acknowledgments

None to declare.

Authors' contributions

Concept: Ö.D.S., A.B., Design: Ö.D.S., A.B., Data Collection or Processing: Ö.D.S., Analysis or Interpretation: Ö.D.S., A.B., Literature Search: Ö.D.S., Writing: Ö.D.S., A.B.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Case Report

J Exp Clin Med
2022; 39(3): 901-903
doi: 10.52142/omujecm.39.3.57

COVID-19 infection may trigger SLE disease: A case report

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Received: 30.01.2022 • **Accepted/Published Online:** 24.06.2022 • **Final Version:** 30.08.2022

Abstract

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease that can affect various body organs, especially the skin, joints, hematopoietic system, kidneys and central nervous system. The signs and symptoms occur depending on the inflammation in the affected tissues. Although many factors are responsible for its aetiology, it is known that some viruses such as Epstein Bar virus may also cause this disease by triggering autoimmunity. Recent studies have revealed that the immune system could be activated due to coronavirus infections and that some autoantibodies could be observed in the blood. A small number of SLE cases activated by COVID infection have been reported in the literature. The present study presented and discussed with the information in the literature a 20-year-old patient with COVID infection, diagnosed with SLE with renal involvement according to EULAR/ACR 2019 criteria by considering histopathological and immunofluorescence findings.

Keywords: lupus erythematosus, systemic, COVID-19, antibodies, autoimmune diseases

1. Introduction

COVID-19 disease, which primarily affects the respiratory system, can manifest itself with various clinical findings ranging from being an asymptomatic carrier to multiorgan failure. After the World Health Organization declared the COVID-19 outbreak a pandemic, no effective treatment for the disease has been found so far. The virus undergoing mutation over time can manifest in a wide range of clinical presentations. Besides the virulence, co-morbidities and immune response are known to be effective in the severity of the clinical state (1, 2). Activation of the immune system and some autoantibodies has been shown in COVID-19 patients (3,4). Additionally, the corrective effect of immunosuppressive treatment on the clinical state has been reported for some critical COVID-19 patients (5). Causes triggering autoimmunity may play a role in the development of SLE. Accordingly, the possibility of COVID-19 disease triggering autoimmunity and playing a role in SLE development can be considered. The literature reported 4 SLE cases triggered by COVID-19 infection (6-9). The present study discussed a recently diagnosed SLE patient with renal involvement and COVID infection. Ours is the first case whose diagnosis was confirmed with immunofluorescence microscopy.

2. Case report

A 20-year-old patient, who was 22-weeks pregnant, applied at the emergency room with complaints of 2-weeks of edema in the lower extremity, knee pain and extensive muscle pain. The patient's health history revealed this to be her first pregnancy, and she had no chronic disease, substance or alcohol use or complaints such as cough, fever, shortness of breath. Her examination findings and blood tests (including urea and creatinine) had been normal in the 12-week routine prenatal visit. We detected pretibial edema in both lower limbs in her examination in the emergency room. Her knee motions were painful, and we found no effusion or redness in the joints. The laboratory investigations showed the following results: creatinine value: 2.49 mg/dl (0.5-1.1), urea: 139 mg/dl (19-49) Na: 131 mEq/L (132-146), potassium: mEq/L6.4 (3.5-5.5) CRP 3 mg/L(0-5) sedimentation rate: 45 mm/h, WBC: 10750 x 109/L, PLT:146000 x 109/L, Hb: 11.8 g/dL, Total protein: 43 gr/l (57-82), Albumin: 20 gr/l(32-48), Uric acid: 10.3 mg/dl(3.1-7.8), Spot urine protein/creatinine ratio: 1387 (200<), Urinalysis proteinuria (4+ protein>), 175 Red blood cells/ high-power field (HPF) , 46 leukocytes/HPF, 3 leucocyte clusters.

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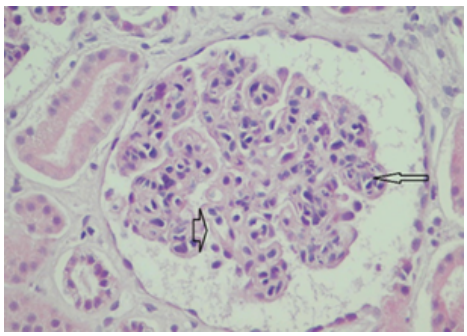

Fig. 1. Thickening in the glomerular tuft and in the glomerular basement membrane, double contour (thick arrow), mesangial cell and matrix increase (long arrow) (H&Ex400).

We detected 24-hour urine output of 150 ml. E. Coli grew in urine culture. We gave intravenous Ertapenem to treat the urinary tract infection. Immunologic test results showed signs of autoimmune disease, including 1:3200 positive antinuclear antibodies (ANA) with a coarse speckled pattern. Anti-ds DNA-ELISA: 460 RU/ml (<100), Anti SS-A: 3+, Anti Ro-52:3+, Anti SS-B: 3+, Nucleosome: 2+, Anti-histone: 3+, Anti-glomerular basement membrane antibody (Anti GMB): negative, Antiphospholipid antibodies: negative, C3c g/L:0.2 (0.9-1.8), C4 g/L: 0.1 (0.1-0.4), MPO ANCA-ELISA: Negative, PR3 ANCA-ELISA negative, P ANCA – ELISA negative, C ANCA-ELISA negative. COVID reverse transcription-polymerase chain reaction (RT-PCR) with a nasopharyngeal swab and COVID-19 IgG+IgM antibody were positive 4.12 (0-0.99). Serology tests results for other viruses were the following: Anti EBV IgM: negative, Anti EBV IgG: negative, Anti-Toxoplasma IgM: negative, Anti-Toxoplasma: IgG negative, Antirubella Ig M: negative, Antirubella IG G: positive, Anti CMV IgM: negative, AntiCMV Ig G: positive, Parvovirus IgM: negative, Parvovirus IgG positive. We detected Grade 1 increase in the parenchymal echogenicity in kidney ultrasound. Meanwhile, the patient's pregnancy ended due to intrauterine fetal death. We performed an ultrasound-guided kidney biopsy for histopathological examination and observed 40 glomeruli and seven arteries in the sections taken. Histopathological findings of kidney tissue revealed thickening of the basement membranes, double contour formation, mesangial cell and matrix increase in more than 50% of the glomeruli, with more prominence in general in the peripheral capillary loops (Fig. 1). We observed inflammatory cell infiltration containing a mild level of lymphocytes in the interstitium and degenerative and regenerative changes in tubular epithelial cells. We also observed thickening in the glomerular basement membrane with PAS-Methanomine Silver and PAS and staining in the immunofluorescence sections with IgG, IgM, C1Q, IgA, C3; C4c; Kappa, lambda (Fig. 2). but no staining with fibrin or albumin. Histopathological and immunofluorescence findings were consistent with diffuse proliferative glomerulonephritis (full house nephropathy) and evaluated as Class 4 lupus nephritis. We diagnosed the patient with SLE according to the European League Against Rheumatism (EULAR) and the American College of Rheumatology (ACR) 2019 criteria (10), gave Methylprednisolone 1gr/day for three days and maintenance steroid treatment after that and started cyclophosphamide to be administered 750 mg once every three weeks. We obtained informed consent from the patient.

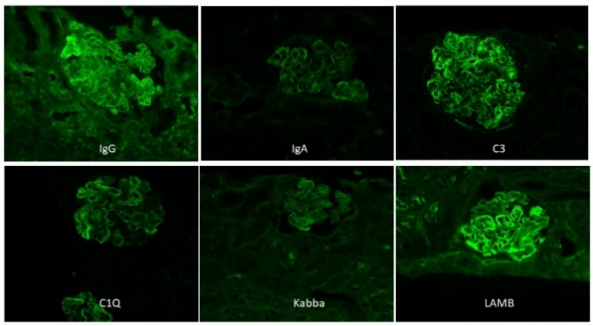

Fig. 2. In immunofluorescence staining, staining is present in the subendothelial area in the glomerular tuft: + 3 with IgG(a), C3c(d), Lambda(g), +2 with C1q(e), +1 with IgM(b), IgA(c), Kappa(f).

3. Discussion

SLE is an inflammatory disease in which autoimmunity, triggered by genetic environmental factors, plays a role in its aetiology, seen more frequently in females (11). Smoking, alcohol use, ultraviolet radiation exposure, vaccines, silica exposure, air pollution and solvent exposure can be counted among the environmental factors triggering SLE development (11,12). Besides these, it has been reported that infectious diseases such as Epstein-Barr virus (EBV) and cytomegalovirus could trigger SLE development (11,13,14).

After the COVID-19 pandemic, newly diagnosed SLE cases triggered by its infection have been reported (6,7,8). The key role of autoimmunity in the etiopathogenesis of SLE and activation of the autoimmune system by COVID-19 disease seem to be the two factors responsible for this relationship. Wang Y et al. detected SARS-CoV-IgG and -IgM antibodies in the serums of %3 of 114 healthy individuals without COVID-19 disease and 32% of 58 SLE patients (15). Another study found SARS-CoV-IgG positivity with a rate of 3% in 66 healthy individuals and 58% SARS-CoV-IgG positivity and 29% SARS-CoV-IgM positivity in 31 SLE patients, while the PT-PCR test was negative for all these patients (16). According to the authors, one possible reason for the false-positive results of SARS-CoV-IgG and IgM antibody in SLE patients is the antigens coated with SARS-CoV and Vero-E6 cells in ELISA methods (16). Because SARS-CoV antibodies cannot be detected in the early stage of the coronavirus disease, an ELISA test 2-3 weeks after the onset of the symptoms is recommended (16,17). In our case, the patient's PT-PCR test was positive when she applied at the hospital. We detected antibodies in her blood one week later with the ELISA test. The positivity of her PT-PCR test suggested that these antibodies were related to COVID-19. Zhou Y et al. reported that they found anti-52 kDa SSA/Ro antibody/25 and anti-60 kDa SSA/Ro antibody in 20% and ANA positivity in 50% of 21 critical

COVID-19 patients. They also reported that autoimmune pneumonia developed in 3 patients (3). The authors have interpreted this situation as a sign of the deterioration of the immune functions in COVID-19 patients. Similarly, we also detected ANA and anti-52 kDa SSA/Ro antibody positivity in our SLE patient with COVID-19, besides anti ds DNA, Anti SS-A, Anti SS-B, Nucleosome and Anti Histone positivity.

In our case, the coexistence of newly developed SLE disease and COVID-19 may be coincidental. However, the involvement of viruses such as EBV in the etiology of SLE, (13) immune system activation, of which the presence is well-known in COVID-19 disease, (1) and the common antibody that can be found in both diseases (3) demonstrated that COVID-19 infection might trigger SLE disease. Prospective controlled studies are needed to determine whether COVID-19 infection plays a role in the etiology of SLE or other autoimmune diseases.

Conflict of interest

The authors declared no conflict of interest.

Funding

The authors declared that they received no funding during the preparation of this article.

Acknowledgments

None to declare.

Authors' contributions

Concept: Y.M., Design: E.A., Data Collection or Processing: N.S., Ş.K.C., Analysis or Interpretation: M.K., B.P., Literature Search: B.P., Writing: EA., M.K.

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Journal of Experimental and Clinical Medicine
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Case Report

J Exp Clin Med
2022; 39(3): 904-906
doi: 10.52142/omujecm.39.3.58

MRI and pathology findings of a mass-like fat necrosis in the breast

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Received: 08.02.2022 • **Accepted/Published Online:** 05.06.2022 • **Final Version:** 30.08.2022

Abstract

Fat necrosis of the breast is a benign inflammatory process and, it has a wide range of findings on Magnetic Resonance Imaging (MRI), some of which are in the malignant spectrum. We present a case of a breast fat necrosis that mimicked malignancies on MRI because of the intense internal enhancement with a type 3 kinetic curve, its relatively large size, and axillary lymphadenopathy.

Keywords: breast, fat necrosis magnetic resonance imaging, biopsy

1. Introduction

Fat necrosis is a benign non-suppurative inflammation of the fat tissue. The most common cause is trauma, whether iatrogenic or non-iatrogenic. Other rarer causes are anticoagulant therapy, radiotherapy, duct ectasia and mastitis (1). Although patients are generally asymptomatic, symptomatic ones present with a breast lump, which can be accompanied by bruise, erythema, tenderness, skin and/or nipple retraction. Palpable abnormality is usually located superficial and periareolar (1-4). Fat necrosis has a wide range of radiologic findings depending on its stage. Both clinical and imaging findings can mimic malignancy. Typically, there are characteristic findings that can suggest fat necrosis but in some rare cases, it is not possible to distinguish fat necrosis from malignancy with clinical and imaging findings. Therefore, in these cases, biopsy is needed to rule out malignancy (3). We would like to report a fat necrosis case that was not possible to distinguish from malignancy on MRI and core biopsy was performed in order to diagnose.

2. Case Report

A 51-year-old woman consulted our hospital complaining of swelling, erythema and nipple discharge on right breast. She had a history of right breast trauma three days before her consultation. Physical examination revealed hyperemia and warmth at right breast. Upon ultrasound examination, increased echogenicity of the fat tissue on right breast and right axillary lymphadenopathy was found and MRI was suggested to evaluate if there's an underlying malignancy. A month later, MRI was performed. MRI revealed an intense heterogeneous enhancing, fat containing solid mass with type 3 enhancement curve (Fig. 1). It was accompanied by diffuse skin thickness, edema on right breast and lymphadenopathy on right axilla. We could not rule out malignancy and suggested biopsy. Two weeks later, an ultrasound guided core biopsy was done, and the result was fat tissue necrosis (Fig. 2). Three months later, the patient underwent a control ultrasound, and it was normal. Written informed consent has been obtained from the patient to publish this paper.

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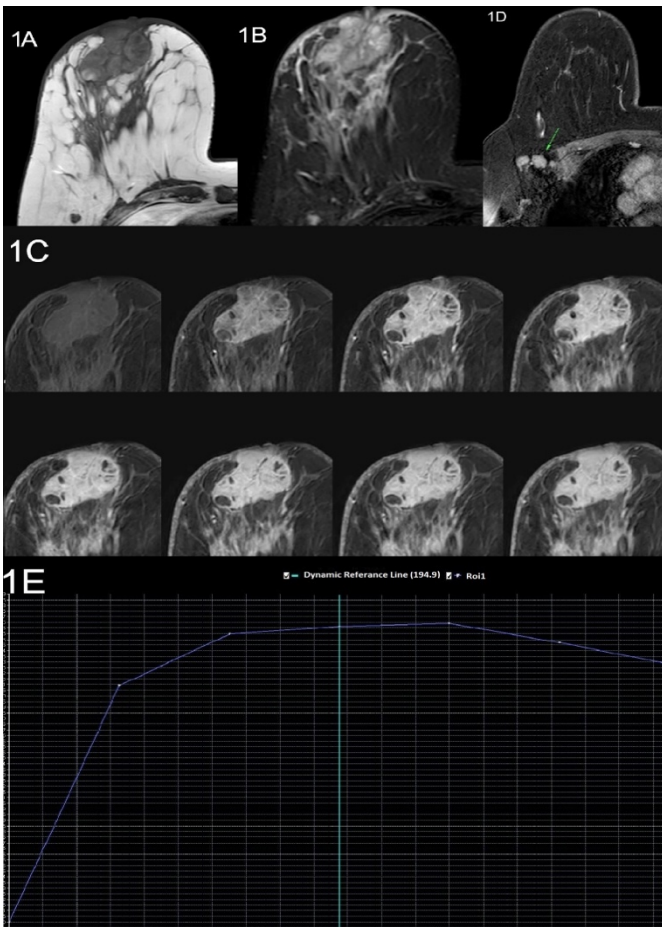

Fig. 1. MRI images; Axial T1-weighted unenhanced (1A), axial STIR (1B), axial T1-weighted fat-saturated contrast-enhanced dynamic images (1C) show approx. 6x4 cm sized heterogeneous enhancing fat containing mass accompanied by diffuse skin thickness, edema on right breast and right axillary lymphadenopathy (1D, green arrow). Kinetic curve of the mass (1E) shows type 3 enhancement curve. Mass extends to the skin and contrast enhancement of the skin adjacent to the mass is increased

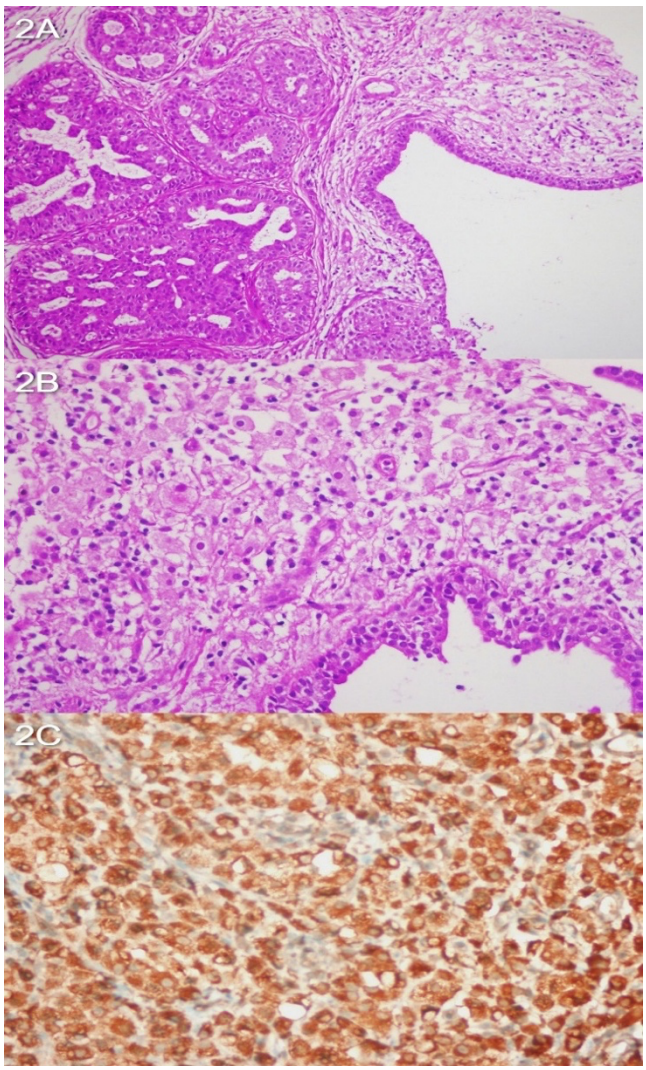

Fig. 2. Fat necrosis Groups of foamy histiocytes around ducts with usual epithelial hyperplasia. H-Ex200(2A), H-Ex400 (2B), CD68 x400 (2C)

3. Discussion

Fat necrosis has a diverse range of radiological findings, some of which are similar to malignancy. Most of the cases, it is easy to diagnose it with trauma history, benign findings that suggest fat necrosis; and a routine annual follow-up is sufficient.

Most of the breast fat necrosis cases in the literature are seen as typical oil cysts with enhancing rim on MRI. Meanwhile, this lesion had a relatively large size, consisting of mostly enhancing solid components and a small amount of fat. It was also accompanied by skin thickening, edema and axillary lymphadenopathy. When we suggested biopsy, we thought of fat-containing malignancies like liposarcoma in the differential diagnosis. Lee et al., (5) reported a similar looking fat necrosis of the upper extremity that mimics liposarcoma.

In the literature, hyperacute inflammatory phase findings are identified as edematous fat tissue, which appears hyperechoic on ultrasound (4). Meanwhile, various findings have been identified for the acute inflammatory phase, some of which are complex cystic lesions, solid lesions with various margin and enhancement features depending on the amount of granulation tissue and severity of fibrosis (4, 6). While findings on ultrasound examination taken a few days after the onset of complaints were consistent with the hyperacute inflammatory phase as defined in the literature, a solid enhancing mass was observed in the MRI taken approximately one month later, and the findings were consistent with the acute inflammatory phase as defined in the literature.

Conflict of interest
None to declare.

Funding
None to declare.

Acknowledgments
None to declare.

Authors' contributions
Concept: A.C., A.V.P., H.A., Design: A.C., A.V.P., Data Collection or Processing: A.C., A.V.P., Analysis or Interpretation: A.C., A.V.P., Y.S., Literature Search: A.C., A.V.P., Writing: A.C., A.V.P., Y.S.

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Journal of Experimental and Clinical Medicine
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Case Report

J Exp Clin Med
2022; 39(3): 907-909
doi: 10.52142/omujecm.39.3.59

Kager's sign; an underestimated radiographic feature in detecting acute Achilles tendon rupture: A report on two cases

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Received: 30.06.2022 • **Accepted/Published Online:** 22.07.2022 • **Final Version:** 30.08.2022

Abstract

This research aimed to show the role of the number of transferred embryos on pregnancy outcomes of the oocyte donation cycles (ODC). This retrospective cohort study included 122 ODCs performed at a private in vitro fertilization (IVF) center between 2020 June - 2021 January. Cases with severe male infertility, tuboperitoneal, and endometrial factors were not included in the study. The median (interquartile range) recipient age was 43 (30–54) years. ODC results revealed that 10.7% of the cases were negative, 4.9% were biochemical pregnancies, and 84.4% were clinical pregnancies. Pregnancy outcomes were checked; miscarriage, preterm, and term delivery rates were 5.7%, 3.9%, and 90.4%, respectively. The rate of recipients for the younger than 40 years was 32%, between the 40–44 years was 27%, and between 45–54 years was 41% respectively. Statistically significant difference was not observed between age groups in terms of endometrial thickness ($p = 0.059$), number of transferred embryos ($p = 0.857$), number of ODC attempt ($p = 0.666$), live birth rate ($p = 0.1$), and other pregnancy outcomes ($p > 0.05$, for all). A total of 96 (78.7%) embryo transfers (ET) resulted in a live birth. In 8.2% (n=10) of cases, single embryo transfer (SET) and in 91.8% (n=112) of cases, double embryo transfer (DET) was performed. The number of embryos transferred was statistically significantly higher among cases that resulted in live births compared to cases without live births ($p = 0.002$). Significant difference was not found in terms of the recipient age ($p = 0.392$), male age ($p = 0.108$), endometrial thickness ($p = 0.478$), and the number of attempt ($p = 0.777$) between cases resulted in live birth or not. The only parameter that affects the live birth rates in ODC is the number of transferred embryos.

Keywords: Oocyte donation cycle, live birth rate, pregnancy outcome, number of transferred embryos

1. Introduction

The Achilles tendon is the most frequently ruptured tendon in the lower extremity, usually associated with sports activities such as basketball, tennis, and diving (1). Injuries caused by abrupt strong plantar flexion or severe dorsiflexion in a plantar flexed foot are frequently noncontact injuries. Acute ruptures are more common in males in their third and fourth decades who participate in sports regularly, and they are commonly misdiagnosed (2). Because the findings of the history and physical examination may be ambiguous (3), radiographic imaging plays a key role in diagnosis in emergency situations. Although Kager's sign has been defined as a radiographic sign of Achilles tendon rupture, its diagnostic efficacy has not been well studied and has not been frequently used in clinical practice (4). Magnetic resonance imaging (MRI) is the gold standard imaging method in the diagnosis of tendinopathies (5), however, it has disadvantages such as being impractical in emergency situations, not being accessible in all facilities, and being expensive. Although bedside ultrasound imaging is increasingly used to assess tendon pathologies (6), it is still operator dependent and not available in many health centers. Due to the advantages, including being easily accessible, rapid and low-cost, radiography should be into focus in the early detection of Achilles tendon rupture. The objective of this report was to present two cases of traumatic Achilles tendon rupture diagnosed early with a positive Kager's sign-on radiographic imaging.

2. Case Report

Case 1

A 55-year-old male patient was referred to the emergency service with acute pain in his left ankle after shifting his weight onto his left leg. There was no audible 'pop,' but he felt sharp pain in his lower leg and couldn't bear weight. He had no previous surgeries or injuries to the affected ankle, and he had no medical history or medications. There was no edema or deformity on the dorsum of the foot or the anterior of the ankle during the physical examination. Tenderness in

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the left retrocalcaneal region was palpable. Thompson test, which has a sensitivity of 96% and a specificity of 93% (7), was negative in our case. The patient's osseous and soft tissues were assessed with lateral radiography of the ankle, and Kager's triangle's deformed edges were observed (Fig. 1).

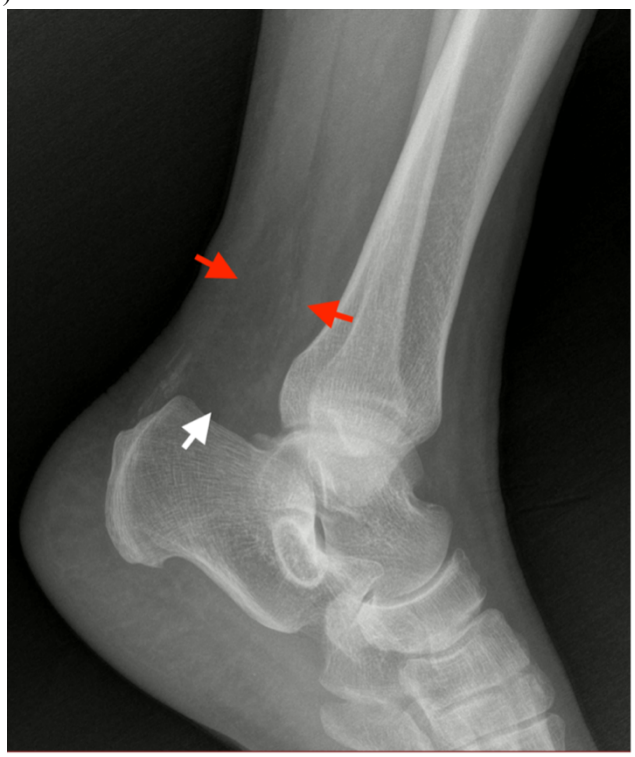

Fig.1. Lateral radiography imaging of the left ankle shows distorted anterior and posterior borders (red arrows) of Kager's triangle with regular inferior border (white arrow).

The patient was diagnosed with acute Achilles tendon rupture without further imaging and was admitted to the hospital with an early operation decision. The patient was placed in a short leg splint with the ankle in limited plantar flexion and admitted to the hospital for surgery. In the operation performed the next day, tendon rupture was surgically confirmed and repaired. The patient recovered completely within two months following the operation.

Case 2

A 23-year-old male patient presented to the emergency service with penetrating trauma in his left ankle from a profile cutting machine. On his physical examination, his vital signs were normal, capillary refill time, skin temperature, and mottling score were in the normal range for both lower limbs. A deep two cm transverse laceration was observed in the posterior left ankle. The limitation of motion in the ankle joint was observed, and the Thompson test was found to be positive. After primary wound closure, a dose of a tetanus vaccine for prophylaxis and empirical antibiotic treatment of 2 g cefazolin and 5 mg/kg gentamicin were given. On lateral ankle radiography, the borders of Kager's fat pad were distorted, but there was no osseous abnormality (Fig. 2a). On Magnetic Resonance Imaging (MRI) which was performed to evaluate the thickness of the tear, a total rupture of the Achilles tendon was observed (Fig. 2b).

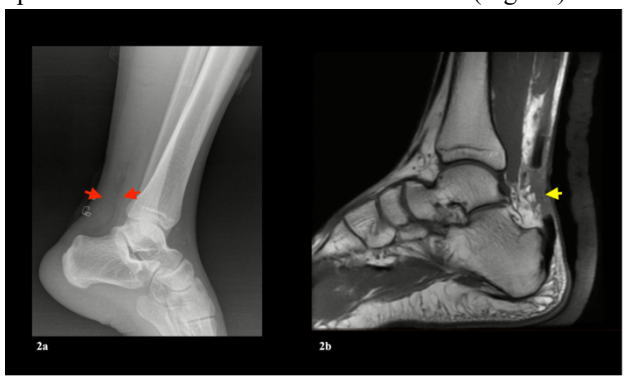

Fig.2. Lateral radiography imaging of the left ankle shows distorted borders of Kager's triangle (red arrows) **(2a).** Magnetic resonance T1W images show a disrupted Achilles tendon with increased thickness and a gap between retracted ends (yellow arrow) **(2b).**

The patient was admitted to the hospital, and the ruptured tendon received surgical repair after 16 hours of injury. The patient recovered completely within four months following the operation.

3. Discussion

Early detection of Achilles tendon rupture is critical since delayed treatment can lead to long-term complications such as ankle joint stiffness and limitation of motion, gait asymmetry or abnormality, and pain. In delayed cases, even if the area between is filled with healing tissue, there is weakness in ankle movement (8).

A positive Thompson test and a noticeable defect around 2-6 cm proximal to the insertion site are common physical examination findings in the diagnosis of Achilles tendon rupture. A quarter of acute Achilles tendon ruptures are misdiagnosed due to a false negative Thompson test, a large hematoma, or plantar flexion caused by extrinsic ankle flexors (9). The significance of recognizing the Achilles tendon rupture by radiography is revealed when the rupture is not detected during a physical examination.

Although magnetic resonance imaging (MRI) is accepted as the gold standard imaging approach for diagnosing Achilles tendon rupture, radiography should be encouraged, which is less expensive and more accessible and can simultaneously evaluate bone, air, and soft tissue.

Kager's triangle is defined as the area filled with adipose tissue, bounded posteriorly by the Achilles tendon, anteriorly by the flexor hallucis longus muscle, and inferiorly by the calcaneus bone (3). Because it is densely packed with fat, Kager's triangle looks like a radiolucent triangle having sharp edges rising beyond the calcaneus on standard lateral ankle radiography. In cases of complete or partial rupture of the Achilles tendon, lateral ankle radiographs show that the soft tissue density increases in the Kager's triangle, its sharp contour disappears, and its borders become unclear and deformed (10). The triangle shrinks, loses transparency, and is

enclosed in a shadowy mesh.

In a study, it was reported that the Kager's sign was positive in the lateral ankle radiograms of all patients with Achilles tendon rupture and confirmed by surgery. During the initial physical examination, 18% of the patients were misdiagnosed, and although all of the patients had Kager's sign, it was not recognized by clinicians (11). Although this suggests that Kager's sign has a good ability to detect Achilles tendon ruptures, clinicians are not much aware of it, and it has not been frequently used in clinical practice.

Surgery is still the most reliable and long-term beneficial method of treatment, and it has been reported that surgery performed within the first 48 hours has superior results (12, 13), highlighting the significance of early diagnosis again.

The clinical use of Kager's sign, which facilitates the radiological diagnosis of Achilles tendon rupture in the early period, especially in emergency departments, should be increased, and further studies should be conducted on its diagnostic value.

Patient's consent

Informed consent was obtained from both of the patients.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

No funding was used for the study.

Acknowledgments

None to declare.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Case Report

J Exp Clin Med
2022; 39(3): 910-912
doi: 10.52142/omujecm.39.3.60

Adamkiewitz syndrome in Marfan syndrome and rehabilitation outcomes-3 cases and review of the literature

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Received: 26.05.2022 • **Accepted/Published Online:** 04.07.2022 • **Final Version:** 30.08.2022

Abstract

Acute Spinal Cord Ischemia Syndrome (ASCIS) is rare but clinically significant. Aortic dissection (AD) is one reason for ischemia of the spinal cord. The artery of Adamkiewicz provides the major blood supply to the anterior thoracolumbar spinal cord, and injury to this artery can cause consequential neurologic damage.Three male patients applied to our rehabilitation clinic with paraplegia. All three had AD due to Marfan Syndrome and clinical findings due to Adamkiewitz artery ischemia. Although we later learned that one of the patients later died from a new ruptured aortic aneurysm, we achieved good motor gains in these rare cases with rehabilitation. Although paraplegia is rare as the initial symptom in AD, vascular etiology should not be forgotten in acute paraplegia. The prognosis of ASCIS is known to be poor, but a good and individualized rehabilitation program is required.

Keywords: Acute Spinal Cord Ischemia Syndrome, Marfan syndrome, paraplegia, rehabilitation

1. Introduction

The spinal vasculature has unique anatomical connections, but there are regions of the spinal cord that can easily become ischemic (1). The artery of Adamkiewicz (AKA), which supplies approximately one-quarter of the spinal cord, is the largest vessel that reaches the spinal cord. AKA mostly originates between T8 and L1 (89%) and has been reported in some people in T4-8 (2). The involvement of the AKA causes ischemia in the anterior regions of the spinal cord, while the posterior cord fed from the posterior spinal artery is less affected. Lesions in T4-6, in particular, are common because this is a 'watershed' area (1).

Acute Spinal Cord Ischemia Syndrome (ASCIS) is rare. However, it is a disease that can cause permanent sequelae. In a study on ASCIS, 16% of cases were associated with aortic pathology (3). Aortic Dissection (AD), whose mortality is 80%, may cause malperfusion or occlusion of segmental arteries feeding the spinal cord, and paraplegia may develop in 2-8% of patients (4,5). Marfan Syndrome (MS) is a genetic disease associated with reduced life expectancy due to the risk of AD and rupture (6). Due to its rarity, 3 MS cases with AD-related AKA Syndrome and their rehabilitation results will be presented here.

2. Case Presentations

2.1. Case 1

A 25-year-old male patient was admitted to our clinic for rehabilitation with complaints of inability to walk. The patient, diagnosed with MS at the age of 14, had a history of surgery due to aortic valve replacement (AVR) and mitral valve replacement (MVR). The patient was admitted to the emergency department due to tearing back pain, an aortic aneurysm rupture was detected, and he was operated. We learned that cerebrospinal fluid (CSF) drainage was inserted in the patient, who developed motor and sensory deficits in the bilateral lower extremity on the postoperative first day, to provide reperfusion in the Medulla Spinalis.

There were a pectus excavatum and an operation scar on the anterior chest wall in the physical examination. The patient had sitting balance. While bilateral lower extremity distal muscle strength was 2/5 globally, right lower extremity proximal muscle strength was 1/5 and left lower extremity proximal muscle strength was 2/5. There was no apparent sensory deficit. We evaluated the patient on the ASIA disorder scale (ABS) D. There was grade 1 spasticity in the lower extremities globally according to the Modified Ashworth Scale. Bilateral patella and Achilles reflexes were hyperactive; pathological reflexes were positive. Routine biochemistry tests were normal.

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We diagnosed the patient with AKA Syndrome in light of the present findings. The patient underwent bilateral lower extremity electrical stimulation, verticalization, cycling exercises, balance and coordination exercises, gait training, breathing exercises, and occupational therapy in our clinic and was discharged to be ambulated with a pair of walking sticks under surveillance. We found from the patient records that the patient died after 1.5 years due to the aortic aneurysm rupture again.

2.2. Case 2

A 25-year-old male patient diagnosed with MS applied to the emergency department due to stabbing pain in his back. The patient was urgently operated for aortic aneurysm rupture and applied rupture repair and aortic valve replacement. External lumbar drainage was applied to provide spinal cord reperfusion. The patient, whose medical stabilization was achieved, was referred to our clinic due to weakness and loss of sensation in the lower extremities.

The general condition of the patient, who was transferred to our service for ASCIS rehabilitation one month after the event, was good, and his systemic examination was normal. The bilateral lower extremity muscle strength was 3/5 in the proximal and 2/5 in the distal. We evaluated the patient's neurological injury level as L2 ABS C. We could not detect the patient's Patella and Achilles tendon reflexes, who had sitting balance and could be ambulated with a wheelchair. or any pathological reflex. There was no incontinence. The patient's Functional Independence Scale (FIM) score was 74 points. We started a rehabilitation program considering the patient's spinal cord damage due to AKA ischemia.

In the rehabilitation program, we gave lower extremity electrical stimulations to the patient and cycling exercises, ambulation training, balance and proprioception exercises, and occupational therapy.

On the 30th day of hospitalization, DTRs were taken as hyperactive, and Babinski's reflex became positive. When he was discharged from the rehabilitation clinic, the patient had ABS C, neurological level L2, and his FIM score reached 84 points. The patient could ambulate up to 40 meters with a pair of soft knee orthoses and a walker.

After two months, the patient was discharged with appropriate anticoagulant treatment and a home exercise program.

2.3. Case 3

A 23-year-old MS patient applied to the emergency department with the complaint of chest pain. Rupture repair with AD diagnosis and external lumbar drainage were performed. On the fifth post-op day, a cardiac pacemaker was implanted in the patient with deep bradycardia. After medical stabilization, the patient was referred to our clinic because of weakness and loss of sensation in the lower extremities.

Three weeks after the event, the patient was transferred to our service with the diagnosis of ASCIS. We performed ABS C and detected neurological level T12. The general condition was good, the systemic examination was roughly normal, the proximal muscle strength of the patient was 3/5, and the distal muscle strength was 2/5. The patient, who was ambulatory at the wheelchair level, had sitting balance. The FIM score was 82 points. The patient did not have pressure sores, had no incontinence and spasticity, could not obtain DTR, and did not have pathological reflexes.

Considering that the patient had ASCIS due to ischemia of the AKA, we initiated a rehabilitation program. We could not give electrical stimulation because he had a cardiac pacemaker. We gave lower extremity strengthening exercises, verticalization with a tilt table, ambulation training, cycling exercises, balance and proprioception exercises, and occupational therapy.

We started pregabalin for neuropathic pain and increased it to 225 mg/day. After a while, the patient's pain subsided; thus, we stopped pregabalin. We evaluated the patient as neurological level L2 and ABS C at the discharge examination. The patient could be ambulated in a short distance with one soft knee orthosis and a walker. The FIM score improved by 13 points.

We advised the patient to continue with the home exercise program and warned him about the risk of falling. He was discharged after three months to be examined in the follow-up.

3. Discussion

The midthoracic part of the spinal cord, which is usually fed by a single anterior radicular artery, is the weakest part of the blood supply; the lower thoracic and lumbar parts are supplied by the anterior radicular arteries, the largest of which is named "AKA" (2).

Spinal cord ischemia usually develops on the basis of atherosclerosis (7). Depending on the infarct site, various spinal cord syndromes may occur. These are Anterior Spinal Artery (ASA) Syndrome, Posterior Spinal Artery Syndrome, Spinal Sulcal Artery Syndrome, "Man-in-the-barrel" syndrome and Syndrome of the AKA (8,9).

Syndrome of the AKA appears as complete transverse spinal cord syndrome. It means that flaccid paresis at the level and spastic paraparesis below the level of infarction, positive pathological reflexes, loss of sensation, and bladder and bowel dysfunction can be seen (8,9). These are similar to our cases' clinical appearance, and except for our third case, we obtained pathological reflexes in all of them.

When we examined their histories, it was remarkable that all of our cases were diagnosed with MS. One of the most critical Ghent criteria for the diagnosis of MS is AD, and the life expectancy is reduced in those with large aortic arch and arch diameters (10). Spinal cord ischemia in dissecting aneurysms is observed at a high rate of 38% (11).

The prognosis of ASCIS is generally not good. In one study, 115 patients with spinal cord ischemia were followed for three years, and approximately 23% of the patients died, 42% of the survivors used a wheelchair, 26% were mobilized with support, and only 32% could walk without support (12). Although we later heard about the death of our first case, all our patients were discharged with support at the end of the rehabilitation program. Ambulatory functional and clinical outcomes, which were likely due to multiple factors, including differences in mean age, etiology of injury, length of follow-up, and most notably, our higher proportion of ASIA A patients, indicating the significant poor prognostic predictor of a severe initial cord injury, differ between 20% and 70% (13).

In conclusion, although paraplegia is rare as the first symptom in AD, vascular etiology should be considered in acute paraplegia. There is no specific treatment other than prevention of complications, treatment of the underlying cause and rehabilitation. Relatively poor prognosis should be recognized, and these cases should be managed in rehabilitation centers.

Conflict of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, or publication of this article.

Funding

The author(s) received no financial support for this article's research, authorship, and/or publication.

Acknowledgments

None.

Authors' contributions

Concept: Z.K.Ü., E.Ü.A., Design: Z.K.Ü., D.C., E.Ü.A., Data Collection or Processing: G.Ç., S.Y.Y., İ.İ., E.Ü.A., Analysis or Interpretation: Z.K.Ü., G.Ç., S.Y.Y., D.C., İ.İ., E.Ü.A., Literature Search: Z.K.Ü., E.Ü.A., Writing: Z.K.U., D.C., E.Ü.A.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Case Report

J Exp Clin Med
2022; 39(3): 913-915
doi: 10.52142/omujecm.39.3.61

Anesthesia management of pregnant with HELLP Syndrome with fetal intrauterine exitus

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Received: 02.06.2022 • **Accepted/Published Online:** 07.07.2022 • **Final Version:** 30.08.2022

Abstract

HELLP syndrome is a serious cause of mortality for pregnant women and requires careful anesthesia management. In this case, anesthesia management and intensive care clinic of a pregnant woman with intrauterine exitus due to preeclampsia and HELLP syndrome is presented.

Keywords: preeclampsia, anesthesia management, HELLP syndrome, intrauterine exitus

1. Introduction

HELLP syndrome is a serious complication of pre-eclampsia characterized by hemolysis, elevated liver enzymes and low platelet count. It can cause serious fetal and maternal morbidity and mortality. In treatment, it is important to give birth as soon as possible (1). Patients with HELLP syndrome are also at risk for multiple organ dysfunction such as pulmonary edema, ablatio placenta, intracerebral hemorrhage, eclamptic convulsions, disseminated intravascular coagulation, and acute renal failure. Perinatal mortality is high. Anesthesia technique is of critical importance in these patients who have a high risk of uncontrollable hypertension, bleeding and multi-organ failure (2, 3). General anesthesia is generally preferred for cesarean delivery in these pregnant women.

In this case, the clinical course of a pregnant woman with intrauterine exitus due to preeclampsia and HELLP syndrome in anesthesia and intensive care is presented.

2. Case Report

A 38-year-old (G4P3) patient at 22 weeks of gestation, with a history of gestational hypertension, was admitted to the emergency department of our hospital with the complaints of nose bleeding and epigastric pain.

In the emergency room, the patient's non-invasive blood pressure (NIBP) was 170/110 mmHg, heart rate (HR) was 97 beats/min, respiratory rate was 20/min, peripheral oxygen saturation (SpO_2) was 98%, and fever was 37C. The patient's admission and postoperative laboratory findings are given in Table 1. Hysterotomy was planned for the patient who did not have fetal heart beat in transvaginal ultrasonography (TVUSG). He was admitted to the intensive care unit for close follow-up with the diagnosis of HELLP syndrome. The patient, whose blood pressure continued to increase despite IV 4 g MgSO4 loading for blood pressure control, 2 g/s infusion and antihypertensive treatment was started in the follow-up, was operated. Since the platelet count was 75 000/mm3, it was decided to apply general anesthesia. Routine monitoring (NIBP, EKG, SpO2) was performed on the patient who was taken to the operating room, and the input values were measured as NIBP: 222/142 mmHg, HR: 116 beats/min, SpO2: 100%. 1mg midazolam was administered for premedication. 1mcg/kg/min remifentanil infusion was started, 2mg/kg propofol and 1mg/kg rocuronium were administered to the patient who underwent preoxygenation before induction. Because of the patient's high blood pressure, IV 20 mg of esmelol was administered before intubation and the NIBP was measured as 170/122 mmHg. Cricoid compression was applied and the patient was intubated with a 7.0 cuffed endotracheal tube. Anesthesia was maintained with 50% O2/air and a minimum alveolar concentration (MAC) of 1 with sevoflurane and 0.4-0.1 mcg/kg/min remifentanil infusion. During t he surgery, NIBP was 168/122- 133/93 mmHg, and HR was 95-83 beats/min. The operation took 40 minutes. No intraoperative complications occurred. The patient did not have any bleeding that required transfusion. The patient was extubated, recurarized with sugammadex and transferred to the intensive care unit. The patient was followed up in the intensive care unit for 24 hours postoperatively, and IV 2g/s maintenance MgSO4 infusion was continued. The patient, whose vital signs were stable during the follow-ups in the intensive care unit, had an average urine output of 100 ml/h and did not have a new

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hypertensive attack, was discharged on the 4th postoperative day with good recovery.

Table 1. Laboratory values of the patient

	Preoperative	Postoperative day 1	Postoperative day 2
Hemoglobin (gr/dl)	12.7	10.2	9
Hematocrit (%)	36	29.5	26.6
Platelets (mm^3)	75.000	79.000	120.000
Prothrombin time	11.6	-	-
Activated partial thromboplastin time	33.2	-	-
INR	0.8	-	-
Alkaline Phosphatase (U/L)	284	227	203
Alanine Amino transferase (U/L)	49	42	30
Aspartate Amino transferase (U/L)	85	69	36
Lactate Dehydrogenase (U/L)	526	464	320
Total Bilirubin (mg/dl)	0.68	0.34	0.31
Direct Bilirubin (mg/dl)	0.38	0.14	0.18
Proteinuria	+1	-	-

3. Discussion

HELLP syndrome is characterized by hemolysis (abnormal peripheral smear or total bilirubin> 20.5 µmol/l), elevated liver enzymes (aspartate amino transferase> 70 IU/l or glutamyl transferase> 70 IU/l), and low platelet count (< 100 000/mm3) and it is a severe form of pre-eclampsia (4, 5).

Although the exact cause of HELLP is unknown, the main underlying cause is the general activation of the coagulation cascade. This leads to microangiopathic hemolytic anemia and platelet depletion. Exposure of liver cells to ischemia explains the elevation in liver enzymes (6). Patients with HELLP syndrome have an increased risk of placental abruption, pulmonary edema, ARDS, ruptured liver hematoma, acute renal failure, cerebrovascular accident, and multiple organ failure (3, 7, 8).

Maternal and neonatal mortality rates are reported as 2-24% and 3-39%, respectively (9, 10). In our case, it was observed that the fetus was intrauterine exitus in the transvaginal ultrasonography (tvUSG) performed in the emergency department. There is a risk of serious maternal mortality if treatment is delayed.

The key point in the anesthesia management of such patients is to control hypertension and eclampsia, and to consider liver and kidney dysfunction and the increase in bleeding tendency (2, 3, 11). The presence of multiple organ dysfunction and coagulopathy in HELLP syndrome may make general anesthesia a safer method than neuraxial anesthesia as long as successful airway management is provided in cesarean sections (12, 13). Presence of thrombocytopenia and coagulopathy may increase the risk of epidural hematoma in neuraxial anesthesia. Although there is no statistical data on the complications of neuraxial blockade in patients with HELLP syndrome in the literature and the platelet count is below the stated value, guidelines recommend a platelet count greater than 100,000 mm3 to minimize this risk (12). Since our patient had a platelet count of 75,000 mm3, we preferred general anesthesia because of the possible risk of epidural hematoma.

General anesthesia is accepted as the preferred method in these patients (2, 3). In general anesthesia, rapid serial intubation technique is used because of the possible risk of difficult airway and aspiration. However, in these patients who already have high blood pressure, harmful hemodynamic changes may occur due to the increased sympathetic response caused by endotracheal intubation. Complications can be minimized at this stage of general anesthesia by administering drugs and procedures that control the hemodynamic response (12). Remifentanil is frequently used to supplement short-term analgesia with cardiovascular stability in high-risk patients (14). In addition, since its metabolism is carried out by plasma cholinesterase, it can be used safely in HELLP cases with liver and kidney dysfunction (15). In our case, remifentanil was used for the induction and maintenance of anesthesia for analgesia. However, since the patient's pre-induction NIBP was very high, such as 222/142 mmHg, esmolol was also used together with remifentanil to reduce the sympathetic response during the intubation phase.

There is no clear consensus on the best approach for anesthesia management in cesarean section cases with HELLP syndrome. There is no official recommendation on when neuraxial anesthesia is safe to administer in these pregnant women with low platelet counts, accompanied by coagulopathies. However, considering the possible complications, the general anesthesia technique in which the airway is secured by intubation with rapid serial induction seems to be a good choice. However, regardless of the preferred anesthesia technique, it should be kept in mind that complications such as eclampsia, pulmonar yedema, heart failure and disseminated intravascular coagulation are still possible in the postpartum period, and most maternal deaths ocur within the first postpartum week (16). Therefore, these patients should be followed up in the intensive care unit after surgery.

Conflict of interest

The authors declared no conflict of interest.

Funding

No funding was used for the study.

Acknowledgments

None to declare.

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Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Case Report

J Exp Clin Med
2022; 39(3): 916-918
doi: 10.52142/omujecm.39.3.62

Large cervical myoma originating from the uterine corpus

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Received: 02.07.2022 • **Accepted/Published Online:** 08.07.2022 • **Final Version:** 30.08.2022

Abstract

This case report aimed to present a case of large cervical myoma that obliterated the ureterovesical area and filled the entire cervix. A 33-year-old patient had a tumoral lesion of approximately 80x60 mm, compatible with myoma, that filled the cervical cavity. On vaginal examination, a barrel-shaped, immobile and large cervix was palpated. A preoperative bilateral double J catheter was applied to the patient. While the uterine cervix was barrel-shaped due to myoma and was palpable from Douglas, it was firmly fixed anteriorly to the bladder and corpus. During bladder dissection, approximately 4 cm defect was formed in the isthmus, and the uterine cavity was entered. A myoma with a diameter of approximately 9 cm, which filled the entire cervix from the uterine isthmus and had a stem attached to the fundus, was observed in the cavity, and the myoma was pulled out from the defect with a tenaculum and removed. Since the anterior cervix could not be reached and bladder dissection could not be achieved, a subtotal hysterectomy was performed. Although they are mainly treated surgically, there is no standard treatment for cervical myomas. Due to its proximity to vital organs, care should be taken against the risk of organ injury.

Keywords: leiomyoma, cervix, hysterectomy, myoma

1. Introduction

Cervical leiomyomas occur rarely, and approximately 1-2% of uterine leiomyomas are cervical (1). The most common symptom is dysmenorrhea. Symptoms of abnormal uterine bleeding and pelvic compression may also develop. It is not surprising that cervical myomas cause compression symptoms due to their proximity to surrounding organs (2).

2. Case Report

A 33-year-old patient was admitted with a complaint of inguinal pain. She had no known chronic, systemic diseases and had 3 cesarean section and tubal ligation surgeries in her history. In the examination with TVUSG, a lesion consistent with a submucous myoma of approximately 4 cm was observed in the isthmic region of the uterus. On pelvic MRI imaging, it was reported that the same lesion was consistent with a well-circumscribed submucous myoma originating from the uterine fundus and extending into the endometrial cavity, measuring 40x88x57 mm at its widest point. In the follow-up of the patient who did not accept the operational procedure 4 months later, the TVUSG examination revealed a tumoral lesion compatible with myoma, approximately 80x60 mm in size, filling the cervical cavity, including the isthmic region. On vaginal examination, a barrel-shaped, immobile and large cervix was palpated. The patient's cervical pap smear was normal and HPV negative. The laboratory examination revealed a Hb value of 7.6 g/dl, platelet of 117000/µL, AST of 35 U/ L, INR of 8.61, total protein of 4.43 g/dl, BG of 181 mg/dl, and creatinine of 1.28 mg/dl. The patient, whose complaint of inguinal pain increased and myoma enlarged, accepted to have the operation. After inserting a bilateral ureteral double-j catheter, the patient's abdomen was entered with a Pfannenstiel incision. On inspection, the uterus fundus and corpus were normal, while the cervical region was globally palpable from the Douglas due to myoma. Anteriorly, the bladder was tightly fixed up to the uterus corpus, and the cervix was inaccessible from the anterior. Bilateral tuba and ovaries were also tightly adhered to the lateral wall of the abdomen and each other. First, the bilateral adnexa were dissected from the lateral wall of the abdomen and released, and a hysterectomy was initiated. During bladder dissection, due to dense adhesion of the lower uterine segment and bladder dome, a defect of approximately 4 cm occurred in the isthmus, and the uterine cavity was entered. A pedunculated, submucous myoma with a diameter of 9 cm extending from the uterine isthmus to the cervix and attached to the fundus was observed in the cavity. The myoma was pulled out from the defect by holding with a tenaculum and removed (Fig. 1, 2). The defect that occurred while trying to remove the dense adhesion in the anterior was determined to belong to the bladder after methylene blue was administered from the catheter, and the defect in the bladder was closed with two layers of 2/0 vicryl sutures. A subtotal hysterectomy was performed since the cervix could not be reached below and bladder dissection could not be achieved.

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Bilateral ureters were dissected, visualization was achieved, and they were observed to be intact. The abdomen was closed after achieving the hemostasis. The patient, who had no complications in the postoperative follow-up, was discharged with the bladder catheter remaining for 10 days.

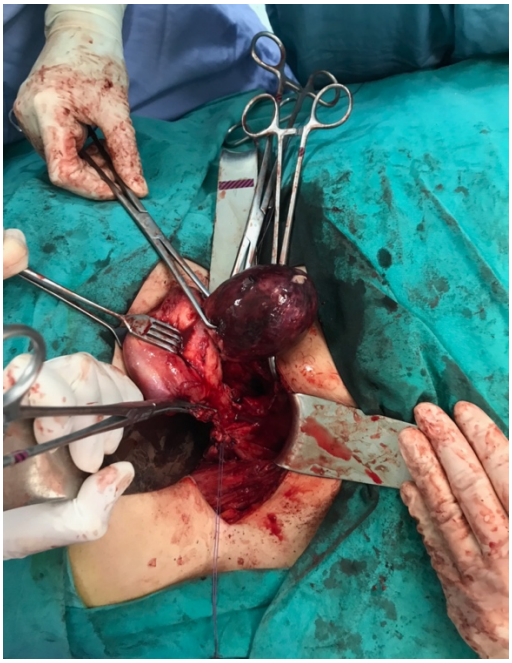

Fig. 1. Large cervical myoma held with a tenaculum

Fig. 2. Large cervical myoma removed from the abdomen

3. Discussion

Cervical myomas are generally classified as subserous (extracervical) and intracervical (intracervical) lesions (3). The anatomical localization of cervical myomas poses a significant challenge in surgical management. It is closely adjacent to the bladder anteriorly, the rectum posteriorly, and the ureters laterally. In cases such as previous pelvic surgery, pelvic inflammatory disease, and endometriosis, severe adhesion to these nearby structures may be encountered, increasing the possibility of organ injury during surgery (4). We had a great suspicion of adhesion since our case had a history of 3 cesarean section operations, and an immobile mass compatible with the frozen pelvis was palpated on vaginal examination. Therefore, we applied a preoperative double J catheter to the patient's ureters. Also, as the size of the cervical myoma increases, it may cause position change, especially by pushing the ureters, and may obstruct the uterine artery and vein (5). Intraoperative hemorrhage risk is also increased due to the proximity of cervical myoma to large vascular structures and vascular hypertrophy due to myoma (6). To minimize bleeding, preoperative use of GnRH agonists (7), uterine artery ligation (8), temporary bilateral uterine artery balloon endovascular ligation (8), and vasopressin injection into myoma (9) have been recommended. However, in very large cervical myomas and the presence of adhesion to the surrounding tissues, it may not be possible to reach the uterine arteries (10). Similarly, cervical myomectomy is difficult to perform due to all these difficulties, and cervical myoma enucleation has also been reported, especially during abdominal hysterectomy in large myomas (11).

In women who want to have children in the future, the location of the leiomyoma on the cervix creates an additional difficulty in the surgical approach. Currently, no standard surgical treatment has been defined for cervical leiomyomas. Therefore, the therapeutic approach depends on the patient's characteristics, fertility desire, and the surgeon's experience. Cases of abdominal hysterectomy, myomectomy, and laparoscopic hysterectomy and myomectomy have been reported in the literature (12).

In addition to surgical treatment, interventional radiology techniques for the treatment of cervical leiomyomas have been reported promising but still limited results. These techniques may be more appropriate in patients who want to preserve the uterus or have contraindications for surgery (13).

Although they are mainly treated surgically, there is no standard treatment for cervical myomas. There are case reports of myomectomy and/or hysterectomy with both laparoscopic and laparotomic techniques in surgical treatment. When choosing a surgical technique, surgical experience, control of the retroperitoneum, the patient's previous surgical history, the size of the myoma, and the patient's expectation of a child should be considered. Particular attention should be paid to the risk of urological injury, especially in patients with a history of previous cesarean sections.

Conflict of interest

The authors declared no conflict of interest.

Funding

No funding was used for the study.

Acknowledgments

None to declare.

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Letter to the Editor

J Exp Clin Med
2022; 39(3): 919-920
doi: 10.52142/omujecm.39.3.63

Effects of COVID-19 pandemic on emergency services

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Received: 18.01.2022 • **Accepted/Published Online:** 29.11.2022 • **Final Version:** 30.08.2022

Dear editor,

We read with great interest Derebey et al.'s article titled "Effects of COVID-19 pandemic on the management of acute cholecystitis: A single tertiary center's experience" (1). We thank the authors for their exciting work investigating the effects of the pandemic on the management of acute cholecystitis, which is a surgical emergency. Additionally, we thank the authors and the editorial board for their courage in publishing this informative and successful article with negative findings. As mentioned in an article named “Highlight negative results to improve science”, published in Nature, highlighting negative results will improve science (2). However, we would like to mention a few points about the impact of the pandemic on emergency service applications.

Emergency services are the first places of application where the emergency medical needs of the patients are met in case of health problems and are one of the most important gates of the health system. Emergency health service includes diagnosis and treatment and coordination between individuals who provide health care and any patient who requires medical, surgical, internal or psychiatric care quickly (3). After the rapidly increasing number of COVID-19 cases and deaths worldwide, governments had to take measures quickly. The increasing number of cases has brought additional burdens to the health system. It has changed the health system, especially during peak periods when the number of cases increases. The importance of triage systems has increased for the effective use of health facilities (4).

Hospitals stopped accepting patients in all clinics during peak periods except for emergency departments. Only emergency and COVID-19 cases were admitted to hospitals. Surgical associations have suggested postponing elective cases, and they have also published recommendations on the priority order of cases (5). On the other hand, the postponement of elective cases and long-term surgical plans have caused additional complications and an additional burden on the emergency department (6).

A decrease in non-COVID-19 emergency admissions is being reported as the number of people hospitalized with COVID-19 increases worldwide. Çıkrıkcı Işık et al. showed in their study a 41.17% decrease in emergency department admissions in Turkey (7). The study of Göksoy et al. revealed a 25% decrease in the number of surgical patients applying to the emergency department during the pandemic period (8).

As a result, it is clear that the pandemic process has caused a change in people's habit of using the emergency service (9). Researchers should be encouraged to conduct new studies to show whether these changes are permanent after the pandemic.

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Letter to the Editor

J Exp Clin Med
2022; 39(3): 920-921
doi: 10.52142/omujecm.39.3.64

Effects of the pandemic on emergency department visits

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Received: 20.03.2022 • **Accepted/Published Online:** 01.07.2022 • **Final Version:** 30.08.2022

Dear editor,

We read with great interest the article titled "The effect of the COVID-19 pandemic on acute appendicitis cases" prepared by Büyükakıncak et al. and published in the 2022 second issue of your journal (1). We thank the authors and editorial board for this article that discusses the non-COVID-19 effects of the pandemic on public health. As emergency medicine specialists, we would like to make a few contributions to the discussion on the impact of the pandemic on emergency department visits.

With the first case seen in Turkey on March 11, 2020, all community and health managers were alert (2). Two new prominent pandemic hospitals were built in our city. Public hospitals also established their own pandemic services. Along with this organizational change, a change was achieved in the standards of patient admission in the emergency department. Emergency services were organized as pandemic rooms and clean rooms. Patients with COVID-19-like symptoms were directed to the pandemic rooms from the hospital entrance. They were treated in these areas to prevent contamination (3). Health workers who met patients at the hospital entrance greeted the patients with protective clothing and triaged according to the patient's symptoms. This situation caused anxiety in society (4). Being in direct contact with patients with COVID-19-like symptoms had also caused anxiety in healthcare workers (5).

Another logical reason for the society's hesitation about applying to the emergency service and the anxiety of the hospital may be the "HES" application developed by the Ministry of Health. With this application, the regions where people with a positive PCR test for SARS-CoV-2 are concentrated are marked in red on the map. During the entire pandemic period, hospitals were marked red in this application (6). Realizing that hospitals are risky areas, the public may have hesitated to come to the hospital, especially those with comorbidities.

The annual number of emergency service applications in Turkey is higher than the country's population. However, emergency service applications suddenly decreased with the announcement of the measures during the pandemic process. Decreased number of applications was observed in emergency diagnoses as well as non-emergency diagnoses. Although the pandemic has placed an extra burden on the health system, the health system in Turkey did not collapse. In fact, despite the closure of non-emergency clinics during the peak periods of the pandemic, our clinical experience has shown that the burden of the emergency service in Turkey during the pandemic period has decreased compared to the pre-pandemic period (6). On the other hand, the closure of non-emergency clinics and the postponement of elective surgeries may have caused non-COVID-19 patients to become more complicated in the emergency department (8). The pandemic has caused new normal in the health organization as well as in community life.

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