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MEDICAL RECORDS-International Medical Journal

Research Article

The Effects of Corona Stimulation on the Osseointegration of Dental Implants: An Experimental Study

Mustafa Ayhan, Humeyra Kocaelli

İstanbul University, Faculty of Dentistry, Department of Oral and Maxillofacial Surgery, İstanbul, Türkiye

Abstract

Aim: Currently, one of the most promising research areas in dental implantology is the exploration of additional procedures to reduce loading time for implants and enhance osseointegration in cases of poor bone quality. Various techniques have been researched and developed for stimulating bone production, including electrical stimulation of the jawbone and surrounding tissues. However, there is limited research on the direct relationship between electrostimulation and osseointegration. This experimental study aims to investigate the effects of corona stimulation (CS) on the rate and quality of osseointegration, as well as its potential to reduce the waiting period for dental implants.

Material and Method: In this experimental protocol, 32 dental implants were inserted into the tibia of four male sheep bilaterally. Implants on the right tibia of each male sheep underwent CS treatment, while the other side served as a control group without any stimulation. The animals were sacrificed on the 15th and 30th days after implantation. Bone segments containing the implants were processed using a noncalcified method. It assessed new bone formation and osseointegration around the dental implants using the undecalcified method and histomorphological analysis. An experienced blinded investigator measured percentages of mineralized bone-implant contact (BIC), bone area (BAr), and bone perimeter (BPm) to evaluate the bone-implant interface. Statistical analyses were performed using SPSS 21 for Windows, with a significance level set at $p<0.05$.

Results: The histomorphometric parameters revealed a significant increase in BIC, BAr, and BPm values in the CS group compared to the control group on both the 15th and 30th days ($p<0.05$). There was no statistically significant difference in BIC ratio between the second and fourth stimulation groups.

Conclusion: The findings of this experimental study suggest that CS may have a positive impact on the early osseointegration period of dental implants.

Keywords: Corona stimulation, dental implant, electrostimulation, histomorphometry, osseointegration

INTRODUCTION

Titanium implants placed in the jawbone typically achieve osseointegration within a few months of the latent phase, as demonstrated in Branemark's 1983 research (1,2). Presently, there is a significant focus within implant research on employing additional techniques to enable early loading of implants and enhancing osseointegration in cases of poor bone quality (3). Various bone-forming methods are under investigation to shorten the osseointegration period and improve success rates in such challenging cases (4). Strategies include enhancing the implant's surface properties, modifying its biochemical and morphological attributes, and boosting bone's inherent healing potential to achieve better bone-implant (BIC) contact both quantitatively and qualitatively (5-7).

Despite numerous studies in this field, current techniques aimed at accelerating postoperative bone healing, reducing prosthetic loading time, or facilitating early loading remain unsatisfactory (8). One such method involves the application of direct or transcutaneous electrostimulation to the bone and surrounding tissues (9). While electrostimulation has shown promise in

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Corresponding Author: Mustafa Ayhan, İstanbul University, Faculty of Dentistry, Department of Oral and Maxillofacial Surgery, İstanbul, Türkiye

E-mail: drmustafaayhan@gmail.com

wound healing and fracture treatments, there is a dearth of literature demonstrating its connection with osseointegration. Transcutaneous electrical nerve stimulation (TENS), a form of electrostimulation, has gained popularity in various treatments in recent years, including expediting wound healing, pain management, and reducing postoperative edema (10).

TENS devices have been shown to exert positive effects on wound healing by stimulating peripheral nerves and vascular structures, increasing blood flow, mitigating edema, providing analgesic effects, and accelerating regeneration. However, their potential impact on osseointegration following implant surgery has not been thoroughly investigated. Furthermore, TENS devices offer ease of use in clinical settings, which enhances patient convenience (11,12).

This research aims to experimentally examine the effect of the Corona Simulation device, a type of TENS device, on the postoperative osseointegration process of dental implants using histomorphometric analysis.

MATERIAL AND METHOD

The experimental protocol commenced following approval from the İstanbul University Animal Experiments Local Ethics Committee, under the reference number 2014/77, on July 18, 2014. Funding for this study was provided by the Scientific Research Project Unit of İstanbul University (BAP project no. 49665). The implants utilized in this research were sourced from Zimmer® (USA). The study comprised four groups: the experimental group and the control group, both of which were euthanized on the 15th and 30th days, respectively.

The Preoperative Preparation

The experimental phase of the study, including the electrostimulation process, took place at İstanbul University Faculty of Veterinary Medicine and involved four rams. Each ram weighed 55±5 kg and was between 12 to 14 months old. They were provided with a diet of concentrated feed (Eriş Fattening Feed - Türkiye) tailored to meet their daily caloric requirements, and they were housed in suitable indoor environments by the İstanbul University Faculty of Veterinary Medicine.

The animals were divided into two groups, with the first group euthanized on the 15th day and the second group on the 30th day of the study. In total, 32 Zimmer® (USA) 3.3×8 mm/Tapered Screw Vent (TSV) implants were applied, with four implants inserted into both the right and left tibias of each ram. The right tibia was designated as the experimental group, while the left tibia served as the control group.

General Anesthesia and Surgical Protocol

The animals were transported from their enclosures to the operating room at the İstanbul University Faculty of Veterinary Medicine Department of Surgery, with the assistance of support staff. For pre-anesthesia, Xylazine HCl (Rompun®, Bayer, Germany) was administered intramuscularly at a dosage of 0.2–0.5 mg/kg, along with intravenous ketamine hydrochloride (Ketalar®, Eczacıbaşı, Türkiye) at a dose of 5 mg/kg. General anesthesia was then induced using isoflurane (Forane®, Abbott. USA). Prior to the surgical procedure, the operative area was shaved and thoroughly cleansed with an antiseptic povidone-iodine solution (Betadine®, Purdue Pharma, USA).

Subsequently, a mid-crestal incision was made near the tibial diaphysis to access the bone, with the removal of both skin and periosteum. At 5 mm intervals, four implants were placed diagonally in the tibia, in proximity to the diaphysis, within the experimental animals. The implant positions were determined using a round steel bur attached to a contra-angle handpiece connected to a physio dispenser, operating at 800–1000 rpm and cooled with sterile saline. Implant cavities, each measuring 8 mm, were created using a 2-mm thick pilot bur followed by a 2.8-mm second bur. Four dental implants (TSV Zimmer®), featuring a diameter of 3.3 mm and a length of 8 mm, were inserted into the tibias of the four rams in bone level position.

Subsequent to the implant placements, subcutaneous tissue closure was achieved using absorbable polyglycolic acid sutures (4.0 Vicryl, Ethicon®, USA), while skin closure was performed with silk sutures (3.0 Doğsan®, Türkiye). All surgical procedures were carried out on the same day by the same surgical team, and the rams were subsequently relocated to a recovery area.

Postoperative Care

A half-dose of the antibiotic Ceftriaxone sodium (1 g) (Iesef®, Ulugay, Türkiye) at a rate of 22 mg/kg was administered intramuscularly every 12 h for 3 days. Subsequently, the sutures were removed one week following the surgical procedure. Throughout the 2 and 4-week recovery periods, the health status of the experimental animals was assessed on a weekly basis. The animals, which were provided with a diet of soft concentrated feed (Erişen Fattening Feed®, Türkiye), were monitored at 6-h intervals daily, post-operation, with particular attention to signs of infection, especially in the wound areas. As a result, all experimental animals completed their recovery period without encountering any complications.

Application of Corona Stimulation

Following the surgery, a postoperative procedure involved applying corona stimulation (CS) in a slow, impulsive mode to the right tibia of the experimental animals using an F3 electrode. This stimulation was administered for 10 min daily over 10 days. In contrast, the left tibia of the experimental animals was designated as the control group (Figure 1).

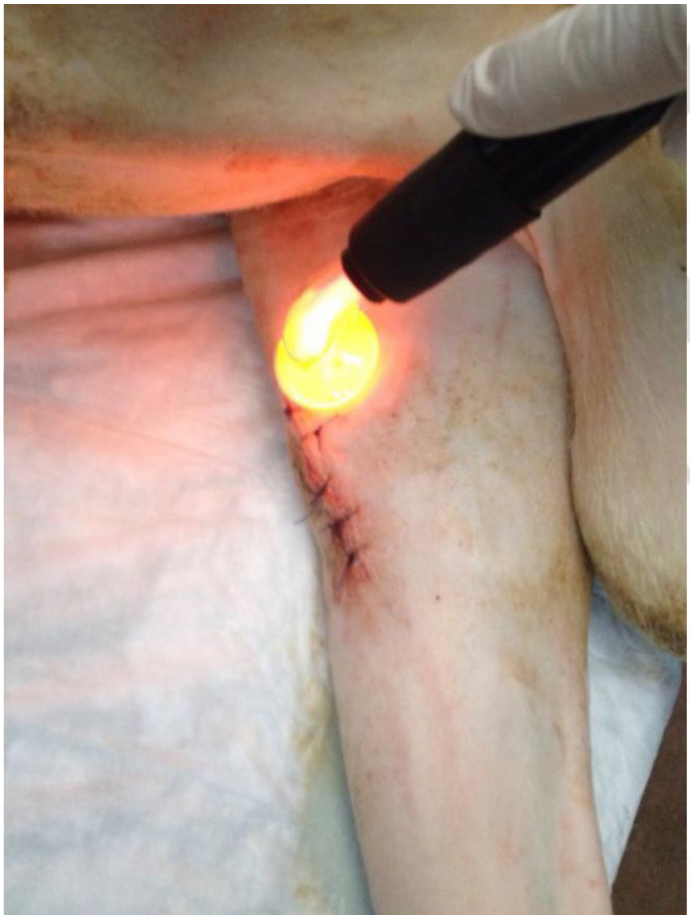

Figure 1. Application of CS in a slow, impulsive mode to the right tibia of the experimental animals

Technical Specifications of the Corona Device

The device in question is a high-voltage glass electrode available in various forms, designed for application on the skin or mucous membranes. These glass electrodes are essentially electron tubes filled with inert gas at low pressure following vacuum sealing. When the device makes contact with the skin, the high voltage at the electrode tips generates a corona discharge. This innovative device was developed by the İstanbul Technical University KOSGEB Technology Development Center.

The corona treatment device operates at a frequency of 22 KHz, boasts a maximum current intensity of 20 mA, a maximum current concentration of 5 mA/cm^2, and a maximum voltage rating of 1200 V. It operates on a 220 V alternating current input and delivers an output voltage ranging from 0.5 to 1.5 KV. This is a monopolar device with a total energy consumption of 40 W, an output energy range from 0 to 35 W, and it generates a sinusoidal current waveform.

Sacrification

Euthanasia procedures were carried out at the İstanbul University Faculty of Veterinary Medicine Department of Pathology. This involved administering intravenous sodium pentobarbital overdose to groups of two experimental animals on both the 15th and 30th days following the surgical procedure.

Before obtaining macroscopic samples from the experimental and control tibia, radiographic images were captured in anteroposterior (A/P) and mediolateral (M/L) projections (Figure 2). Subsequently, under a light microscope at 40× magnification (Olympus DP70, Tokyo, Japan), measurements were taken and recorded for the bone-to-implant contact (BIC), bone area (BAr), and bone circumference (BPm). This was accomplished using a semi-automatic image analysis program (Image Processing and Analysis in Java, Imaje J 1.46 j Version, Wayne Rasband, USA) after staining the sections with Toluidine blue.

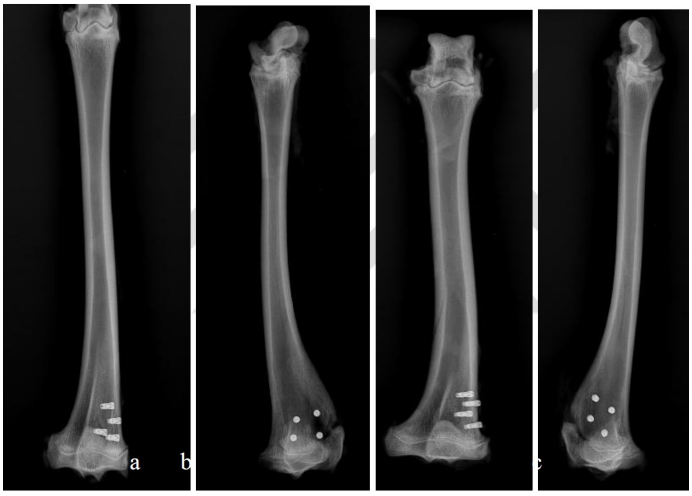

Figure 2. Radiographic images of tibia in experimental (left) and control (right) group

Histomorphometric Evaluation

The experimental and control groups were immersed in containers filled with 10% formalin to ensure thorough sample fixation. The bone thickness around the implant did not exceed 3–4 mm. After a 24-h fixation period, the implants underwent dehydration using ethyl alcohol, followed by plastic infiltration using a methylmethacrylate historesin solution (Technovit® 7200 VLC, Kulzer & CO. GmbH, Friedrickdorf, Germany).

Following the embedding of the samples and acrylic polymerization, parallel surfaces were prepared for the initial cutting of the blocks. The implant sections were then split using the Exakt 300 CP and a diamond saw, resulting in a final thickness of 200 µm. These sections were subsequently stained with Toluidine blue and examined under a light microscope at 40× magnification (Olympus DP70, Tokyo, Japan). Measurements for BIC, bone area (BAr), and bone circumference (BPm) were obtained using a semi-automatic image analysis program (Image Processing and Analysis in Java, Imaje J 1.46 j Version, Wayne Rasband, USA) (Figure 3).

The BIC value represents the ratio of the entire implant surface length to the length of the bone tissue in contact with the implant surface, expressed in percentage (%). The BAr value is the sum of bone areas located between the implant threads, measured in square millimeters (mm²). Additionally, the BPm value corresponds to the total

circumference of the bone tissue formed between the threads of the implants, measured in millimeters (mm).

For the histopathological assessment of the sections, the preparations used in the histomorphometric analysis of the experimental protocol were examined at the İstanbul University Faculty of Medicine, Department of Basic Medical Sciences, Department of Pathology. Upon comparing the 5 µm thick preparations used for histomorphometric analysis with the 100 µm thick sections containing implants, it was determined that the latter were not suitable for histological evaluation to obtain statistically significant results. Therefore, the study included only the results from histomorphometric analysis.

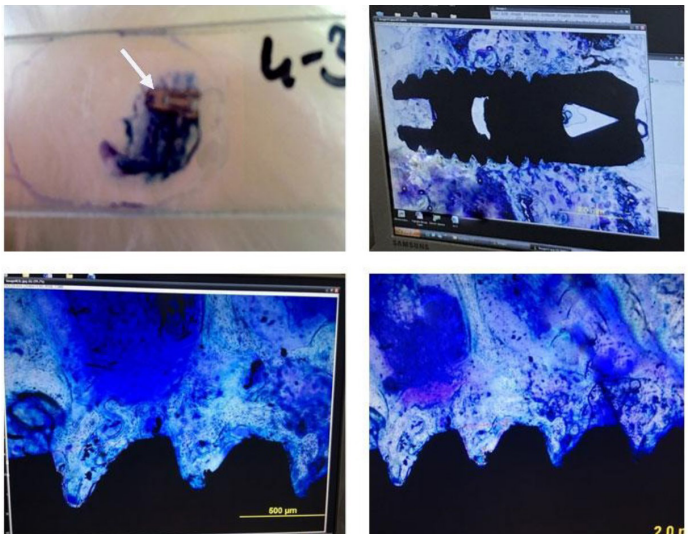

Figure 3. The implant sections were split resulting in a final thickness of 200 µm and examined under a light microscope at 40× magnification

Statistical Evaluation

Statistical analyses were conducted at the İstanbul University Faculty of Medicine, Department of Basic Medical Sciences - Public Health, using IBM SPSS (Statistical Package for Social Sciences for Windows, Version 21.0, Armonk, NY, IBM Corp.). In this study, descriptive statistical methods, including median, minimum-maximum, and standard deviation, were employed and assessed through the Mann-Whitney U test.

To compare parameters that did not exhibit a normal distribution, as well as quantitative data, the groups were analyzed based on median, mean, ±standard deviation, and minimum-maximum values. In the Mann-Whitney U test, the significance level was determined by the median value, and statistical significance was considered at the $p<0.05$ level.

RESULTS

In this research, the impact of coronal stimulation on the osseointegration process of 32 dental implants was investigated. It conducted histomorphometric assessments of the osseointegration process, with implant samples being sacrificed on both the 15th and 30th days. The study comprised four primary groups, each containing eight implants: the 15th-day experimental group (n=8), the 15th-day control group (n=8), the 30th-day experimental group (n=8), and the 30th-day control group (n=8) (Figures 4 and 5).

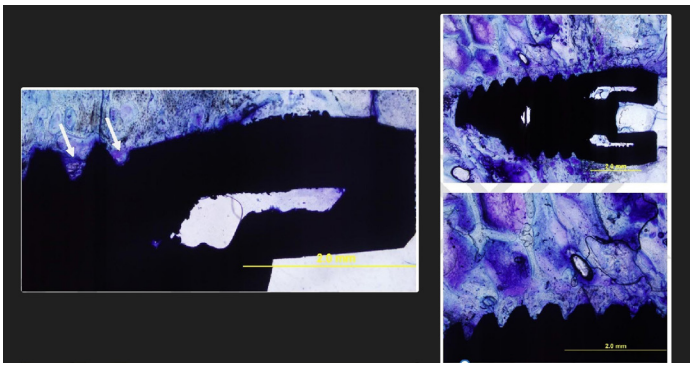

Figure 4. 15th day control (left) and experiment (right) group

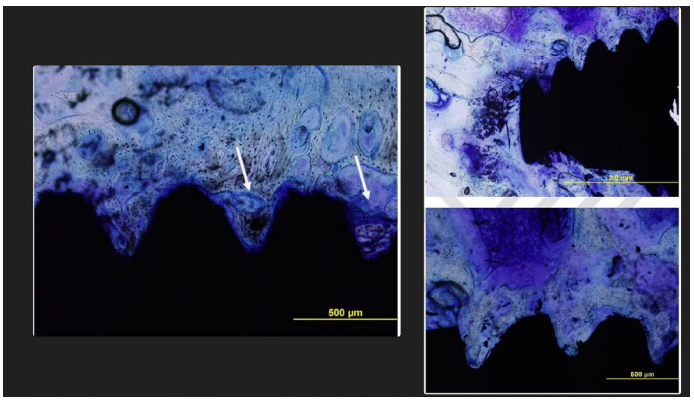

Figure 5. 30th day control (left) and experiment (right) group

In this histomorphometric analyses, three key parameters were assessed: BIC, bone area (BAr), and bone circumference (BPm).

The measurements derived from histomorphometric sections were computed using a specialized computer-aided image analysis program, specifically Image Processing and Analysis in Java, Imaje J 1.46j Version, developed by Wayne Rasband in the USA.

The BIC value represents the percentage obtained by dividing the entire implant surface area by the area of bone tissue in direct contact with the implant surface.

The BAr value quantifies the area of bone formation located between the implant threads, and its unit of measurement is square millimeters (mm^2).

The BPm value, on the other hand, signifies the cumulative length of the bone tissue circumference formed between the implant threads, and its unit of measurement is millimeters (mm).

The exponent symbols {a, b, c, d, e, f, x} used in group comparisons are presented, with significance values determined for {a, b, c, d, e, f} being $p<0.05$, while the significance value for x is $p>0.05$.

When the BIC values between the experimental and control groups on the 15th day were compared, a notable

difference in significance values emerged. Specifically, the BIC value on the 15th day was statistically higher in the experimental group that underwent CS (p=0.001) compared to the control group.

Similarly, when the BIC values between the experimental and control groups on the 30th day were compared, it observed a significant difference. Specifically, the BIC value on the 30th day was statistically higher in the experimental group that underwent CS (p=0.001) compared to the control group.

Similarly, when the BAr values between the experimental and control groups on the 15th day were compared, a significant difference was observed. Specifically, the BAr value on the 15th day was statistically higher in the experimental group that underwent CS (p=0.005) compared to the control group.

Likewise, when the BAr values between the experimental and control groups on the 30th day were compared, a significant difference in significance values emerged. Specifically, the BAr value on the 30th day was significantly higher in the experimental group that underwent CS (p=0.001) compared to the control group.

In a similar vein, when the BPm values between the experimental and control groups on the 15th day were compared, a notable difference in significance values emerged. Specifically, the BPm value on the 15th day was statistically higher in the experimental group that underwent CS (p=0.001) compared to the control group.

Similarly, when the BPm values between the experimental and control groups on the 30th day were compared, a significant difference in significance values was evident. Specifically, the BPm value on the 30th day was statistically higher in the experimental group that underwent CS (p=0.002) compared to the control group (Table 1).

Table 1. Histomorphometric analysis of groups

Groups	n	BIC (%)	BAr (mm²)	BPm (mm)
Day/groups	32	**Mean±SD;med; (min-max)**	**Mean±SD;med; (min-max)**	**Mean±SD;med; (min-max)**
15th day experiment	8	67.71±5.07, 67.50 (61.73-76.25)[ax]	0.56±0.23, 0.47 (0.32-0.91)[b]	6.94±1.22, 6.70 (5.51-9.20)[c]
15th day control	8	35.99±6.09, 35.81 (29.27-48.40)[a]	0.24±0.16, 0.18 (0.13-0.65)[b]	4.07±0.84, 3.99 (2.73-5.20)[c]
30th day experiment	8	68.33±7.97, 70.04 (55.47-78.15)[dx]	0.84±0.27, 0.89 (0.49-1.14)[e]	8.83±1.08, 8.67 (7.54-10.85)[f]
30th day control	8	45.63±8.48, 41.96 (36.72-56.80)[d]	0.34±0.06, 0.35 (0.21-0.43)[e]	5.60±1.51, 5.73 (3.31-8.18)[f]

DISCUSSION

Various forms of electrical stimulation have been utilized for several years to expedite wound healing in both soft and hard tissues (13). Furthermore, they have found application in post-surgical pain and edema control, neuralgiform pain treatment in the craniofacial region, acute fracture management, correction of non-union fractures, periodontal disease treatment, dental procedure anesthesia, and the management of chronic and acute pain in the maxillofacial region (14-17). In recent years, they have also been employed to enhance the osseointegration of dental implants, thus reducing healing time, with ongoing research continually adding to the existing literature (18-21).

In particular, research dedicated to enhancing bone integration through electrostimulation has brought about notable advancements in this field. An initial study by Bassett et al. (22) posited that weak electrical currents could initiate osteogenesis and provided evidence of the beneficial impacts of direct electrical currents on bone formation. Subsequent studies have consistently affirmed the effectiveness of direct electrical currents in stimulating and augmenting osteogenesis (23-25).

In addition to direct electrical currents, non-invasive techniques such as alternating current, electromagnetic fields, and TENS have also displayed positive effects on bone healing. A study by Ciombor et al. (26) underscored the advantageous impact of electromagnetic fields on bone formation, highlighting the potential of non-invasive approaches. Schwartz et al. (27) conducted research on stem cells and demonstrated that pulsed electromagnetic fields (PEMF) enhanced the osteoblastic differentiation of mesenchymal cells, particularly in the presence of BMP-2. Similarly, Sun et al. (28) observed that the application of electromagnetic fields accelerated the proliferation and differentiation of bone marrow stem cells.

The favorable influence of electrical stimulation on mesenchymal cell proliferation has garnered popularity in the context of improving the osseointegration of dental implants. In their study, Gittens et al. (29) examined the impact of electrical stimulation on cell differentiation within an experimental cell culture model. Their results revealed that electrical stimulation augmented the differentiation of MG63 osteoblasts and the production of local factors. Additionally, they observed that the effect of applied polarized electricity was voltage-dependent, with a more pronounced increase in osteoblast differentiation noted at higher potential differences.

Diniz et al. (30) conducted research on the impact of electromagnetic field stimulation on osteoblast maturation in a cell culture setting. Their study demonstrated that electromagnetic fields expedited osteoblast proliferation and differentiation but hindered the formation of bone-like tissue during the mineralization phase. Jansen et al. (31) explored the initial effects of electromagnetic fields on the metabolism and differentiation of human bone marrow stromal cells. Their findings suggested

that electromagnetic fields enhanced mineralization and promoted cell proliferation.

As a consequence, electrostimulation has emerged as a prominent area of research for enhancing the osseointegration of dental implants, particularly during the initial phases of healing. The results indicate that electrostimulation expedites healing in the early stages and fosters osseointegration. Nevertheless, the long-term effects are intricate and warrant further investigation.

In this study, the results revealed that the experimental groups exhibited significantly higher values in comparison to the control groups, thus reinforcing the favorable impact of coronal stimulation on the osseointegration of dental implants. When comparing the experimental groups at 2 and 4 weeks, the absence of significance in BIC values indicates that coronal stimulation expedites early-stage healing and osseointegration. These findings align with prior studies that have similarly identified electrostimulation as having a substantial influence on wound healing and early-stage osseointegration (30-33).

CONCLUSION

This study marks the inaugural experimental exploration of employing the Coronally Stimulated Implant Device (CSID) in dental implant procedures. In this study, results have statistically substantiated the efficacy of CSID during the initial phases of osseointegration. Specifically, the absence of statistical disparities in BIC values between the experimental and control groups at 15 and 30 days implies that CSID exerts a favorable influence on both the early osseointegration's quality and quantity.

Based on these findings, the utilization of CSID could prove advantageous in scenarios where early implant loading is under consideration, particularly for patients with systemic health concerns. Given the straightforward application of this device in oral and maxillofacial surgery, its translation into clinical settings appears practicable. Moreover, owing to its ability to alleviate edema and pain effectively, it may help diminish the necessity for post-implantation medication.

Financial disclosures: Funding for this study was provided by the Scientific Research Project Unit of İstanbul University (BAP project no. 49665). The implants utilized in this research were sourced from Zimmer® (USA).

Conflict of Interest: *The authors have no conflicts of interest to declare.*

Ethical approval: The experimental protocol commenced following approval from the İstanbul University Animal Experiments Local Ethics Committee, under the reference number 2014/77, on July 18, 2014.

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MEDICAL RECORDS-International Medical Journal

Research Article

Anti-Leukemic Effect of Malachite Green-Mediated Photodynamic Therapy by Inducing ER Stress in HL-60 Cells

Metin Caliskan[1], Gulsen Bayrak[2], Sercin Ozlem Caliskan[3]

[1]Uşak University, Faculty of Medicine, Department of Medical Biology, Uşak, Türkiye
[2]Uşak University, Faculty of Medicine, Department of Histology and Embryology, Uşak, Türkiye
[3]Uşak University, Faculty of Medicine, Department of Biophysics, Uşak, Türkiye

Abstract

Aim: Our objective is to explore the relationship between the anti-leukemic impact of malachite green-mediated photodynamic therapy (PDT) and the induction of endoplasmic reticulum (ER) stress in acute promyelocytic leukemia cells (HL-60).
Material and Method: For one hour the cells were incubated with different concentrations (3.125, 1.56, 0.78, 0.39, 0.195, 0.0975, 0.04875 μM) of malachite green and then were exposed to 0.47 mW/cm^2 irradiance and 0.84 J/cm^2 fluence for 30 minutes. Also, HL-60 cells were exposed to PDT with light only and both in the presence or absence of malachite green. MTT assay was used to determine cell viability, and immunocytochemical staining was used to detect the expression of ER stress markers Protein Kinase R-like ER Kinase (PERK) and Glucose-regulated protein 78 (GRP78).
Results: The cell viability of the treatment group (combination of malachite green and light) was significantly decreased compared to the malachite green, control group, and light control. Moreover, immunocytochemical staining scores showed that PERK and GRP78 were significantly upregulated in the treatment group compared with other groups.
Conclusion: Our results indicate that ER stress may contribute to the cytotoxicity occurring in HL-60 cancer cells after malachite green-mediated PDT. Future studies will be crucial in shedding light on the molecular mechanisms underlying ER stress that may occur after PDT. These findings lay the foundation for further investigations in this area.

Keywords: Acute myeloid leukemia, malachite green, photodynamic therapy, ER stress, Protein Kinase R-like ER Kinase, Glucose-regulated protein 78

INTRODUCTION

Acute Myeloid Leukemia (AML), which causes symptoms associated with bone marrow failure and infiltration of organs, is a highly aggressive malignancy of leukocytes. If left untreated, AML invariably leads to fatality, and potentially life-threatening complications can swiftly emerge even in initially asymptomatic patients (1). Chemotherapy, radiotherapy, and allogeneic stem cell transplantation are the main current treatments for leukemia; However, these therapies can cause serious side effects, such as normal cell cytotoxicity, drug resistance, and increased risk of infection, during or after treatment (2). Therefore, there has been a greater focus on seeking physical alternative approaches such as light and sound.

The basis of photodynamic therapy (PDT) is cell death as a result of a series of photochemical/photophysical reactions that occur when light activates photosensitizers at the appropriate wavelength in the presence of oxygen. The activated photosensitizer in the unstable excited structure transfers the excess energy to the surrounding molecules via two types of mechanisms. The sensitizer One is the direct energy transfer to molecular oxygen, which forms singlet oxygen, and the other is the transfer of energy to an electron or proton, which produces reactive

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Corresponding Author: Sercin Ozlem Caliskan, Uşak University, Faculty of Medicine, Department of Biophysics, Uşak, Türkiye
E-mail: srn.ozlem@gmail.com

oxygen species (ROS) (3). While it is acknowledged that both possibilities contribute to cell death, the specific mechanism that predominantly leads to cell death varies depending on the presence of oxygen, the concentration of substrates, and especially the photosensitizer used (4). Singlet oxygen and/or hydroxyl radicals generated through photosensitizer activation are highly reactive and have a very short lifespan (0.04 microseconds). As a result, they are effective within a very small region (0.02 μm) (5). These parameters suggest that PDT is a localized treatment. PDT has serious advantages over traditional cancer therapies as it can be repeated in multiple doses and is a minimally invasive and localized therapy method. There is no risk of cancer cells developing resistance to PDT (6).

ER is the primary organelle in the cell that performs many cellular functions such as maintenance of cellular homeostasis, intracellular Ca^{2+} storage, protein folding, modification, and assembly (7). PDT causes apoptosis as a result of oxidative stress through the production of large amounts of ROS, and ER stress, which is one of the mechanisms underlying cancer cell death, as a result of the induction of GRP78, a chaperone in the ER and critical modulator of the unfolded protein response (UPR), through the accumulation of unfolded proteins (8-11). Under non-stress conditions, PERK, an ER membrane protein with luminal stress-sensing domains, forms a complex with a significant amount of the ER chaperone immunoglobulin binding protein. This interaction keeps PERK inactive, and when ER stress occurs, it becomes active and phosphorylates factors that will reduce new protein translation (12). In addition, excessive protein accumulation in the ER lumen results in an increased need for proteins essential for protein folding (11,13). The safety and effectiveness of PDT largely depend on photosensitizers, which are considered an important factor with specificity and phototoxicity. Malachite green is a triarylmethane dye with an absorption band at approximately 617 nm (14). Triarylmethane dyes are of interest as antimicrobial and anticancer agents due to their selective localization and structural properties (15). In this study, we examined the effect of malachite green-mediated PDT on ER stress in HL-60 cells.

MATERIAL AND METHOD

Cell Culture

HL-60 cells were cultured in RPMI 1640 (+) L-glutamine medium supplemented with 1% Penicillin-Streptomycin and 10% FBS in 25cm^2 flasks in a humidified and 5% CO_2 incubator at 37°C. In laminar airflow, the culture medium was renewed every 2-3 days.

Photosensitizer

In this study, malachite green, which has a cationic structure, was used as a photosensitizer. Malachite green stock solution was prepared in PBS. Final experimental malachite green concentrations were determined as 3.125, 1.56, 0.78, 0.39, 0.195, 0.0975, and 0.04875 μM. Cells ($1x10^5$) were exposed to malachite green at 37°C for one hour.

Study Design

In this study, 4 different groups were studied using different concentrations of malachite green.

Group 1: Control: No exposure (malachite green or light)

Group 2: Light control: Only exposure to red light for 30 minutes

Group 3: Malachite green: Only exposure to all concentrations of malachite green for one hour

Group 4: Malachite green mediated PDT: One hour of exposure to malachite green followed by 30 minutes of exposure to red light from a distance of 10 cm

Malachite Green Mediated Photodynamic Therapy Using Red Light

After exposure to malachite green for one hour, centrifugation was performed at 250 g for five minutes and the supernatant was discarded to remove any remaining free malachite green and fresh PBS was added over the cell sediment. Centrifugation, discarding the supernatant, and adding fresh PBS were repeated 3 times in all groups. The light source utilized was an LED system (O'melon Omega Led) comprising 283 units across three panels emitting red light at a wavelength of 640 nm, which is optimal for activating malachite green. A power meter (Newport, USA) was used to measure light output; An irradiance of 0.47 mW/cm^2 was quantified and a fluence of 0.84 J/cm^2 in 30 minutes was calculated. Following the applications, fresh medium was added to all groups and incubated at 37°C for 24 hours.

Analysis of Cell Viability of HL-60 Cells by the MTT

3-(4,5-Dimetiltiazol-2 yl)-2,5-Diphenyltetrazolium Bromide (MTT) is a water-soluble tetrazolium salt that, if degraded by the dehydrogenase enzyme in viable cell mitochondria, is converted into a soluble formazan. Cells were assessed by a spectrophotometric method using MTT solution in 96 microplates. After treatment, the MTT reagent was applied equally to all groups, followed by a 24-hour incubation period. The solubilization buffer was added and left to incubate at 37°C overnight. Cell viability percentages were calculated using optical density (OD) measurements taken from each well in the wavelength range of 550 to 600 nm using a microplate spectrophotometer.

ER Stress Analysis

Immunocytochemical Markers

The cells were centrifuged and then the pellet was spread on the slides and then the slides were allowed to dry. The cells were then fixed with cold methanol. After fixation, slides were washed with PBS and were kept in for 10 minutes with 3% hydrogen peroxide to block

endogen peroxidase. Then the slides were washed with PBS and after dropped onto the slides normal goat serum (Invitrogen- 50062Z) for blocking for 8 minutes. After blocking, the slides were incubated overnight at +4°C with primary antibodies: Anti-PERK (1:100, bs2469R; Bioss) and anti-GRP78 BiP/HSPA5 (1:100, PB9640; Boster). Following this incubation period, the slides were treated with anti-rabbit IgG secondary antibody (1/200, Thermo Scientific, 65-6140) for 30 minutes, followed by a thorough PBS wash. Subsequently, Horseradish peroxidase (HRP, 1/200, Thermo Scientific, 43-4323) was introduced and allowed to incubate for an additional 10 minutes at room temperature in the dark. The reaction was developed using chromogen diaminobenzidine (DAB, Abcam, ab64238). After washing the slides with distilled water, they were mounted with entellan. Finally, the slides were examined under a light microscope (Olympos BX50) and images were captured using the attached camera. For immunocytochemical scoring, 100 cells were counted in 4 different areas at X400 magnification and the staining intensities of these cells were scored as strong (++++), medium (+++), weak (++), or absent (+). All slides were evaluated by the same histologist.

Statistical Analysis

One-way analysis of variance (ANOVA) was used to determine our data to assess potential differences between at least two groups (IBM SPSS Statistics 25, USA). ANOVA followed by the Tukey test was used as a post-hoc analysis for further evaluation. Statistical significance was accepted as p-value≤0.05.

RESULTS

Cytotoxic Activity of Malachite Green and Malachite Green Mediated PDT in HL-60 Cells

The results showed that malachite green-mediated PDT exposure caused a significant decrease in the viability of HL-60 cells. Cell viability at all concentrations of the malachite green-mediated PDT group was significantly lower than the same concentrations of the malachite green and other groups (p<0.001). Cell viability percentages of control, light control, and malachite-mediated PDT groups were determined as 95.2±1.70%, 92.4±1.15%, 75.6±3.59%, 74.8±1.65%, 72.9±1.95%, 68.6±0.72%, 60.5±1.94%, 52.1±2.85%, and 36.2±2.15%, respectively. The results demonstrated that malachite green-mediated PDT significantly increased the cytotoxicity in HL-60 cells, and cell survival is positively correlated with malachite green concentration. There was no significant difference in cell viability between the control and light control groups (p=0.797). In the malachite green group, cell viability percentages were determined as 87.4±0.92%, 85.1±2.66%, 85.8±2.04%, 79.4±0.95%, 77.3±2.08%, 71.6±1.52%, and 63.3±1.52% from low to high concentration, respectively. It was observed that the cell viability of malachite green group was higher than the malachite mediated PDT group (Figure 1).

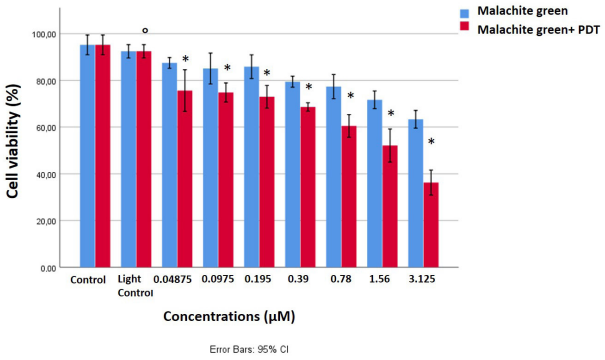

Figure 1. Evaluation of cytotoxicity after treatment with control, light control, MG and MG-mediated PDT. The data represent the means±standard deviations (SDs) of 3 independent experiments. * indicates statistically significance compared to control group (p<0.001); º indicates not statistically significance compared to control group (p>0.05), Error bars 95% confiedence interval

Examination of Immunostaining Markers of ER Stress after Malachite Green, Malachite Green- Mediated PDT

Immunocytochemistry analysis was performed by examining the changes in GRP78 and PERK staining in control, light control, malachite green, and malachite green-mediated PDT groups. In the control group, GRP78 (Figure 2I, 3H) and PERK (Figure 4I, 5H) were found to be weakly stained. There was no significant difference in staining intensity when the control and light control groups were compared (Figure 2H, 4H). In both the malachite green group and the malachite green-mediated PDT group, both GRP78 and PERK staining intensities increase as the malachite green concentration increases (Figure 3A-H and Figure 5A-H) (Figure 2A-H and Figure 4A-H). As shown in Figure 6, both GRP78 and PERK staining showed a significant increase in the malachite green-mediated PDT group compared to the malachite green group, light control group, and control group (p<0.001, Figure 6A-D).

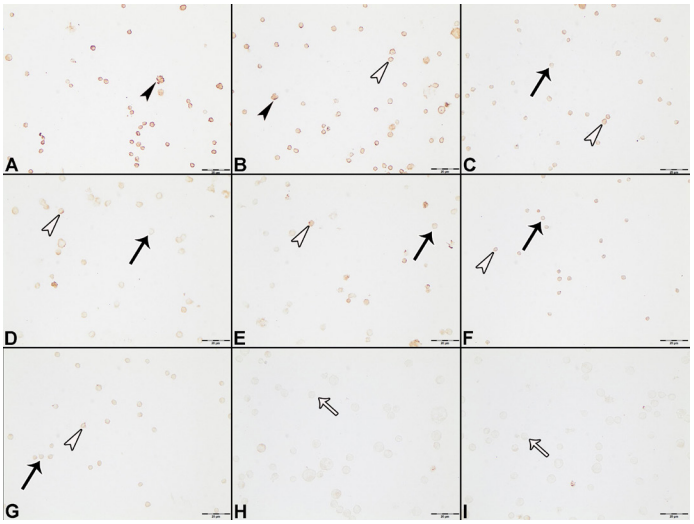

Figure 2. GRP78 immunostaining of Malachite green mediated PDT groups. 3.125µM Malachite green mediated +PDT (**2A**); 1.56µM Malachite green mediated +PDT (**2B**); 0.78µM Malachite green mediated +PDT (**2C**); 0.39µM Malachite green mediated +PDT (**2D**); 0.195µM Malachite green mediated +PDT (**2E**); 0.0975µM Malachite green mediated +PDT (**2F**); 0.04875µM Malachite green mediated +PDT (**2G**), light control (**2H**) and control (**2I**). Strong staining (black arrowhead), medium staining (white arrowhead), weak staining (black arrow), absent staining (white arrow). All photos imagination X400

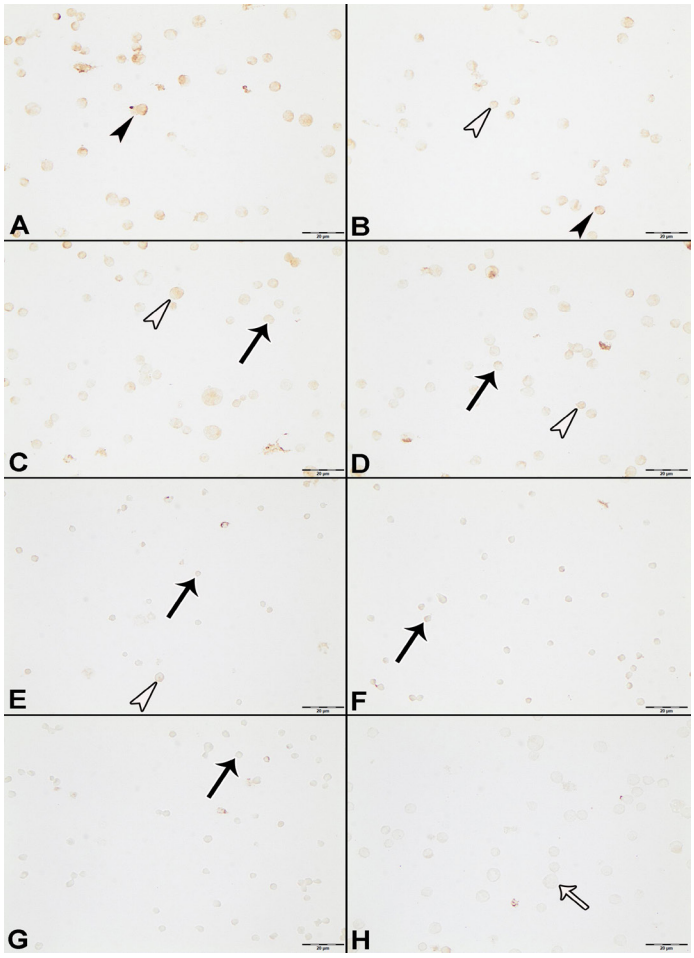

Figure 3. GRP78 immunostaining of malachite green groups. 3.125µM malachite green (**3A**); 1.56µM malachite green (**3B**); 0.78µM malachite green (**3C**); 0.39µM malachite green (**3D**);0.195µM malachite green (**3E**); 0.0975µM malachite green (**3F**); 0.04875µM malachite green (**3G**) and control (**3H**). Strong staining (black arrowhead), medium staining (white arrowhead), weak staining (black arrow), absent staining (white arrow). All photos imagination X400

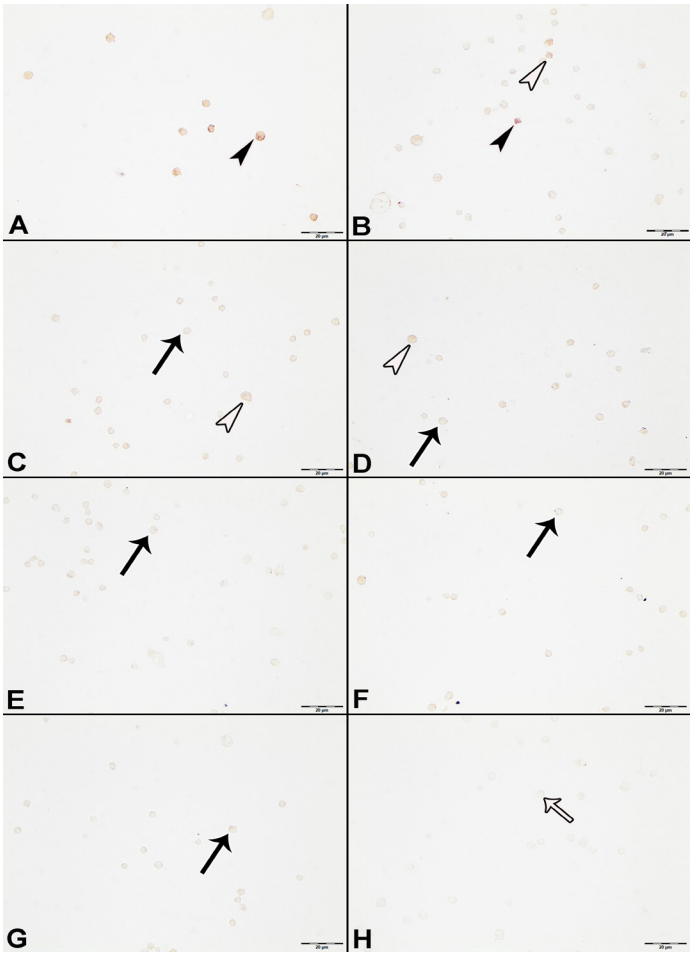

Figure 5. PERK immunostaining of malachite green groups. 3.125µM malachite green (**5A**); 1,56µM malachite green (**5B**); 0.78µM malachite green (**5C**); 0.39µM malachite green (**5D**); 0.195µM malachite green (**5E**); 0.0975µM malachite green (**5F**); 0.04875µM malachite green (**5G**), and control (**5H**). Strong staining (black arrowhead), medium staining (white arrowhead), weak staining (black arrow), absent staining (white arrow). All photos imagination X400

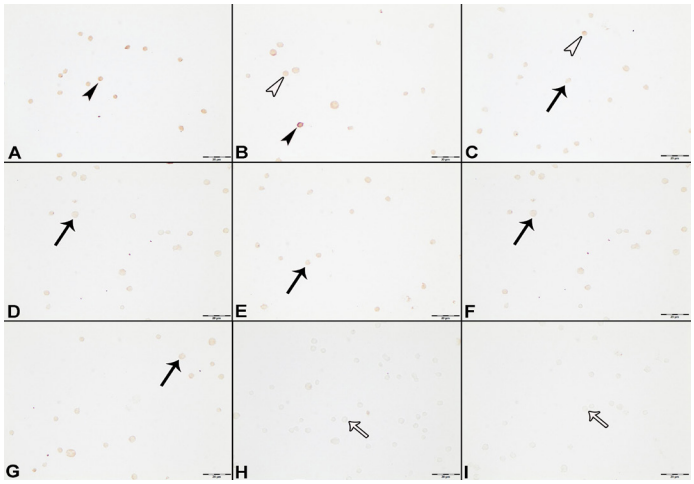

Figure 4. PERK immunostaining of malachite green mediated PDT groups. 3.125µM malachite green mediated PDT (**4A**); 1.56µM malachite green mediated PDT (**4B**); 0.78µM malachite green mediated PDT (**4C**); 0.39µM malachite green mediated PDT (**4D**);0.195µM malachite green mediated PDT (**4E**); 0.0975µM malachite green mediated PDT (**4F**); 0.04875µM malachite green mediated PDT (**4G**), light control (**4H**) and control (**4I**). Strong staining (black arrowhead), medium staining (white arrowhead), weak staining (black arrow), absent staining (white arrow). All photos imagination X400

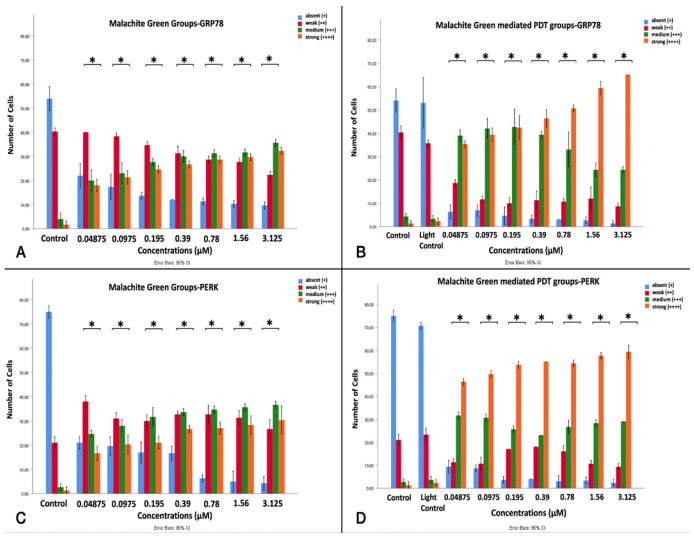

Figure 6. Statistical analysis of GRP78 malachite green groups (**6A**), GRP 78 malachite green mediated PDT groups (**6B**), Statistical analysis of PERK malachite green groups (**6C**), PERK malachite mediated PDT groups (**6D**). *Significance according to the control groups. The data represent the means±standard deviations (SDs) of 3 independent experiments. Error bars 95% confiedence interval

DISCUSSION

The classical treatments for leukemia come with specific drawbacks. Transplantation of allogeneic hematopoietic stem cells may cause significant risks for both the recipient and the donor. Chemotherapy can cause severe and various side effects and may lose its effectiveness as a result of drug resistance. High-dose radiotherapy may not eliminate cancer cells and may cause various side effects during or after treatment (16). PDT stands out as a minimally invasive treatment method for a wide range of malignancies. It is the subject of clinical research both as a treatment and as a complementary therapeutic procedure to other treatments (13). Although previous studies have shown that acute monocytic leukemia and chronic myeloid leukemia cells can be effectively killed using a variety of classical photosensitizers such as zinc phthalocyanine, Nile blue, 5-aminolevulinic acid (5-ALA), hypericin, malachite green, and Photolon® (17-22), the use of PDT It has received relatively less interest in the treatment of leukemia. The results of our study showed that malachite green-mediated PDT caused a significant decrease in the proliferation of HL-60 cells compared to both controls and malachite green.

ER stress induced by therapeutic agents contributes to cancer cell death and often occurs simultaneously with oxidative stress. However, the molecular mechanisms that will explain the relationship between ROS and apoptosis caused by ER stress are not yet known (23). Apart from therapeutic agents, PDT provides cancer cell death by creating harmful levels of ROS in the tumor tissue (13). Buytaert et al. have reported that hypericin, which is localized in the ER membrane and activated by light, produces ROS, which rapidly depletes ER Ca2+ stores, resulting ultimately in apoptotic cell death and mitochondrial dysfunction (24).

PDT-induced ER stress is a cause of cancer cell death (10). When ER stress is excessive, it initiates the ER-associated apoptotic pathway, leading to cellular apoptosis (25,26). ROS promotes the expression of apoptotic proteins by triggering ER stress, thus causing apoptosis, and is one of the most important executors of apoptosis (27). Chirante et al. have shown that following lipophilic copper(II) phthalocyanine (Pc9)-mediated PDT on CT26 colorectal cancer cells, the expression of various ER chaperones such as Hsp90, Hsp110, calnexin, and GRP78/BIP, increased. Additionally, they demonstrated that ER stress provided to apoptotic cell death by activating the mitochondria-dependent apoptotic pathway (28). Firczuk et al. have shown that Photofrin®-mediated PDT induces upregulation of GRP78 protein expression in Du145 prostate cancer cells (11). Zuo et al. reported that pheophorbide a-based PDT on HOS human osteosarcoma cells could induce cell apoptosis and increase GRP78 expression (29). Moserova and Kralova reported in their study on HL-60 acute promyelocytic leukemia and 4T1 mouse mammary carcinoma cells that the activation of the PERK pathway is a crucial trigger point for the ER stress induced by mTPP(EG)4-mediated PDT (30). It has been reported that the PERK plays a role in inducing autophagy or apoptosis in tumor cells during ER stress and after PDT treatment in many studies (31,32).

In our study, we observed upregulation of PERK and GRP78, markers of cell death and ER stress, in HL-60 cells following malachite green-mediated PDT. We conclude that further studies on ER stress may contribute to the success of treatment.

Limitations

This study has several limitations. One limitation is that our study is an in vitro study and it is unclear what the outcome will be in the patient. Another limitation is that not all proteins involved in the UPR response were examined. Finally, the mechanism that causes cell death has not been demonstrated. The strength of our study is that it provides preliminary information for the development of treatment focused on ER stress in malachite-mediated PDT.

CONCLUSION

We observed that malachite green-mediated PDT caused ER stress in HL-60 cells. Therefore, malachite green-mediated PDT may be a treatment option or an adjunct method in the treatment of acute myeloid leukemia through ER stress-induced cytotoxicity. In conclusion, a significant relationship was observed between cell death and ER stress markers. We are planning studies to observe the underlying molecular mechanisms.

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Conflict of Interest: *The authors have no conflicts of interest to declare.*

Ethical approval: *Since the methodological structure of the study is a "cell culture study", it does not require ethics committee approval in accordance with the World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research on Humans".*

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Research Article

Investigation of Functional Disability, Pain, And Quality of Life in Patients with Cervical Radiculopathy by Gender

Aydin Sinan Apaydin[1], Musa Gunes[2], Metehan Yana[2]

[1]Karabük University, Faculty of Medicine, Department of Neurosurgery, Karabük, Türkiye
[2]Karabük University, Faculty of Health Sciences, Department of Physiotherapy and Rehabilitation, Karabük, Türkiye

Abstract

Aim: This study aimed to compare pain, functional limitation, disability, and quality of life in females and males with cervical radiculopathy and to evaluate their relationship.
Material and Method: A total of 111 patients of both genders, 81 (72.98%) females and 30 (27.02%) males, were included in the study. Pain and numbness of the patients were evaluated with the Numeric rating scale (NRS), neck disabilities with the Neck disability index (NDI), and quality of life with the EuroQol Five-Dimensions– 3-Level (EQ-5D-3L). Functional limitation caused by cervical radiculopathy was assessed with the Cervical radiculopathy impact scale (CRIS).
Results: The average age of the patients was 45.59±11.00. While the severity of neck and arm pain was similar between genders ($p>0.05$), numbness radiating to the arm and hand was more severe in females ($p=0.027$). Also, female's quality of life outcomes was worse than males ($p<0.05$). However, there was no difference between genders in CRIS subheading scores ($p>0.05$). This study determined a moderate negative correlation between CRIS symptoms and quality of life parameters and a moderate-weak correlation between CRIS symptoms with pain and numbness ($p<0.01$).
Conclusion: This study determined that female's disability and quality of life were worse. In addition, the severity of numbness females feel in the upper extremities is higher. Additionally, functional limitation is associated with pain and quality of life in patients with cervical radiculopathy.

Keywords: Cervical radiculopathy, functional disability, pain, quality of life

INTRODUCTION

Radiculopathy may be elaborated as the pathology in which progression along a spinal nerve and its processes is limited or blocked. Clinically, pain and paresthesia occurring in a single extremity and radiating along a nerve root suggest radiculopathy. Symptoms may also include muscle weakness and loss of sensation. Cervical radiculopathy is a clinical syndrome caused by compression of the cervical nerve nerves. It is a condition of fractures due to underlying dysfunction in the cervical spine nerves, nerve roots, or both (1). It is most distinguished from spondylotic changes such as cervical disc herniation and proliferation of bone cells in this area, which lead to nerve root loosening and inflammatory changes (2).

It manifests itself with pain as the initial symptom. When the pain spreads from the neck to the shoulder and arm, cervical radiculopathy, which causes sensory complaints and motor weakness, should be suspected. Cervical radiculopathy occurs in the weakness of a nerve root, accompanied by sensory and motor complaints or reflex changes depending on the condition of the affected nerve (3). Cervical radiculopathy is observed in both genders and varies mostly between 50–54 (4). The incidence rate of cervical radiculopathy was 83.2/100.000/year. Depending on gender regime, it is more common in males, 107.3/100.000/year, 63.5% (5). The performance of cervical radiculopathy is less than that of lumbosacral radiculopathy. A prevalence study stated that while cervical radiculopathy was 3.5/1000, lumbosacral radiculopathy was 9.8/1000 (6).

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Corresponding Author: Aydin Sinan Apaydin, Karabük University, Faculty of Medicine, Department of Neurosurgery, Karabük, Türkiye
E-mail: dr.sinanapaydin@yahoo.com

The most common cause of cervical radiculopathy is foraminal spinal nerve compression in the foraminal area (70–75%). The factors that cause this are disc degeneration that narrows the neurological foramen due to loss of distribution in the vertebra or intervertebral disc, posterolateral herniation of the intervertebral disc, and cervical spondylosis due to degenerative changes in the vertebral body, the joints occurring in the anterior or the facet joints in the posterior (7). Causes of cervical radiculopathy vary depending on age. While disc herniations and acute damage are frequently responsible in the young population, foraminal changes due to osteophyte and spondylosis are frequently responsible in older ages (8).

Unlike axial neck pain, radiculopathy often presents with unilateral pain. This may be replicated with ipsilateral dermatome. However, a diagnosis of cervical radiculopathy can be made even if there is no pain in the arm. In addition, the pain can spread to the arm and cause loss of sensation along the dermatome. Additionally, weakness along the myotome where it spreads may accompany patients. It has been reported that radicular pain is most caused by C7 root involvement, followed by C6 and C8 levels, respectively (9). Therefore, surgery, inflammatory drugs, steroid injections, and physical therapy are recommended for treatment in patients. Surgical treatment often significantly improves pain and limitation when other treatments have failed (1,3,4).

Degenerative changes in the spine observed with aging and neck posture and nerve damage and compression in individuals with disc herniations may cause radicular pain. However, previous studies clearly stated the relationship between pain caused by regularly developing cervical radiculopathy and functional deficit, disability, and quality of life due to expanding radiculopathy (1-3). In addition, the lack of a general outcome study evaluating the functional limitation caused by radicular pain has led to a deficiency in evaluating functional limitation (10). The Cervical Radiculopathy Impact Scale (CRIS) is a newly developed scale that uses this ability to evaluate the functional limitation of radicular pain. This potential extends the gap between pain, functional disability, disability, and quality of life with cervical radiculopathy (10,11). However, the relationship between functional limitation due to cervical radicular pain and clinical symptoms has not been adequately examined. Additionally, this limitation due to radiculopathy may differ between genders. In a study, Singh et al. showed that females with cervical radiculopathy had more disability (12). However, the results were insufficient since it was studied in a small sample. Therefore, the difference between genders remained unclear. The hypotheses of this study are that women with cervical radiculopathy have more pain and limitation and that functional limitation is associated with pain and quality of life. Within the scope of this research, this study aimed to elucidate the relationship between pain, functional disability, disability, and quality of life in patients with cervical radiculopathy.

MATERIAL AND METHOD

This study, which is a cross-sectional observational study, included patients who referred to the neurosurgery outpatient clinic of Karabük University Training and Research Hospital and were diagnosed with cervical radiculopathy. Eighty-one females and 30 males were included in the study. The inclusion criteria for patients were as follows: age over 18 years, with nerve root compression in the cervical region, with or without neck pain consistent with a magnetic resonance imaging (MRI) diagnosis, radiating pain neck or upper extremity, showing nerve root compression, including numbness or paresthesia. Patients showing clinical and radiological symptoms were included. Patients with severe neurological deficits, spine malignancies, cervical surgery within the last 12 months, and pregnant patients were excluded from the study.

All procedures followed were by the ethical standards of the committee responsible for human experimentation and the Declaration of Helsinki. The study was approved by the University Ethics Committee (2023-KAEK-111), and informed consent was obtained from all study participants.

Patients who met the inclusion criteria were asked to answer the survey face-to-face. Through the questionnaire, patients' age, gender, height, weight, duration of symptoms, affected side, etc., information was recorded. The severity of pain and numbness of the patients was evaluated using the numeric rating scale (NRS), functional limitation using the Cervical Radiculopathy Impact Scale (CRIS), disability using the Neck Disability Index (NDI), and quality of life using the EuroQol five-dimensions – 3-level scale (EQ-5D-3L).

Outcome Measure

Numeric Rating Scale

The NRS was used to measure the severity of pain. Scores on the scale range from 0 to 10. A score of 0 indicates no pain and 10 indicates unbearable pain. In the study, NRS was used for three different assessments: neck pain (NRS-neck), pain radiating to the arm (NRS-arm), and numbness in the finger, hand, or arm (NRS-numbness) (11).

Cervical Radiculopathy Impact Scale

The CRIS is a scale that evaluates functional limitations in patients with cervical radiculopathy (10). The CRIS Turkish version was used in this study (11). This scale consists of 3 subheadings and includes a total of 21 questions. First title: The symptoms comprised nine items covering pain in the neck, shoulder, and arm/hand/fingers, as well as related to tingling, loss of strength, and stiffness in the neck. Another title is Energy and postures (6 items), and the 3rd subheading consists of items related to functional limitations due to pain and symptoms in actions and activities (6 items).

Neck Disability Index

The NDI is a scale that evaluates the impact of neck pain on daily living activities. This scale consists of

10 sections, including severity of pain, personal care, weightlifting, reading, headache, concentration, work life, driving, sleep and leisure activities. Each section consists of 6 responses scored between 0 and 5. Patients select the option that best suits them from each section. An increase in points indicates an increase in disability. The test scores were collected at the end of the survey, and the patients' deficiencies were determined. Turkish validity and reliability were tested by Telci et al. in 2009 (13).

The EuroQol Five-Dimensions – 3-Level

The individual's quality of life was evaluated using the Turkish version of the EQ-5D-3L. This scale consists of two subheadings, and the first subheading is the EQ-5D index scale. An index score between -0.59 and 1 is calculated from the first subheading of the scale. In the index score, 0 points indicate death, 1 point indicates perfect health, and negative scores indicate closed consciousness (14). The second subtitle of the scale is the EQ-5D-3L Visual Analog Scale (VAS) scale. It is a VAS containing values between 0 and 100 that evaluate the health status of individuals on the same day. In the scale, individuals' quality of life scores ranging from 0 to 100 are noted, and as the score increases, the quality of life increases (15).

Statistical Analysis

The number of patients to participate in the study was determined using the Gpower program (G*Power Universität Düsseldorf: Psychologie). According to the data obtained from the pilot study, it was calculated that for a significant correlation between pain and functional disability (r=0.318), 95% confidence interval, and 95% power, at least 102 people would need to participate in the study. Considering that there may be data loss in the research, at least 112 people must participate for the 10% cut-off point.

The study evaluated the normal distribution of the data using the Shapiro-Wilk test and graphs. Analysis of qualitative variables was performed with the chi-square test and data were presented as numbers and percentages (%). Normally distributed numerical variables were shown with mean and standard deviation, and non-normally distributed ones were shown with median, minimum, and maximum values. Spearman correlation test was used for correlation in the statistical analysis of the data obtained at the end of the research. Correlation coefficients r>0.89 were considered very strong correlation, 0.70–0.89 as strong correlation, 0.40–0.69 as medium correlation, and 0.20-0.39 as weak correlation (16). Statistical significance was evaluated at p<0.05 level.

RESULTS

This study is a cross-sectional, observational study conducted. A total of 114 patients with cervical radiculopathy were screened, and three patients were excluded. Two patients had undergone cervical spine surgery last year, and one was unwilling to participate. The study was completed with 111 patients. 81 (72.98%) of the patients were female, 30 (27.02%) were male, and the average age was 45.59±11.00 (Table 1). The mean ages and BMI of males and females were similar (p>0.05). A comparison of the demographic characteristics of the patients is given in Table 1.

Table 1. Demographic and physical characteristics of the patients

	Females (n=81)	Males (n=30)	Total (n=111)	p
	X±SD Med (Min-Max)	X±SD Med (Min-Max)	X±SD Med (Min-Max)	
Age, years	46.38±10.75	43.43±11.55	45.59±11.00	0.211
Height, cm	160 (148-175)	174 (161-184)	162 (148-184)	**<0.01**
Weight, kg	73.91±14.29	80.73±12.72	75.76±14.15	**0.024**
BMI, kg/m²	29.02±5.36	27.23±4.06	28.54±5.08	0.101
Duration of symptoms, month	15 (3-72)	12 (3-60)	14 (3-72)	0.426
Comorbidities, n (%)				
No diseases	53 (65.4%)	21 (70%)	74 (66.7%)	0.650
Diabetes	4 (4.9%)	1 (3.3%)	5 (4.5%)	0.717
Hypertension	25 (30.9%)	6 (20.0%)	31 (27.9%)	0.257
Heart diseases	4 (4.9%)	3 (10%)	7 (6.3%)	0.330
Dominant hand side, n (%)				
Right	70 (86.4%)	25 (83.3%)	95 (85.6%)	0.681
Left	11 (13.6%)	5 (16.7%)	16 (14.4%)	
Affected side, n (%)				
Right	28 (34.6%)	6 (20.0%)	34 (30.6%)	0.230
Left	30 (37.0%)	16 (53.3%)	46 (41.4%)	
Bilateral	23 (28.4%)	8 (26.7%)	31 (27.9%)	

BMI: body mass index, SD: standard deviation

When genders were compared, while the neck and arm pain intensity of males and females were similar (p>0.05), numbness extending to the arms, hands, and fingers was significantly higher in females (p=0.027). Additionally, there was no difference between the functional limitation levels of the genders (p>0.05).

Female patients had more disabilities and a worse quality of life than males (p<0.05). The evaluation results for the groups are presented in Table 2. A moderate negative correlation was observed between CRIS symptoms and quality of life parameters, and a moderate-weak correlation between CRIS symptoms with pain and numbness (p<0.01). A negative correlation was also detected between pain and numbness with quality-of-life parameters. According to the evaluation results, the relationship obtained in patients with cervical radiculopathy is elaborated in Table 3.

Table 2. Pain, numbness, functional limitation, neck disability, and health-related quality of life scores of the patients

	Females (n=81)	Males (n=30)	Total (n=111)	p
	X±SD Med (Min-Max)	X±SD Med (Min-Max)	X±SD Med (Min-Max)	
NRS-neck	6 (2-9)	5.5 (2-9)	6 (2-9)	0.175
NRS-arm	5 (2-8)	4 (2-8)	5 (2-8)	0.285
NRS-numbness	4 (2-6)	3 (2-5)	3 (2-6)	**0.027**
CRIS				
Symptoms subscale	61.00±17.06	57.87±17.62	60.16±17.19	0.396
Energy and postures subscale	62.50 (4.17-95.83)	64.58 (8.33-100)	62.50 (4.17-100)	0.089
Actions and activities subscale	29.16 (0-100)	25 (0-91.67)	29.26 (0-100)	0.630
NDI	20 (7-42)	17 (4-42)	19 (4-42)	**0.021**
EQ-5D-3L index score	0.62 (0.09-0.86)	0.71 (0.09-0.81)	0.68 (0.09-0.86)	**<0.01**
EQ-VAS	50 (20-85)	60 (10-90)	50 (10-90)	**0.011**

NRS: numeric rating scale, CRIS: cervical radiculopathy impact scale, NDI: neck disability index, EQ-5D-3L: EuroQol Five-Dimensions – 3-Level, VAS: visual analog scale, SD: standard deviation

Table 3. The relationship between pain, numbness, neck disability, health-related quality of life, and functional disability in cervical radiculopathy

	Variables		1	2	3	4	5	6	7	8
1	**NRS-neck**	r	1							
		p	-							
2	**NRS-arm**	r	0.659**	1						
		p	<0.01	-						
3	**NRS-numbness**	r	0.581**	0.556**	1					
		p	<0.01	<0.01	-					
4	**NDI**	r	0.534**	0.392**	0.410**	1				
		p	<0.01	<0.01	<0.01	-				
5	**EQ-5D index score**	r	-0.309**	-0.256**	-0.315**	-0.650**	1			
		p	<0.01	<0.01	<0.01	<0.01	-			
6	**EQ-VAS**	r	-0.666**	-0.527**	-0.468**	-0.719**	0.561**	1		
		p	<0.01	<0.01	<0.01	<0.01	<0.01	-		
7	**CRIS-symptoms subscale**	r	0.386**	0.438**	0.252**	0.615**	-0.501**	-0.524**	1	
		p	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	-	
8	**CRIS-energy and postures subscale**	r	0.453**	0.369**	0.284**	0.413**	-0.310**	-0.400**	0.408**	1
		p	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	-
9	**CRIS-actions and activities subscale**	r	0.455**	0.399**	0.319**	0.516**	-0.395**	-0.506**	0.336**	0.403**
		p	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

NRS: numeric rating scale, CRIS: cervical radiculopathy impact scale, NDI: neck disability index, EQ-5D-3L: Euro Qol Five-Dimensions – 3-Level, VAS: visual analog scale r: Spearman correlation coefficient (*p<0.05; **p<0.01)

DISCUSSION

This study showed that females with cervical radiculopathy had more numbness in their upper extremities, worse disability, and poorer quality of life. However, the functional limitation they experienced due to radiculopathy was similar between genders. In addition, pain, functional limitation, disability, and quality of life are interrelated in patients with cervical radiculopathy.

Cervical spondylosis refers to degenerative changes resulting from aging in most adult populations. Spondylotic changes may also be seen in many asymptomatic adults. Radiculopathy develops due to the narrowing of the intervertebral foramen (17). The degenerative process of the cervical spine is divided into three distinct stages: (i) dysfunction, (ii) instability, and (iii) stabilization. Dysfunction occurs between the ages of 15 and 45. At this stage, radial and circumferential tears may occur in the annulus, accompanied by localized synovitis in the facet joint. Instability can occur in individuals between the ages of 35 and 70. This stage is characterized by facet joint degeneration and degradation of the internal disc with progressive resorption. This leads to stabilization, the final stage of the process that most commonly occurs after age 60. At this stage, hypertrophic bone develops around the facet joints and the disc and supports the spine. Additionally, each spinal segment may be at a different degenerative stage. While one level completes the dysfunction phase, the stabilization phase may begin at the other level. While disc herniations occur due to the dysfunction and instability phase, spinal stenosis occurs due to the late instability phase. Also, it may be due to the early stabilization phase due to bone overgrowth and disc space narrowing (18).

Evaluation methods are gaining importance in determining the effective treatment in radiculopathy, the presence of symptoms thresholding the problem, and excluding these problems. Patients with cervical radiculopathy may experience increased disability symptoms due to pain radiating to their neck, especially their arms. Studies have shown that patients with radiculopathy are restricted due to pain, and their disability symptoms increase (19). However, the evaluations focused on neck pain rather than arm and extremity pain and were insufficient to conduct a comprehensive evaluation caused by radiculopathy (20).

The CRIS, developed by Gartner et al., measures the functional limitation in patients with cervical radiculopathy due to pain reflected in the upper extremity, tingling, and additional sensory interference due to arm and neck involvement. It provides more objective information for evaluations (10). A study evaluating radiculopathy due to cervical disc herniation showed that CRIS better expresses the functional limitations of patients (12). Similarly, a study on radiculopathy due to cervical disc herniation determined that radiculopathy provided clearer information with a higher score on the CRIS compared to the neck disability questionnaire (NDQ) and that females had more functional disability (21). However, since radiculopathy causes pain and weakness in the extremities and necks of individuals, the lack of evidence regarding functional disability is noteworthy.

Although quality of life is one of the parameters that show the health status perceived by patients, measurements of this parameter are used to evaluate the effects of disease and/or injury on the activities of individuals. It is stated that these questionnaires, which the patients themselves answer, are measurement methods that reflect their health status very well. Wang et al. stated that the quality of life was significantly affected in people with chronic symptoms due to neck pain (22). However, the number of studies examining the quality of life and disability level in patients with isolated radiculopathy and the relationship between these parameters is limited. A study conducted in this context found that in the presence of radiculopathy due to disc herniation, the quality of life and the level of disability related to upper extremity function were affected more than in patients without radiculopathy (23). It has been stated that in patients with radiculopathy, the disability may result from neck pain and deficiencies in upper extremity functions. However, since this functional limitation and reduced quality of life in patients with radiculopathy may be affected by ageing, the lack of studies examining the relationship between these parameters is noteworthy (23).

Pain may also spread to both the neck and extremities in patients with radiculopathy, causing a decrease in the quality of life and functional limitations. In patients with radiculopathy due to disc herniation, neck and arm pain may vary from moderate to severe, increasing the level of disability (24). This study observed that neck disability and quality of life were worse in females than males. The results were like the findings of Oe et al., who found more disability in females than males (25). This difference is thought to be caused by female's lower physical activity levels and sedentary behavior (25). However, many physical parameters, such as repeated and increased workload during daily activities, cause posture disorders in females and increase the pressure on the cervical region. In addition, degeneration in the intervertebral disc and structures surrounding the spine with age may lead to the progression of disability. This condition results in compression of the nerves exiting the cervical spine. It is also stated that the cervical vertebrae's linear and areal dimensions may differ in females than in males. Therefore, females are more susceptible to cervical soft tissue and overuse injuries. When anatomical structures are examined, bone mineral density is lower in females. Consequently, they may be more prone to damage than males (9). However, this study determined that the functional limitation due to cervical radiculopathy was similar between genders. This may be associated with males being more active but with a high expectation level due to injury or fear of losing work capacity and productivity due to re-injury. Therefore, a detailed evaluation of functional limitation and the factors affecting it in patients with cervical radiculopathy is essential in the clinical follow-up process.

CONCLUSION

This study determined that females' disability and quality of life were worse than males. In addition, functional limitations with pain and quality of life are interrelated in patients with cervical radiculopathy. Pain and numbness have also been found to be associated with quality of life. Therefore, investigating gender differences and evaluating functional limitations in patients with cervical radiculopathy is essential in the clinical follow-up process.

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Research Article

Can Mecsina Hemostopper, which has a Cytotoxic Effect on Mcf-7 Cells, be Considered an Anticarcinogenic Agent due to its Immunological Properties?

Mustafa Cicek[1], Mehmet Kemal Tumer[2], Alpgiray Turgut[3]

[1]Kahramanmaraş Sütçü İmam University, Faculty of Medicine, Department of Medical Biology, Kahramanmaraş, Türkiye
[2]Alanya Alaaddin Keykubat University, Faculty of Dentistry, Department of Oral and Maxillofacial Surgery, Antalya, Türkiye
[3]Erzurum Technical University, Faculty of Science, Department of Molecular Biology and Genetics, Erzurum, Türkiye

Abstract

Aim: Mecsina is used as a hemostatic agent such as Ankaferd and Tranexamic acid. To struggle with breast cancer, which is a serious public health issue, new, effective, and less toxic therapeutic agents are needed. Hence, it is aimed to compare and evaluate the effects of Mecsina and Ankaferd, both of which contain natural biomolecules in their structure, and synthetic Tranexamic acid on MCF-7 cells.

Material and Method: For this study, MCF-7 immortalized cell lines were commercially purchased. The cells, 5000 cells per flask for each different dose group, were distributed to the 9 groups (mecsina 1:1, 1:2, 1:10, 1:50, 1:100, 1:200, 1:500, distilled water administered negative and control without any administration). Cytotoxicity, ELISA cytokine levels were evaluated, and flow cytometric analyzes were performed for each group using the XTT analysis method, after 24 hours of incubation.

Results: A significant difference was observed between different doses of drug administration groups of Mecsina Hemostopper hemostatic agent in MCF-7 cells ($p<0.001$). Besides, cytokine levels were found to be significantly higher than those of other possible therapeutic agents.

Conclusion: Mecsina Hemostopper has been found to have anti-tumoral activity in MCF-7 cancer cell lines by producing a hemostatic effect.

Keywords: MCF-7, cytotoxicity, flow cytometry, CD4+, CD8+, mecsina

INTRODUCTION

Breast cancer is one of the most common malignant diseases in women today, and its prevalence is increasing rapidly due to the stress of modern life (1). Since surgical resection, radiotherapy and chemotherapy are among the limited treatment options for breast cancer, new chemopreventive agents that can effectively prevent or manage breast cancer are urgently needed (2). Ankaferd Blood Stopper (ABS) is a hemostatic agent containing 5 different plant extracts. It is known to be an effective hemostopper produced for use in the fields of medicine and dentistry and routinely used in and after surgical procedures (3). Each of these plants that constitute the content of ABS has different effects on endothelium, blood cells, cell proliferation, angiogenesis and cellular mediators (4). Tranexamic acid, known to be a fibrin adhesive, is a hemostatic agent that increases vascularization and granulation tissue (5).

Today, antihemorrhagic agents actively used in the medical field can prevent bleeding by using different mechanisms of action (6). "Mecsina Hemostopper®" is created from herbal ingredients such as Mentha arvensis, Urtica angustifolia, Vitis vinifera, Hypericum perforatum, Syzygium aromaticum, Glycyrrhiza glabra extract, Alpinia officinarum. And this compound provides very important erythrocyte aggregation by forming a protein network. It has been proven with the help of electron microscopy that it binds to fibrinogen in the area exposed to the application, forming a protein network, and that erythrocytes are arranged in a roll in this network.

CITATION

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Corresponding Author: Alpgiray Turgut, Erzurum Technical University, Faculty of Science, Department of Molecular Biology and Genetics, Türkiye

E-mail: agiraytrgt@gmail.com

Therefore, the hemostasis effect has also been observed (7). The main therapeutic effect of hemostatic agents such as Anakaferd, Mecsina and Tranexamic Acid is their hemostatic activity by regulating the protein network. This study aimed to compare the immunological, apoptotic, antiproliferative and cytotoxic effects of Tranexamic Acid, Ankaferd Blood Stopper, which is routinely used as a hemostatic agent during and after surgical procedures, and Mecsina Blood Stopper, a new hemostatic agent. and to examine their possible positive or negative effects on MCF-7 cancer cells.

MATERIAL AND METHOD

Cell Culture

In this study we designed, MCF-7 cells were commercially obtained from ATTC (American Type Culture Collection). DMEM and F-12 (containing 10% Fetal serum) were used as media for the cells. We provided the growth medium for the cells under environmental conditions containing 95% humidity and 5% CO_2 at 37°C. We also multiplied the cells in 25 cm^2 flasks. It was then extracted from the vial surface using 0.05% trypsin-EDTA solution. During passage, cells were transferred at a ratio of 1:2 cells per new passage. The culture medium was changed every two days after passage. After staining with Sigma brand Trypan Blue (0.05%) dye, the amount of live/dead cells was determined with the Celeromics cell counter. After reaching a sufficient number of cells, the cells were distributed into 3 groups (drug, negative treated with distilled water, and untreated control), with 5000 cells per vial for each drug. It was preserved using a DMSO-based cryopreservation protocol during the stocking process. Additionally, as is known, ethics committee approval is not required for cell culture studies. We did not receive ethics committee approval for our study.

Cell Proliferation Assay

10,000 cells were seeded in a 96-well plate for this assay. The appropriate dose was found in the cells after 24 hours of incubation by administering the drug at doses of 1:10, 1:50, 1:100, 1:200, and 1:500.

The ELISA Study

Control cells and 1:2, 1:10 dosages of drug administered MCF-7 cells were seeded in a 96-well plate at 10,000 cells.

Cell extract was obtained after 24 hours of drug treatment. In our study, we obtained the "GeneAll ProtinEx total protein extraction solution" kit by applying the manufacturer's protocol to measure antibody levels in both monolayers and supernatants. The supernatants were used for ELISA plates after measurement with Nanodrop. YEHUA Interleukin 1beta (IL-1b) (YHB1720Hu), IL-6 (YHB1747Hu) and TNF-alpha (YHB3112Hu) kits were used in the ELISA study. After the standards of the study were prepared and pipetted according to the kit protocol, the color change was measured at 450 nm wavelength after 2 hours (Eliza Device). A linear curve was created according to the Optical Density (OD) values. Concentrations were then calculated by writing the standard concentrations and OD values of the samples to the equation in the graph.

In Vitro Cytotoxicity Assays

Ankaferd, Tranexamic acid, and Mecsina Hemostopper were used in the treatment of cells. Cells and media were cultured into plates. In 6-well culture plates containing the groups we formed during the assays, we monitored the generation of 5-10 million cells. The study was started once the target number was achieved (24-48 hours). Cells were transferred to 96-well cell culture plates at 5000 per well. For 48 hours in the incubator, the cells were allowed to adhere. The XTT solution and the activation solution were mixed together. XTT was activated by mixing 25 microliters of Activation solution with 5 milliliters of XTT agent. 50uL of active XTT was taken and added to 100ul of culture medium in cell-coated wells. Cells were kept in the incubator for 24 hours. In this phase, it was analyzed at 450 nm wavelength at 2nd, 4th, 6th, 8th, 12th, and 24th hours, and the cytotoxic effect that would arise due to the dose differential between the groups was examined.

Flow Cytometric Analysis

Flow cytometric analysis was performed in Atlas Biotechnology Laboratory (Ankara, Türkiye). The intracellular cytokines TNF alpha, IL-1B, and IL-6, which are located in CD4 + and CD8 + T cells, were flow cytometrically examined separately.

Statistical Analysis

When evaluating the data, Shapiro-Wilk test is used to determine whether the variables are normally distributed, two-way analysis of variance (univariate ANOVA) is used for analyzes based on normally distributed variables and drug groups, and Tukey HSD test is used for effects in dose groups and multiple comparison tests (post-hoc). Three different tests were used: Tamhane T2 test and Dunnett test. Statistical parameters were evaluated using Mean±SD. Statistical significance was accepted as $p<0.05$. Inhibitor concentration (IC50) values were calculated based on dilution ratios. IBM SPSS version 22 (IBM SPSS for Windows version 22, IBM Corporation, Armonk, New York, United States) and R.3.3.2 software were used to evaluate the data.

RESULTS

Cell Proliferation and Viability (%) Assessment

The dose-related cytotoxic effect of Mecsina, Ankaferd and Tranexamic Acid on MCF-7 breast cancer cell proliferation was analyzed by XTT assay (Table 1). At the end of 24 hours, cell viability rates of Ankaferd, Mecsina and Tranexamic Acid were determined at 100%, 50%, 10%, 2%, 1%, 0.05% and 0.02% concentrations. According to optical density values, the inhibition of all agents at these concentrations was compared with both the control group and each other (Table 1). It was observed that all agents showed cytotoxic effects on MCF-7 cells and there was a significant difference between the dose groups both within and between groups ($p<0.001$). All three agents showed the greatest cytotoxic effect at 100% concentration. Additionally, Mecsina Hemostopper showed a significant cytotoxic effect at concentrations of 1%, 0.05% and 0.02%.

Tranexamic Acid showed a cytotoxic effect on MCF-7 in almost all dose groups except 2% concentration (p<0.001). Almost no cytotoxic effect was observed for Ankaferd at 10%, 2% and 1% concentrations, and for Mecsina at 50% and 10% concentrations (p<0.001). Additionally, when all dose groups were evaluated, the highest IC50 concentration was observed in Ankaferd, while the lowest IC50 concentration was observed in Mecsina and Tranexamic Acid (Table 2).

Table 1. Demonstration of cytotoxicity and proliferation values of Mecsina, Ankaferd and Tranexamic Acid on breast cancer cells

		Mecsina hemostopper	Ankaferd blood stopper	Tranexamid acid	
	Concentration	Mean±SD	Mean±SD	Mean±SD	p
	Control	100.00±0.00	100.00±0.00	100.00±0.00	
	1/500	35.46±22.35**	47.57±32.05**	14.52±3.36**	0.271
	1/200	40.06±26.86**	59.97±38.15	18.72±0.04**	0.250
	1/100	38.65±27.42**b	120.97±7.29a.c	39.18±3.42**b	p<0.001*
MCF-7	1/50	58.95±1.63**b.c	116.67±1.12a.c	72.30±1.41**a.b	p<0.001*
	1/10	102.60±3.08b.c	81.19±0.94a.c	22.64±7.02**a.b	p<0.001*
	1/2	96.07±2.65b.c	3.56±0.31**a	6.20±2.65**a	p<0.001*
	1/1	6.04±1.82**b.c	0.94±0.02**a	0.73±0.25**a	p<0.001*
	p	p<0.001*	p<0.001*	p<0.001*	

Univariate;α:0.05;Post-hoc:Dunnett test;Tukey Test; Tamhane T2 Test;*The difference is statistically significant;**The difference compared to the control group is statistically significant; The difference according to the a Mecsina group is statistically significant; The difference compared to the b Ankaferd group is statistically significant; The difference according to c Tranexamid Acid Group is statistically significant

Table 2. Demonstration of IC50 values of Mecsina, Ankaferd and Tranexamic Acid on breast cancer cells

IC50	Mecsina Hemostopper	Ankaferd Blood Stopper	Tranexamid Acid
MCF-7	2.345µl	26.81µl	1.345µl

ELISA Evaluation

The immunological effects of Ankaferd, Mecsina, and Tranexamic Acid intracellular cytokines on MCF-7 breast cancer cells were evaluated both within and between groups at 1/2 and 1/10 concentrations of each cytokine (Table 3). According to the data obtained, TNF-α and IL-1B, both of which are pro-inflammatory cytokines, and the IL-6, anti-inflammatory cytokine, were found to have significant differences both for each agent separately within the group, as well as across the groups. While a significant increase was observed in TNF-α and IL-1B levels of Mecsina in 1/10 concentration compared to Ankaferd and Tranexamic Acid, IL-6 levels decreased (p<0.05). At 1/2 concentration, statistical significance was observed both between and within groups (Table 3) (p<0.05).

Table 3. Evaluation of proinflammatory and anti-inflammatory cytokines levels of Mecsina, Ankaferd and Tranexamic Acid on breast cancer cells by ELISA method

			MCF-7						
			Mecsina blood stopper ankaferd blood stopper		Tranexamid acid blood stopper		Ankaferd blood stopper		
Group	Cell	Dilution	Mean	±SD	Mean	±SD	Mean	±SD	P
IL-1B		Control	100.00	±0.00	100.00	±0.00	100.00	±0,00	
	Supernatant	1/2	123.00	±2.83**b	112.00	±1.41**a.c	129.00	±2.83**b	0.014*
		1/10	127.50	±2.12**b.c	102.00	±0.00a	106.00	±1.41a	0.001*
		P	0.002*		0.001*		0.001*		
TNF-α		Control	100.00	±0.00	100.00	±0.00	100.00	±0.00	
	Supernatant	1/2	4.00	±1.41**b.c	98.48	±0.74a.c	109.98	±1.39**a.b	p<0.001*
		1/10	125.48	±2.09**b	102.03	±0.33**a.c	121.48	±2.09**b	0.002*
		P	p<0.001*		0.011*		0.002*		
IL-6		Control	100.00	±0.00	100.00	±0.00	100.00	±0.00	
	Supernatant	1/2	81.40	±1.41**c	85.20	±1.41**c	116.00	±0.00**a.b	p<0.001*
		1/10	85.30	±2.12**c	87.70	±0.71**c	106.00	±1.41**a.b	0.002*
		P	0.002*		0.001*		0.001*		

Univariate;α:0.05;Post-hoc:Dunnett test;Tukey Test; Tamhane T2 Test; *The difference is statistically significant; **The difference compared to the control group is statistically significant; The difference according to the a Mecsina group is statistically significant; The difference compared to the b Ankaferd group is statistically significant; The difference according to c Tranexamid Acid Group is statistically significant

FLOW CYTOMETRY Results in CD4 + and CD8 + T Cells

Expressions of TNF alpha, IL-1B, and IL6, which are intracellularly released cytokines of CD4+ and CD8+ cells, were compared to the anti-hemorrhagic agents Ankaferd, Mecsina, and Tranexamic acid, as well as control groups of their various concentrations, each other, and their measurements at different times (Table 4).

Table 4: Evaluation of the levels of proinflammatory and anti-inflammatory cytokines on breast cancer cells of Mecsina. Ankaferd and Tranexamic Acid in CD4 and CD8 cells by Flowcytometry method

				Mecsina hemostopper		Ankaferd blood stopper		Tranexamid acid		
			Dilution	Mean	±SD	Mean	±SD	Mean	±SD	p
MCF-7 cell line 6 hours	TNF-α	CD4+	Control	100.00	±0.00	100.00	±0.00	100.00	±0.00	
			1/2	102.35	±0.21$^{**b.c}$	100.02	±0.01^{a}	100.65	±0.21^{a}	**0.002***
			1/10	102.70	±0.42**	102.30**	±0.28	101.60	±0.28**	0.100
			p	**0.004***		**0.001***		**0.010***		
		CD8+	Control	100.00	±0.00	100.00	±0.00	100.00	±0.00	
			1/2	104.00	±0.71$^{**b.c}$	100.20	±0.14^{a}	100.95	±0.21^{**a}	**0.006***
			1/10	106.55	±0.49^{**c}	103.50	±0.71^{**c}	101.35	±0.21$^{**a.b}$	**0.018***
			p	**0.005***		**0.006***		**0.009***		
	IL-6	CD4+	Control	100.00	±0.00	100.00	±0.00	100.00	±0.00	
			1/2	360.00	±28.28$^{b.c}$	220.00	±21.21^{**a}	195.00	±7.07^{**a}	**0.008***
			1/10	829.00	±35.36$^{b.c}$	630.00	±28.28^{**a}	565.00	±21.21^{**a}	**0.006***
			p	**0.001***		**0.001***		**p<0.001***		
		CD8+	Control	100.00	±0.00	100.00	±0.00	100.00	±0.00	
			1/2	360.00	±14.14$^{**b.c}$	185.00	±±7.07^{**a}	175.00	±7.07^{**a}	**0.001***
			1/10	635.00	±21.21$^{**b.c}$	365.00	±21.21^{**a}	420.00	±28.28^{**a}	**0.003***
			p	**0.001***		**0.001***		**0.001***		
	IL-1B	CD4+	Control	100.00	±0.00	100.00	±0.00	100.00	±0.00	
			1/2	100.0	±0.01^{c}	101.35	±0.21**	103.00	±0.71^{**a}	**0.014***
			1/10	107.00	±0.28$^{**b.c}$	102.70	±0.28$^{**a.c}$	105.45	±0.49$^{**a.b}$	**0.003***
			p	**0.014***		**0.002***		**0.004***		
		CD8+	Control	100.00	±0.00	100.00	±0.00	100.00	±0.00	
			1/2	100.65	±0.21$^{b.c}$	103.60	±0.71^{**a}	104.75	±0.35^{**a}	**0.007***
			1/10	102.20	±0.28^{**c}	104.50	±0.71^{**c}	107.30	±0.71$^{**a.b}$	**0.008***
			p	**0.004***		**0.009***		**0.001***		
MCF-7 cell line 24 hours	TNF-α	CD4+	Control	100.00	±0.00	100.00	±0.00	100.00	±0.00	
			1/2	103.55	±0.35^{**c}	104.50	±0.71^{**c}	115.50	±1.41$^{**a.b}$	**0.002***
			1/10	124.55	±0.49$^{**b.c}$	112.00	±1.41^{**a}	116.20	±1.41^{**a}	**0.005***
			p	**0.002***		**0.002***		**0.001***		
		CD8+	Control	100.00	±0.00	100.00	±0.00	100.00	±0.00	
			1/2	107.50	±1.41**	108.50	±0.71**	111.50	±2.12**	0.154
			1/10	129.50	±0.71**	112.50	±2.12**	112.80	±1.41**	0.202
			p	**0.004***		**0.005***		**0.006***		
	IL-6	CD4+	Control	100.00	±0.00	100.00	±0.00	100.00	±0.00	
			1/2	111.00	±1.41^{**c}	119.00	±2.83^{**c}	131.00	±1.41$^{**a.b}$	**0.005***
			1/10	116.50	±2.12$^{**b.c}$	187.50	±4.24$^{**a.c}$	167.00	±2.83$^{**a.b}$	**0.001***
			p	**0.003***		**0.001***		**p<0.001***		
		CD8+	Control	100.00	±0.00	100.00	±0.00	100.00	±0.00	
			1/2	111.00	±1.41^{**c}	109.50	±0.71^{**c}	143.50	±2.12$^{**a.b}$	**p<0.001***
			1/10	165.00	±2.83^{**b}	152.50	±3.54^{**a}	147.00	±2.83**	**0.023***
			p	**0.001***		**0.001***		**p<0.001***		
	IL-1B	CD4+	Control	100.00	±0.00	100.00	±0.00	100.00	±0.00	
			1/2	114.00	±2.83**	116.50	±3.54**	114.50	±0.71**	0.650
			1/10	121.10	±1.41**	119.50	±2.83**	117.30	±2.12**	0.618
			p	**0.004***		**0.009***		**0.002***		
		CD8+	Control	100.00	±0.00	100.00	±0.00	100.00	±0.00	
			1/2	113.50	±2.12^{**b}	121.00	±1.41$^{**a.c}$	112.00	±1.41**b	**0.024***
			1/10	125.50	±1.41^{**b}	122.90	±2.12$^{**a.c}$	115.00	±1.41^{**b}	**0.031***
			p	**0.003***		**0.001***		**0.002***		

Univariate; α:0.05;Post-hoc:Dunnett test;Tukey Test; Tamhane T2 Test; *The difference is statistically significant; **The difference compared to the control group is statistically significant; The difference according to the a Mecsina group is statistically significant; The difference compared to the b Ankaferd group is statistically significant; The difference according to c Tranexamid Acid Group is statistically significant.

Table 4: Evaluation of the levels of proinflammatory and anti-inflammatory cytokines on breast cancer cells of Mecsina. Ankaferd and Tranexamic Acid in CD4 and CD8 cells by Flowcytometry method

				Mecsina hemostopper		Ankaferd blood stopper		Tranexamid acid		
			Dilution	Mean	±SD	Mean	±SD	Mean	±SD	p
MCF-7 cell line 72 hours	TNF-α	CD4+	Control	100.00	±0.00	100.00	±0.00	100.00	±0.00	
			1/2	312.00	±14.14**c	300.00	±7.07**c	370.00	±14.14**a.b	**0.020***
			1/10	690.00	±28.28**	625.00	±35.36**	620.00	±28.28**	0.543
			p	**0.001***		**0.001***		**p<0.001***		
		CD8+	Control	100.00	±0.00	100.00	±0.00	100.00	±0.00	
			1/2	370.00	±28.28**	320.00	±14.14**	380.00	±21.21**	0.132
			1/10	830.00	±70.71**	765.00	±21.21**	794.00	±14.14**	0.064
			p	**0.003***		**0.001***		**p<0.001***		
	IL-6	CD4+	Control	100.00	±0.00	100.00	±0.00	100.00	±0.00	
			1/2	112.00	±1.41**b.c	105.00	±0.71**a	107.30	±0.71**a	**0.013***
			1/10	119.50	±2.12**b.c	110.50	±1.41**a	111.00	±1.41**a	**0.021***
			p	**0.002***		**0.003***		**0.003***		
		CD8+	Control	100.00	±0.00	100.00	0.00	100.00	±0.00	
			1/2	104.50	±0.71**b	109.00	±1.41**a.c	104.50	±0.71**b	**0.032***
			1/10	126.30	±0.71**b	111.80	±1.41**a.c	106.20	±1.41**b	**0.031***
			p	**0.004***		**0.004***		**0.014***		
	IL-1B	CD4+	Control	100.00	±0.00	100.00	±0.00	100.00	±0.00	
			1/2	420.00	±14.14**c	370.00	±21.21**c	213.00	±7.07**a.b	**0.002***
			1/10	720.00	±21.21**c	625.00	±28.28**c	338.00	±28.28**a.b	**0.001***
			p	**0.001***		**0.001***		**0.002***		
		CD8+	Control	100.00	±0.00	100.00	±0.00	100.00	±0.00	
			1/2	420.00	±21.21**c	283.00	±35.36**c	198.00	±14.14**a.b	**0.007***
			1/10	730.00	±28.28**b.c	725.00	±35.36**a	282.00	±28.28**a	**0.001***
			p	**0.001***		**0.001***		**0.005***		

Univariate; α:0.05;Post-hoc:Dunnett test;Tukey Test; Tamhane T2 Test; *The difference is statistically significant; **The difference compared to the control group is statistically significant; The difference according to the a Mecsina group is statistically significant; The difference compared to the b Ankaferd group is statistically significant; The difference according to c Tranexamid Acid Group is statistically significant.

An increase in the expression of all intracellular cytokines was observed in Mecsina BS-administered cell lines at 6th, 24th, and 72nd hours compared to Ankaferd BS administration in CD4 + T cells at 1/2 and 1/10 concentration ($p<0.05$). TNF-α and IL-6 levels in CD8 + T cells increased in the Mecsina-administered group relative to the other groups at 1/2 and 1/10 concentrations at the 6th hour, while IL-1B levels decreased ($p<0.005$). In comparison to the other groups, all cytokine levels at 1/10 concentration increased significantly in the Mecsina application considering the 24th hour ($p<0.05$). The levels of TNF-α and IL-6 cytokines were increased at 1/10 concentration ($p<0.05$), and the levels of IL-1B were significantly higher in the Mecsina-administered group compared to the other groups at 1/2 and 1/10 concentrations at the 72nd hour ($p<0.05$) (Table 4).

DISCUSSION

Breast cancer is the most common type of cancer in women worldwide, and its incidence increases by an average of 0.4% every year. The financial impossibilities brought about by this situation increase our search for low-cost new drugs and methods as researchers (8). The chemotherapeutic effect of existing drugs is known. However, not every patient reacts the same to these drugs, and clear answers, positive or negative, are not known about their side effects on healthy cells (9). For these reasons, it leads to the search for low-cost, fast-acting and non-toxic agents from natural bioflavonoids to treat this disease. In our study, the cytotoxicity of the anti-hemorrhagic agent Mecsina BS, which is obtained from natural compounds with limited information about its efficacy and cytotoxicity in the literature, and its effectiveness on MCF-7 cells, has been tried to be compared with another natural anti-hemorrhagic agent, Ankaferd BS, and a synthetic anti-hemorrhagic agent, Tranexamic Acid. The fact that our study is the first in this field, as determined by literature searches, increases its clinical significance. It is a condition related to tumor cell formation, decreased apoptosis and uncontrolled cell growth. For this reason, the use of cytotoxic drugs, which aim to activate apoptotic pathways and also reduce cell proliferation, is among the studies carried out for cancer treatment (10). Due to the significant side effects of chemotherapeutic drugs, researchers have recently experimented on the use of natural compounds and discovered that these compounds have high efficacy, low toxicity, and fewer side effects (11). Since the precursors of agents that induce apoptosis are

natural compounds, natural compounds are also used to induce apoptosis in human cancer cells (12,13). In this study, we aimed to evaluate the apoptotic, antiproliferative and cytotoxic effects and effectiveness of different doses of Mecsina and Ankaferd, prepared from standard plants, and Tranexamic Acid, a synthetic anti-hemorrhagic agent, on breast cancer. In this context, different doses of Mecsina, Ankaferd and Tranexamic Acid were applied to MCF-7 cells and the XTT viability test was evaluated depending on dose and time. Compared to Ankaferd, Mecsina showed a significant cytotoxic effect on MCF-7 cells at doses of 1% and lower. Tranexamic acid showed this effect at doses of 2% and lower. TNF alpha plays a key role in the activation of pro-inflammatory cytokines and leukocyte adhesion molecules. Increasing TNF-α levels increases NF-kB expression and inflammatory response (14). TNF alpha remarks the activity in the early stage of the disease. IL-1B functions as the major pro-inflammatory cytokine in the development of the Systemic Inflammatory Response Syndrome (SIRS) response (15). TNF alpha stimulates fibroblasts to release collagenase, increases vascular permeability, induces the release of cytokines such as IL-1β and IL-6, increases the production of adhesion molecules, affects the production of factors involved in osteoclast differentiation, and also increases bone resorption by showing a synergistic effect with IL-1 (16). It has been shown that, by inducing the expression of proinflammatory genes and activating stromal and immune cells, IL-1B can initiate inflammation and increase tumor activity, as well (15). In a study, increased levels of IL-1B were linked to tumor invasiveness and a poor prognosis (17). In another study, IL-1β was found to inhibit the growth of MCF-7 cells and was thought to achieve this feature together with TNF-α and IL-6 (18). IL-6 is a multifunctional cytokine that has a wide range of humoral and cellular immune effects in relation to inflammation, host defense, and tissue damage. It is released from fibroblasts in response to inflammatory stimuli such as IL-1B and TNF-α. Its biological effects include the proliferation of T cells, induction of bone resorption by synergistic effect with IL-1β (19). This is the first study to compare ABS, Mecsina, and Tranexamic Acid on MCF-7 cancer cells in the literature. In the study, changes in TNF-α, IL-1B, and IL-6 levels of possible therapeutic agents administered to MCF-7 cells and which of these agents had more anti-tumoral activity were evaluated. While the levels of TNF-a and IL-1B, which cause apoptotic cell death, were significantly higher in Mecsina application at 1/10 concentration compared to other agents, IL-6 levels were significantly lower. According to our findings, Mecsina, at a concentration of 1/10, was found to be more effective on MCF-7 cells than other therapeutic agents.

T cells are divided into three classes according to their immunological effects: helper cytotoxic T cells (Tc), regulatory T cells, and T cells (Th) (20). These cells secrete cytokines and play a role in the differentiation of activated CD4+ T cells. In contrast, Tc cells play a role in the differentiation of activated cytotoxic CD8 + T cells (21). In a study using T cell immunotherapy on MCF-7 cells, CD8 + and CD4 + T cells, which have strong antitumor effects, were selected for immunotherapy (22). It has been pointed out that subpopulations of T cells play a synergistic role in the regulation of the immune response (23). It is known that immune level adequacy is an important risk factor in the development of cancer biology and cancer prognosis, in this study, the effects of Mecsina and Ankaferd, which are bioactive compounds, and Tranexamic Acid, a synthetic molecule, on CD4 + and CD8 + T cells were investigated. It has been reported that TNF-α can induce apoptotic cell death through p38 MAPK activation. This kinase has been shown to phosphorylate Bcl-2, resulting in increased caspase-3 expression. 9 activation (24). In the study, we think that Mecsina and Ankaferd mediate the induction of apoptosis against MCF-7 cells and this may be due to an indirect effect through TNF-α. In particular, it was observed that cell viability decreased significantly. Since TNF-α activity caused by 1/10 Mecsina application increased its expression in CD8+ cytotoxic T cells. IL-6 is a molecule that induces different biological reactions depending on the function of the target cell. Additionally, it stimulates proliferation in CD8+ cells. The pleiotropic cytokine IL-1B facilitates cancer progression in various tumor types (24). Since cytokines have a significant effect on the proliferation and activation of lymphocytes, flow cytometric measurements were made of their regulatory effects on the expression of IL-1, IL-6 and TNF-α in lymphocytes activated by Mecsina, Ankaferd and Tranexamic Acid. The expression of TNF-α, IL-6, and IL-1B in CD8+ T cells in the Mecsina-treated group was found to be significantly higher than in the Ankaferd and Tranexamic Acid-treated group. It was observed that all these cytokines stimulated cytotoxic activity in MCF-7 tumor cells, especially with the application of 1/10 Mecsina.

When the results are evaluated together, it was determined that Mecsina Hemostopper has an anti-tumoral activity by producing a hemostatic effect on MCF-7 cancer cell lines. In comparison to other possible agents, Mecsina has been shown to have strong immune-enhancing, tumor-selective, and inhibitory properties. In addition, though Mecsina has been shown to be beneficial in the treatment and prevention of human cancers, more molecular studies are required to discover the molecular mechanism of this condition.

CONCLUSION

In this study, since new, effective and less toxic treatment agents are needed to combat breast cancer, which is a serious public health problem, it is known to be used as hemostatic agents in the literature, such as Mecsina, Ankaferd and Tranexamic acid. In our study, we aim to compare and evaluate the effects of Mecsina and Ankaferd, both of which contain natural biomolecules in their structure, and synthetic Tranexamic acid on MCF-7 cells. Cytotoxicity, ELISA cytokine levels were evaluated on commercially available MCF-7 cells, and

flow cytometric analyzes were performed using the XTT analysis method for each group after 24 hours of incubation. A significant difference was observed in MCF-7 cells between different dose drug application groups of Mecsina Hemostopper hemostatic agent ($p<0.001$). Additionally, it was determined that cytokine levels were significantly higher than other possible therapeutic agents. As a result, Mecsina Hemostopper was found to have anti-tumoral activity in MCF-7 cancer cell lines by producing a hemostatic substance.

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MEDICAL RECORDS-International Medical Journal

Research Article

Determination of the Frequency of Celiac and Autoimmune Thyroid Diseases in Children and Adolescents Diagnosed with Type 1 Diabetes Mellitus

Ilknur Kaba[1], Samet Can Demirbas[1], Havva Nur Peltek Kendirci[2]

[1]Hitit University Faculty of Medicine, Department of Child Health and Diseases, Çorum, Türkiye
[2]Hitit University Faculty of Medicine, Department of Pediatrics, Department of Pediatric Endocrinology, Çorum, Türkiye

Abstract

Aim: Celiac disease (CD) and autoimmune thyroid disease (AITD) are more common in individuals with Type 1 diabetes mellitus (T1DM). Hypothyroidism; has been associated with increased risk of hypoglycemia, reduced growth, and untreated CD with reduced bone mineral density has been associated with skeletal problems. It was aimed to screen the frequency of children and adolescents with T1DM in terms of CD and AITD.

Material and Method: The patients diagnosed with T1DM in July 2015-March 2022 were retrospectively analyzed. The patients' age, gender, age at diagnosis, anti-TPO, anti-TG, Islet Cytoplasmic Antibodies (ICA), Insulin Autoantibodies (IAA), Glutamic Acid Decarboxylase (GADA) antibodies and accompanying autoimmune disease were investigated.

Results: Chronological age was 12.5±4.4 (2.5-21.3), diagnosis of T1DM age 8.5±4.3 (1.0-17.5), duration of diabetes 4.0±3, 199 patients with T1DM aged 5 (0.0-18.1) years were included. 52.3% (n=104) of the cases were female. While the frequency of CD is 23.2% (n=10), the presence of anti-TPO and anti-TG antibodies is 6.9% (n=3) in patients whose diabetes diagnosis age is less than 5 years. Both CD and the presence of thyroid autoantibodies were more common in girls (73.3%, 68%).

Conclusion: We found that the frequency of CD and AITD in our patients with T1DM was higher than in the general population, the frequency of CD increased in patients with a younger age at diagnosis of T1DM, anti-TPO antibodies was observed with advancing age at diagnosis, and both conditions were more common in females.

Keywords: Adolescent, autoimmune thyroiditis, celiac disease, child, type 1 diabetes

INTRODUCTION

Type 1 diabetes mellitus (T1DM) is an autoimmune disease that specifically results in insulin deficiency as a result of an exaggerated immune response to β-cell autoantigens. The most common autoimmune diseases in patients with T1DM; autoimmune thyroid disease (AITD) is followed by Celiac disease (CD) (1-6).

Graves disease and Hashimoto's thyroiditis (HT) are the most common autoimmune thyroid diseases (7). Hyperthyroidism is more common in Graves disease and hypothyroidism is more common in HT. Genetic predisposition and environmental factors play a role in the etiology of autoimmune thyroid disease (8,9). While the prevalence of AITD in children and adolescents is 0.3-1.1%, it has been determined that it is seen at a higher rate in children with T1DM and this rate is approximately 3-8% (10). The prevalence of AITD increases up to 20% with age, and hypothyroidism is observed in most of the patients (11).

Celiac disease is an autoimmune small bowel disease caused by persistent sensitivity to gluten found in wheat, rye and barley in genetically susceptible individuals (12). CD is more common in individuals with T1DM than in the general population, and its prevalence is reported to be

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Corresponding Author: Ilknur Kaba, Hitit University Faculty of Medicine, Department of Child Health and Diseases, Çorum, Türkiye
E-mail: ilknurkaba@yahoo.com

1-10% in children and adolescents with T1DM (13). The risk of celiac disease increases as the age at diagnosis of diabetes decreases, and this risk is more pronounced especially in children with T1DM under the age of 5 (14).

Hypothyroidism has been associated with an increased risk of hypoglycemia and decreased growth, while untreated CD has been associated with skeletal problems with reduced bone mineral density. In this study, determining the frequency of CD and AITD in children and adolescents followed up with the diagnosis of T1DM; it was aimed to evaluate the relationship between growth, glycemic control, age, gender and puberty. It is necessary for the patient to screen for comorbidities and to diagnose and treat them at an early stage.

MATERIAL AND METHOD

The patients diagnosed with T1DM in July 2015-March 2022 were retrospectively examined. Age, gender, age at diagnosis, duration of T1DM, presence of antithyroid peroxidase (TPO Ab), antithyroglobulin (TG Ab) antibodies, and the concomitant autoimmune disease were investigated.

Ethics committee approval for the study was obtained from the Hitit University Faculty of Medicine Clinical Research Ethics Committee decision no: 2022-27 and taken on:31/03/2022.

Statistical Analysis

Statistical analysis IBM SPSS Statistics It was carried out with the software for Windows 22.0. Pearson's chi-square test or Fisher's exact test was used to compare qualitative variables when the expected frequency was less than 5 cells. The Shapiro-Wilk test was used to determine whether the numerical variables were normally distributed, and the t-test in independent groups was used to compare the normally distributed variables in 2 groups used. Mann Whitney U test was used to compare the non-normally distributed variables in 2 groups. A p value of <0.05 was considered statistically significant.

RESULTS

199 patients with T1DM were included. Mean chronological age was 12.5±4.4 (2.5-21.3) years, mean diabetes duration was 4.0±3.5 (0.0-18.1), and 52.3% (n=104) of the study were female. While 84.9% (n=169) of the patients had no accompanying disease, 7.5% (n=15) had CD, 12.8% (n=25) had TPO Ab, 6.5% (n=13) had TG Ab, CD and AITH were present in 0.5% (n=1). The ages at diagnosis of the cases are shown in Table 1.

While the frequency of CD is 23.2% (n=10), the presence of TPO Ab and TG Ab were 6.9% (n=3) in those with diabetes diagnosis younger than 5 years. The frequency of CD was 3.3% (n=5), the presence of TPO Ab was 14.1% (n=22), and the presence of TG Ab was 6.4% (n=10) in those who were 5 years or older (Table 2). When the gender distribution is examined; the female sex ratio was 73.3% (n=11) in CD, 68% (n=17) in patients with TPO Ab, and 61.5% (n=8) in patients with TG Ab.

Table 1. T1DM, CD diagnosis and thyroid autoantibody detection ages of the cases

Diagnosis ages	n (%)	mean±SD	min-max
T1DM diagnosis age (years)	199 (100)	8.5±4.3	1.0-17.5
Celiac disease diagnosis age (years)	15 (7.5)	6.6±3.8	2.0-14.7
Age at detection of anti-TPO (years)	25 (12.8)	10.9±3.2	4.9-14.0
Anti-TG detection age (years)	13 (6.5)	10.1±3.3	4.9-13.9

Table 2. Frequency of CD and thyroid autoantibodies detection according to T1DM diagnosis age group

		T1DM diagnosis age		
		≤5 years (n=43) (n) (%)	>5 years (n=156) (n) (%)	Total (n=199) (n) (%)
Celiac disease	Yes	10 (23.2)	5 (3.3)	15 (7.5)
	No	33 (76.8)	151(96.7)	184 (92.5)
TPO Ab	Yes	3 (6.9)	22 (14.1)	25 (12.8)
	No	40 (93.1)	134 (85.9)	174 (87.2)
TG Ab	Yes	3 (6.9)	10 (6.4)	13 (6.5)
	No	40 (93.1)	146 (93.6)	186 (93.5)

DISCUSSION

We found that 7.5% of our patients with T1DM had CD, 12.8% had TPO Ab, 6.5% had TG Ab, and 0.5% had CD and AITH. In addition, while the frequency of CD is 23.2% and the presence of TPO and TG antibodies is 6.9% in patients with T1DM diagnosis age younger than 5 years; the frequency of CD is 3.3%, the presence of TPO Ab is 14.1%, and the presence of TG Ab antibody is 6.4% in diabetes diagnosis age were 5 years or older.

Patients diagnosed with T1DM, an autoimmune disease that results in insulin deficiency as a result of an exaggerated immune response to β-cell autoantigens, are more likely to develop other autoimmune diseases than the general population (15). While it is recommended to

screen for common conditions such as AITD and CD at regular intervals in T1DM patients, it is recommended to screen for other less common autoimmune diseases in the presence of symptoms (16).

Current guidelines of the American Diabetes Association (ADA) recommend testing for antithyroid peroxidase, antithyroglobulin antibody and thyroid function tests in children and adolescents immediately after diagnosis of T1DM. If thyroid function tests are normal, it is recommended to be checked every 1-2 years. if the patient develops symptoms such as thyroid dysfunction, thyromegaly, abnormal growth rate or an unexplained glycemic variation should be checked earlier (17). The International Pediatric and Adolescent Diabetes Association (ISPAD) recommends screening for thyroid stimulating hormone (TSH) and anti-TPO Ab in the diagnosis of diabetes, and then every two years in asymptomatic individuals and in the absence of goiter or in the absence of thyroid autoantibodies, otherwise more frequent evaluations should be made (18).

Celiac disease develops against gluten found in wheat, rye and barley; It is an autoimmune enteropathy that occurs in genetically predisposed individuals and is characterized by inflammation of the small intestine, villous atrophy and malabsorption (19). The clinical picture of CD may be silent in the absence of gastrointestinal signs or symptoms, so it is important to be screened (20). If there are no symptoms in children with T1DM, the ADA recommends measuring Tissue Transglutaminase Immunoglobulin A level at the time of diagnosis and then repeating it 2 and 5 years later (17). It is recommended by ISPAD that screening should be performed during the diagnosis of T1DM and every 1-2 years there after (18).

The incidence of T1DM does not differ significantly between females and males. In a study conducted in our country, it was determined that 51.4% of the cases were male and 48.6% were female (21). In our study, the gender distribution of the cases was found to be 52.3% female and 47.7% male, consistent with the literature. Studies have reported that the frequency of autoimmune thyroiditis in children and adolescents with T1DM varies between 7.3% and 21.6% (22-25). In our country, the frequency of AITD in children and adolescents with T1DM was found to be 6.2% (26), 11.9% (27), 12% (28) and 12.8% (29). We found the presence of TPO Ab in 12.8% of our cases, and the presence of TG Ab in 6.5% of our cases, consistent with the data of our country. In studies involving children with T1DM, the frequency of thyroid autoantibodies was found to be high in females (11,22,23,27), and Hashimoto's thyroiditis occurs mostly after puberty begins (22,23). Consistent with this finding; in our study, the female sex ratio was found to be 68% in patients with TPO Ab and 61.5% in patients with TG Ab. The mean age of TPO Ab detection was 10.9 years, and the mean age of TG Ab detection was 10.1 years in our cases. It was observed that the presence of autoantibodies increased with age (The presence of TPO and TG antibodies was 6.9% in those with diabetes diagnosis age 5 years and younger, while the presence of TPO Ab in those with diagnosis age over 5 years was 14.1%, TG Ab presence was found to be 6.4%). Similar to our study, many studies have found that the frequency of thyroid autoantibodies increases as age and duration of diabetes increase (30). The presence of thyroid autoimmunity significantly increases the risk of deterioration in thyroid functions in individuals with T1DM, and this risk is higher in children than in adults (10). However, the low number of patients in need of treatment in children with T1DM and Hashimoto's thyroiditis suggests that screening should be evaluated in terms of cost-benefit (27).

CD and T1DM are autoimmune diseases that share common genetic variants and are both chronic, and similar to other autoimmune diseases, the combination of genetic susceptibility and environmental factors play a role in the formation of both diseases (31). Compared to the general population, the children with T1DM have 15 times more celiac serology positivity and up to 10 times more biopsy-proven CD. Prevalence of biopsy-proven CD varies between 1.6% and 16.4% (32). In our study, the frequency of biopsy-proven CD was found to be 7.5%, which is similar to the rate of 6.7% found in the study conducted by Esen et al. in our country (29). The substantial genetic overlap between celiac disease and T1DM explains the increased prevalence of CD in T1DM patients compared with healthy individuals. Polymorphisms related to the HLA-II-DQ and DR alleles are responsible for the risk of co-development of T1DM and CD. Almost 90% of celiac patients have HLA-DQ2 or HLADQ8 with the DR3 haplotype, which is also shared 60-70% by T1DM (33). The genetic overlap between T1DM and CD (including HLA and non-HLA) highlights common pathogenic mechanisms and explains the increasing prevalence of comorbid diseases. Recent genome-wide association studies (GWAS) have identified single-nucleotide polymorphisms associated with autoimmune diseases, including T1DM and CD. CD and T1DM-associated genes discovered in GWAS show variable effect sizes and effects directions. There are also gene-environment interactions that likely increase risk, though not yet identified (34). Genetic and environmental factors that play a common role in the pathogenesis of both diseases may be the reason for the intercommunal differences in the frequency of CD in individuals with T1DM.

As the age at diagnosis of diabetes decreases, the risk of CD increases, and children diagnosed with T1DM have a higher risk of developing CD, especially around the age of five. (31). In our study, the mean age at diagnosis of CD was 6.6 years, and the frequency of CD was 23.2% in patients with diabetes diagnosis of 5 years and younger, and 3.3% in patients with a diagnosis of more than 5 years. This shows that there is a direct correlation between insulin deficiency and the duration of gluten sensitivity. In various studies conducted on children and adolescents with T1DM and CD association, gender distribution was found to be different. While it was reported that it was

seen more frequently in girls and in boys in some studies, no gender difference was found in some studies (32). In our study, it was observed that most of our cases with T1DM and CD coexistence were female (73.3%).

In addition to publications in the literature reporting that there is a weak or no relationship between CD and thyroid autoimmunity and studies that found a significant relationship between the two diseases (35). Tissue transglutaminase antibody positivity was reported at a rate of 6.4-7.8% in patients with Hashimoto's thyroiditis (35-37) and TPO Ab positivity of 10.5-14.6% in patients with CD (36, 37). In addition, the risk of developing thyroid autoimmunity is 3 times higher in children diagnosed with CD (38). Ventura et al. showed that the frequency of thyroid autoantibodies is high in patients with CD and that these antibodies disappear with a gluten-free diet (39,40). It is thought that this relationship results from the common genetic background of CD and autoimmune thyroiditis or environmental factors that affect tolerance to their own tissues (40). We detected the presence of thyroid autoantibodies and CD in only 0.5% of the cases. The fact that the mean chronological age of our cases was 12.5 years and the mean diabetes duration was 4.0 years may cause our sample not to reflect the true frequency of this association.

The limitations of our study are its retrospective design and single center experience. Multicenter and long-term follow-up cohort studies will more accurately reflect national data.

CONCLUSION

In conclusion; we detected in our patients with T1DM, the frequency of CD and AITD is more frequent than in the general population, the frequency of CD increases in patients with a younger age at diagnosis of T1DM, but the presence of thyroid antibodies occurs with advancing age at diagnosis, and both conditions are more common in females. Longer-term studies with larger patient groups will guide the development of new screening strategies.

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MEDICAL RECORDS-International Medical Journal

Research Article

Is There a Relationship between Insulin Resistance and Eosinophil, Inflammatory Parameters Neutrophil to lymphocyte ratio, C-Reactive Protein Values?

Meltem Yigit, Ozgur Olukman

İzmir Bakırçay University Çiğli Training and Research Hospital, Department of Pediatric, İzmir, Türkiye

Abstract

Aim: The relationship between insulin resistance and inflammation and atopy is a matter of curiosity and various studies have been conducted. Study results show differences. We aimed to evaluate the relationship between insulin resistance and inflammation and atopy using C-Reactive Protein (CRP) and Neutrophil to lymphocyte ratio (NLR) as eosinophils and inflammatory indices and to determine whether the low-cost hemogram can be used as a reliable marker.
Material and Method: Between May 2022 and November 2023, 1566 children admitted to the Pediatrics outpatient clinics of İzmir Bakırçay University Çiğli Training and Research Hospital were retrospectively screened. A total of 349 pediatric patients without any known chronic disease and with complete blood count, CRP and HOMA-IR levels were retrospectively included in the study.
Results: Individuals with HOMA-IR<2.5 were considered as control group and those with HOMA-IR≥2.5 were considered as children with insulin resistance. As a result of Mann-Whitney-U test performed according to HOMA-IR groups, age and NLR parameters were found to be higher in the group with insulin resistance. This result was statistically significant ($p<0.05$). The results were not significant in eosinophil ($p=0.324$) and CRP ($p=0.352$) parameters.
Conclusion: NLR value may be a credible predictive marker of insulin resistance. Inexpensive and simple complete blood count measurement may help in the early diagnosis of Type 2 DM and in the design of treatments. In addition, due to the increasing risk of insulin resistance with age, education on conscious nutrition can be given in schools during adolescence.

Keywords: Neutrophil-to-lymphocyte ratio, Insulin resistance, Inflammation, eosinophils, CRP

INTRODUCTION

Obesity has become an increasingly common health problem in both children and adults (1,2). The HOMA-IR value is an important tool for detecting and measuring insulin resistance (IR) indicator. Obesity and IR are often directly proportional. Obesity in general is a health problem that negatively affects the whole system and even leads to metabolic consequences.

IR is known to be one of the main causes of Diabetes Mellitus Type 2 (T2DM), but the mechanism of IR is not fully understood. Some studies have shown that there may be an association between IR and inflammation (3,4). IR may trigger allergic diseases and inflammation through different mechanisms. In individuals with high IR, plasma IL-6 level was found to be high, which also increased in inflammation (5).

Eosinophils are immune cells associated with parasitic infections and allergic reactions. They are produced from multipotent hematopoietic stem cells in the bone marrow. They are involved in the pathology of chronic inflammation directly or by producing inflammatory cytokines (6).

The prevalence of both obesity and asthma has increased rapidly in recent years and the association of these two diseases is remarkably high. Although some studies have shown that aeroallergen sensitization is higher in obese children, no clear information has been found. Mechanisms

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Corresponding Author: Meltem Yigit, İzmir Bakırçay University Çiğli Training and Research Hospital, Department of Pediatric, İzmir, Türkiye
E-mail: meltemdr@yahoo.com

that may explain this relationship include increased chest wall weight and/or reduced lung volumes due to pro-inflammatory mediators released by adipocytes (7,8).

NLR is a recently popular and frequently used parameter in subclinical inflammation. It is calculated by the ratio of neutrophil and lymphocyte percentages in hemogram analysis (9,10). Increased neutrophil to lymphocyte ratio as a result of increased neutrophil count and decreased lymphocyte count is considered as an indicator of immune response and inflammation. NLR is also used to measure the severity of the disease (11). NLR has become an inexpensive and reliable method used to determine both cardiac and non-cardiac inflammation (11,12). More studies needed to understand the relationship between IR and NLR.

CRP is a protein that indicates tissue damage and inflammation and is produced by the liver. CRP measurement, which is used as an inflammatory marker, is a simple and inexpensive test.

Some studies have shown that IL-6, TNF-alpha and CRP levels are higher in obese patients, but the number of studies is not sufficient and more studies are needed. In a study by Park et al. it was reported that there was a direct relationship between CRP and body mass index and between IL-6 and body fat ratio (14).

In this study, we tried to determine the relationship of peripheral eosinophil percentage with IR and whether elevated inflammatory indices suggest IR by using a simple and inexpensive test of complete blood count and CRP measurement.

MATERIAL AND METHOD

Study Population and Design

The study was conducted with the decision of the Local Non-interventional Ethics Committee 1233 dated 11.10.2023. It was conducted in the pediatrics department of a training and research hospital. In the study, 1566 children aged 3-17 years were retrospectively screened and 349 children with complete blood count, CRP and HOMA-IR tests and without known chronic diseases were included in the study. As a result, the study was conducted on 160 males and 189 pediatric subjects. The blood findings of the individuals were retrospectively selected randomly from the hospital archive system and those with HOMA-IR level <2.5 were selected as control group and those with HOMA-IR level ≥2.5 were selected as insulin resistant group. Eosinophil, CRP and NLR values were also included in the study. Demographic information of the individuals was also recorded. Children with a history of chronic disease were excluded from the study as exclusion criteria.

Statistical Analysis

The conformity of the data to normal distribution was tested with the Anderson Darling test, which is a normality test. Median, minimum and maximum values were included in the descriptive statistics of the data. Mann Whitney-U test was used for analysis between two groups. The relationship between the data was revealed by Spearman rho correlation analysis and the level of HOMA-IR determination of the parameters was revealed by ROC analysis. SPSS 21 package program was used for statistical analysis with $p<0.05$.

RESULTS

As a result of the normality test, it was found that all parameters did not show normal distribution according to HOMA-IR groups ($p\geq0.05$). Descriptive statistics of the control and insulin resistant groups are given in Table 1.

As a result of Mann-Whitney-U test, age, NLR and HOMA-IR parameters were found to be significantly different between insulin groups ($p<0.05$). There was no significant difference in eosinophil ($p=0.324$) and CRP ($p=0.352$) parameters.

The relationship between the parameters and the degree of relationship were evaluated by Spearman rho correlation test and a moderate significant relationship was found between HOMA-IR and age. Very weak correlations were found between CRP and eosinophils, NLR and age, eosinophils and CRP, and HOMA-IR and NLR (Table 2).

The effect of the parameters was evaluated by ROC analysis and it was found that the age parameter had the highest effect, followed by the NLR parameter (Figure 1, Table 3).

Table 1. Descriptive statistics according to insulin groups

Parameters	Group	Minimum	Median	Maximum
Age	Control	3.00	7.00	17.00
	IR	3.00	13.00	17.00
Eosinophil	Control	0.00	2.60	40.00
	IR	0.10	2.40	13.00
CRP	Control	0.30	0.56	207.00
	IR	0.30	0.66	45.00
NLR	Control	0.34	1.07	26.00
	IR	0.47	1.46	12.15
HOMA-IR	Control	0.10	0.90	2.40
	IR	2.50	3.95	17.00

Table 2. Spearman rho correlation table				
Parameters	**Age**	**Eosinophil**	**CRP**	**NLR**
Eosinophil	-0.088 0.102			
CRP	-0.037 0.486	-0.138 **0.010**		
NLR	0.331 **0.000**	-0.230 **0.000**	0.227 **0.000**	
HOMA-IR	0.636 **0.000**	-0.008 0.878	-0.010 0.849	0.281 **0.000**

Table 3. ROC performanses					
Parameters	**AUC (95%)**	**Cutt off**	**p**	**Sensitivity**	**Specificity**
Gender	0.452 (0.392-0.513)	1.50	0.124	49	41
Age	0.821 (0.777-0.865)	10.50	**0.000**	73	76
Eosinophil	0.472 (0.411-0.533)	2.55	0.369	47	49
CRP	0.529 (0.468-0.590)	0.58	0.352	53	53
NLR	0.647 (0.589-0.705)	1.293	**0.000**	63	63

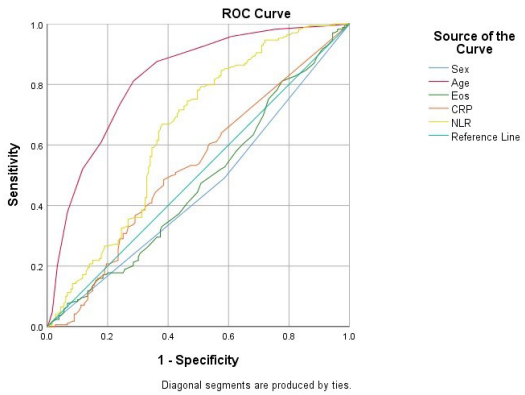

Figure 1. ROC Curve

DISCUSSION

Eosinophils are produced from multipotent hematopoietic stem cells in the bone marrow. Immune cells, including eosinophils, play a role in the pathology of chronic inflammation, either directly or by producing inflammatory cytokines (6).

There are some studies investigating the effects of asthma, atopy and insulin resistance. However, these studies have shown inconsistent results.

Lee et al. found a significant association between insulin resistance and total IgE in premenopausal women, but not in men or postmenopausal women (15).

In a Danish study, IR was directly associated with asthma-like symptoms (16). In a study by Ma et al. on adults in the USA, insulin resistance has not been found to be a risk factor for allergies (17). In a study by Zhu et al. on 9111 Chinese adults, the percentage of eosinophils was inversely correlated with the risk of T2DM (18). In another study conducted in the United States, the association between IR and asthma was found to be significant (19). The differences between these results were thought to be due to differences in the study population. In our study, no significant association was found between eosinophil percentage and IR.

There is chronic inflammation in the mechanism of many diseases, however, many studies have shown that chronic inflammation is involved in the pathogenesis of T2DM. This suggests that IR and inflammation may be related and there may be a potential relationship between IR and inflammatory markers NLR and CRP. Inflammatory factors such as CRP, TNF-α, IL-6 and MCP-1 are elevated in patients with T2DM and this is thought to be due to chronic inflammation. Chronic inflammation results in high neutrophil counts (20). IR was significantly associated with increased NLR in the study by Lou et al. (21). In the study of Atlı et al. CRP levels were found to be significantly higher in obese patients compared to non-obese patients, but no statistically significant difference was found in NLR and PLR values. (22). In a study by Karakaya et al. NLR was significantly higher in obese patients with IR was detected (23). In a study conducted by Sarıcam on 274 patients, the relationship between insulin resistance and inflammatory parameters was found to be significant (24).

In a study conducted by Gelaye et al. on Peruvian adults, a significant association between high insulin resistance and increased CRP was found (25). Kim et al. found a significant positive association between IR and elevated CRP in a study group with a sample of 36 patients in 2011 (26). In our study, no significant correlation was found between IR and CRP. However, a significant direct correlation was found between NLR and IR. Inflammatory markers such as CRP and NLR may be a helpful method for early diagnosis of T2DM, but more research is needed to confirm this observation.

The study by Yang et al. showed that IR increased with increasing age (27). In a study by Strazhesko et al. it

was shown that HOMA-IR was inversely associated with telomere length (28). Because of the shortening telomere length with increasing age and the inverse relationship between IR and telomeres, this resulted in a direct relationship between age and IR. This suggests that the probability of increasing IR increases with increasing age. However, more studies are needed on this subject. In our study, a significant relationship was found between IR and age. The probability of IR increased with increasing age.

CONCLUSION

High NLR value may be a suitable predictive marker of IR. However, more studies are needed because of different results in the studies on the relationship between CRP and eosinophil percentage and IR. Detection of an elevated NLR value by complete blood count measurement, which is an simple and inexpensive test, may help in the early diagnosis of T2DM. Due to the significant direct correlation between IR and age, proper nutrition education and counseling is especially important during adolescence.

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Research Article

The Effect of Surgical Nurses' Attitudes Towards Evidence-Based Practices on Patient Safety Culture: Descriptive and Relationship-Seeking Study

Gurkan Kapikiran[1], Yasin Cetin[2], Cansu Agrali[3], Esra Unal[4]

[1]Malatya Turgut Özal University, Faculty of Health Sciences, Department of Emergency Aid and Disaster Management, Malatya, Türkiye
[2]Adıyaman University, Faculty of Health Sciences, Department of Nursing, Adıyaman, Türkiye
[3]Osmaniye Korkutata University, Faculty of Health Sciences, Department of Midwifery, Osmaniye, Türkiye
[4]Osmaniye Korkutata University, Faculty of Health Sciences, Department of Nursing, Osmaniye, Türkiye

Abstract

Aim: This study was aimed to evaluate the relationship and the effect of surgical nurses' attitudes to evidence-based nursing (EBN) practices on patient safety culture (PSC).
Material and Method: The study was descriptive and correlational. The sample consisted of 364 nurses working in the surgical departments of a university hospital. Data were collected by using individual information form, attitude towards EBN scale and PSC scale.
Results: The average total score of PSC of surgical nurses was 3.60±0.38 and the average total score of attitudes towards EBN practice was 60.07±6.01. There was a strong positive correlation between EBN practices and PSC (r=0.705, p=0.000). In the regression analysis, it was seen that the attitude towards EBN practices, which was the predictor variable, explained approximately 49.7% of the variance in the predicted variable of PSC (F=358.175; R^2=0.497; p=0.000).
Conclusion: It was determined that the attitudes of surgical nurses towards EBN practices and PSC were positive. It was found that surgical nurses with higher attitudes towards EBN practices had more positive attitudes towards PSC. Although PSC is important for nurses working in all clinics, it is more important for nurses working in surgical clinics. Therefore, it is recommended that working conditions should be improved and in-service trainings should be planned in line with the latest evidence findings.

Keywords: Patient safety, patient safety culture, evidence-based nursing practices, surgical nurses

INTRODUCTION

Patient Safety (PS) is defined as a concept that aims to reduce the risk of harm to individuals while receiving healthcare services and determines the quality of care services (1). This is considered a critical issue that should be handled carefully by healthcare institutions and healthcare personnel and is directly related to efforts to prevent medical errors (2,3). According to data published by the World Health Organization (WHO), one out of every ten patients in the world is exposed to medical errors and is faced with health problems as a result of this situation (2,4). Accurate diagnosis, appropriate treatment and safe storage of patient information has a crucial role in the prevention of medical errors (5,6). In addition, a collective safety culture should be developed in all health care institutions and health personnel to prevent medical errors. For the development of this culture, it is of great importance to create educational content including audit and quality control strategies based on academic foundations (1,5-7). The increasing global interest in improving quality and safety in healthcare both increases efforts to update training programmes for healthcare professionals and encourages policy-making regarding clinical practice (8). Surgical areas are an area where patient safety is emphasised and handled with care as an area where patients experience one of the most sensitive and high-risk treatment processes (9). The role of surgical

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Corresponding Author: Gurkan Kapikiran, Malatya Turgut Özal University, Faculty of Health Sciences, Department of Emergency Aid and Disaster Management, Malatya, Türkiye
E-mail: gurkankpkrn@gmail.com

nurses is critical in ensuring patient safety at every stage of surgical interventions. Therefore, evidence-based nursing (EBN) methods and procedures applied by surgical nurses constitute one of the cornerstones of patient safety culture (PSC). EBN practice refers to the integration of scientific evidence and the latest research into clinical practice, which is critical for PSC (10). Clinical procedures and care standards applied by surgical nurses are updated in line with the constantly developing scientific knowledge in the field of health services. EBN practices have a crucial role in this process and form the basis of clinical decisions to ensure that patients have a safer and more effective surgical experience (11). Attitudes of surgical nurses towards EBN practice are critical for the adoption and implementation of this innovative perspective (12).

PS should be at the centre of health services. For this purpose, surgical nurses' acceptance and use of EBN practices may contribute to the prevention of adverse events. However, the attitudes of surgical nurses towards EBN practices may change under the influence of many factors. The effect of these factors on PSC has not been completely determined in studies. The study investigated the effect of surgical nurses' attitudes towards EBN practices on PSC.

MATERIAL AND METHOD

Study Type

The study was descriptive and correlational type.

Research Design and Participants

The study samples comprised of 564 surgical nurses employed in a university hospital between July 2022 and June 2023. It was determined that a minimum of 229 surgical nurses should be reached by power analysis with 5% margin of error and 95% confidence interval. However, the data collection process was extended in the study and it was aimed to reach the entire population and the study was completed with 364 surgical nurses. The participation rate in the study was 64%.

Inclusion criteria;

- Being a nurse,
- Working in surgical department for at least 6 months,
- To be willing to participate in the Research.

Exclusion criteria;

- Surgical nurses who incompletely completed the data collection form were excluded.

Data Collection and Measures

The aim of the study was declared and the necessary consent was obtained within the scope of the Declaration of Helsinki. The questionnaire form created by the researchers was collected through Google Form.

Data Collection Tools

Individual information form, attitudes towards EBN scale, PSC scale were used for data collection. It took approximately 10 minutes to complete the data collection form.

Individual Information Form

The individual information form was prepared by analysing the relevant literature (1-8,13,14) and consisted of 12 questions questioning the socio-demographic characteristics of the individual and research-specific information.

Attitude Scale towards Evidence-Based Nursing (ATEBNS)

The scale was developed by Ruzafa-Martinez and co-authors (15). It's Turkish validity and reliability were examined by Ayhan and co-authors (16). It consists of 15 items, 3 sub-dimensions and 5-point Likert structure. Scores between 15 and 75 points can be obtained from the scale. The higher the score, the more positive the attitude towards EBN. While the alpha reliability coefficient of the scale developed in Turkish was 0.86, this value was 0.96 in this study.

Patients Safety Culture Scale (PSCS)

PSCS was developed by Türkmen and co-authors (17). The scale consists of 51 items, 5 sub-dimensions and a 4-point Likert scale. Scored between 1 and 4 points, the lower the score on the scale indicates a negative PSC and the higher the score indicates a positive PSC (17). The alpha reliability coefficient of the scale developed by Türkmen and co-authors was 0.97 and this value was calculated as 0.77 in this study.

Statistical Analysis

The study data were analysed using IBM SPSS version 23.0. Skewness and Kurtosis tests were performed, determining that the data adhered to a normal distribution. Independent two-group t test was employed to compare two independent groups while a one-way analysis of variance test was used for comparing more than two independent groups. Pearson correlation of scales and linear regression analysis was used for regression analysis. The level statistical significance was set at $p<0.05$.

Ethical Endorsement

Ethics committee approval was received from the Social and Human Sciences Ethics Committee at Adıyaman University on 16th February 2022 (Decision no: 218). After obtaining institutional and ethics committee approval, the principles of the Declaration of Helsinki were adhered to and the participants were informed on the first page of the Google Forms and their consent was obtained. They were informed that they could withdraw from the study at any point.

RESULTS

The mean age of the surgical nurses included in the study was 30.83±5.16, 54.9% were married, 79.7% were bachelor's degree, 75.8% had an income equal to their expenses, 71.2% lived in the city, 46.7% worked in surgical clinics, 39.6% had been working as a nurse for 0-5 years, 73.1% had been working in their current clinic for 0-5 years, 72% worked 40 hours per week, 81.9% worked in shifts (both day and night), 93.4% chose the nursing profession willingly (Table 1).

Table 1. Socio-demographic characteristics of nurses

Personal characteristics	(Mean±SD)	Min-Max	Patient safety culture	Evidence-based nursing practice
Age	30.83±5.16	22-50	r: -.094, p: 0.072	r: -.105, p: 0.054
	n	**%**	**Mean±SD**	**Mean±SD**
Gender				
Female	207	56.9	3.56±0.43	59.37±7.02
Male	157	43.1	3.66±0.28	60.99±4.17
			t: -2.629, **p: 0.009***	t: -2.736, **p: 0.007***
Marital Status				
Married	200	54.9	3.62±0.36	59.86±6.53
Single	164	45.1	3.58±0.39	60.32±5.31
			t: . 844, p: 0.399	t: .-733, p: 0.464
Education Level				
High school	26	7.1	3.63±0.32	61.73±3.09
Associate degree	20	5.5	3.42±0.55	57.35±9.38
Bachelor's degree	290	79.7	3.60±0.36	60.03±5.90
Post graduate	28	7.7	3.82±0.25	62.25±1.48
			F: 4.418, **p: 0.005***	F: 3.513, **p: 0.015***
Economic Situation				
Income < expense	32	8.8	3.29±0.43	57.28±5.90
Income ≈ expense	276	75.8	3.63±0.38	60.25±4.06
Income > expenditure	56	15.4	3.64±0.25	60.80±6.01
			F: 12.481, **p: 0.000***	F: 4.051, **p: 0.018***
Where you lived with your family for the longest time				
Village	30	8.2	3.54±0.46	60.26±5.65
Town	28	7.7	3.49±0.52	58.82±8.05
City	259	71.2	3.64±0.31	60.53±5.25
Metropolitan	47	12.9	3.50±0.52	58.17±8.15
			F: 3.158, p: 0.250	F: 2.521, p: 0.058
Which surgical unit				
Surgical clinics	170	46.7	3.74±0.20	62.44±1.07
Surgical intensive care units	133	36.5	3.53±0.44	58.41±8.26
Operating room	61	16.8	3.37±0.46	57.09±5.83
			F: 27.735, **p: 0.000***	F: 29.808, **p: 0.000***
How many years have you been a nurse?				
0-5 years	144	39.6	3.63±0.35	60.58±4.76
6-10 years	121	33.2	3.60±0.39	60.10±6.21
11 years and over	99	27.2	3.55±0.39	59.29±7.24
			F: 1.339, p: 0.263	F: 1.357, p: 0.259
How many years have you been working in the clinic?				
0-5 years	266	73.1	3.58±0.39	60.01±6.07
6-10 years	82	22.5	3.67±0.34	60.25±6.27
11 years and over	16	4.4	3.57±0.25	60.06±3.27
			F: 1.723, p: 0.180	F: .049, p: 0.953
Weekly working time				
39 hours or less	45	12.4	3.71±0.25	61.75±2.05
40 hours	262	72.0	3.56±0.39	60.32±5.31
41 hours and over	57	15.6	3.60±0.38	60.43±6.01
			F: 4.821, **p: 0.009***	F: 2.374, p: 0.095
Working times				
Continuous daytime	66	18.1	3.95±0.13	61.98±6.41
Shift (Both day and night)	298	81.9	3.52±0.37	59.65±3.03
			t: -15.516, **p: 0.000***	t: -4.428, **p: 0.000***
The status of choosing nursing willingly?				
Yes	340	93.4	3.64±0.33	60.77±4.78
No	24	6.6	3.02±0.49	50.16±11.11
			t: 6.137, **p: 0.000***	t: 4.645, **p: 0.000***

Min: minimum, Maks: maximum, SD: standart deviation, *p<0.05

It was concluded that the mean scores of those who chose the nursing profession willingly were higher in both PS and EBN practices and were statistically significant (p<0.05). According to the educational status, it was seen that as the educational level increased, both PSC and EBN practices total scores increased and were statistically significant (p<0.05). According to the department, it was observed that nurses working in surgical clinics had higher attitudes towards both PSC and EBN practices compared to nurses working in intensive care units and operating rooms, and this was statistically significant (p<0.05). It was concluded that the mean score of PSC was higher and statistically significant in those whose weekly working hours were less than 39 hours (p<0.05). According to the working time, it was observed that the mean scores of both PSC and EBN practice were higher and statistically significant in continuous daytime workers compared to shift workers (p<0.05) (Table 1).

The results of the study display the mean scores for the total score and sub-dimensions of the PSCS and attitudes towards EBN scale, as well as the correlation analysis, which can be found in Table 2. The mean value of the total score of the PSCS was 3.60±0.38, with the highest mean score of 3.68±0.41 recorded in the sub-dimension of employee training. The mean score for attitudes towards EBN practices was 60.07±6.01, with the beliefs and expectations sub-dimension recording the highest mean score of 34.04±2.55 points (Table 2). The study revealed a strong positive correlation between EBN practices and PSC (r=0.705, p=0.000). In other words, adherence to EBN practices and their implementation in patient care by surgical nurses resulted in improved patient safety behaviours. Table 2 shows that there is a moderate positive relationship between the "employee behaviour" subcategory of PSCS and the EBN practices indicator (r=0.687, p=0.000) (Table 2).

Table 2. Scale mean scores and correlation analysis

Variables	Mean±SD (Min-Max)	PSCS (Total)	Management and leadership	Employee behavior	Unexpected event and error reporting	Employee training	Maintenance environment	Attitude Towards Evidence-Based Nursing (EBN) Scale	Beliefs and expectations towards EBN	Emotions about EBN	EBN implementation intention
Patient Safety culture PSCS (Total)	3.60±0.38 (2.14-4)	1									
Management and leadership	3.54±0.39 (1.71-4)	.923**	1								
Employee behavior	3.61±0.43 (1.79-4)	.963**	.850**	1							
Unexpected event and error reporting	3.63±0.45 (1.60-4)	.911**	.782**	.877**	1						
Employee training	3.68±0.41 (1.57-4)	.870**	.705**	.808**	.801**	1					
Maintenance environment	3.64±0.40 (2-4)	.867**	.695**	.803**	.788**	.793**	1				
Attitude Towards Evidence-Based Nursing (EBN) Scale (Total)	60.07±6.01 (28-63)	.705**	.631**	.687**	.667**	.669**	.573**	1			
Beliefs and expectations towards EBN	34.04±2.55 (22-35)	.511**	.451**	.495**	.469**	.521**	.410**	.760**	1		
Emotions about EBN	15.23±1.84 (7-16)	.556**	.522**	.537**	.496**	.551**	.406**	.684**	.755**	1	
EBN implementation intention	15.88±2.29 (7-17)	.568**	.503**	.549**	.508**	.563**	.480**	.767**	.762**	.759**	1

Min: minimum, Max: maximum, SD: standart deviation, *p<0.05

A simple linear regression analysis was conducted to evaluate the impact of attitudes towards EBN practices on PSC, with statistically significant outcomes (F: 358.175, p: 0.000). The explanatory power of the model is determined by the R^2 value, which was calculated to be 0.497. As such, the predictor variable attitude towards EBN practices elucidates almost 50% of the variability observed in the predicted variable of PSC (R^2: 0.497). The beta coefficient of the predictor variable incorporated in the regression model was ß: 0.705 as shown in Table 3.

Table 3. Regression analysis results for the patient safety culture scale of th e evidence-based nursing practice scale

Dependent variable	Independent variable	ß	t	p	F	Model (p)	R	Adjusted R^2
Patient Safety Culture	Constant	0.916	6.410	0.000	358.175	0.000	0.705	0.497
	Evidence Based Nursing Practices	0.045	18.926	0.000				

DISCUSSION

The use of EBN practices in nursing practices has a very important place in making decisions based on continuously updated scientific knowledge in the professionalisation process of the nursing profession (18). EBN provides continuous access to new scientific information, effective application of the latest research evidence on the patient and safe care delivery in PS (19). The results of the impact of the attitudes of nurses working in surgical clinics towards EBN practices on PSC were discussed in line with the literature.

According to the findings obtained, the average age of surgical nurses was 30.83±5.16 years, 56.9% were female, 54.9% were married, the majority of nurses are undergraduate graduates, 39.6% had been working as a nurse for 0-5 years, 73.1% had been working in the same clinic for 0-5 years, the majority of nurses are worked in shifts, 72% worked 40 hours per week, most nurses are chose the nursing profession willingly, and 79.9% did not follow EBN practices (Table 1). The data obtained are similar to the results of the studies published in the literature (20,21).

This study concluded that the attitudes of nurses working in surgical clinics towards EBN were at a high level with a score of 60.07±6.01 (Table 2). Considering the studies in the literature in which attitudes towards EBN were examined, Köse et al. found the total scores of the EBN practices scale to be 55.46±9.80 (22), Danacı et al. 62.90±8.90 (20), Doğan et al. 60.68±8.62 (23), Karakoç et al. 58.23±9.34 (18), Dikmen et al. 57.20±9.06 (24), Şen and Yurt 51.33±5.18 (13,18,20-25). The attitudes towards EBN examined in the literature were found to be similar to the findings of our study (13,18,20-25). However, in another study of nurses, which examined their attitudes towards the application of research findings in practice, the attitudes of nurses were found to be negative and it was concluded that the most important obstacle was lack of time (26). In a different study in the literature, it was reported that nurses' attitudes towards EBN practices were negative (27). Considering both our study and other recent studies, the studies concluded in recent years show that the attitude towards EBN has increased positively (13,18,20-25). It is thought that the presence of EBN courses in education curricula, the use of EBN research findings in in-service training, increased opportunities for post-graduate education, easier access to information and increased number of open access journals contribute to the attitude towards EBN over time. The utilization of evidence in nursing practice presents an opportunity to professionalise the nursing field as well as a vital aspect of enhancing society's health and implementing PS issues at a high level (28). Using the latest research findings that have evidence value in EBN practices contributes to the provision of more effective, more efficient and safer patient care (19).

In the study, the mean PSCS score for nurses working in surgical clinics was 3.60±0.38. The highest mean score obtained for the PSCS was 3.68±0.41 in the employee training sub-dimensions, whereas the lowest mean score was 3.54±0.39 in the management and leadership sub-dimensions. As seen in the description of the PSCS, when it is considered that the mean score approaching 4 is positive on PSC (17) the data obtained in this study show that PSC is at a high level (Table 2). When the studies on PSC in the literature were examined, Kapıkıran et al. found 3.36±0.21 (29), Karaca and Arslan found 3.09±0.38 and 2.86±0.69 (14), Ertürk et al. found 2.81±0.40 (30), Erdağı and Özer found the lowest 2.45±0.61 and the highest 2.68±0.54 (31) in their study conducted with nurses in two different hospitals. When reviewing other studies utilising diverse measuring tools in the literature, a positive attitude towards PS was observed among nurses who worked in surgical centres (32,33). It was observed that the findings of the studies on PSC examined in the literature were similar to the findings of this study (14,29-33) In the studies in the literature where PSC is analysed, it can be said that the mean PSC scores are higher among nurses working in surgical units (29,30). This situation can be explained by the fact that many of the parameters evaluated within the scope of PS in hospitals are actively fulfilled in surgical clinics. Surgical nurses who adhered to EBN practices and implemented them in patient care exhibited a greater degree of PSC attitudes ,according to observations (r=0.705, p=0.000) recorded in Table 2. In the literature, Sevinç Turaç and Top concluded that EBN practices had a moderate (r=0.430) effect on PSC in their study on nurses (34). Sonğur et al. reported that EBN practices had a moderate (r=0.418) effect on PSC in their study on nurses (35). When the literature was examined, it was observed that the findings of the studies examining the effect of attitude towards EBN practices on PSC were similar to the findings of this study (34,35). However, it is suggested that the high Correlation coefficient found in the study as compared to literature may be attributed to the fact that the study was carried out exclusively in surgical clinics.

CONCLUSION

The attitudes of surgical nurses towards EBN practices and PSC were found to be positive. It was concluded that surgical nurses with high attitudes towards EBN practices had more positive attitudes toward surgical PSC. Although PSC is important for nurses working in all clinics, it is more important for nurses working in surgical clinics. Therefore, it is recommended that working conditions should be improved, training nurses who follow EBN practices up to date and in-service trainings should be planned in line with the latest evidence findings.

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Research Article

Gossypin Regulated Doxorubicin-Induced Oxidative Stress and Inflammation in H9c2 Cardiomyocyte Cells

Yener Yazgan[1], Betul Yazgan[2]

Kastamonu University, Faculty of Medicine, Department of Biophysics, Kastamonu, Türkiye
Kastamonu University, Faculty of Medicine, Department of Physiology, Kastamonu, Türkiye

Abstract

Aim: Doxorubicin (DOX), an anthracycline, is widely used in chemotherapy due to its effectiveness in fighting many cancers. Experimental and clinical studies prove that this drug damages non-targeted tissues (including cardiomyocytes) and reduces patients' quality of life during and after DOX treatment. The discovery of potent compounds as a protective tool to slow cardiomyocyte damage during the use of anti-cancer drugs such as DOX is crucial for both more effective cancer treatment and to improve patient's quality of life. Gossypin (GOS) is a flavonoid with several important properties, such as anti-cancer, analgesic, antioxidant, and anti-inflammatory. GOS shows supportive effects against oxidative stress and inflammation by activating antioxidant defense enzymes.
Material and Method: For the study, four groups were formed from H9c2 embryonic cardiomyocyte cells as Control, DOX (1 µM, 48 h), GOS25 (25 µg/ml, 48 h), and GOS50 (50 µg/ml, 48 h). In the study, Total antioxidant and oxidant status (TAS and TOS), levels of the inflammatory cytokines IL 1 beta and 6, and TNF α, lipid peroxidation levels as malondialdehyde (MDA), glutathione peroxidase (GSHPx), and glutathione (GSH) levels in the H9c2 embryonic cardiomyocyte cells were determined.
Results: The results showed that DOX treatment caused cell toxicity in the embryonic cardiomyocyte cells and increased TOS, IL 1 beta and 6, TNF α, and MDA levels while decreasing TAS, GSH, and GSHPx levels. This situation improved with GOS treatment.
Conclusion: As a result, it was determined that GOS treatment showed a protective effect in the DOX-induced cell toxicity model in H9c2 embryonic cardiomyocyte cell lines.

Keywords: Oxidative stress, inflammation, H9c2 cardiomyocyte cell, gossypin, doxorubicin

INTRODUCTION

Doxorubicin (DOX), an anthracycline and one of the most effective anti-cancer drugs since the 1960s, is widely used in chemotherapy and is effective against many types of cancer, including blood cancer, sarcoma, and cancer (1). However, experimental and clinical studies have shown that DOX is toxic to non-target organs and reduces the quality of life during and after treatment (2). Known harmful side effects such as myelosuppression, cardiotoxicity, brain, kidney, and liver toxicity, and alopecia limit the clinical use of this drug (3-5).

It is unknown which factors are involved in the mechanisms of cardio-toxicity induced by DOX. Reports in the literature suggest that oxidative stress and inflammation may be important mechanisms underlying cardiotoxicity (6,7). Increased reactive oxygen species (ROS) have been reported to cause lipid peroxidation, organelle damage, and cellular signaling imbalances in various cells, including cardiomyocytes (8-11). In addition, some reports that increased oxidative stress with DOX exposure increase the release of pro-inflammatory cytokines such as interleukin 1 beta (IL 1 beta) and tumor necrosis factor alpha (TNF α) (6,12).

The discovery of potent compounds as a protective tool to slow down myocyte damage during the use of anti-cancer drugs such as DOX is crucial both for more effective cancer treatment and for improving patients' quality of life. Gossypin (GOS) is a flavonoid with various essential properties such as anticancer, analgesic, antioxidant, and anti-inflammatory (13,14). GOS shows its supportive

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Corresponding Author: Yener Yazgan, Kastamonu University, Faculty of Medicine, Department of Biophysics, Kastamonu, Türkiye
E-mail: yener8275@hotmail.com

effects against oxidative stress and inflammation by activating antioxidant defense enzymes (15). Although DOX is an effective chemo-therapeutic agent, cardio-toxicity is known to contribute to patient mortality (11). There are many experimental and clinical studies in the literature investigating how to reduce the cardio-toxic effects of DOX (10,11,16,17). Cardio-protective effects of GOS have been reported in a limited number of in vitro and in vivo experimental studies (13,18). However, whether GOS can ameliorate the cardio-toxic effects of DOX, which is widely used in many cancers, has not been investigated. Therefore, this study was designed to examine whether GOS has protective effects against DOX-induced cardio-toxicity in a dose-dependent manner in the H9c2 cell line. This cell line is a cardio-myoblast derived from embryonic rat heart tissue and is widely used in heart disease research (13,19,20).

In this study, we evaluated the protective effects of GOS on rat H9c2 embryonic cardiomyocytes by determining the oxidant/antioxidant status (TAS, TOS, GSHPx activation, GSH, and lipid peroxidation levels). In addition, we aimed to evaluate the inflammation status in myocytes by investigating inflammatory cytokines IL 1 beta and 6 and TNF α levels in H9c2 embryonic cardiomyocyte cells.

MATERIAL AND METHOD

Chemicals and ELISA Kits

GOS (Cat; 652-78-8) was purchased from Sigma Aldrich C. (USA/St Louis). DOX (Cat; T1020) was purchased by TargetMol (Target Molecule Corp., USA). IL 6 (Sunred, Cat; SRB-T-83168), IL 1 beta (Sunred, Cat; SRB-T-83324), and TNF α (Sunred, Cat: SRB-T-82883) were purchased from SunRed Biotechnology Company (SRB) Ltd (Shanghai, China). Total Oxidant and Total Antioxidant Capacity (TOS and TAS) ELISA kit was obtained from Rel Assay (Gaziantep/Türkiye).

Cell Culture and Experimental Groups

A growth medium was prepared for the cells used in the research according to the instructions provided by the seller. FBS (cytiva Cat; SV30160.03) (10%) and penicillin/streptomycin (biosera Cat; LM-A4118) (1%) were added to equivalent volumes of DMEM (biowest Cat; L0064) as growth medium contents. Cells were cultured in 25 cm^2 culture flasks in an incubator at 37°C under a 5% CO_2 atmosphere. H9c2 embryonic cardiomyocyte cell lines were divided into four groups and incubated according to the experimental procedure. DOX and GOS were freshly prepared on the experimental days. The following incubation procedure was applied to the experimental groups. After the incubation period was completed, the cells were washed with fresh 1xPhosphate Buffered Saline (Biochrom/Germany), and 0.25% Trypsin-EDTA (Sigma-Aldrich) was applied to separate the cells from the flask floor. After completing the experimental steps, analyses were performed for all groups.

For the study, H9c2 cardiomyocyte cells in the experiment were divided into four groups as follows.

Control group (n=5), the cardiomyocyte cells were kept in a culture medium for 48 hours without treatment.

DOX group (n=5), the cardiomyocyte cells were incubated with DOX (1 µM for 48 h) (11,21).

GOS25 group (n=5), cardiomyocyte cells in this group were pretreated with GOS (25 µg/ml) 3 hours before DOX treatment and then incubated with DOX (1 µM) for 48 hours (13).

GOS50 group (n=5), cardiomyocyte cells in this group were pretreated with GOS (50 µg/ml) 3 hours before DOX treatment and then incubated with DOX (1 µM) for 48 hours (13).

Preparation of Cells Homogenates

For each group, cells were transferred into separate sterile falcon tubes and centrifuged according to the kit procedure (1000 rpm and 20 min). After centrifugation, the supernatants on the top of the falcon tubes were removed with the help of an automatic pipette, the cells were suspended in PBS, and a cell suspension with a density of approximately 1×10^6 cell/ml was obtained. The cell structure was lysed (PBS) by freeze-thaw repetition, and the mixture was centrifuged (4000 rpm, 10 min) after removal of cytoplasmic components. The supernatant remaining at the top of the falcon tubes was removed with pipettes and taken in Eppendorf tubes for analysis. The Bradford protein assay kit (Merck-Millipore) measured total protein levels in the groups.

Analyses

Measurement of Total Oxidant-Antioxidant and Inflammatory Cytokines Levels in the H9c2 Embryonic Cardiomyocyte Cells

TOS and TAS and inflammatory cytokines (IL 6 and 1 beta and TNF α) levels in H9c2 embryonic cardiomyocyte supernatants were determined using ELISA kits. For the analyses, supernatants were first incubated (37°C, 60 min) by the protocols specified by the companies for commercial kits and then placed in 96-well plates with automatic pipettes. The supernatant and standard samples placed on the plate were incubated for 60 minutes, followed by washing steps, and then staining solutions were added and incubated (15 min). A stop solution was added at the end of all these procedures, and absorbance values were read on an ELISA (BioTek Epoch™) microplate spectrophotometer (22).

Measurement of Glutathione/Glutathione Peroxidase and Lipid Peroxidation Levels in the H9c2 Embryonic Cardiomyocyte Cell

Lipid peroxidation activity, which is known as malondialdehyde (MDA) release in DOX-induced cell toxicity in H9c2 embryonic cardiomyocyte cells, was determined by thiobarbituric acid (TBARS) reaction in a highly sensitive spectrophotometer (V-730 UV-Visible

Spectrophotometer, Japan) according to the method of Placer et al. All cell groups were reconstituted with 1/9 (2.25 ml) TBARS solution. The experiment used a mixture of 0.25 ml phosphate buffer and 1/9 of TBARS as a blind. Samples and blind were kept in 100 °C water for 20 minutes (23,24). It was then cooled on ice and centrifuged (1000 g, 5 min). The upper pink liquid was taken with an automatic pipette and read against the blind in a spectrophotometer at 532 nm wavelength in a 1 cm light transmission cuvette. The standard was standard: 1, 1, 1, 3, 3 tetraethoxy propane solution prepared in the same proportions. Values were determined as µmol/g protein.

GSH levels of H9c2 embryonic cardiomyocyte cells were determined spectrophotometrically (412 nm) using the Sedlak and Lindsay method (25). H9c2 embryonic cardiomyocyte cells (10^6 cells per mL) were transferred to sterile falcon tubes with the help of an automatic pipette and centrifuged to separate the proteins after mixing with 10% trichloroacetic acid. After centrifugation, 0.1 ml of the supernatant remaining on the falcon tube was taken and placed in a glass tube, 0.5 mL 5.5-dithiobis (2-nitrobenzoic acid), 2 mL phosphate buffer (pH 8.4), and 0.4 mL distilled water were added. The resulting sample was read (412 nm) in a spectrophotometer. Values were determined as µmol/g protein.

GSHPx levels of H9c2 embryonic cardiomyocyte cells were determined spectrophotometrically at 412 nm by the method of Lawrence and Burk (26). GSHPx activity was expressed as international units (IU) oxidized glutathione/g protein.

Statistical Analysis

All data are expressed as mean±standard deviation in this study, and data analysis was performed by one-way ANOVA using SPSS. For all data with a statistically significant difference, the post-hoc Tukey test was used. p≤0.05 was considered to be statistically significant.

RESULTS

Effect of Gossypin on TOS and TAS Levels in H9c2 Embryonic Cardiomyocyte Cells

It is shown in Figure 1 that GOS treatment modulated the decrease in TAS and increase in TOS levels as a result of DOX treatment in H9c2 embryonic cardiomyocyte cells. A significant increase in TOS levels (Figure 1A) was observed in the DOX-treated group was compared to Control, GOS25, and GOS50 groups (p≤0.05), and in parallel, a significant decrease in TAS levels (Figure 1B) was observed in the DOX-treated group was compared to Control, GOS25, and GOS50 groups (p≤0.05). The decrease in TAS and increase in TOS levels after DOX treatment in H9c2 embryonic cardiomyocyte cells were regulated by GOS treatment. Significant results were obtained in TAS and TOS levels in H9c2 embryonic cardiomyocyte cells pretreated with 25 and 50 µg/ml of GOS. However, the 50 µg/ml of GOS further regulated the DOX-induced and disrupted oxidant/antioxidant balance.

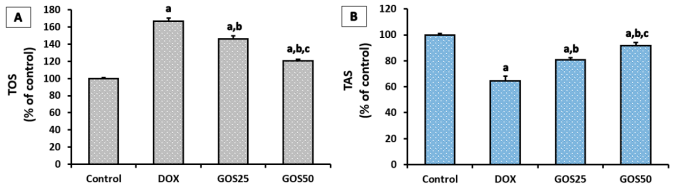

Figure 1. Effect of GOS on TOS (**1A**) and TAS (**1B**) levels in H9c2 embryonic cardiomyocyte cells after DOX-induced cell toxicity (mean±SD). ([a]p≤0.05 vs Control group, [b]p≤0.05 vs DOX group, [c]p≤0.05 vs GOS25 group)

Effect of Gossypin on Inflammatory Cytokines Levels in H9c2 Embryonic Cardiomyocyte Cells After Doxorubicin-induced Cell Toxicity

GOS treatment modulated DOX-induced IL 6 and 1-beta, and TNF α levels in the H9c2 embryonic cardiomyocyte cells are shown in Figure 2. When the DOX-induced treated group was compared to the Control, GOS25, and GOS50 groups between the groups, it was observed that the IL 1 beta, IL 6 (Figure 2A-2B), and TNF α (Figure 2C) levels increased considerably (p≤0.05). The increase in IL 1 beta and 6 and TNF α levels after DOX treatment in H9c2 embryonic cardiomyocyte cells was regulated by GOS treatment. Significant results were obtained in IL 6 and 1 beta, and TNF α levels in H9c2 embryonic cardiomyocyte cells pretreated with 25 and 50 µg/ml doses of GOS; however, the 50 µg/ml dose of GOS further regulated the DOX-induced and disrupted inflammation balance.

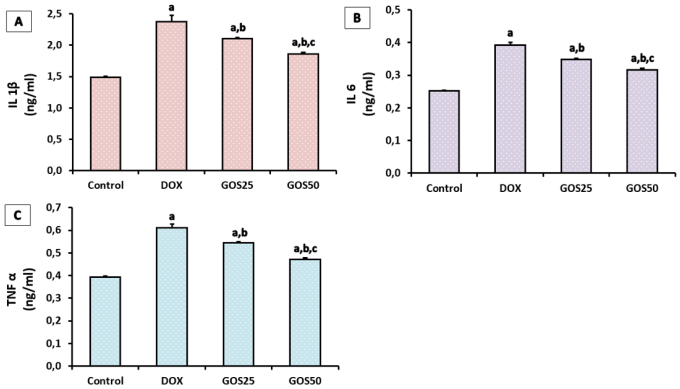

Figure 2. Effect of GOS on IL 1β (**2A**), IL 6 (**2B**), and TNF α (**2C**) levels in H9c2 embryonic cardiomyocyte cells after DOX-induced cell toxicity (mean±SD). ([a]p≤0.05 vs Control group, [b]p≤0.05 vs DOX group, [c]p≤0.05 vs GOS25 group)

The Gossypin Treatment Attenuated the Doxorubicin-induced Changes in GSH, GSHPx, and MDA Levels

The changes in GSH, GSHPx, and MDA levels in cells against DOX-induced cell toxicity of GOS in the groups formed were measured with the spectrophotometrically (V-730 UV-Visible Spectrophotometer, Japan). It is shown in Figure 3 that GOS treatment modulated the increase in oxidative stress (MDA) and decrease in antioxidant (GSH and GSHPx) levels as a result of DOX treatment in H9c2 embryonic cardiomyocyte cells. When the DOX-induced treated group was compared to the Control, GOS25, and GOS50 groups, it was observed that the GSH levels (Figure 3A) and GSHPx levels (Figure 3B) decreased considerably (p≤0.05). MDA levels (Figure 3C) were significantly

increased between the groups when the DOX-induced treated group was compared to the Control, GOS25, and GOS50 groups (p≤0.05). The decrease in GSHPx and GSH levels and increase in MDA levels after DOX treatment in H9c2 embryonic cardiomyocyte cells were regulated by GOS treatment. Significant results were obtained in GSH, GSHPx, and MDA levels in H9c2 embryonic cardiomyocyte cells pretreated with 25 and 50 µg/ml doses of GOS. However, the 50 µg/ml dose of GOS further regulated the DOX-induced and disrupted oxidant/antioxidant balance.

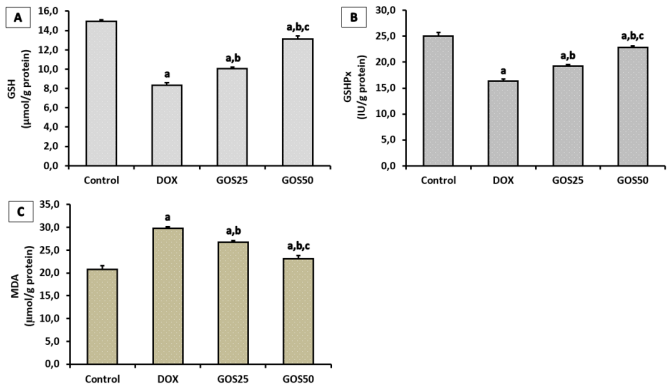

Figure 3. Effect of GOS on GSH (**3A**), GSHPx (**3B**), and MDA (**3C**) levels in H9c2 embryonic cardiomyocyte cells after DOX-induced cell toxicity (mean±SD). ([a]p≤0.05 vs Control group, [b]p≤0.05 vs DOX group, [c]p≤ 0.05 vs GOS25 group)

DISCUSSION

DOX is a vital drug widely used in cancer treatment since the 1960s (1,7). However, its cardiotoxic effects limit its use. It has been emphasized that the cardio-toxic effects of DOX are multifactorial and cause functional disorders in cardiomyocyte cells (27). Based on the physiopathological mechanisms of DOX-induced cardio-toxicity, it may be possible to reduce the side effects of the drug. In this study, we investigated whether GOS administration is protective against the cardio-toxic effects of DOX. For this purpose, we applied GOS at two different doses (25 µg/ml and 50 µg/ml) in H9c2 cells. Our results showed that DOX treatment-induced cardiotoxicity in H9c2 cardiomyocytes and that GOS treatment ameliorated the cardiac damage at both doses, but to a greater extent at the 50 µg/ml dose.

One of the most widely accepted damage mechanisms for DOX-induced cardio-toxicity is the activation of damage mechanisms due to increased oxidative stress (5,6,28). Shaker et al. reported a significant increase in lipid peroxidation and a reduction in TAS levels in the rat heart after DOX administration (6). Similarly, it has been reported that there is a decrease in GSH and GSHPx levels in rats administered DOX and that limonin, known for its antioxidant properties, is beneficial in preventing cardiac damage (29). In another study, Yıldızhan et al. found that DOX therapy caused a significant decrease in the levels of GSHPx and GSH in the serum and samples of heart tissue. In contrast, selenium administration with antioxidant effects caused a considerable increase in GSH and GSHPx levels (7). DOX treatment was shown to increase lipid peroxidation levels, particularly in heart tissue, in another experimental study. However, it decreased GSH and GSHPx levels (30). In another in vivo and in vitro study, heart tissue from mice exposed to oxidative stress and an H9c2 embryonic cardiomyocyte cell line showed a significant reduction in superoxide dismutase (SOD) and GSH activity (13). Our data showed that DOX treatment significantly increased lipid peroxidation and TOS levels and significantly decreased TAS levels in H9c2 embryonic cardiomyocyte cells, consistent with previous studies. Similar to previous studies, the reduction in DOX-induced GSH and GSHPx activity demonstrated the depletion of cardiac antioxidant enzymes. Cinar and colleagues reported an increase in SOD activity and GSH levels in mice and H9c2 embryonic cardiomyocyte cells and a reduction in lipid peroxidation levels when GOS was administered in various dosages (13).

GOS is an essential flavonoid with potent antioxidant and anti-inflammatory properties (14). GOS shows its supportive effects against oxidative stress and inflammation by activating antioxidant defense enzymes (15). Cinar and colleagues reported that SOD activity and GSH levels increased and lipid peroxidation levels decreased in mice and H9c2 embryonic cardiomyocyte cells in a study testing the antioxidant effects of various doses of GOS (13). In the same study in which they applied GOS at doses of 25, 50, and 100 µg/ml, they found that the most effective amount of GOS in H9c2 cells was 50 and 100 µg/ml. In our study, we found that GSH, GSHPx, and TAS were significantly increased in the GOS25 and GOS50 groups compared to the DOX group. Furthermore, GOS25 and GOS50 significantly reduced lipid peroxidation and TOS levels compared to the DOX group. Thus, we found that GOS pre-treatment reduced DOX toxicity in myocytes at both doses (25 and 50 µg/ml).

We predict the activation of pro-inflammatory cytokines in cardiac tissue in the physiopathological process of DOX-induced cardio-toxicity. It has been demonstrated in clinical and experimental studies that DOX therapy causes myocardial inflammation (6,31-33). There have been important reports showing that DOX therapy induces the release of various pro-inflammatory cytokines, including TNF α, and causes several inflammatory responses in the myocardium (6,34). We demonstrated significant increases in cardiac IL 1 beta and 6 and TNF α in the DOX group compared to the control group, in line with these reports. The results of this study support the critical role of inflammation in the pathogenesis of DOX-induced cardio-toxicity. When these results are evaluated with the oxidative parameters obtained in this study, they suggest that increased inflammatory markers may be associated with impaired antioxidant capacity (low TAS, high TOS levels) and lipid peroxidation. Yıldızhan et al. reported increased IL 1 beta, TNF α, and ROS levels with significantly reduced GSH and GSHPx levels in the DOX group, consistent with the results of this study (7).

The anti-inflammatory effects of GOS have been reported in the literature (14,18). In a study investigating the

curative impact of GOS against gentamicin-induced nephron-toxicity in rats, inflammatory cytokines in renal tissues were reduced by (TNF α and IL 6) GOS therapy (15). In another study, IL 1 beta and TNF α mRNA expression induced by H_2O_2 in L929 fibroblast cells was significantly reduced with different doses of GOS treatment (25 and 50 µg/mL) (35). Our data showed that DOX treatment in an embryonic cardiomyocyte cell line caused a significant increase in IL 1 beta and 6 and TNF α. Against DOX toxicity, we found that GOS pre-treatment reduced these inflammatory cytokines. In our study, in the GOS25 and GOS50 groups, IL 1 beta and 6 and TNF α levels decreased significantly in a dose-dependent manner, more effectively in the GOS50 group than in the DOX group. This suggests that GOS treatment may have a beneficial effect on inflammation in heart muscle tissue.

Thus, we demonstrated the protective effect of GOS on heart tissue using an H9c2 embryonic cardiomyocyte cell line. In conclusion, the findings in the literature and the results of this study emphasize that GOS, a potent antioxidant and anti-inflammatory agent, can be used as a cardio-protective agent against DOX toxicity.

CONCLUSION

It is known that the cardio-toxic effects of DOX, which is widely used in cancer treatment, are multifactorial and cause functional disorders in cardiac cells. Based on the physiopathologic mechanisms of DOX-induced cardio-toxicity, it is vital to find therapies to reduce the side effects of the drug.

This study demonstrated DOX-induced cellular toxicity in H9c2 embryonic cardiomyocyte cells by disrupting the oxidant/antioxidant balance and increasing the release of inflammatory cytokines. In addition, critical preclinical data were provided to the literature by determining the curative efficacy of GOS. While these data indicate the possibility of using natural substances such as GOS to reduce damage during cancer treatment, more preclinical research is needed.

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Conflict of interest: *The authors have no conflicts of interest to declare.*

Ethical approval: *The current study has no study with human and human participants. The study is not subject to ethics committee approval. Ethics Committee Approval is not required for cell culture studies.*

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MEDICAL RECORDS-International Medical Journal

Research Article

Accessory and Sesamoid Bones in the Body: A study on their Size and Presence

Muhammet Degermenci[1], Ali Aygun[2], Halil Yilmaz[1], Adem Tokpinar[1], Orhan Bas[1]

[1]Ordu University, Faculty of Medicine, Department of Anatomy, Ordu, Türkiye
[2]Ordu University, Faculty of Medicine, Department of Emergency Medicine, Ordu, Türkiye

Abstract

Aim: Accessory and sesamoid bones are hidden anatomical structures that fulfil a wide variety of functions in the human body. Therefore, studying the nature, formation and dimensions of these structures is of great importance for the medical literature. In our study, we aimed to measure the presence and area (size) of these ossicles.
Material and Method: Our study was performed retrospectively on the images of individuals who applied to Ordu University and whose radiological images were obtained. The presence of accessory and sesamoid ossicles (present/absent) and their sizes (measured using the PACS system) were evaluated on radiographs and Computed Tomography (CT) images. The results of the ossicles were divided into gender, age, and bilateral groups. Statistical analyzes were performed with the SPSS program.
Results: The most common and largest accessory ossicles in the foot were os peroneum (18.2%), os naviculare accessoria (17.4%), os trigonum (12.1%) and os intermetatarsarum (7.8%). Sesamoid ossicles at the level of the first metatarsophalangeal (mtp) joint of the foot and the first metacarpopgalangeal (mcp) joint of the hand were found to be 100% common. The second most common sesamoid bone in the hand and foot was at the fifth mtp (22.22%) and mcp (68.53%) joints. In the hand, the most common accessory ossicles were os triangulare (6.08%), os radiale externum (2.60%) and os centrale (1.73%). Accessory ossicles (fabella: 19%, cyamella: 17.5%) were found in 25.5% of the knee.
Conclusion: Accessory and sesamoid ossicles are most commonly found in the hand, foot, and knee. The presence and size of these ossicles can be both beneficial and harmful. Sesamoids provide mechanical benefit, whereas accessory ossicles can be mistaken for fractures and may lead to unnecessary medical conditions. Knowing the dimensions of these ossicles allows them to be clinically differentiated from avulsion fractures.

Keywords: Accessory ossicles, sesamoid ossicles, morphometry, radiograph

INTRODUCTION

Accessory bones in the body are variational bones that are formed by separation from the main bone during the development of the skeletal system and are of congenital origin. They are usually formed when the secondary ossification centre fails to join the primary ossification centre. They are usually round or oval in shape and have smooth edges. Although they do not cause pain under normal conditions, they may cause pain due to fractures, dislocations, bony deformities, irritation of soft tissues and overuse. Fractures and dislocations are the most common manifestations of disorders of these ossicles. In the presence of trauma, these bones may be misdiagnosed as avulsion fractures and may restrict movement. Therefore, the localization and dimensions of the accessory bones should be well known to avoid misdiagnosis. Misdiagnoses cause excessive workload, additional costs, and unnecessary treatment services (1,2).

Accessory bones around the foot and ankle are commonly seen. In the current literature, many accessory ossicles have been described in the foot. Some of these bones have not been adequately described or some of them have been described by many people. Musculoskeletal injuries in this region are commonly seen in patients presenting to the emergency department due to trauma. It is important for physicians to be aware of the presence of these bones and to differentiate them from fractures in radiographs or

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Corresponding Author: Muhammet Degermenci, Ordu University, Faculty of Medicine, Department of Anatomy, Ordu, Türkiye
E-mail: mdegermenci@yahoo.com.tr

tomography images taken in emergency departments or clinics. Therefore, knowing the normal and/or abnormal variations of bone structures in the lower extremity and various radiological pitfalls that may cause confusion is the primary objective of our study (1,3).

The most common accessory ossicles in the foot and ankle are os trigonum, os peroneum and os naviculare accessoria. The os trigonum is formed by the separation of the lateral posterior process of the talus and should not be confused with a fracture of this process (Shepherd's fracture). Os peroneum is located within the tendon of the peroneus longus muscle within the cuboid tunnel. Accessory navicular bone is formed by the separation of the tubercle of navicular bone in the medial part of the navicular bone (4-6).

Os intermetatarsarum is usually located dorsal to the bases of the first and second metatarsal bones. The secondary calcaneal bone is an accessory ossicle arising from the anterior process of the calcaneus. The os vesalianum pedis is formed by the separation of the secondary ossification centre of the fifth metatarsal bone and is located within the tendon of the peroneus brevis muscle (7).

The term sesamoid is derived from the Latin word 'sesamum' and means sesame. A sesamoid bone is a small round bone embedded in a tendon or joint capsule. Sesamoid bones are typically found where a tendon passes over a joint. They prevent friction between the tendon and the joint, protect the tendon and increase the biomechanical effect of the tendon by changing its direction of pull. Most sesamoid bones begin as cartilaginous nodules that undergo endochondral ossification in early to late childhood, between the ages of 3 and 12 years. Sesamoid bones are generally thought to result from an interaction between mechanical and biological factors (2,8,9). The number and size of these bones can vary from person to person and are of great importance for athletes, dancers and other people involved in physical activity.

The largest sesamoid bone is the patella and is located within the tendon of the quadriceps muscle. In the foot, there are usually two sesamoid bones (sometimes 2-3 in pieces) at the connection of the first metatarsal bone with the big toe. Both are located within the tendon of the flexor hallucis brevis muscle. One is located on the lateral side of the first metatarsal and the other on the medial side. Some people have only one sesamoid on the first metatarsal bone (10).

There is a sesamoid bone called fabella embedded in the tendon of the lateral head of the gastrocnemius muscle behind the lateral condyle of the femur behind the knee. It is found in 10-30% of humans. It can be single-parted or multi-parted. Cyamella is rarely seen in humans and is found in the tendon of the popliteus muscle. The presence of fabella and cyamella usually does not cause knee pain or discomfort, but it can sometimes lead to knee problems in some athletes or individuals engaged in physical activity. Sesamoiditis, an inflammation of the sesamoid bones, is common in dancers and is caused by inflammation or irritation of the tendons surrounding these bones (11).

MATERIAL AND METHOD

Ethical approval was obtained from Ordu University Clinical Research Ethics Committee (2023/103) and Ordu Provincial Health Directorate. Computed Tomography (CT) and radiography images to be used in the study were obtained from Ordu University Training and Research Hospital PACS system. Individuals with fractures and chronic bone disorders in their extremities were not included in the study. Radiological images of the foot, ankle and knee were evaluated. The frequency of accessory and sesamoid bones (present/absence) and the size (area) of the bones with the cursor were calculated. The frequency of accessory and sesamoid bones according to age and gender and the bilateral differences were determined.

Statistical Analysis

Statistical analysis of the data was performed with IBM SPSS 28. All the measurements are expressed as the Mean±SD, and the incidence of each type is described in terms of numbers and percentages. The homogeneity of variance was performed using the Kolmogorov-Smirnov test. Mann-Whitney U test was applied for gender comparison in nonparametric data. $p<0.05$ was considered statistically significant.

RESULTS

Foot and ankle images (45 right foot, 30 left foot and 40 bilateral feet) of a total of 115 individuals were evaluated. The mean age of the individuals is 45.65±16.76 (14-86 years) and of these, 54 were male (47%) and 61 were female (53%). 55 patients had no accessory bone (47.82%), while 60 patients had at least one accessory bone (52.17%). In 16 bilateral feet (7 males, 9 females), no accessory bone was found (40%). No accessory bone was found in 25 right feet (55.55%) and 14 left feet (46.66%) in unilateral feet (Table 1).

On an individual basis, the most commonly identified accessory bones were os peroneum (18.2%), os naviculare accessoria (17.4%), os trigonum (12.1%) and os intermetatarsarum (7.8%). On a foot basis, os peroneum (14.83%), os naviculare accessoria (14.83%), os trigonum (9.03%) and os intermetatarsarum (0.64%) were the most common accessory bones (Figure 1). Accessory bones were found in 55.55% (30) of males and 50.81% (31) of females. The frequency of accessory bones in the foot is shown in Table 1.

In our study, in addition to the normal accessory ossicles reported in the literature, bipartite os trigonum* (2 feet) and bipartite os naviculare accessoria** (1 foot) were

found. These pairs of bones are usually one large and one small (Figure 2).

Table 2 shows the dimensions of the accessory bones found in the foot, their minimum and maximum values according to gender and bilateral averages. The largest accessory bone was os trigonum (83.83 mm^2), followed by os peroneum, os intermetatarsarum and os naviculare accessoria. Although accessory ossicles showed a steady increase with age, this increase was not found to be regular due to individual differences. The sizes of males were found to be larger than those of females (Graphic 1).

Table 1. Frequency of accessory bones in the foot

Accessory bones	Genders (54 Men, 61 Women)						Total (115)
	Men (36)		Women (39)		Men (18)	Women (22)	
	Right (21)	Left (15)	Right (24)	Left (15)	Bilateral (40)		
Os peroneum	5	3	5	6	1	1	21 (18.2%)
Os naviculare accessoria	6	1	4	6	1	2	20 (17.4%)
Os trigonum	2	4	7	1	-	-	14 (12.1%)
Os intermetatarsarum	4	3	-	1	-	1	9 (7.8%)
Os calcaneus secundarius	2	-	-	1	-	1	4 (3.5%)
Os vesalianum pedis	-	1	1	1	-	-	3 (2.6%)
Os supranaviculare	-	1	2	-	-	-	3 (2.6%)
Os supratalare	-	-	-	1	1	-	2 (1.7%)
Os cuboideum secundarium	1	-	-	1	-	-	2 (1.7%)
Os subfibulare	-	-	1	-	-	-	1 (0.9%)
Os talotibiale	-	1	-	-	-	-	1 (0.9%)
Os infranaviculare	-	-	-	1	-	-	1 (0.9%)
Os intercuneiforme	1	-	-		-	-	1 (0.9%)
Os tuberis calcanei	-	-	-	1	-	-	1 (0.9%)

Table 2. Areas (sizes) of accessory bones in the foot (mm^2)

Accessory bones	Men (54)			Women (61)		
	Min	Max	Right - Left (means)	Min	Max	Right - Left (means)
Os peroneum	11.07	60.03	23.89-27.82	3.42	44.12	9.63-16.65
Os naviculare accessoria	5.27	58.10	21.78-15.19	2.68	40.12	13.26-13.77
Os trigonum	33.68	83.83	71.09-58.77	13.5	68.62	43.95-13.50
Os intermetatarsarum	3.75	59.87	24.62-13.43	3.95	32.85	30.11-18.40
Os calcaneus secundarius	16.34	22.48	19.41	2.91	34.42	17.78-18.66
Os vesalianum pedis	4.48		4.48	8.96	27.13	27.13-8.96
Os supranaviculare	10.37		10.37	11.91	12.05	11.98
Os supratalare	9.98	10.11	10.11-9.98	4.54		4.54
Os cuboideum secundarium	3.35		3.35	7.37		7.37
Os subfibulare	-	-	-	29.26		29.26
Os talotibiale	24.77		24.77	-	-	-
Os infranaviculare	-	-	-	12.48		12.48
Os intercuneiforme	3.99	3.99	-	-		-
Os tuberis calcanei				22.56		

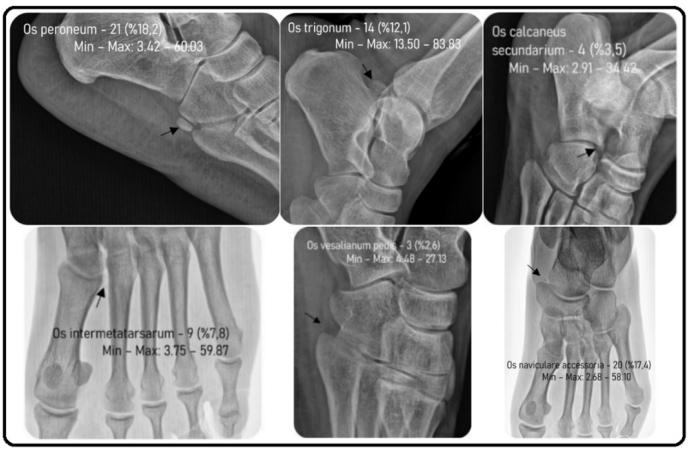


Figure 1. The most widely found accessory ossicles and their sizes in our study on foot radiographs

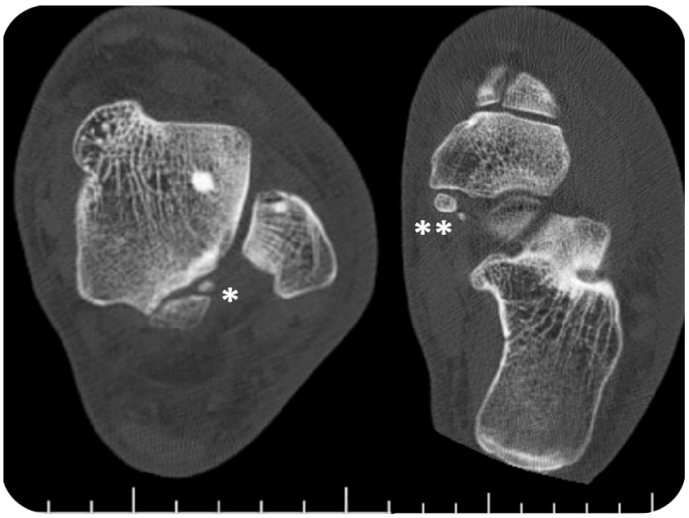
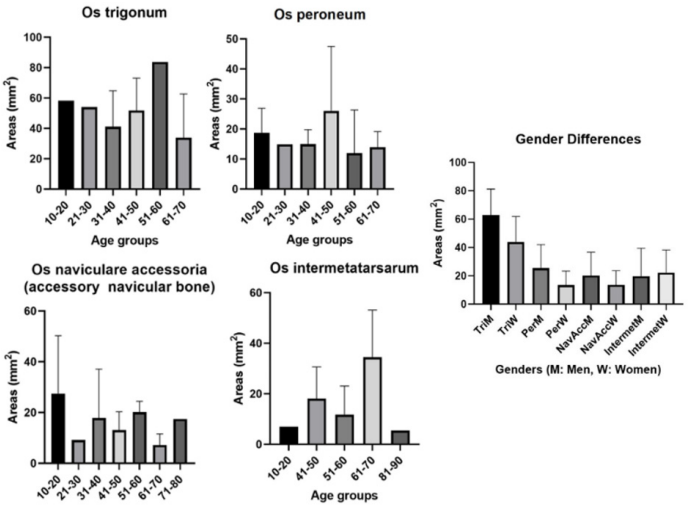


Figure 2. Bipartite os trigonum and bipartite os naviculare accessoria on axial CT section



Graphic 1. Comparison of the sizes of the most common accessory ossicles according to age and sex

Sesamoid Ossicles in the Foot

Sesamoid bones are small ossicles located at the level of the metatarsophalangeal joints in the foot and increase the insertion angle of the joint and provide better movement. In our study, sesamoid bones at the level of the right (Rmtp) and left metatarsophalangeal joints (Lmtp) and sesamoid ossicles at the level of the interphalangeal (interp) joints were evaluated.

Sesamoid ossicles were found in the lateral (Lat) and medial (Med) parts of the first metatarsophalangeal joint (mtp1) in all 84 right and 69 left feet. In 7.14% of the right and 8.69% of the left feet, these medial ossicles were divided into proximal (Prox) and distal (Dis) ossicles. These ossicles have different sizes depending on the age, bone development and gender. Figure 3 shows the frequency and mean size (mm^2) of sesamoid and accessory ossicles (mtp1, mtp2, mtp3, mtp4 and mtp5) in the foot. Accordingly, the largest sesamoid bone in the foot belonged to Rmtp1 Lat (97.84±26.47 mm^2). This was followed by Rmtp1 Med, Lmtp1 Lat, and Lmtp1 Med. The largest sesamoid bone was found to be Rmtp1 Lat with 181.49 mm^2. The smallest sesamoid ossicle was Rmtp5 (3.26 mm^2). The least common sesamoid ossicles were Rmtp3 (1.19%) and Rmtp4 (1.19%).

Figure 3. Frequency and size of the sesamoid bones commonly found in the foot (A and C: right foot; B and C: left foot) and of the sesamoid bones not commonly found (E, F and G).

Different from these sesamoid ossicles, we detected variational sesamoid ossicles including lateral and medial ossicles on the fifth metatarsophalangeal joint in 6 feet (Figure 3G), medial and lateral ossicles on the first interphalangeal joint in 3 feet (Figure 3E), proximal and distal separation of the lateral ossicle on the first metatarsophalangeal joint in 2 feet, lateral and medial ossicles on the fifth proximal interphalangeal joint in 1 foot (Figure 3F).

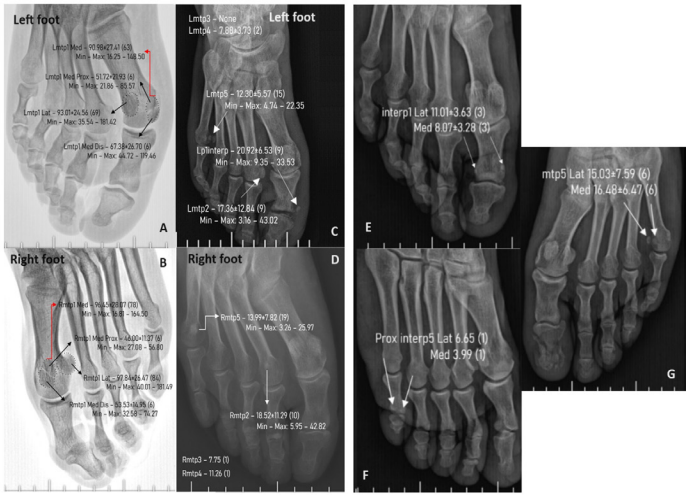


Figure 3. Frequency and size of the sesamoid bones commonly found in the foot (A and C: right foot; B and C: left foot) and of the sesamoid bones not commonly found (E, F and G)

Accessory Bones in the Knee

In our study, bilateral knees of 200 individuals were evaluated. Of these, 68 were male and 132 were female with a mean age of 52.56±15.79 years (14-88 years). The incidence and dimensions of primary accessory bones of fabella and cyamella in the knee were evaluated. 149 (74.5%) of 200 participants had no accessory bones in the knee.

Fabella was found in 38 (19%) right and 35 (17.5%) left knees. In bilateral evaluation, fabella was found only in the right knee of 4 participants and only in the left knee of 1 participant. In the bilateral evaluation of 200 individuals, cyamella were found in 11 right knees and 12 left knees. Only 1 individual had cyamella only on the left side.

The minimum, maximum and average dimensions of fabella and cyamella in the right and left knee are shown in Figure 4. As a result of the measurements, it was observed that fabella and cyamella accessory ossicles in the knee increased with age. In addition, the sizes of these bones were determined to be larger in male individuals and a statistically significant difference (*p<0.05) was found between men and women in cyamella bones (Graphic 2). These findings provide an important contribution to research on bone development and sex differences.

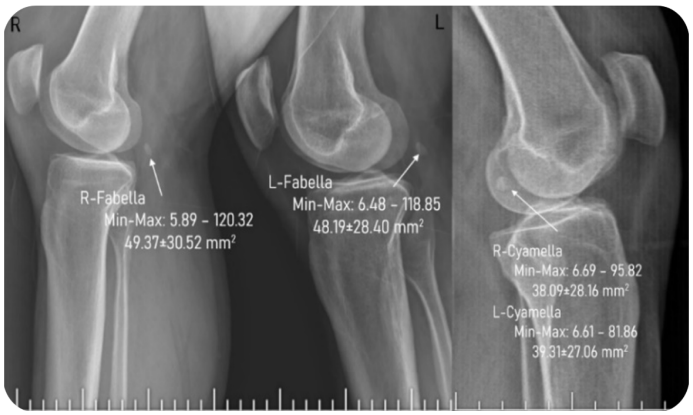
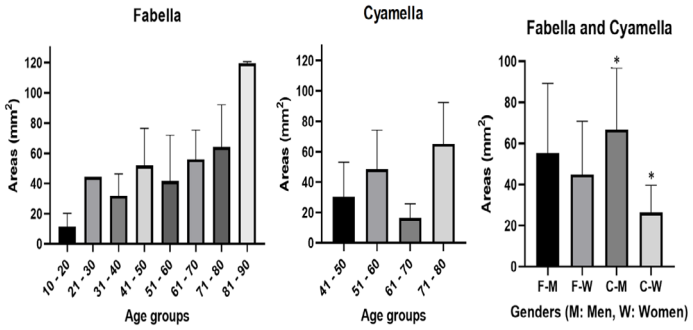


Figure 4. Accessory and sesamoid ossicles identified on lateral knee radiographs



Graphic 2. Comparison of accessory bones in the knee according to age and sex (F: fabella, C: cyamella)

Sesamoid Ossicles in the Hand

In our study, 143 hand images of a total of 115 individuals, including 45 right hands, 42 left hands and 28 bilateral hands, were evaluated. The mean age of the individuals is 43.50±19.06 (14-88 years) and of these, 49 were male (42.60%) and 66 were female (57.40%). Sesamoid ossicles on the metacarpophalangeal joint (mcp1, mcp2, mcp3, mcp4 and mcp5) and interphalangeal joint were analysed on the images. The lateral and medial sesamoid ossicles on the first metacarpophalangeal joint (mcp1=hallux sesamoids) were found in all hands. The largest sesamoid ossicle found in the right hand was the lateral sesamoid ossicle (Rmcp1 Lat) on the metacarpophalangeal joint (23.47±7.68 mm²). The average size of the sesamoid ossicles in the hand is shown in Figure 5B.

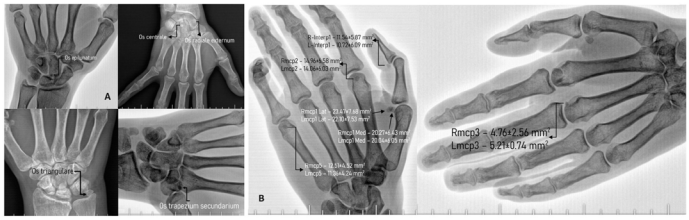


Figure 5. The frequency and mean size of sesamoid (B) and accessory (A) ossicles in the right and left hand

The most common sesamoid ossicles in the hand were mcp1 (100%), left mcp5 (72.85%), right mcp5 (64.38%) and right mcp2 (53.42%). When analysed individually, the largest sesamoid ossicle was found to be Lmcp1 lateral (49.40 mm²), and the smallest sesamoid ossicle was found to be left mcp4 (2.60 mm²). Table 3 shows the frequency of sesamoid ossicles in the hand and their minimum and maximum dimensions. Sesamoid ossicles in the hand increase significantly with age, but this increase is not large due to individual differences.

Accessory Bones in the Hand

The presence and dimensions of the accessory ossicles in the hand were evaluated and the most common accessory ossicle was os triangulare. Seven different accessory ossicles were found, os centrale (1.73%-11.13 mm²), os triangulare (6.08%-9.21 mm²), os radiale externum (2.60%-9.30 mm²), os epilunatum (0.86%-6.60 mm²), os vesalianum manus (0.86%-6.45 mm²), os epitrapezium (0.86%-1.27 mm²) and os trapezium secundarium (0.86% - 12.90 mm²) (Figure 5A).

Tablo 3. The frequency and minimum-maximum size of sesamoid ossicles in the hand

	Frequency of occurrence		Minimum - Maximum	
	Right hand	Left hand	Right hand	Left hand
Mcp1 lateral	(73)-100%	(70)-100%	3.45-47.11	3.30-49.40
Mcp1 medial	(73)-100%	(70)-100%	3.62-38.72	3.59-35.74
Mcp2	(39)-53.42%	(31)-44.28%	3.69-31.45	6.11-27.56
Mcp3	(3)-4.10%	(3)-4.28%	2.84-7.68	4.57-6.03
Mcp4	-	(1)-1.42%	-	2.60
Mcp5	(47)-64.38%	(51)-72.85%	4.51-20.62	3.18-22.26
Interp1	(24)-32.87%	(29)-41.42%	2.54-26.80	3.92-32.40

Mcp: metacarpophalangeal joint; interp1: first interphalangeal

DISCUSSION

The accessory bones and sesamoid bones are notable for being mysterious anatomical structures that perform a wide range of functions in the human body. These bones are found in the body in unique shapes and have a variety of functions. It is therefore of great importance to the fields of medicine and biology to study the nature, occurrence, and dimensions of these important structures (9).

The functions of the accessory and sesamoid ossicles are primarily mechanical. Accessory ossicles often act as pulleys, changing the direction and magnitude of muscle forces, or as shock absorbers, protecting joints from excessive forces. Sesamoid ossicles reduce friction and increase the mechanical advantage of tendons, improving their ability to transmit force and maintain joint stability (12).

Although they are generally recognised as anatomical variations, their presence can have clinical implications. Understanding the anatomy, function and clinical significance of these ossicles is crucial for the accurate diagnosis and treatment of associated conditions. Further research in this area may shed more light on the prevalence and variability of accessory and sesamoid ossicles among populations. Although there are many studies in the literature measuring the presence of accessory and sesamoid bones, there are not studies examining the areas (sizes) covered by these ossicles and their distribution according to age and gender. In our study, we tried to fulfil this deficiency.

The first discovery of these structures was made by cadaver dissection, and later, many studies have been carried out with these ossicles with imaging. The prevalence of accessory bones in the foot varies considerably according to the population studied and ranges from 21% to 49.2% (9,13). In a study conducted in a Korean population, accessory ossicles were found in 49.2% of healthy, asymptomatic Korean adults. Although we reported a high rate of accessory ossicles in our study, we identified at least one accessory ossicle in 52.18% (60/115). In studies in the literature, this rate was reported as 18.3%, 26.1% and 40.2% with different rates (14-17).

Although there are approximately 40 accessory bones, os trigonum is one of the most common accessory ossicles. Os trigonum syndrome is the pathology of this ossicle. It may occur with hyperflexion of the ankle (18,19). As we found in our study, it may be bipartite in some individuals. This variation should be noted as it may have radiographic appearances similar to a fracture of the posterior talar process (20). Its frequency varies between 1-25% in the literature (7,14-17,21). Candan B. et al. evaluated the diameters of os trigonum on radiological images and showed its mean length as 10.21 mm and mean width as 6.53 mm.

Os peroneum was first described in the "Fabrica" of Andreas Vesalius and is found in 9% to 26% of the general population. It has an oval, triangular or round shape and can be bipartite or multipartite. It is one of the largest accessory bones (40 mm^2) and its size range is very wide. In our study, a prevalence of 18.2% was found similar to the literature. In a study (22), the mean area was reported as 2.48mm^2 on the right and 2.70 mm^2 on the left, and the minimum area was found to be 3.42 mm^2 in our study.

Os naviculare accessoria, also called os tibiale externum, is the third most common accessory ossicle in our study. Its reported prevalence varies between 4 and 34% (23,24). Although it was found bilaterally in 3 patients in our study, it may be bilateral up to 50% according to some studies (5). According to Geist classification, it may be triangular or heart-shaped with dimensions ranging from 2 mm to 12 mm (25). In our study, it contains an area of approximately 2.70-58 mm^2.

In addition to these most common ossicles, other accessory ossicles: os intermetatarseum, os calcaneus secundarius, os vesalianum, os supranaviculare, os supratalare, os cuboideum secundarium, os subfibulare, os talotibiale, os infranaviculare, os intercuneiforme and os subtibiale are rarely reported in the literature (Table 4).

Table 4. The frequency of accessory bones in the foot reported in the literature

	Our study (115)	Kır et al. (277)	Cıllı et al. (464)	Candan et al. (1651)	Kalbouneh et al. (1000)	Total range
Accessory bones (Total)	52.17%	45.4%	18.3%	26.1%	40.2%	21%-49.2%
Os peroneum	18.2%	16.6%	31.8%	5.8%	11.5%	9%-26%
Os naviculare accessoria	17.4%	65.8%	28.2%	7.9%	13.7%	4%-34%
Os trigonum	12.1%	11.9%	23.5%	9.8%	15.4%	7%-25%
Os intermetatarsarum	7.8%	2.3%	1.2%	0.12%	0.2%	1%-7%
Os calcaneus secundarius	3.5%	-	-	0.42%	0.3%	0.4%-11%
Os vesalianum pedis	2.6%	7.1%	5.9%		1.1%	<1%
Os supranaviculare	2.6%	-	3.5%	0.36%	0.7%	~1%
Os supratalare	1.7%	-	2.4%	0.48%	0.3%	0.2%-2.4%
Os cuboideum secundarium	1.7%	-	-	-	-	~0.1%
Os subfibulare	0.9%	-	-	0.42%	0.6%	0.2%-6.6%
Os talotibiale	0.9%	3.9%	-	-	0.4%	<0.5%
Os infranaviculare	0.9%		3.5%	-	0.3%	
Os intercuneiforme	0.9%	2.3%	-	-	-	
Os tuberis calcanei	0.9%					

Sesamoid bones provide mechanical advantage during flexion of the fingers by reducing friction and strengthening the adjacent soft tissues (4). The medial and lateral hallux sesamoids are embedded in the medial and lateral tendons of the flexor hallucis brevis tendon. The size and shape of the hallux sesamoids vary considerably. In our study, the mcp1 lateral sesamoid (93.01 mm^2) was found to be larger than the medial (90.98 mm^2), and in addition, medial hallux sesamoid being in two parts as proximal and distal, which is reported with a rate of 2.7% in the literature (1), was found in 6 individuals (8.69%) in our study. Since it is difficult to distinguish bipartite medial hallux sesamoid radiologically, size difference as we measured in our study will be the solution to this situation. The fractured medial hallux sesamoid will appear larger than the lateral one, but as we stated in our study, the lateral hallux sesamoid is larger than the medial one. Knowing the normal dimensions, especially at the first presentation to the hospital, will reduce the likelihood of fracture. The incidence of mtp2 (12.92%), mtp3 (0.68%), mtp4 (2.04%) and mtp5 (23.12%) sesamoid ossicles was similar to the literature (1,4,26).

In humans, most of the sesamoid ossicles begin to develop in early and late childhood. Dharap et al. (27) reported that ossification of the first finger starts at the age of 10-11 years and is completed at the age of 13-14 years. Sesamoid ossicles are known to have important functions, such as protecting the tendon from damage and, in some cases, increasing the efficiency or mechanical advantage of associated muscles. If we consider that the sesamoid bones in the foot are particularly useful in walking, the sesamoids in the hand may have benefits such as increasing the strength of hand movements and providing a benefit in grasping an object with the fingers.

The incidence and size of sesamoid bones in the hand may vary depending on factors such as race, sex, and region. In our study, lateral and medial sesamoid ossicles at the level of the metacarpophalangeal joint of the thumb were seen in all individuals (73, 100%). In the literature, this rate varies between 98-100% (8,27). Similar to mtp1 sesamoids in the foot, the lateral sesamoid ossicle was found to be larger in mcp1 (Rmcp1 Lat: 23.47 mm^2, Lmcp1 Lat: 22.10 mm^2). In our study, the frequencies of mcp2, mcp3, mcp4 and mcp5 were 48.95%, 4.19%, 0.70% and 68.53%, respectively. Similar to our study, the frequency of mcp2 and mcp5 was reported as 42.3% and 41.1%, respectively, in the study by Amar E et al. (8).

Seki Y. et al. (28) reported the frequency of interp1 sesamoid ossicle as 67% in their study in which they analysed the sesamoid ossicles on the interphalangeal joint of the hand, while this rate was 37.06% in our study. In our study, os triangulare (6.08%) and os radiale externum (2.60%) were the most common accessory ossicles in the hand. Amar et al. (8) reported the most common os ulnostyloideum (1.13%) and os triangulare (0.67%).

The term 'Fabella' originated from the Latin term 'faba', meaning bean. In humans, it is more common in men than in women, and in older individuals than in younger ones. Bilateral presence is more common and unilateral is less common, and in individual cases fabellae are equally likely to be found on the right or left knee. Fabella and cyamella are anatomically similar sesamoid ossicles. Akkoç et al. (29) reported its presence in 38.8% of Turkish population and found the thickness, width, and length of fabella to be 3.84 mm, 6.04 mm, and 6.23 mm, respectively. In our study, the incidence of fabella was similarly 36.5% and the mean size was 49.37 mm^2 on the right and 48.19 mm^2 on the left.

CONCLUSION

According to the results of our study, the frequency of accessory and sesamoid ossicles in the hand, foot and knee was found to be compatible with the literature. Accessory and sesamoid ossicles are first visualised on radiographs and CT scans in the hospital. In our study, we measured the size (area) of these ossicles to differentiate them from avulsion fractures. There is no similar study in the literature. The physician can predict that the fragment separated from the main bone may be an accessory bone or a fracture with the size values in our study. With these results in our study, we thought that we could help clinicians and contribute to the literature.

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Conflict of Interest: *The authors have no conflicts of interest to declare.*

Ethical approval: Ethical approval was obtained from Ordu University Clinical Research Ethics Committee (2023/103) and Ordu Provincial Health Directorate for obtaining radiographic images from the hospital PACS system.

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MEDICAL RECORDS-International Medical Journal

Research Article

The Effect of Uterocervical Angle on Treatment Efficacy in First-Trimester Pregnancy Terminations

Hasan Yilmaz Akin[1], Tayfur Cift[2], Suleyman Serkan Karasin[3], Zeynep Toksoy Karasin[4]

[1]Bursa İnegöl Public Hospital, Department of Obstetrics and Gynecology, Bursa, Türkiye
[2]Bursa Esentepe Private Hospital, Department of Obstetrics and Gynecology, Bursa, Türkiye
[3]Health Sciences University Bursa Yüksek İhtisas Training and Research Hospital, Department of Obstetrics and Gynecology, Bursa, Türkiye
[4]VM Medical Park Bursa Hospital, Department of Obstetrics and Gynecology, Bursa, Türkiye

Abstract

Aim: We aimed to evaluate the effectiveness and usability of the uterocervical angle, which we examined ultrasonographically before misoprostol treatment, which we used in first-trimester pregnancy terminations, and its relationship with the abortion time.
Material and Method: This prospective study includes 207 pregnant women diagnosed with an ex-fetus in utero hospitalized for medical termination in a single center. These patients were divided into two groups patients whose treatment was completed in the first cycle and who needed additional cycles. The characteristics of all pregnant women, abortion times, and misoprostol doses used were compared with uterocervical angle and cervical length and analyzed between groups.
Results: The mean age of the patients in the entire study group was 30.1±6.3, the median uterocervical angle was 112 degrees, and the cervical length was 36 mm. Increasing cesarean number and increasing uterocervical angle degree were positively correlated with increasing abortion time. The number of cesarean sections and the degree of uterocervical angle differ significantly between the group whose treatment was completed in the first cycle and the groups that needed additional cycles. In the treatment groups with elevated uterocervical angle degrees, the first cycle was higher than the successful group. Additional dose and cycle requirements arise if the uterocervical angle is >110 degrees.
Conclusion: Evaluation of the uterocervical angle in first-trimester medical terminations may guide the clinician in the early completion of treatment. With this evaluation before medical treatment, the duration of hospitalization can be shortened, and the need for surgical intervention for patients can be reduced.

Keywords: Pregnancy, first trimester, prenatal diagnosis, misoprostol, abortion missed

INTRODUCTION

Abortion is among the most common complications in pregnancy. It is the most common cause of vaginal bleeding in the first and second trimesters (1). It can be evaluated into two groups spontaneous or induced abortion according to how it occurs. According to the World Health Organization (WHO) definition in 1977, The expulsion of a fetus and its appendages before the 20th gestational week or less than 500 grams out of the uterine cavity is abortion (2).

In clinical pregnancy follow-up, measurement of β-hCG and progesterone levels, abdominal/transvaginal ultrasound (tvusg) evaluation is performed. The threshold level of β-hCG, at which the intrauterine gestational sac should be strictly monitored, is called the "differential zone" (3). For transvaginal ultrasonography, β-hCG is 2000-3000 mIU/mL, while intrauterine gestational sac monitoring is expected (4). With transvaginal ultrasonography, the embryonic pole is seen from the sixth week. Crown-rump length (CRL) is the measurement of the echogenic area between the embryonic head and rump poles. CRL increases by an average of 1 mm per day (5).

Spontaneous abortion, which has a wide range, is around 15-40%, and most of them occur before the 12th gestational week. Approximately 30-50% of conceptions and approximately 15% of clinically diagnosed pregnancies

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Corresponding Author: Suleyman Serkan Karasin, Health Sciences University Bursa Yüksek İhtisas Training and Research Hospital, Department of Obstetrics and Gynecology, Bursa, Türkiye
E-mail: sskarasin@icloud.com

result in miscarriage. Chromosomal abnormalities are present in 70 percent of pregnancy losses before 20 weeks (6,7). Medical abortion is the termination of pregnancy by using pharmaceuticals to induce a process similar to a miscarriage. Mifepristone (8), misoprostol (9), or combinations are frequently used globally in the medical approach.

In our clinical experience, we have applied a misoprostol regimen following the International Federation of Gynecology and Obstetrics (FIGO) guidelines for many years, and we standardize the treatment protocols (10,11). The efficacy of misoprostol is affected by many factors such as gestational age, route of administration, dose, and dose range (12-15).

It is still controversial to apply direct dilatation & curettage or to wait for the medical treatment process in cases of missed abortion in the first trimester and early weeks. In this study, we aimed to measure the uterocervical angle (UCA) ultrasonographically to evaluate the effectiveness of misoprostol treatment for the termination of fetuses diagnosed in utero ex during the first trimester of pregnancy. With this pre-treatment evaluation, we can avoid possible side effects of the drug (diarrhea, vomiting, headache, fatigue, breast tenderness, and fever) and unnecessary medical treatment and increase patient comfort by reducing possible complications and length of hospital stay.

In this way, we aimed to evaluate the reliability and usability of the UCA and its relationship with the abortion time.

MATERIAL AND METHOD

This research article was a prospective study at the Health Sciences University Bursa Yüksek İhtisas Training and Research Hospital Gynecology and Obstetrics department. Approved by the local ethics committee with the decision number 2011-KAEK-25 2020/09-06, it covers 207 pregnant women diagnosed with intrauterine ex fetus between 01 November 2020 - 30 April 2021 for a medical termination. Both written and verbal consent was obtained from all of our patients.

First trimester (under 13 weeks), pregnant women aged 20-40 years were included in the study. Under 20 and over 40 years of age, with any comorbidity (diabetes mellitus, hypertension, malignancies, impaired bleeding diathesis, etc.), with a history of uterine anomalies with signs of acute infection, and a fetus with anomaly were out of the study.

We added cervical length and UCA measurements in addition to traditional ultrasonographic measurements (gestational week, gestational sac, crown-rump Length, fetal cardiac activity, location of gestational sac, placentation measurements). The UCA is the segment measured between the lower uterine part and the cervical canal, generating a measurable angle as in the literature (16). The calipers were placed where the anterior and posterior lips of the cervix connect the internal and external os along the endocervical canal. The first line was from the internal os to the external os. If the cervix was curved, the first line was also drawn from the internal os to the external os straight. Then, a second line was drawn to delineate the lower uterine segment. This line was traced up the anterior uterine segment to a distance allowed by the preloaded image. Ideally, the second line would reach 2-3cm up the lower uterine segment to show an adequate measurement. The anterior angle in between the two lines was measured with a protractor.

During the measurement, the patients were standardized, and the measurements were made on the same gynecological examination table and with the same ultrasound device. All dimensions were applied by an experienced sonographer (A.Y.H.) on the same ultrasound with a 5-MHz transabdominal transducer (Ultrasound System Voluson S6; Europe - EAGM). At the same time, the bladder and rectum were empty.

Misoprostol (200 micrograms per tablet) therapy is standardized and routinely used in our department, as well as recommended in World Health Organization (WHO) and FIGO 2017 clinical guidelines (10,17). Four tablets vaginal misoprostol were applied to the group without a previous cesarean section, three vaginal misoprostol was applied to those who had a previous cesarean section, and two vaginal misoprostol was administered to those who had a previous cesarean section. The first dose of misoprostol, determined after the ultrasonography procedure was completed, was applied to the patients on the gynecological examination table. Repeated doses were administered vaginally in the patient's bed to patients who did not abort within the first 8 hours.

The amount and doses of misoprostol used after the patients aborted were recorded in the case report forms and the hours from the patient orders. Curettage procedures were performed under sterile conditions and anesthesia by carmen cannulas for the patients who had an incomplete abortion in the control tvusg. After the procedure, the patients were discharged in good general condition and had no bleeding. The patient group included in the study was divided into 2. The first group of patients whose treatment was completed during the first 8 hours of treatment; The patients who needed additional cycles for treatment constituted the second group. Then, following the treatment protocol, the patients were divided into three groups according to the aborted cycle at 8-hour intervals and reevaluated.

Statistical Analysis

Windows-based SPSS 24.0 statistical analysis program (SPSS Inc., USA) was used for appropriate statistical analysis. Variables were analyzed using visual (histograms, probability graphs) and analytical methods (Shapiro-Wilk and Kolmogorov-Smirnov) to determine whether the data showed a normal distribution. Variables mean±standard deviation (X±SD), mean difference between groups, 95%

confidence interval (95% CI), median (min-max), frequency (n) and percentage (%). Mann-Whitney U test was used to compare non-normally distributed variables in a two-group analysis. Variables containing more than two groups were analyzed with Kruskal Wallis tests, as they did not show normal distribution. Spearman tests were performed to show the correlations between non-normally distributed variables. In the first cycle of successful prediction, binary logistic regression analysis analyzed independent predictors of misoprostol treatment. Model compatibility was found to be significant at $p<0.05$. The degree of UCA was found to have a higher predictive effect than the number of vaginal delivery parameters, and receiver curve characteristic analysis (ROC analysis), which could determine the cutoff value, was applied. However, we divided the volunteers into three groups according to their aborted cycle at 8-hour intervals. By multinomial regression analysis, the degree of UCA was significantly predictive in the groups that aborted in the first 8 hours compared to the groups that aborted after 16 hours.

RESULTS

The descriptive analysis showing the demographic and clinical characteristics of cases terminated with Misoprostol in first trimester pregnancies is shown in Table 1. The mean age in the entire study group was 30.1±6.3. According to the head-rump distance, the median number of pregnancies in the study group was 3, and the median gestational age was 55 days. The median value of the UCA degree, which we evaluated ultrasonographically, was 112 degrees and the cervical length was 36 mm, while the median abortion time of the patients was 10.4 hours. While the number of patients who aborted in the first 8-hour treatment cycle was 86 (40%), The number of patients whose treatment was not completed in the first two cycles and who required additional treatments was determined as 59 (28.5%). All of the findings are shown in Table 1.

Table 1. Analyzes of demographic and clinical characteristics of the participants included in the study

Characteristics of pregnant women and clinical findings	Participants (n=207)
	Median (min-max)
Age (years)	30.1±6.3
Body mass index	26.2 (17.1-43)
Parity	2 (0-9)
Gestational sac diameter (mm)	33.5 (10-81)
Crown-rump length (mm)	15 (2-63.1)
Gestational age according to crown-rump length (days)	55 (40-90)
Cervical length (mm)	36 (10-52)
Uterocervical angle (degrees)	112 (32-172)
Misoprostol dosage (tablets)	6 (2-16)
Time to miscarriage (hours)	10.4 (2.5-30.1)
First cycle abortion (n; %)	86 (41.5%)
Abortion in the second cycle (n; %)	62 (30%)
Abortion in other cycles (n; %)	59 (28.5%)

mm: millimeter, n: number, min: minimum, max: maximum

First-trimester pregnant groups terminated with Misoprostol were divided into two groups according to the abortion time and analyzed in Table 2. Accordingly, the first group of the groups whose treatment was completed during the first 8-hour treatment period; The groups that needed additional cycles for treatment formed the second group. Accordingly, the number of cesarean section deliveries ($p=0.004$) and the degree of UCA ($p=0.002$) differed significantly between the two groups. As the degree of UCA increases, the need for additional misoprostol cycles increases statistically and clinically.

Table 2. Comparison of clinical findings according to additional dose misoprostol requirement

	First cycle successful group (n=86)	Group requiring additional doses (n=121)	
	Median (min–max)	Median (min–max)	p
Age (years)	29±4.8	31±5.3	0.32
Body mass index	26.6 (17-43)	25.6 (18.5-39.4)	0.31
Parity	2 (0-9)	2 (0-8)	0.81
Number of vaginal births	1 (0-8)	1 (0-8)	0.09
Number of cesarean births	0 (0-2)	0 (0-2)	0.004
Gestational sac (mm)	34 (15-81)	32.8 (10-81)	0.43
Crown-rump length (mm)	16 (2-63)	14 (2.4-63.1)	0.52
Gestational age (days)	57 (40-90)	55 (40-89)	0.47
Cervical length (mm)	36 (10-46)	35 (21-52)	0.31
Uterocervical angle (degrees)	110 (32-172)	115 (50-160)	0.002

Descriptive analyzes are presented using mean±SD, median (min-max) and (n;%) for normally, non-normally distributed and categorical variables, respectively. Student's t test and Mann-Whitney U test $p<0.05$ was considered significant. mm: millimeter, n: number, min: minimum, max: maximum

Binary logistic regression analysis was evaluated between the two groups to determine the most compatible independent variable that could predict termination success in the first treatment cycle. Accordingly, the first-dose misoprostol successful group was accepted as the reference category. The UCA was significantly higher in the repeated dose groups than in the reference group (p=0.009). Each 1-degree increase in the UCA increases the need for additional cycles 1.02 times (Table 3).

Receiver operating characteristic curves (ROC curves) were created for the degree of UCA, which predicted successful termination with Misoprostol. The area under the curve (AUC), sensitivity (sen.), specificity (spe.), and Youden index were calculated. According to the first dose successful group, a cut-off value was determined for the UCA. Accordingly, if the UCA of the patient, which we evaluated ultrasonographically, is >110 degrees, additional dose and cycle requirements arise with a probability of 63%, a sensitivity of 60%, and a specificity of 55.5% (Table 4, Figure 1)

Table 3. Logistic regression model for additional dose misoprostol requirement

	B	Wald	Odds ratio	95% CI	p
Additional dose misoprostol requirement	0.018	6.850	1.018	1.005-1.032	0.009
	-0.151	2.539	0.860	0.714-1.035	0.111

CI (95%); confidence interval, Wald: test statistic value. Binary logistic regression was used because the dependent variable consisted of 2 groups. Enter method is used. The first cycle successful group was taken as the reference category. Variables that were found to be significant in the previous analysis and with a type 1 error margin level close to 0.05 were included in the analysis. Hosmer-Lemeshow model fit was found p<0.05

Table 4. ROC analysis table of uterocervical angle degree in terms of additional cycle needs in misoprostol treatment success

Area under ROC curve (95% CI)	P	Cut-off (Youden)	Sensitivity	Specificity	PPV	NPV
0.633 (0.558–0.709)	0.001	110	60%	55.5%	55.6%	46.1%

CI: confidence interval, PPD: positive predictive value, NPV: negative predictive value

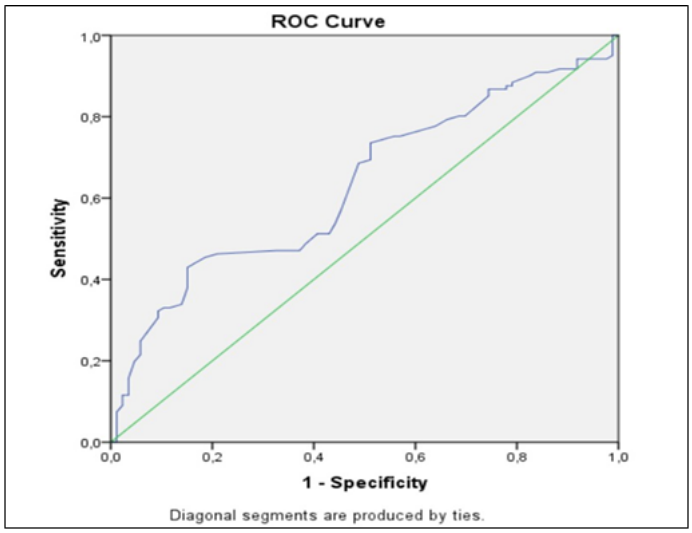

Figure 1. ROC analysis curve of uterocervical angle degree in terms of prolonged treatment in misoprostol treatment success

DISCUSSION

This study examined patients who were administered misoprostol for medical abortion. Our evaluations found that as the degree of UCA increased, higher doses of misoprostol were needed, and a more extended treatment period was required. The number of pregnancies and the number of previous cesarean sections were associated with the degree of UCA. It is seen that the UCA decreases as the gestational sac size and cervical length increase. Increasing cesarean section number and increasing UCA degree were positively correlated with increasing abortion time.

Epidemiologically, about 90% of recurrent pregnancy losses are experienced in the first trimester. Among the causes of abortion, there are many reasons such as medical disorders, environmental factors, uterine or cervical anatomical problems (18).

The cervical length is the most commonly used ultrasonographically used parameter that gives information about the cervix. However, in recent years, the angle measurement between the lower uterine segment and the cervical canal has become popular in many subjects, especially in predicting birth and preterm labor (16,19).

The angulation (UCA) between the uterus and cervix during pregnancy due to structural changes and pressure can give information about labor. This measurement plays a decisive role in estimating the abortion period of the patients.

Depending on the angle of inclination in the pregnant uterus, the cervical canal closes with pressure at a narrow angle or opens with compression in case of a wide-angle (20). The relationship between this change in cervical angle and preterm birth may also be valuable for predicting first trimester abortions.

In a study conducted on multiparous women in the literature, UCA and cervical length were measured by transvaginal ultrasonography before treatment in second-trimester pregnancy terminations. Later, an intracervical Foley catheter was placed in the patients, and then an oxytocin infusion was given. After the patients were aborted, the termination times were compared. It was found that the UCA had a significant effect in patients who aborted in the first 24 hours compared to those who

aborted in the first 48 hours (19).

In another study conducted on primiparous women, transvaginal ultrasonography before treatment in second-trimester pregnancy terminations measured UCA and cervical length. Then, misoprostol treatment was applied to the patients, and the abortion times of the patients were compared. Patients who aborted in the first 24-hour period had higher UCAs than those who aborted late. In this study, groups with UCA >105 were given a shorter-term misoprostol treatment. Therefore, less medication was required, and it was shown that they aborted in a shorter time (21).

The fact that both cervical length and UCA can be measured with the same method or even from the same ultrasound image allows both to be evaluated simultaneously. Many studies on the advantages of UCA measurement have been published recently and continue to be published.

In a study by Pruksanusak et al.; The reliability of UCA and cervical length measurements were compared. The UCA and cervical length were measured separately by three specialists for the 16-24 weeks of pregnant women who participated in the study. Accordingly, UCA measurements showed less variability compared to cervical length measurements. It was stated that UCA measurement is more reliable than cervical length measurement (22).

In a prospective controlled study in which Sallam et al. investigated the measurement of UCA before embryo transfer, measuring the UCA with ultrasound prior to embryo transfer improves clinical pregnancy and implantation rates in patients undergoing in-vitro fertilization and intracytoplasmic sperm injection (23).

In a retrospective case-control study by Sochacki et al., UCA measurement was made and statistically evaluated in the first and second trimesters. A significant difference was found between the median UCA of women who gave birth preterm and the UCA of women who gave birth at term. It was observed that the probability of spontaneous preterm delivery increased in those with a wider angle, and the UCA increased as the gestational week progressed. Despite the limitations of this study, it showed that UCA measured in the first trimester might be a predictive parameter for spontaneous preterm delivery (20).

Our study has some limitations. The first of these is that the study was single-centered. The other one is interobserver variability, which cannot be evaluated because a single clinician performed ultrasonography during the study.

We excluded pregnant women with chronic diseases, uterine anomalies, signs of acute vaginal or cervical infection, previous pelvic surgery, and fetuses with anomalies. The fact that we excluded all pregnant women with non-standardization findings from the study constituted the study's strengths. At the same time, to standardize all patients, they were evaluated with the same ultrasound device on the same gynecological table while the bladder and rectum were empty. The relationship between UCA and abortion duration in second-trimester medical terminations has been investigated, and no comprehensive research has yet been conducted to evaluate UCA in first-trimester medical terminations. In this respect, our work is a candidate to be a valuable and preliminary work.

CONCLUSION

In conclusion, evaluating the UCA for first-trimester medical terminations may guide the clinician, especially in terms of early completion of the treatment. With this evaluation before medical treatment, the duration of hospitalization can be shortened, and the need for surgical intervention for patients can be reduced. Evaluating UCA before medical treatment may be valuable for patients, we plan to terminate with misoprostol treatment.

In order to understand the clinical importance and role of the UCA and obtain more precise data, studies with larger patient groups are needed to support this study.

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Ethical approval: *Approved by the local ethics committee with the decision number 2011-KAEK-25 2020/09-06.*

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MEDICAL RECORDS-International Medical Journal

Research Article

Bibliometric Analysis of Articles on Methanol Poisoning in Web of Science Database

Pelin Koca[1], Hanefi Ozbek

İzmir Bakırçay University, Faculty of Medicine, Department of Pharmacology, İzmir, Türkiye

Abstract

Aim: Methanol, which is a type of toxic alcohol, can cause poisoning through voluntary or involuntary exposure. It is known that the frequency of methanol poisoning has increased especially recently. The aim of this research is bibliometric analysis of scientific studies conducted on methanol poisoning in Web of Sciences (WoS) database.

Material and Method: Studies in Web of Science database including the words "methanol" or "methyl alcohol" in their title and the words "poisoning" or "poison" or "toxicity" or "toxic" or "overdose" as the topic were reviewed as of 21.08.2023. "Web of Science Categories", "Web of Science Index" and "Document Types" categories were used as filtering options. Language of article, year of publication, the journal in which it was published, the publishing company to which the journal is affiliated, authors of the article, institutions of authors, the country where the research was conducted, whether financial support was received and citation status of the research were analysed and evaluated.

Results: A total of 548 articles were included in the research. It was found that language of most of the studies were English (n=536; 97.8%) and they were published in journals that were in Science Citation Index-Expanded (SCI-E) (n=451; 82.3%) indices. It was found that the highest number of articles were published in 2020 and the country in which the highest number of articles was published was the United States of America (n=143; 22.7%).

Conclusion: The present research performs a bibliometric analysis of articles on methanol poisoning in WoS database. The data found in this research can be a valuable source for other researchers and a guide future studies.

Keywords: Methanol poisoning, bibliometric study, Web of Science

INTRODUCTION

Methanol, which is also known as methyl alcohol (CH3OH), is an industrial solvent and also an odorless, colorless, toxic alcohol found especially in bootleg, paint removers and antifreezes. It can lead to voluntary (suicide, illegal alcohol consumption) or involuntary poisoning (1,2). The incidence of methyl alcohol poisoning has increased dramatically in Iran, especially after 2018 (3).

Gastrointestinal absorption of methanol in the body is quick (shorter than 10 minutes) and its volume of distribution is large (0.7L/kg). It is metabolized in the liver and only 2-5% is excreted via the kidney. Methanol poisoning may occur after through oral, dermal or inhalation intake. Adults are intentionally abuse this substance by using methanol instead of ethyl alcohol (ethanol, C2H5OH) or they are frequently exposed to methanol in window washer fluid for suicidal purposes. It is stated that especially alcoholic or suicidal individuals or young children who are just discovering their surroundings are at risk. Lethal dose of methanol has been indicated as approximately 30-240 mL or 1 gr/kg. Toxic effects of methanol occur 12-24 hours after exposure. Methanol is converted to formaldehyde and formic acid in the liver and causes various clinical symptoms and signs. While moderate methanol poisoning may cause non-specific symptoms such as abdominal pain, nausea and acidosis, severe methanol poisoning may cause multiple organ failure (acute renal failure, liver failure, dysrhythmias, coma), and papilledema (edema of the optic nerve), and especially visual disturbances such as appearance of snow, blurred vision or blindness. Methanol poisoning may result in coma, irreversible central system

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Corresponding Author: Pelin Koca, İzmir Bakırçay University, Faculty of Medicine, Department of Pharmacology, İzmir, Türkiye

E-mail: pelinozkan19@gmail.com

damage or death (2-6). It is difficult to diagnose methanol poisoning and requires highly suspicious anamnesis. Diagnosis is based on clinical signs and symptoms and supported by examining the serum methanol level, if available at the health institution. Symptomatic treatment is essential. Ethanol or fomepizol is used as antidote in methanol poisoning. Hemodialysis is an extracorporeal method that can be preferred to remove methanol and formic acid from the body (6,7).

For both scientists and the companies that provide financial support to them, it is very important how valuable a scientific research is. However, it is quite difficult to determine the value of a scientific research. Bibliometric analyses are important research methods that systematically assess scientific activity in a particular field of research over a specific time interval. Therefore, they provide both quantitative and qualitative information about the functioning of this field of research. Bibliometric analyses allow us to examine the impact of studies and to learn about new trends. Especially in the field of medicine, they also make some information about unusual symptoms or signs and rare diseases more accessible. Recent research has shown that with the development of interdisciplinary methodologies and increasing technological advances in analysing big data, bibliometric analyses have become more popular and are being performed more frequently than in the past (8-10).

Methanol poisoning is a preventable and important public health problem. There are no bibliometric studies conducted on methanol poisoning. The aim of this research is to access and bibliometrically analyse studies on methanol poisoning in Web of Sciences (WoS) database.

MATERIAL AND METHOD

In order to achieve the aim of the research, search engine of Web of Sciences (WoS) database was used and studies containing the words “methanol” or “methyl alcohol” in the title and at the same time having the words “poisoning”, “poison” or “toxicity”, “toxic” or “overdose” as the topic were analysed. Studies included in the WoS database related to methanol poisoning until 21.08.2023 were evaluated retrospectively.

The categories “Web of Science Categories”, “Web of Science Index” and “Document Types” were used from the filtering options in WoS database. Articles in Science Citation Index (SCI), Science Citation Index-Expanded (SCI-E) and Emerging Sources Citation Index (ESCI) included in “toxicology”, “pharmacology, pharmacy”, “medicine general internal”, “emergency medicine” or “medicine research experimental” categories were included in the research. The articles included in the research were evaluated by analysing the language of article, year of publication, the journal in which it was published, the publishing company to which the journal is affiliated, authors of the article, institutions of authors, the country where the research was conducted, whether financial support was received and citation status of the research.

Statistical Analysis

The data obtained from WoS were transferred to Microsoft Office Excel and analysed with SPSS-18 (SPSS INC., Chicago, IL, USA) statistical package program. The results of descriptive analyses were presented as number (n) and percentage (%).

Ethics Committee Approval

Approval was obtained from İzmir Bakırçay University Non-interventional Clinical Research Ethics Committee with the decision number 2023/1182.

RESULTS

Among all studies included in the WoS database, all studies on methanol poisoning published until 21.08.2023 were researched. A total of 2.853 publications including any one of the words “poisoning” or “poison” or “toxicity” or “toxic” or “overdose” as the topic and those including “methanol” or “methyl alcohol” in the title were accessed. By using a filtering method, 548 articles were found in SCI, SCI-E, ESCI indices which were in “toxicology” or "pharmacology, pharmacy” or “medicine general internal” or “emergency medicine” or “medicine research experimental” categories (Figure 1).

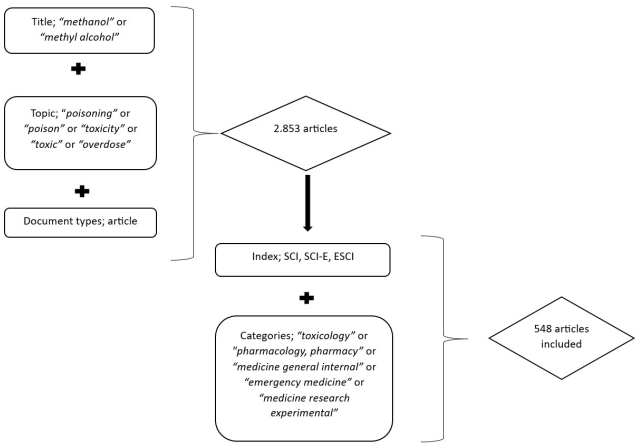

Figure 1. Filtering method used on the WoS database

The language of publication of the articles included in the research were English (n=536; 97.8%), French (n=4; 0.7%) and Turkish (n=4; 0.7%), respectively. While the total number of citations to these publications was 5.334, average number of citations per publication was 17. The information for the top 10 articles that had the most citations is shown in Table 1. It was found that 373 (68.0%) of the articles included in the research received financial support from a total of 188 institutions during research and/or publication. It was found that among the institutions that provided financial support, United States Department of Health Human Services (HHS) (n=41; 10.9%), National Institutes of Health (NIH) (n=40; 10.7%), National Institute of Environmental Health Sciences (NIEHS) (n=25; 6.7%) were the institutions that supported the highest number of studies, respectively (Table 2).

Table 1. The most cited articles

No	Articles	Total number of citations
1	Brent, J., McMartin, K., Phillips, S., Aaron, C., & Kulig, K. (2001). Fomepizole for the treatment of methanol poisoning. New England Journal of Medicine, 344(6), 424-429.	227
2	McMartin, K. E., Ambre, J. J., & Tephly, T. R. (1980). Methanol poisoning in human subjects: role for formic acid accumulation in the metabolic acidosis. The American journal of medicine, 68(3), 414-418.	203
3	Hovda, K. E., Hunderi, O. H., Tafjord, A. B., Dunlop, O., Rudberg, N., & Jacobsen, D. (2005). Methanol outbreak in Norway 2002–2004: epidemiology, clinical features and prognostic signs. Journal of internal medicine, 258(2), 181-190.	164
4	Paasma, R., Hovda, K. E., Tikkerberi, A., & Jacobsen, D. (2007). Methanol mass poisoning in Estonia: outbreak in 154 patients. Clinical toxicology, 45(2), 152-157.	150
5	Jacobsen, D., & McMartin, K. E. (1997). Antidotes for methanol and ethylene glycol poisoning. Journal of Toxicology: Clinical Toxicology, 35(2), 127-143.	143
6	Brent, J. (2009). Fomepizole for ethylene glycol and methanol poisoning. New England Journal of Medicine, 360(21), 2216-2223.	121
7	Paine, A. J., & Dayan, A. D. (2001). Defining a tolerable concentration of methanol in alcoholic drinks. Human & experimental toxicology, 20(11), 563-568.	119
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Table 2. Institutions providing financial support

Institutions		Number (n)	Percentage (%)*
1	United States Department Of Health Human Services (HHS)	41	10.9
2	National Institutes Of Health (NIH)	40	10.7
3	Nih National Institute Of Environmental Health Sciences (NIEHS)	25	6.7
4	Ministry Of Health Czech Republic	13	3.5
5	Canadian Institutes Of Health Research Cihr	9	2.4
6	Charles University In Prague	7	1.9
7	Department Of Science Technology India	6	1.6
8	European Union Eu	5	1.3
9	National Natural Science Foundation Of China Nsfc	5	1.3
10	Nih National Eye Institute Nei	4	1.0
Total		**373**	**100.0**

*: Organized by total number of supported articles

It was found that 451 (82.3%) of the articles included in the research were published in journals indexed in SCI-E, while 97 (17.7%) were published in journals indexed in ESCI. When the journals in which articles were published were analysed, it was found that the highest number of articles (n=32; 5.9%) were published in Journal of Ethnopharmacology, which was followed by Clinical Toxicology (n=23; 4.3%), Human Experimental Toxicology (n=15; 2.8%) and Pharmaceutical Biology (n=15; 2.8%). The list of journals which published the highest number of articles on methanol poisoning according to WoS database is shown in Table 3. When the publishing companies to which these journals were affiliated were analysed, it was found that Elsevier (n=153; 27.9%), Taylor&Francis (n=66; 12.0%) and Wiley (n=43; 7.8%) stood out (Table 4). When the countries in which studies were conducted were analysed, it was found that the highest number of studies were conducted in the United States of America (n=143; 22.7%), followed by India (n=46; 7.3%), Türkiye (n=37; 5.9%) and Iran (n=35; 5.6%) (Table 5).

Table 3. Publishing tools of articles

	Journals	Number (n)	Percentage (%)*
1	Journal of Ethnopharmacology	32	5.9
2	Clinical Toxicology	23	4.3
3	Human Experimental Toxicology	15	2.8
4	Pharmaceutical Biology	15	2.8
5	Toxicology and Applied Pharmacology	15	2.8
6	Journal of Toxicology Clinical Toxicology	13	2.4
7	Tropical Journal of Pharmaceutical Research	12	2.2
8	Teratology	10	1.9
9	Toxicology	10	1.9
10	Basic Clinical Pharmacology Toxicology	8	1.5
Total		**536**	**100.0**
Conference			
1	Annual Meeting of the North American Congress of Clinical Toxicology	2	13.3
2	10th International Symposium on Biological Monitoring Isbm	1	6.7
3	19th International Neurotoxicology Conference	1	6.7
4	35th Annual Meeting of the Society of Toxicology	1	6.7
5	3rd European Congress of Toxicologıc Pathology	1	6.7
6	3rd International Conference on Innovations in Cancer Research and Regenerative Medicine	1	6.7
7	49th Annual Meeting of the Teratology Society	1	6.7
8	7th International Congress of Toxicology	1	6.7
9	Annual Meeting of the Association for Research in Vision and Ophthalmology	1	6.7
10	Meeting of The North American Congress of Clinical Toxicology	1	6.7
11	Natıonal Toxicology and Clinical Toxıcology Symposium	1	6.7
12	North American Congress of Clinical Toxicology	1	6.7
13	Southeastern Regional Meeting of the Socıety for Academic Emergency Medicine	1	6.7
14	Meeting of the North American Congress of Clinical Toxicology	1	6.7
Total		**15**	**100.0**
Books			
	Advances in Experimental Medicine and Biology	1	100.0
Total		**1**	**100.0**

*: Each group was organized by the total number of articles within it

Table 4. Publishing companies to which the journals were affiliated

	Publishing Companies	Number (n)	Percentage (%)*
1	Elsevier	153	27.9
2	Taylor & Francis	66	12.0
3	Wiley	43	7.8
4	Springer Nature	31	5.7
5	Sage	20	3.6
6	Marcel Dekker Inc	14	2.5
7	Pharmacotherapy Group	12	2.2
8	Lippincott Williams & Wilkins	11	2.0
9	Mdpi	8	1.5
10	Aves	7	1.3
	Others	183	33.5
Total		**548**	**100.0**

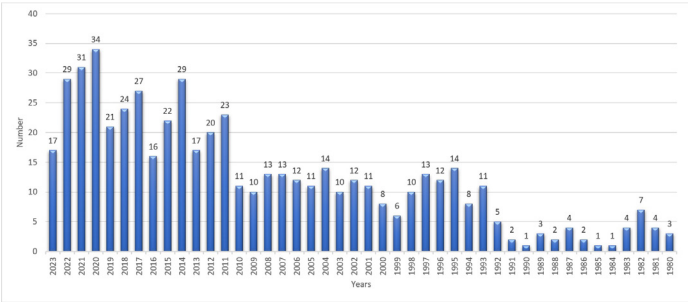
*: Organized by total number of articles

Table 5. Countries where studies are most frequently conducted			
Countries		**Number (n)**	**Percentage (%)***
1	**United States of America**	143	22.7
2	**India**	46	7.3
3	**Türkiye**	37	5.9
4	**Iranian**	35	5.6
5	**Nigeria**	33	5.2
6	**Canada**	30	4.8
7	**Czech Republic**	29	4.6
8	**Malaysia**	21	3.3
9	**Norway**	20	3.2
10	**Chinese**	16	2.5
Others		220	34.9
Total		**630**	**100.0**
*: Organized by total number of articles			

When the authors were analysed, it was found that Sergey Zakharov (n=24; 3.3%) was the author with the highest number of studies, followed by Tomas Navratil (2.5%) with 19 articles, Knut Erik Hovda (2.2%) with 16 articles, and Daniela Pelclova (2.08%) with 15 articles (Table 6). The institutions to which the authors were affiliated were mostly from Charles University Prague (n=28; 3.7%) and General University Hospital Prague (n=28; 3.7%) (Table 7). It was found that the highest number of studies were conducted in 2020 (n=34; 6.2%) and 2021 (n=31; 5.6%) (Graphic 1).

Table 6. Number of articles published by authors			
Authors		**Number (n)**	**Percentage (%)***
1	**Zakharov, S.**	24	3.3
2	**Navratil, T.**	19	2.6
3	**Hovda, K.E.**	16	2.2
4	**Pelclova, D.**	15	2.1
5	**Vaneckova, M.**	14	1.9
6	**Diblik, P.**	14	1.9
7	**Kotikova, K.**	13	1.8
8	**Seidl, Z.**	12	1.7
9	**Rogers, J.M.**	10	1.4
10	**Nurieva, O.**	9	1.2
Others		575	79.9
Total		**721**	**100.0**
*: Organized by total number of articles			

Table 7. Institutions affiliated with the authors			
Institutions		**Number (n)**	**Percentage *(%)**
1	**Charles University Prague**	28	3.7
2	**General University Hospıtal Prague**	28	3.7
3	**University of Oslo**	20	2.6
4	**Czech Academy of Sciences**	19	2.5
5	**J Heyrovsky Institute of Physical Chemistry of The Czech Academy of Sciences**	19	2.5
6	**United States Environmental Protection Agency**	19	2.5
7	**University Of North Carolina**	16	2.1
8	**Shahid Beheshti Unıversıty Medical Sciences**	15	2.0
9	**University Of North Carolina Chapel Hill**	15	2.0
10	**Egyptian Knowledge Bank Ekb**	12	1.6
Others		573	74.8
Total		**764**	**100.0**
*: Organized by total number of articles			



Graphic 1. Number of articles by year

DISCUSSION

The aim of scientific research on methanol poisoning is to define the variety of clinical signs and symptoms that may occur in the patient and/or to decrease mortality and morbidity by increasing the success of treatment. The contribution of these studies to literature is evaluated by citations. A total of 548 articles were included in this research and the total number of citations to these articles were 5.334, while the average number of citations per article was 17. These values obtained with the increasing number of studies especially after 2010 show the contribution of studies conducted on methanol poisoning to literature.

It was found that 68.0% of the articles included in the research had received financial support from 10 institutions in total. The institutions that provided the most support were found as HHS, NIH, NIEHS, respectively. The fact that more than 2/3 of the studies conducted on methanol poisoning were financially supported by large institutions was considered to indicate that scientific research on methanol poisoning is given the required importance by these institutions. It can be said that if these studies continue to receive financial support, the number and quality of research to be conducted on the subject will increase and also the points that are not still fully explained in the process from diagnosis to treatment related to methanol poisoning will be enlightened.

As a result of the filters used in the research, it was found that a total of 548 articles were published on methanol poisoning from 1980 to 21.08.2023 and studies are published on the subject almost every year, although the number varies. Graph 1 shows a significant increase in the number of articles published especially in the last 10-13 years. It was found that the highest number of articles conducted was 34 (6.20%) in 2020, followed by 31 (5.66%) articles in 2021, 29 (5.29%) articles in 2022 and 29 (5.29%) articles in 2014.

When the journals in which the articles included in the research were published were examined; it was found that Journal of Ethnopharmacology (n=32, 5.9%), Clinical Toxicology (n=23, 4.3%) and Human Experimental Toxicology (n=15, 2.8%) were the journals the articles were most frequently published, while Elsevier (n=153, 27.9%), Taylor & Francis (n=66, 12.0%) and Wiley (n=43, 7.8%) were the publishing companies the journals were affiliated to. It can be said that these journals and the publishing companies they were affiliated to contributed more to literature when compared with the other journals and publishing companies on methanol poisoning. Based on these data, it can be thought that the recognition and reputation of these journals and publishing companies in toxicology community is the reason of preference by authors.

When the countries from which articles on methanol poisoning were sent were examined, it can be seen that the countries which contributed most to this subject were the United States of America (n=143, 22.7%), India (n=46, 7.3%) and Türkiye (n=37, 5.9%). It can be said that a significant number of studies that contributed to literature are from Türkiye. Table 6 and Table 7 show the authors and the institutions of authors in studies. It was found that Zakharov, S. (n=24, 3.3%), Nevratil, T. (n=19, 2.6%) and Hovda, K.E. (n=16, 2.2%) were the authors who conducted the highest number of studies on methanol poisoning. When the institutions of authors were examined, the most frequent institutions were found as Charles University Prague (n=28, 3.7%), General University Hospital Prague (n=28, 3.7%) and University of Oslo (n=20, 2.6%). Considering the number of articles these authors and institutions contributed to literature, it can be thought that this situation may encourage other researchers and also institutions.

CONCLUSION

As a conclusion; with the analyses we conducted on methanol poisoning by using WoS database, we analysed the language, publication year, journal, publishing company of the journal, article authors, institution of the author, the country in which the research was conducted, whether financial support was received and citations. The present research is the first bibliometric research conducted on methanol poisoning by using WoS database. This research may be inspiring for bibliometric studies on other poisoning. It can be said that the data obtained from the research is a source of reference and compass for further studies to be conducted on methanol poisoning.

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MEDICAL RECORDS-International Medical Journal

Research Article

Is Post-COVID 19 Vaccination Antibody Level Related to Happiness and Stress Hormones?

Cihat Ucar[1], Faruk Disli[2], Tugce Atcali[3], Sedat Yildiz[2]

[1]Malatya Turgut Özal University, Faculty of Medicine, Department of Physiology, Malatya, Türkiye
[2]İnönü University, Faculty of Medicine, Department of Physiology, Malatya, Türkiye
[3]Bingöl üniversity, Faculty of Veterinary Medicine, Department of Physiology, Bingöl, Türkiye

Abstract

Aim: The level of antibody production in those vaccinated against coronavirus can be affected by many different situations. There is an important balance between immune response, stress and emotional state. However, it is not known how this situation affects antibody production after vaccination. This study aimed to investigate the correlation between the antibody response induced by the COVID-19 vaccine and the hormones cortisol, a marker of the stress axis, and serotonin, a marker of happiness.
Material and Method: Serum cortisol and serotonin levels were analyzed in those who tested positive (n=40) and negative (n=40) for Anti-SARS CoV-2 IgG induced by vaccination. Anti-SARS CoV 2 IgG, cortisol, and serotonin levels were determined by using the ELISA method. The data were compared using the Mann-Whitney U test. The value of $p<0.05$ was considered as statistically significant.
Results: Cortisol level (42.2±26.2 ng/ml) and serotonin level (414±246 ng/ml) were determined in subjects who tested positive for Anti-SARS CoV-2 IgG, while cortisol level (45.1±34.5 ng/ml) and serotonin level (372±209 ng/ml) were determined in subjects who tested negative for Anti-SARS CoV-2 IgG. There was no statistical difference or correlation between cortisol and serotonin levels in those with positive and negative levels of Anti-SARS CoV-2 IgG ($p>0.05$).
Conclusion: Consequently, no effect of the stress parameter cortisol and the happiness parameter serotonin, was found in vaccine-induced immunization. It is considered that the different antibody responses in individuals may vary depending on other factors.

Keywords: COVID 19 vaccination, anti-SARS CoV 2 IgG, cortisol, serotonin

INTRODUCTION

In December 2019, a group of patients in Wuhan, Hubei Province, China, were identified with pneumonia of unknown cause. On 7 January, 2020, the Chinese Centers for Disease Control and Prevention identified a new beta-coronavirus in lower respiratory tract samples from patients with pneumonia (1). This novel coronavirus was later named “severe acute respiratory syndrome coronavirus-2”. (SARS-CoV-2). Unlike other coronavirus outbreaks, the virus is easily transmitted from person to person, primarily through inhalation, droplets, respiratory secretions, and direct contact. From the first day of its spread until 17 August 2021, the virus has infected more than 273 million people and caused 5.3 million fatalities (2).

A strong immunity develops after the COVID-19 infection. This results in the formation of virus-specific antibodies. Vaccination is recommended for the formation of immunity in those, who have not been infected, and considered the only solution for disease prevention. Therefore, a better understanding of the development of humoral immunity in response to infection in individuals who have been exposed to disease or who have been vaccinated is required. This is because the development of an antibody response is affected by many factors. When the studies are reviewed, it is observed that the antibody response in individuals who have survived the disease or who have been vaccinated is different (3-5). It has been reported that an increase in Anti-SARS CoV-2 immunoglobulin-G (IgG) is proportional to the rise in the severity of the disease in infected individuals, while Anti-SARS CoV-2 IgG is found to a lesser extent in asymptomatic/mild individuals and those who have been treated with immunosuppressive

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Corresponding Author: Cihat Ucar, Malatya Turgut Özal University, Faculty of Medicine, Department of Physiology, Malatya, Türkiye
E-mail: ucarcht@gmail.com

medications (3,6). Different studies have also reported that antibody responses differ depending on age (7), gender (8), and hormones (9,10). Antibody responses have been found to be different between adults and children (11,12). Not only are different antibody responses evoked after SARS-CoV-2 infection, but different antibody responses are also elicited in vaccinated individuals who have not been infected. Vaccines approved by the World Health Organization are administered all over the world. In Türkiye, mRNA-based and inactivated vaccine forms are available. Vaccines have the ability to produce antibodies through different mechanisms. This difference may induce changes in the formation of antibodies in humans (4). Studies have shown that neutralizing antibodies against COVID-19 are produced after vaccination. However, it is not yet known how much these neutralizing antibodies are produced in each individual and how protective they are.

There is an important balance between the immune system, stress, and emotional state. A study reported that psychological stress is vital to the severity and recurrence of acute respiratory diseases (13). Long-term increases in stress hormones can trigger inflammatory responses by suppressing the cellular and humoral immune systems (14). Recent studies have reported differences in the immune responses of COVID-19 patients (15). Stress leads to activating the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system (ANS) (16). As a result of HPA activation, the release of cortisol as an end product in the bloodstream increases (16). There is a bidirectional correlation between emotional state and serotonin. Increased serotonin hormone may affect the central nervous system and autonomic nervous system, leading to immune modulation (17). Peripheral serotonin is a potent immune modulator and affects various immune cells through its receptors (18). The hormone serotonin directly affects B lymphocytes through its receptors on B lymphocytes (18). However, the roles of serotonin hormone in immune cell functions or the formation of the immune response are not yet known.

This study aimed to investigate the correlation between the antibody response induced by the COVID-19 vaccine and the hormones cortisol, a marker of the stress axis, and serotonin, a marker of happiness.

MATERIAL AND METHOD

Study Design and Participants

The study protocol was conducted in accordance with the principles of the Declaration of Helsinki and approved by the scientific ethics committee (Protocol# 2022/3161). All written consent was obtained from the participants. 80 participants were included in the study. All participants were vaccinated with Sinovac/CoronaVac in two doses at 21-day intervals. Anti-SARS CoV-2 IgG was measured from serum samples collected 21 days after the second dose. Two groups were formed: 40 people who tested positive for antibodies (antibody positive) and 40 people who tested negative for antibodies (antibody negative). Cortisol and serotonin were then measured in the serum of all participants in both groups.

Enzyme-Linked Immunosorbent Assay for Anti-SARS CoV-2 IgG

For the detection of anti-SARS-CoV-2 IgG, a commercial enzyme-linked immunoassay (ELISA) test (QuantiCOR, Y Immunotek A.S.) was used. This test was approved for quantification of COVID 19 IgG antibodies in human sera by the Ministry of Health of Türkiye, General Directorate of Public Health, Department of Microbiology Reference Laboratories and Biological Products, which follows the criteria outlined by the World Health Organization (WHO). Optical density (OD) ratios were calculated by dividing the OD at 450 nm by the OD of the cut-off included in the kit. The calculated cut-off index (COI) was used as a relative measure for the titer of antibodies in serum. For IgG response, a COI of >1.0 was considered positive (19). Samples from both groups were studied on the same test plate.

Enzyme-Linked Immunosorbent Assay for Cortisol

Following thawing, samples were centrifuged at 4000 g for 10 min and the supernatant was used for ELISA analyses as reported by Ozgocer et al. (20). Samples were diluted 5x with assay buffer and assayed in duplicate. Briefly, cortisol-BSA stock solution was diluted with carbonate buffer and added to a 96-well micro titer plate at 200 µL/well. Following incubation overnight at +4°C and they were washed 5 times with wash buffer using eight-channel pipette. Binding sites not occupied by the coating antigen were blocked by the blocking buffer (200 µL/well) for 2 h at 37°C. Following washing steps (5 times), standard solutions or samples (40 µL/well) and diluted primer antibody (antiserum) (40 µL/well) were added in duplicate and incubated at 37°C for 45 min. Following washing 5 times, biotinylated anti-rabbit antibody was added (100 µL/well) and the plate was incubated at 37°C for 30 min. The plate was washed 5 times and the streptavidin peroxidase solution (100 µL/well) was added and the plate was incubated for 15 min at +4°C. Then, the plate was washed again for 5 times and the substrate solution (150 µL/well) was added and incubated in dark for 10 min. Following incubation, stop solution (50 µL/well) was added and the absorbance was measured at 450 nm using a microplate reader. Samples from both groups were studied on the same test plate.

Enzyme-Linked Immunosorbent Assay for Serotonin

Serotonin was measured using a test kit from DRG. Before running the test, serum was acylated and the samples were prepared. To do this, 25 ul of each of serum, control, and standards were added into 1.5 ml Eppendorf tubes, and 500 ul of acylation buffer and 25 ul of acylation reagent were added into them and incubated for 15 minutes at room temperature.

Pipette 25 ul of the acylated standartds, controls and samples into the appropirate wells of the serotonin microtiters strips. Pipette 100 ul of the serotonin

antiserum into all wells. Incubate 1 h at room temperate. Dsicard the contents of the wells. Wash the plate 3 x by adding 300 ul of wash buffer, discarding the content and blotting dry each time by tapping the inverted olate on absorbent material. Pipette 100 ul of the cojugate into all wells and incubate 15 minute at room temperate. Discard the contents of the wells. Wash the plate 3 x by adding 300 ul wash buffer, discarding the content and blotting dry each time by tapping the inverted olate on absorbent material. Pipette 100 ul of the substrate into all wells and incubate 15 minute at room temperate (avoid exposure to direct sunlight). Add 100 ul of the stop solution to each well. Read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 450 nm. Samples from both groups were studied on the same test plate.

Statistical Analysis

All statistical analyses were conducted using Minitab program (MINITAB 19, PA, USA). The normal distribution of the data was evaluated with the Normality Test. Mann Whitney U test was used to compare groups. The correlations were calculated with Spearman Rho coefficient. The data were presented as mean±standard deviation. $p<0.05$ was considered as statistically significant.

RESULTS

Table 1 presents the results of Anti-SARS CoV-2 IgG measured in those who were vaccinated with the COVID-19 vaccine and the cortisol and serotonin levels in the groups that tested positive and negative for IgG. There was no statistical difference in cortisol and serotonin levels in the groups that tested positive and negative for Anti-SARS CoV-2 IgG ($p>0.05$).

Table 2 shows the correlations between IgG, cortisol, and serotonin levels in the group that tested positive for Anti-SARS CoV-2 IgG. Table 3 presents the correlations between IgG, cortisol, and serotonin levels in the group that tested negative for Anti-SARS CoV-2 IgG. There was no statistically significant correlation between Anti-SARS CoV-2 IgG, cortisol, and serotonin levels in both groups ($p>0.05$).

Table 1. Levels of anti SARS-CoV-2 IgG, cortisol and serotonin in participant

	COVID 19 Ab (+) (n=40)	COVID 19 Ab (-) (n=40)	p value
Anti-SARS-CoV-2 IgG	6.18±4.05	0.65±0.02	0.000
Cortisol (ng/ml)	42.23±26.22	45.16±34.53	0.642
Serotonin (ng/ml)	414±246	372.6±209.3	0.433

Ab titer: antibody index, (serum/cutoff ratio) x10; positive >1.0. The data represents mean±standard deviation

Table 2. Correlations between Anti-SARS-CoV-2 IgG, cortisol and serotonin in COVID-19 Ab (+)

Variables	Cortisol (ng/ml)	Serotonin (ng/ml)
Anti-SARS-CoV-2 IgG	0.037 >0.05	0.244 >0.05
Cortisol (ng/ml)		-0.117 >0.05

In each cell, upper value is R-squared, lower values is p

Table 3. Correlations between Anti-SARS-CoV-2 IgG, cortisol and serotonin in COVID-19 (-)

Variables	Cortisol (ng/ml)	Serotonin (ng/ml)
Anti-SARS-CoV-2 IgG	0.058 >0.05	0.187 >0.05
Cortisol (ng/ml)		-0.120 >0.05

In each cell, upper value is R-squared, lower values is p

DISCUSSION

In this study cortisol and serotonin hormones were measured in those who were not infected with COVID-19 and who tested positive and negative for anti-SARS CoV 2 IgG with two doses of the Sinovac vaccine. Correlations between all parameters were also checked. No statistically significant difference was found between cortisol and serotonin levels in the subjects who tested positive for COVID-19 IgG and those who tested negative. No correlation was found between anti-SARS CoV 2 IgG, cortisol, and serotonin in both groups.

The COVID-19 pandemic has raised panic and anxiety all over the world. Our lifestyle and habits have dramatically changed. Unlike other viral infections, the COVID-19 pandemic has been depicted by the media as a peculiar hazard that heightens fear, tension, and anxiety. Such circumstances have profoundly affected both the immune

system and the neuroendocrine system. The central nervous system, the endocrine system, and the immune system are interrelated. It is widely recognized that stress impairs the immune system (21). Evidence shows that high levels of chronic psychological stress reduce antibody responses to vaccination (22,23). Besides chronic stress, a lifestyle that fosters negative personality traits has also been associated with poor antibody responses in young and healthy individuals (24,25). Acute stress, on the other hand, has been reported to increase antibody responses. A pandemic such as COVID-19 increases anxiety in humans, which would inevitably have an effect on the generation of antibody responses. No correlation was detected between the antibody responses induced by the vaccine, and cortisol, which is considered a stress parameter in this study. Therefore, the correlation between stress and antibody responses seems to depend on other factors. Even if the vaccine was administered, the absence of an antibody response was not found to be correlated with cortisol.

Serotonin is associated with the state of happiness and is used as an anti-depressant (18). However, the correlation between serotonin and antibody responses has not yet been clarified. There is a serotonin receptor on B lymphocytes responsible for the antibody response (18). Therefore, there may be a possible correlation between serotonin and antibody responses. However, the literature includes no studies on the correlation between serotonin and antibody responses in humans. There are a few studies on animals in which serotonin does not affect (17), reduces (26), or increases the antibody response (27). The effect of the fear and anxious state induced by the COVID-19 pandemic on the antibody response has not been known. Therefore, this study analyzed the serotonin levels in individuals who were vaccinated but had different antibody levels. However, no difference was found in serotonin levels.

CONCLUSION

Consequently, no effect of the stress parameter cortisol and the happiness parameter serotonin on vaccine-induced immunization was found. It is considered that different antibody responses in individuals may vary depending on other factors.

Strength and limitation of the study: The time of collection of serum samples could not be standardized for all participants.

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MEDICAL RECORDS-International Medical Journal

Research Article

Investigation of the Antiepileptic Effect of (R)-(-) and (S)-(+) Carvone in Penicillin-Induced Epileptiform Activity Model

Ozge Beyazcicek, Sumeyye Altun, Ersin Beyazcicek, Serif Demir

Düzce University, Faculty of Medicine, Department of Physiology, Düzce, Türkiye

Abstract

Aim: Epilepsy affects approximately 70 million people worldwide. While many drugs can prevent seizures, they have a limited impact on preventing or curing the disease. In this perspective, natural compounds, especially monoterpenes derived from medicinal plants, have been investigated in epilepsy models, such as carvone (CAR). The principal constituent of peppermint oil, (R)-(-)-carvone (R-CAR), and the primary component in cumin and dill seed oils, (S)-(+)-carvone (S-CAR), find diverse applications in cosmetics, food, and pharmaceutical formulations. This study aims to investigate the antiepileptic effects of the natural compounds S-CAR and R-CAR in penicillin (PEN)-induced experimental epilepsy model in rats.

Material and Method: In the research, 91 male Wistar rats were used. The rats were grouped into 3 main groups as common groups, pre-penicillin groups and post-penicillin groups. The main groups were divided into a total of 13 subgroups. Electrocardiogram recording was taken from rats. At the end of the experiment, the latency of the first epileptiform activity (EA), spike-wave frequency (SWF), and spike-wave amplitude (SWA) of the EA were analyzed.

Results: S-CAR and R-CAR administered before penicillin prolonged the latency to the onset of the first EA. S-CAR and R-CAR administered before penicillin decreased SWF. 100 mg/kg doses of S-CAR and R-CAR injected 30 minutes after penicillin administration decreased SWF. While 200 mg/kg dose of R-CAR administered before penicillin decreased SWA in a time-dependent manner, 100 mg/kg dose of S-CAR administered after penicillin decreased SWA.

Conclusion: These findings indicate that carvone could exhibit both protective and therapeutic effects in the management of epilepsy.

Keywords: Carvone, epilepsy, penicillin, rat

INTRODUCTION

Epilepsy stands as one of the prevalent neurological disorders, distinguished not only by recurring unprovoked seizures but also by frequent associated somatic and psychiatric comorbidities (1,2). Epilepsy has negative socioeconomic consequences not only for patients but also for families and society (1,3). Epilepsy affects 70 million people worldwide (4,5). Approximately 20-30% of epilepsy patients are resistant to current antiepileptic drugs (AEDs). Therefore, there demand for drugs that are effective against drug-resistant seizures, have a low negative side effect profile, have favorable side effect profiles, especially in terms of neurological and psychiatric effects, and are cost-effective (6).

Carvone (CAR) is mostly obtained from the essential oils of plants of the Mentha genus. CAR (C10H14O), a ketone monoterpene, is isolated in (R)-(-) and (S)-(+) isomeric forms (7). CAR, which has a wide range of uses, has become popular, especially in the pharmaceutical industry (8). Studies have reported the anti-inflammatory (9), antioxidant (10), antinociceptive (11), antispasmodic (12), and neuroprotective effects of CAR (13).

There is an increasing search for the development of effective drugs with a combined effect, aiming to overcome the problems of polypharmacy that are specific to multiple diseases. In this context, special attention is paid to natural compounds such as terpenoids, which can bind to different pharmacological targets in the body. Moreover, terpenoids and their derivatives enhance penetration by affecting the ordered structure of biological membranes (14,15).

Compounds found in essential oils such as CAR have

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Corresponding Author: Ersin Beyazcicek, Düzce University, Faculty of Medicine, Department of Physiology, Düzce, Türkiye

E-mail: beyazcicek13@gmail.com

pharmacological effects on molecules involved in the mechanism of epilepsy (NMDA, Glycine, GABA...) (16-18). In addition, they affect resting membrane potential dynamics by modulating voltage-gated sodium and calcium channels (19). In epilepsy treatment, the ability of target molecules to cross the blood-brain barrier (BBB) is taken into consideration. Molecules smaller than 400 Da and lipophilic molecules easily cross the BBB (20). Therefore, CAR in essential oils also meets these criteria. This study aimed to electrophysiologically investigate the acute effects of S-CAR and R-CAR on penicillin-induced experimental epilepsy model in rats.

MATERIAL AND METHOD

Animals

Rats were obtained from Abant Izzet Baysal University (AIBU) Experimental Animal Research and Application Center. Male Wistar rats (n=91) weighing 230±30 g were kept in the laboratory at room temperature of 23°C, 60±5% humidity and 12:12 light-dark cycle, with free food and water intake. Ethical approval for the study was obtained from the AIBU Animal Research Local Ethics Committee with code number 2013/08.

Experimental Groups, Drugs, and Administration Routes

In the study, chemically purchased carvone (Sigma-Aldrich Missouri, USA) was administered intraperitoneally (i.p.) at doses of 100 mg/kg and 200 mg/kg. Urethane (Sigma-Aldrich Missouri, USA) at a dose of 1.25 g/kg i.p. was used as an anesthetic and 99% dimethyl sulfoxide (DMSO; Loba Chemie, India) was used as a solvent. Penicillin G potassium salt (I.E. Ulagay, Türkiye) used to induce epilepsy was administered intracortically (i.c.) at 500 IU in 2 µl volume. All drugs were prepared daily.

Rats were divided into 3 main groups: Common groups, pre-penicillin groups, and post-penicillin groups. The groups were then divided into subgroups as shown in Table 1. Only R-CAR, Only S-CAR, Sham, and DMSO groups were formed to determine whether the substances or surgical procedures caused any epileptic activity.

Table 1. Experimental groups, substances and routes of administration

	Subgroups	Substance	Dose	Route	n
Common groups	Sham	-	-	-	7
	Only R-CAR	(R)-(-) CAR	200 mg/kg	i.p.	7
	Only S-CAR	(S)-(+) CAR	200 mg/kg	i.p.	7
	DMSO (Solvent)	DMSO	1 ml/kg	i.p.	7
	Penicillin (PEN)	PEN	500 IU	i.c.	7
Pre-penicillin groups	Pre-R-CAR100	(R)-(-) CAR+PEN	100 mg/kg +500 IU	i.p.+i.c.	7
	Pre-S-CAR100	(S)-(+) CAR+PEN	100 mg/kg+500 IU	i.p.+i.c.	7
	Pre-R-CAR200	(R)-(-) CAR+PEN	200 mg/kg +500 IU	i.p.+i.c.	7
	Pre-S-CAR200	(S)-(+) CAR+PEN	200 mg/kg+500 IU	i.p.+i.c.	7
Post-penicillin groups	Post-R-CAR100	PEN+(R)-(-) CAR	500 IU+100 mg/kg	i.c.+i.p.	7
	Post-S-CAR100	PEN+(S)-(+) CAR	500 IU+100 mg/kg	i.c.+i.p.	7
	Post-R-CAR200	PEN+ (R)-(-)CAR	500 IU+200 mg/kg	i.c.+i.p.	7
	Post-S-CAR200	PEN+(S)-(+) CAR	500 IU+200 mg/kg	i.c.+i.p.	7

Surgical Procedure and Induction of Epileptiform Activity

In all groups, each animal was anesthetized with 1.25 g/kg urethane. The rats were then fixed to a stereotaxic frame (Harvard Instruments, MA, USA) in the supine position. Following the head shave, an incision was made along the midline of the scalp from front to back. Subsequently, the bony portion over the left cerebral cortex was delicately thinned with a drill and carefully removed. The penicillin-induced EA model was established using a previously described method (20-22). In brief, epileptic activity was induced by administering 500 IU/2 µl of penicillin intracortically at 2 mm lateral to the bregma line, 1 mm anterior, and 1.2 mm cortical depth using a Hamilton microsyringe (701N, Hamilton Co., Reno, NV, USA).

Electrophysiological Recordings

Two silver-silver chloride ball electrodes were placed in the somatomotor cortex area lateral to the Bregma line on the left hemisphere. The reference electrode was fixed to the right ear of the rats. Recording coordinates were set as follows: the first electrode was placed 1 mm anterior to the Bregma line and 2 mm lateral to the sagittal suture, and the second electrode was placed 5 mm posterior to the Bregma line and 2 mm lateral to the sagittal suture. After the electrodes were placed, electrocorticography (ECoG) recordings were obtained using the PowerLab/8SP system (ADInstruments Pty Ltd, NSW, Australia). A 5-minute baseline activity recording was taken after the electrodes were placed. Following the baseline activity recording, substances specified in Table 1 were administered intraperitoneally (i.p.) to all groups except for the post-penicillin groups. In the pre-penicillin groups, 30 minutes after substance administration, intracortical penicillin G application was performed, and ECoG recordings were taken for an additional 120 minutes. For the post-penicillin groups, after placing the electrodes, a 5-minute baseline

activity recording was obtained. Following the baseline activity recording, intracortical penicillin G application was performed. Thirty minutes after penicillin administration, substances specified in Table 1 were administered i.p. and ECoG recordings were taken for another 120 minutes.

Statistical Analysis

Recorded data from each animal were used to automatically calculate the onset latency of the first EA, spike-wave frequency, and spike-wave amplitude using software. Epileptiform activity recordings were analyzed after being segmented into ten-minute intervals. The initial onset time of EA, spike-wave frequency, and spike-wave amplitude measurements were assessed for each interval. Data were presented as mean±SD. GraphPad Prism 8 was utilized for all statistical analyses. The normal distribution of the data was assessed using the Shapiro-Wilk test. Two-way repeated measures ANOVA followed by LSD post-hoc test was employed for the evaluation of other data obtained from the groups. A p-value <0.05 was considered statistically significant.

RESULTS

Carvone Administration did not cause any EA in Groups not Induced Epilepsy with Penicillin

In the study, only (R)-CAR, Only (S)-CAR and DMSO administration did not have any EA effect on basal activity in the groups not stimulated with penicillin. Similarly, no discharge of epileptic activity was observed in the Sham group (Figure 1).

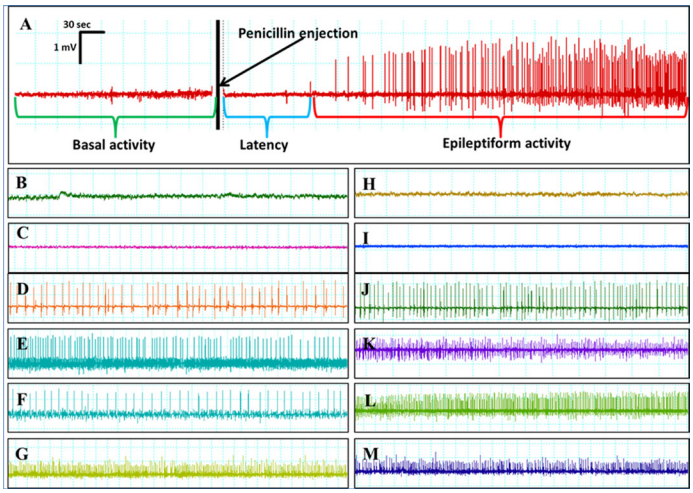

Figure 1. Representative samples of ECoG records from groups. **1A:** PEN group; **1B:** Sham group; **1C:** DMSO groups; **1D:** Pre-R-CAR100 group; **1E:** Pre-S-CAR100 group; **1F:** Pre-R-CAR200 group; **1G:** Pre-S-CAR200 group; **1H:** Only R-CAR200 group; **1I:** Only S-CAR200 groups; **1J:** Post-R-CAR100 group; **1K:** Post-S-CAR100 group; **1L:** Post-R-CAR200 group; **1M:** Post-S-CAR200 group

Carvone Prolongs Time to the Onset of the First EA

When the groups were compared according to the time of onset of the first EA, a statistical difference was detected between the groups (P<0.001) (Figure 2). When the groups were analyzed in more detail, the mean time to onset of the first EA in the Pre-R-CAR100, Pre-S-CAR100, Pre-R-CAR200, and Pre-S-CAR200 groups was statistically longer than that in the PEN groups (p<0.001, p=0.007, p<0.001 and p<0.001, respectively). Similarly, the mean time to onset of the first EA in the Pre-R-CAR100, Pre-R-CAR200, and Pre-S-CAR200 groups was statistically higher than in the Pre-S-CAR100 group (p<0.001, p<0.001 and p<0.001, respectively). The mean time to onset of the first EA in the Pre-S-CAR200 group was statistically higher than in the Pre-R-CAR100 group (p=0.040).

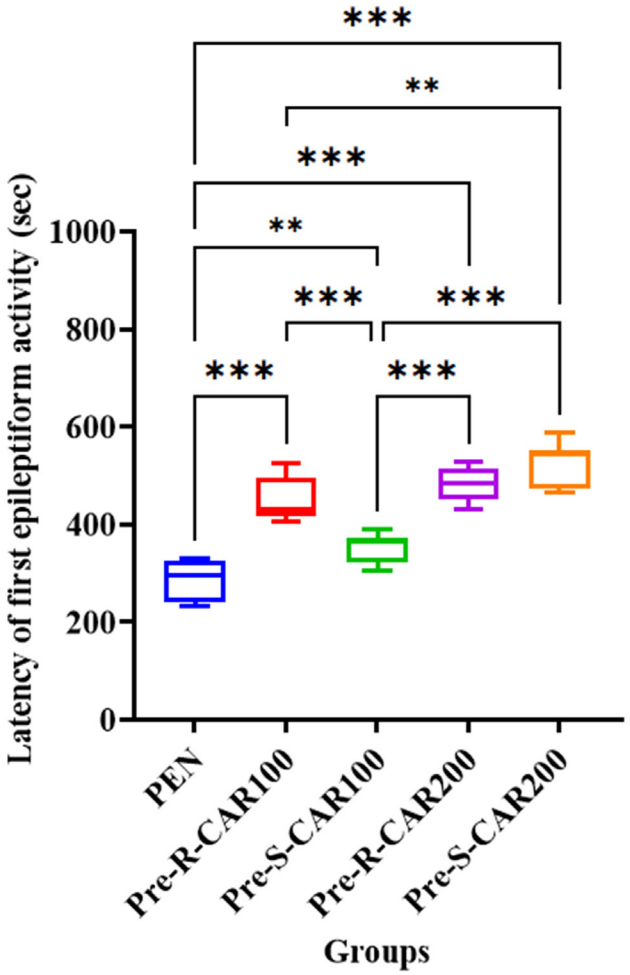

Figure 2. Latency of the first epileptiform activity in pre-penicillin groups (*p<0.05, **p<0.01, ***p<0.001)

Carvone Administration Reduces the SWF

No EA was detected in ECoG recording measurements during the basal activity recordings obtained from the penicillin injection groups. After penicillin administration, a certain number of spike-wave frequency values were obtained in 12 different measurements taken in ten-minute periods (Figure 1A and Figure 3). The mean SWF values of the Pre-R-CAR100 and Pre-R-CAR200 groups were lower than the PEN group in 12 different time periods taken during 0-120 minutes of recording (p<0.05) (Figure 3A). Furthermore, the mean SWF values of the Pre-R-CAR200 group were less than Pre-S-CAR100 group (p<0.01). The mean SWF values of the Pre-S-CAR200 group were lower than the PEN group except for the time periods 11-20, 51-60, and 61-70 (p<0.05). Furthermore, the mean SWF values

of the Pre-S-CAR200 group were lower than the Pre-S-CAR100 group in the measurements taken between 0-80 minutes (p<0.05).

ECoG recordings obtained as a result of carvone administration 30 minutes after penicillin injection were analyzed for 120 minutes. The 120-minute recordings were divided into 12 different time periods of ten minutes. A statistically significant distinction was observed among the groups (p=0.004) (Figure 3B). The mean SWF values of the Post-R-CAR100 and Post-S-CAR100 groups were lower than the PEN group in 12 different time periods taken during 30-150 minutes of the recording (excluding 30-40, 101-110, 131-140, and 141-150 time periods) (p<0.05). In 81-90, 111-120, and 121-130 time periods, the mean SWF values of the Post-R-CAR200 groups were lower than the PEN group (p=0.020, p=0.040, and p=0.040, respectively). Moreover, the mean SWF values of the Post-S-CAR200 group were lower than the PEN group in the 41-50, 61-70, and 71-80 time periods (p=0.020, p=0.020, and p=0.020, respectively). In the 51-60 and 61-70 time periods, the mean SWF values of the Post-R-CAR100 group were lower than the Post-R-CAR200 group (p=0.049 and p=0.030).

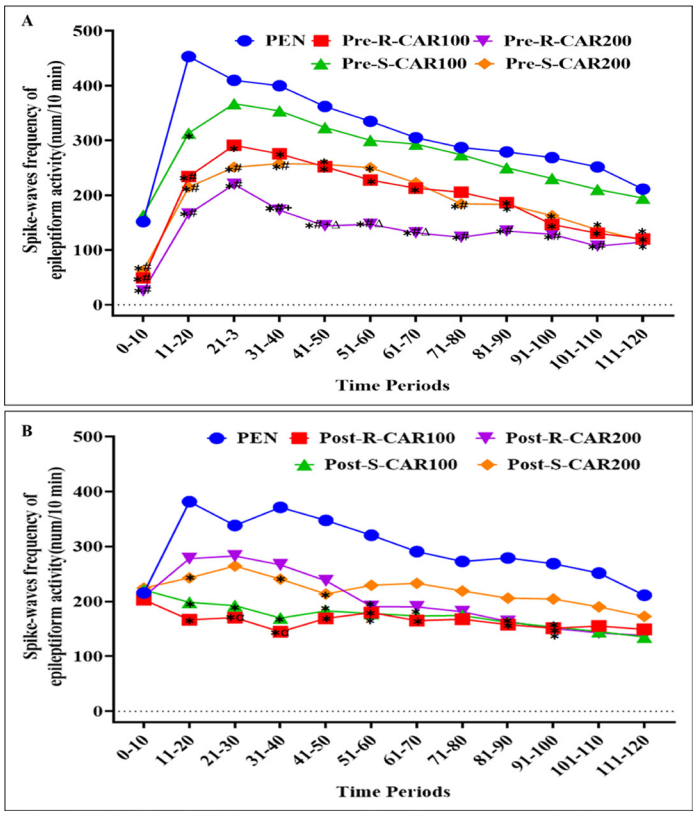

Figure 3. Mean of the time-dependent spike-wave frequency of epileptiform activity (number/min) obtained from the recording of rats. **3A:** pre-penicillin groups and **3B:** post-penicillin groups. (*Significant compared to PEN group; #Significant according to Pre-S-CAR100 group; +Significant compared to the Pre-R-CAR100 group; ΔSignificant compared to Pre-S-CAR200 group)

Effect of Carvone Administration on the Total SWF

The mean total spike wave frequency counts of the groups before penicillin injection were evaluated during 120 minutes of ECoG recording after penicillin administration. According to the results of the comparison of the groups in terms of the mean total SWF counts, a statistically significant distinction was observed among the groups (p<0.001) (Figure 4A). In terms of total SWF, the mean SWF values of the Pre-R-CAR100, Pre-R-CAR200, and Pre-S-CAR200 groups were lower than those of the PEN group (p=0.002, p<0.001 and p=0.002, respectively). Likewise, the mean SWF levels of the Pre-R-CAR100, Pre-R-CAR200, and Pre-S-CAR200 groups were lower than the Pre-S-CAR100 group (p=0.010, p<0.001 and p=0.009, respectively). However, there was no statistically significant difference between the groups in terms of total SWF value in ECoG recordings obtained as a result of carvone administration 30 minutes after penicillin injection (p=0.230) (Figure 4B).

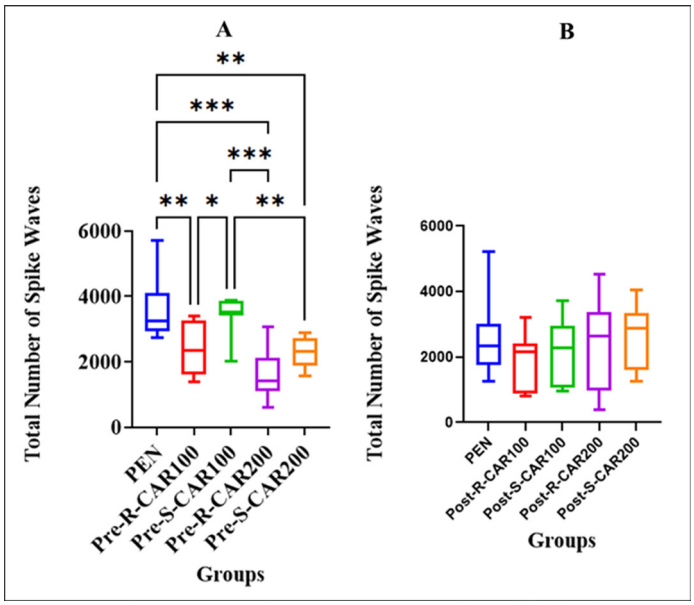

Figure 4. Display of total spike-wave number of pre-penicillin groups (**4A**) and post-penicillin groups (**4B**) (*p<0.05, **p<0.01, ***p<0.001)

Effect of Carvone Administration on SWA

Descriptive statistics of the SWA values measured at different times from ECoG recordings obtained from the groups before penicillin injection and the results of the comparison of the groups are given in Figure 5A. Except for the 0-10 time interval of the recording (p=0.051), the mean SWA values of the Pre-R-CAR200 group were statistically lower than the Pre-S-CAR100 group (p<0.05) in 12 different time intervals taken during 120 minutes of recording. In addition, the mean SWA values of the Pre-S-CAR200 group were statistically lower than the Pre-S-CAR100 group in the 31-40 and 61-70 time periods (p=0.040 and p=0.049). No statistically significant difference was found between the other groups (p>0.050).

ECoG recordings obtained as a result of carvone administration 30 minutes after penicillin injection were analyzed for 120 minutes. The 120-minute recordings were divided into 12 different time periods of ten minutes. A statistically significant distinction was observed among the groups (p<0.001) (Figure 5B). The mean SWA values of the Post-S-CAR100 group were lower than those of the PEN and Post-S-CAR200 groups in 12 different time periods

taken during 30-150 minutes of the recording (p<0.05). The mean SWA values of the Post-S-CAR100 group were lower than the Post-R-CAR100 group in the time periods 61-80, 91-100, and 121-150 (p<0.05). In addition, the mean SWA values of the Post-S-CAR100 group were lower than the Post-R-CAR200 group in the time periods between 51-90 minutes (p<0.05).

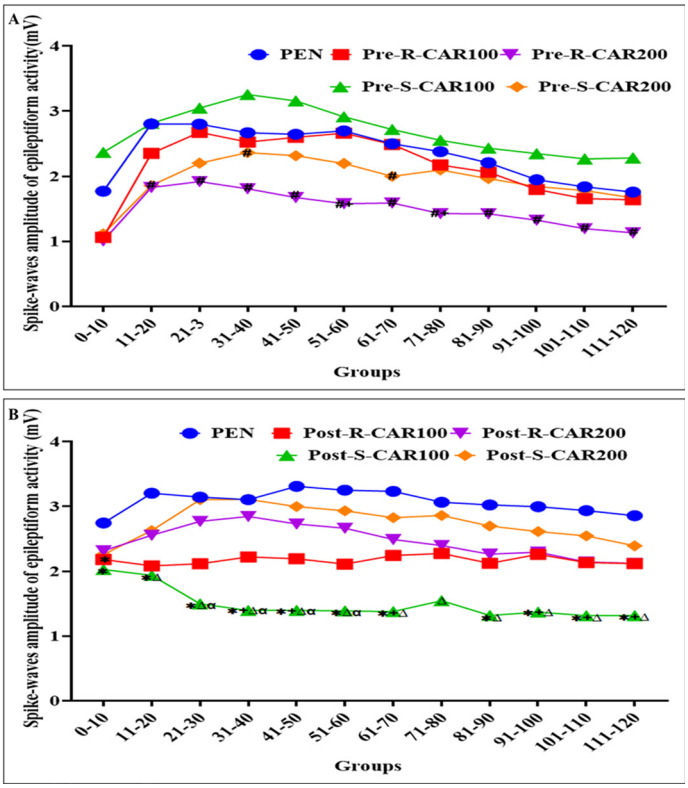

Figure 5. Mean of the time-dependent spike-wave amplitude of epileptiform activity (mV) obtained from the recording of rats. **5A:** pre-penicillin groups and **5B:** post-penicillin groups. (*Significant compared to PEN group; #Significant according to Pre-S-CAR100 group; +Significant compared to the Pre-R-CAR100 group; ΔSignificant compared to Pre-S-CAR200 group; αSignificant compared to Pre-S-CAR200 group)

DISCUSSION

In this study, the antiepileptic effects of 100 and 200 mg/kg doses of S-CAR and R-CAR were investigated in the penicillin-induced EA model. No EA was found in ECoG recordings taken for 120 minutes in the DMSO and Sham groups. These data are consistent with the literature (20-22). Similarly, no EA was observed in the only R-CAR and only S-CAR groups. This finding was not compared due to the lack of data in the literature.

In a study testing the effects of CAR on the latency of the first EA, it was reported that 200 mg/kg S-CAR prolonged the seizure onset time in PTZ and picrotoxin (PTX)-induced seizure models in mice, but 200 mg/kg R-CAR had no effect (23). In studies conducted with cyano-carvone (CC), a synthetic derivative of CAR, the protective effect of CAR was tried to be demonstrated. CC was investigated in the pilocarpine (PILO)-induced epilepsy model in mice. They reported that CC (25, 50, or 75 mg/kg) delayed the onset of the first epileptic seizure (24). In another similar study, the effects of CC against PILO, PTZ, and PTX-induced seizures were investigated (25). They reported that CC prolonged the time to onset of the first EA in all three models. In another study with PTZ, it was reported that CAR prolonged latency (26). In the present study, both doses (100 and 200 mg/kg) of S-CAR and R-CAR administered before penicillin prolonged the latency to onset of the first EA. Especially 200 mg/kg S-CAR and R-CAR doses prolonged the latency to the onset of the first EA approximately two-fold compared to the PEN group.

In a few studies with CC, the effects of carvone on seizure frequency were investigated. Costa et al. reported that CC at doses of 25, 50, or 75 mg/kg reduced seizure frequency in a PILO-induced epilepsy model (24). In another study testing the effect of CAR in the PTZ model, they reported that 10 and 20 mg/kg doses significantly reduced seizure frequency (26). In the present study, S-CAR (200 mg/kg) and R-CAR (100 and 200 mg/kg) administered before penicillin reduced time-dependent SWF. Especially 200 mg/kg R-CAR dose decreased SWF the most. However, SWF values of 100 mg/kg S-CAR dose were similar to the PEN group. Both doses (100 and 200 mg/kg) of R-CAR administered before penicillin decreased total SWF. The 100 mg/kg doses of S-CAR and R-CAR injected 30 minutes after penicillin administration decreased SWF.

In this study, 200 mg/kg dose of R-CAR administered before penicillin decreased SWA over time, while 100 mg/kg dose of S-CAR administered after penicillin decreased SWA. No comparison was made because there were no similar studies in the literature.

The induction of EA by penicillin is actively involved in cortical pyramidal cells. In the penicillin induced epilepsy, potentials relying on both GABAA and GABAB receptors play a role in the abrupt depolarization shifts observed in cells (27). Direct application of penicillin to the cortex inhibits GABA receptors, similar to the effect of bicuculline. Consequently, the suppressed GABA activity initiates EA that begins locally but progresses to generalize by disrupting the brain's inhibitory system. Research indicates that penicillin reduces intracellular Cl- influx by binding to subunits of GABAA receptors (28). Other studies have reported that penicillin binds to the chlorine receptor, preventing the channel from opening (29). Penicillin binds to the benzodiazepine binding site and causes convulsions (21). The primary target of penicillin is the β-subunit of the GABAA receptor, to which GABA binds. It is hypothesized that penicillin binds to the GABA binding site with a β-lactam ring, preventing GABA from binding to this site (30). While GABA levels in the brain were not investigated in this study, it has been demonstrated that CAR, administered at various doses, can reverse penicillin-induced EA. This suggests that CAR may act by increasing GABA levels in the penicillin-induced epilepsy model. It is conceivable that CAR not only affects GABAA receptors but also diminishes the release of excitatory neurotransmitters from excitatory neurons by acting on GABAB receptors. However, there is currently no literature available to corroborate this information.

CONCLUSION

In conclusion, carvone inhibited epileptiform activity, possibly by modulation of the GABAergic system. These results suggest that carvone may have both protective and therapeutic effects in the treatment of epilepsy. However, additional studies are required to determine its clinical use.

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Conflict of interest: The authors have no conflicts of interest to declare.

Ethical approval: Ethical approval for the study was obtained from the AIBU Animal Research Local Ethics Committee with code number 2013/08.

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MEDICAL RECORDS-International Medical Journal

Research Article

Dexmedetomidine and Fentanyl in Endotracheal Intubation: A Comparative Analysis of Hemodynamic and Intubation Responses

Aysegul Torun Goktas[1], Feriha Temizel[2], Gulten Arslan[3], Metin Ozseker[3], Husnu Suslu[4], Leyla Saitoglu[5]

[1]Giresun Training and Research Hospital, Department of Anaesthesiology and Reanimation, Giresun, Türkiye
[2]İstanbul Ataşehir Avicenna Hospital, Anaesthesiology and Reanimation Clinic, İstanbul, Türkiye
[3]İstanbul Kartal Lütfi Kırdar Training and Research Hospital, Department of Anaesthesiology and Reanimation, İstanbul, Türkiye
[4]Maltepe University, Faculty of Medicine, Department of Anaesthesiology and Reanimation, İstanbul, Türkiye
[5]Retired from İstanbul Kanuni Sultan Süleyman Training and Research Hospital, Department of Anaesthesiology and Reanimation, İstanbul, Türkiye

Abstract

Aim: Endotracheal intubation, a critical procedure in anaesthesia, can induce significant hemodynamic fluctuations, posing risks, especially to patients with cardiovascular concerns. This study compares the effects of dexmedetomidine and fentanyl, two agents commonly used to mitigate these responses, on endotracheal intubation conditions and associated hemodynamic changes.
Material and Method: Conducted at tertiary care training and research hospital, this study involved 60 patients aged 40-60, all classified American Society of Anesthesiologists (ASA) I-II, undergoing elective upper and lower extremity surgeries. Excluding patients with contraindicating conditions, the subjects were divided into two groups to receive either dexmedetomidine or fentanyl, along with propofol and vecuronium, for induction. Hemodynamic parameters were continuously monitored, and intubation conditions were assessed using the Cooper scoring system.
Results: The study found that both dexmedetomidine and fentanyl effectively stabilised hemodynamic parameters during intubation. However, the fentanyl group displayed significantly higher total scores on the Cooper intubation conditions scale, indicating more favourable conditions for endotracheal intubation in terms of ease and patient comfort.
Conclusion: While both dexmedetomidine and fentanyl are effective in maintaining hemodynamic stability during endotracheal intubation, fentanyl demonstrates a slight advantage in optimising intubation conditions. This distinction offers valuable insight for anesthesiologists in tailoring anaesthetic strategies and balancing patient safety with procedural efficiency in surgical settings.

Keywords: Dexmedetomidine, fentanyl, endotracheal intubation, hemodynamic responses, surgical anaesthesia

INTRODUCTION

Endotracheal intubation stands as a cornerstone procedure in anesthesiology, pivotal for maintaining patient airway patency during surgical interventions. This procedure, while routine, is not without its complexities and challenges, particularly in the context of hemodynamic stability (1). The act of intubation often triggers a cascade of physiological responses, primarily sympathetic activation, leading to fluctuations in heart rate and arterial pressure. These responses are not merely transient occurrences; they bear significant implications, especially for patients with pre-existing cardiovascular or cerebrovascular conditions (2). Thus, the quest for optimal anaesthetic agents that can mitigate these hemodynamic perturbations while ensuring effective and safe intubation conditions is a topic of ongoing clinical and academic interest.

In this background, dexmedetomidine and fentanyl emerge as two significant pharmacological agents. Dexmedetomidine, a selective α2 adrenoceptor agonist, is esteemed for its sedative, analgesic, and anxiolytic properties, with a notable feature of not depressing respiratory function (3). Its mechanism, centred around the α2 adrenoreceptors, offers a pathway to reducing sympathetic outflow, thus potentially stabilising hemodynamic responses during intubation (4,5). On the other hand, fentanyl, a potent opioid, is renowned for its analgesic efficacy. Beyond pain control, fentanyl's influence on the central nervous system translates into

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Corresponding Author: Aysegul Torun Goktas, Giresun Training and Research Hospital, Department of Anaesthesiology and Reanimation, Giresun, Türkiye
E-mail: aysegultorungoktas@yahoo.com

blunting of the stress response to intubation, which could yield benefits in terms of hemodynamic management (6). However, the selection between these two agents is not straightforward, as each brings its own profile of benefits and limitations.

Understanding the comparative effects of dexmedetomidine and fentanyl on endotracheal intubation conditions and hemodynamic responses is not just an academic pursuit; it has tangible implications for clinical practice. Anesthesiologists frequently grapple with the choice of these agents, seeking to balance efficacy with safety, particularly in patients with specific vulnerabilities. Therefore, this study aims to dissect and compare the impacts of dexmedetomidine and fentanyl when used in the context of endotracheal intubation. By doing so, it seeks to provide evidence-based insights that can guide clinical decision-making, ultimately enhancing patient outcomes in the diverse landscape of surgical anaesthesia.

MATERIAL AND METHOD

The study protocol was approved by the local ethics committee of Giresun Training and Research Hospital. Informed patient consent was waived due to the retrospective design of the study. This study was guided by the relevant ethical principles of the Declaration of Helsinki, revised in 2013. The study was conducted on 60 patients aged between 40 and 60, all in ASA I-II physical condition, at tertiary care training and research hospital for elective upper and lower extremity surgeries. Excluded from the study were patients with higher ASA classifications, complex intubation criteria, age outside 40-60, uncontrolled hypertension, cardiovascular or pulmonary diseases, hepatic or renal dysfunctions, psychiatric treatments, chronic opioid use, hypersensitivity to opioids or propofol, and liver or kidney failure. Dexmedetomidine and fentanyl groups were created with data obtained from patient file records of anesthesiologists who used only one of these drugs. Each patient was evaluated and consented to a day before surgery. In the operating room, they received a 20 gauge intravenous line with 0.9% NaCl infusion and underwent noninvasive monitoring without premedication. After preoxygenation, patients were divided into two groups for administering either fentanyl or dexmedetomidine, followed by propofol and vecuronium for induction. Anesthesia was maintained with a mix of N2O, oxygen, and sevoflurane. Intubation conditions were assessed using Cooper scoring (Table 1). Vital signs were continuously monitored and recorded at various stages of the procedure. After surgery, intravenous tramadol was administered, and anaesthetic gases were replaced with 100% oxygen. Neostigmine and atropine were used post-operation to reverse the effects of muscle relaxants.

Table 1. Cooper scoring system

Score	Jaw relaxation	Vocal cords	Response to intubation
0	Poor (impossible)	Closed	Severe coughing or bucking
1	Minimal (difficult)	Closing	Mild coughing
2	Moderate (fair)	Moving	Slight diaphragmatic movement
3	Good (easy)	Open	None
Total score: Excellent (8-9), Good (6-7), Fair (3-5), Poor (0-2)			

Statistical Analysis

Number Cruncher Statistical System (NCSS) 2007 & PASS 2008 Statistical Software (Utah, USA) programs were used for statistical analyses. In addition to descriptive statistical methods (Mean, Standard deviation), the Student t-test was used to compare parameters with normal distribution between two groups, and the Whitney U test was used to compare parameters without normal distribution between two groups. Paired sample t-test was used for intra-group comparisons of normally distributed parameters. The chi-square test was used to compare qualitative data. Significance was evaluated at $p<0.05$ level.

RESULTS

The study was conducted with 60 patients, 30 in the Dexmedetomidine group and 30 in the Fentanyl group, who were to undergo upper and lower extremity surgery. The ages of the patients ranged between 40 and 60 years, with a mean age of 50.3±7.4 years. 61.7% (n=37) of the patients were female and 38.3% (n=23) were male. There is no statistically significant difference between age, weight, height, ASA and gender ($p>0.05$) (Table 2).

There was no statistically significant difference between the systolic arterial blood pressure (SABP) levels before induction, before intubation, 1st, 3rd, 5th, 10th, and 15th minutes after intubation ($p>0.05$). In the dexmedetomidine group, statistically significant decreases were observed in SABP levels at the 1st, 3rd, 5th, 10th, and 15th minutes after intubation compared to pre-induction SABP levels ($p<0.05$). In the fentanyl group, statistically significant decreases were observed in SABP levels before intubation, 1st, 3rd, 5th, 10th, and 15th minutes after intubation compared to SABP levels before induction ($p<0.05$) (Table 3).

In the dexmedetomidine group, statistically significant decreases were observed in diastolic arterial blood pressure (DABP) levels before intubation, 1st, 3rd, 5th, 10th, and 15th minutes after intubation compared to pre-induction DABP levels ($p<0.05$). In the fentanyl group, there was no statistically significant change in DABP levels at 1st min after intubation compared to pre-induction DABP levels, and statistically significant decreases were observed in DABP levels at 3rd, 5th, 10th, and 15th minutes after intubation compared to pre-induction DABP levels ($p<0.05$) (Table 4).

Table 2. Comparison of characteristics of patients between two groups

Patients' characteristics		Dexmedetomidine (n: 30)		Fentanyl (n: 30)		p
		Number	%	Number	%	
Gender$^{x^2}$	Female	9	30.0	14	46.7	0.184
	Male	21	70.0	16	53.3	
ASA$^{x^2}$	I	17	56.7	18	60	0.793
	II	13	43.3	12	40	
		Mean±SD		Mean±SD		
Aget		50.4±8.4		50.3±6.4		0.973
Weight^{t}		73.3±10.5		73.1±12.3		0.955
Height^{t}		162±9.6		162.6±23.4		0.897

*p<0.05, **p<0.01, x^2: Chi-square test (Categorical data), t: student T test, Med: median, SD: standart deviation

Table 3. Evaluation of SABP according to groups

SABP (mm Hg)	Dexmedetomidine (n=30)	Fentanyl (n=30)	p
	Mean±SD	Mean±SD	
Before induction	138.1±7.0	135.1±4.9	0.065
Before intubation	121.3±14.2	117.5±8.8	0.211
1st min after intubation	127.6±9.7	131.8±10.7	0.118
3rd min	125.7±10.5	123.0±9.2	0.292
5th min	128.0±6.8	124.5±6.8	0.053
10th min	127.9±7.2	124.4±6.6	0.059
15th min	128.0±6.5	123.5±10.9	0.057
p value	Dexmedetomidine (n=30)	Fentanyl (n=30)	
Before induction – before intubation	**0.001***	**0.001***	
Before induction – 1st min after intubation	**0.001***	**0.048***	
Before induction – 3rd min	**0.001***	**0.001***	
Before induction – 5th min	**0.001***	**0.001***	
Before induction – 10th min	**0.001***	**0.001***	
Before induction – 15th min	**0.001***	**0.001***	

*p<0.05, t: student T test, SD: standart deviation, min: minute

Table 4. Evaluation of DABP according to groups

DABP (mm Hg)	Dexmedetomidine (n=30)	Fentanyl (n=30)	p
	Mean±SD	Mean±SD	
Before induction	86.3±8.4	82.7±7.4	0.065
Before intubation	73.6±9.5	72.2±7.8	0.547
1st min after intubation	79.0±7.0	82.7±7.4	0.055
3rd min	74.5±7.6	75.6±7.5	0.588
5th min	77.6±7.9	74.0±8.3	0.093
10th min	77.8±7.5	74.2±7.9	0.080
15th min	78.4±6.0	75.1±7.7	0.071
p value	Dexmedetomidine (n=30)	Fentanyl (n=30)	
Before induction – before intubation	**0.001***	**0.001***	
Before induction – 1st min after intubation	**0.001***	0.754	
Before induction – 3rd min	**0.001***	**0.001***	
Before induction – 5th min	**0.001***	**0.001***	
Before induction – 10th min	**0.001***	**0.001***	
Before induction – 15th min	**0.001***	**0.001***	

*p<0.05, t: student T test, SD: standart deviation, min: minute

There was no statistically significant difference between the pre-induction, pre-intubation, and post-intubation 1st, 3rd, 5th, 10th, and 15th minutes mean arterial blood pressure (MABP) levels between the groups (p>0.05). In the dexmedetomidine group, statistically significant decreases were observed in the pre-intubation, post-intubation 1st, 3rd, 5th, 10th and 15th minutes MABP levels compared to the pre-induction MABP levels (p<0.05) (Table 6). In the fentanyl group, no statistically significant change was observed in the MABP levels at the 1st minute after intubation compared to the pre-induction MABP levels. Statistically, significant decreases were observed in the MABP levels at the 3rd, 5th, 10th and 15th minutes after intubation compared to the pre-induction MABP levels (p<0.05) (Table 5).

There was no statistically significant difference between the groups in pre-induction, pre-intubation, and post-intubation 1st, 3rd, 5th, 10th, and 15th minutes peak heart rate (PHR) levels (p>0.05). In the dexmedetomidine group, statistically significant decreases were observed in pre-intubation, post-intubation 1st, 3rd, 5th, 10th, and 15th minutes PHR levels compared to pre-induction PHR levels (p<0.05). In the fentanyl group, statistically significant decreases were observed in PHR levels at 1st, 3rd, 5th, 10th, and 15th minutes after intubation compared to pre-induction PHR levels (p<0.05) (Table 6).

Table 5. Evaluation of MABP according to groups

DABP (mm Hg)	Dexmedetomidine (n=30)	Fentanyl (n=30)	p
	Mean±SD	Mean±SD	
Before induction	86.3±8.4	82.7±7.4	0.065
Before intubation	73.6±9.5	72.2±7.8	0.547
1st min after intubation	79.0±7.0	82.7±7.4	0.055
3rd min	74.5±7.6	75.6±7.5	0.588
5th min	77.6±7.9	74.0±8.3	0.093
10th min	77.8±7.5	74.2±7.9	0.080
15th min	78.4±6.0	75.1±7.7	0.071
p value	Dexmedetomidine (n=30)	Fentanyl (n=30)	
Before induction – Before intubation	**0.001***	**0.001***	
Before induction – 1st min after intubation	**0.001***	0.754	
Before induction – 3rd min	**0.001***	**0.001***	
Before induction – 5th min	**0.001***	**0.001***	
Before induction – 10th min	**0.001***	**0.001***	
Before induction – 15th min	**0.001***	**0.001***	

*p<0.05, t: student T test, SD: standart deviation, min: minute

Table 6. Evaluation of PHR according to groups

PHR (beats/min)	Dexmedetomidine (n=30)	Fentanyl (n=30)	p
	Mean±SD	Mean±SD	
Before induction	80.9±10.1	80.4±6.8	0.824
Before intubation	68.3±9.8	72.8±8.2	0.077
1st min after intubation	70.8±5.7	74.0±8.7	0.105
3rd min	68.8±8.3	72.8±8.9	0.077
5th min	69.5±9.0	72.5 ±8.3	0.182
10th min	72.5±8.4	73.5±6.6	0.612
15th min	72.3±8.7	73.2±6.8	0.658
p value	Dexmedetomidine (n=30)	Fentanyl (n=30)	
Before induction – before intubation	**0.001***	**0.001***	
Before induction – 1st min after intubation	**0.001***	**0.001***	
Before induction – 3rd min	**0.001***	**0.001***	
Before induction – 5th min	**0.001***	**0.001***	
Before induction – 10th min	**0.001***	**0.001***	
Before induction – 15th min	**0.001***	**0.001***	

*p<0.05, t: student T test, SD: standart deviation, min: minute

Oxygen saturation (SPO2) levels maintained stability throughout the operation in both groups, with no significant changes observed across different time intervals (p>0.05). Additionally, end-tidal carbon dioxide (etCO2) levels remained consistent and did not show statistically significant differences in either group at any observed time intervals (p>0.05).

One notable finding was in the assessment of intubation conditions using Cooper scoring. The Fentanyl group displayed statistically significantly higher total intubation conditions scores compared to the Dexmedetomidine group (p<0.05). This suggested a higher proportion of patients in the Fentanyl group experienced excellent intubation conditions, while the Dexmedetomidine group predominantly had good intubation conditions.

DISCUSSION

The intricate relationship between anaesthetic agents and their physiological impact during endotracheal intubation is a focal area in anesthesiology, underscored by our study's findings. Though a routine procedure, endotracheal intubation often elicits a sympathoadrenergic response, leading to cardiovascular stress (7). This phenomenon, particularly critical in patients with cardiac ischemia or cerebrovascular conditions, has been well-documented, as in studies by Saitoh et al., highlighting the imperative for effectively managing these hemodynamic changes (8).

Our research, focusing on dexmedetomidine and fentanyl, contributes to this area by providing a comparative analysis of their effects on hemodynamic responses during intubation. Dexmedetomidine's efficacy, as evidenced in our study, aligns with the growing body of literature advocating for its use in anaesthetic practice due to its minimal respiratory depression and stabilising influence on hemodynamics, as supported by findings from Özköse et al., Dyck et al., and Başar et al. (9-11). Meanwhile, fentanyl's effective suppression of hemodynamic responses without significant side effects, resonating with the work of Salihoğlu et al. and Myless et al., underlines its utility in surgical anaesthesia (12,13).

Methodologically, our study faced limitations such as a confined demographic range and a specific surgical context, which may influence the generalizability of the findings. Future research could expand on these aspects, exploring varied patient populations and surgical settings to validate and extend our results.

Clinically, the insights from this study have profound implications. The nuanced understanding of how dexmedetomidine and fentanyl modulate cardiovascular responses could guide anesthesiologists in selecting the most appropriate agent, particularly in patients with pre-existing cardiovascular conditions (14). The preference for fentanyl in scenarios demanding smoother intubation processes, as suggested by our findings, could enhance patient comfort and procedural efficiency.

The field would benefit from further research exploring the long-term outcomes of using these agents, their interactions with other medications, and their effects in more diverse patient cohorts. Such studies would enrich our understanding and help develop more refined anaesthetic protocols.

Study Limitations

This research, while insightful, has its limitations. The study's sample size and demographic concentration may limit the extrapolation of results to a broader population, as it was conducted within a single medical centre and possibly lacked diversity in patient profiles. The focus on short-term hemodynamic and intubation responses also means that the longer-term effects of the anaesthetic agents were not explored. Additionally, the study design did not incorporate blinding, potentially introducing bias in assessing outcomes. High-risk patients and those with complex medical histories were excluded, which might restrict the applicability of our findings to these patient groups. The reliance on specific drugs for induction and maintenance alongside dexmedetomidine and fentanyl could have influenced the results, and variability in intubation techniques may have introduced additional outcome variability. Lastly, the absence of a comparative analysis with other anaesthetic agents limits the scope of understanding the relative efficacy of dexmedetomidine and fentanyl in a wider anaesthetic context.

CONCLUSION

This study demonstrates that both dexmedetomidine and fentanyl effectively manage hemodynamic and intubation conditions during endotracheal intubation, with fentanyl slightly outperforming in terms of intubation conditions. These findings guide anesthesiologists in choosing suitable agents, highlighting the need for tailored approaches in anaesthesia to optimise patient safety and outcomes within the study's limitations.

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Ethical approval: *The study was conducted in accordance with the Helsinki Declaration principles and was approved by our Corporate Ethics Committee, Giresun Training and Research Hospital (2023/KAEK-164).*

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MEDICAL RECORDS-International Medical Journal

Research Article

Investigation of Endoplasmic Reticulum Stress and Apoptosis Caused by Malachite Green-Mediated Sonodynamic Therapy in HL60 Cells

Metin Caliskan[1], Sercin Ozlem Caliskan[2], Gulsen Bayrak[3]

[1]Uşak University, Faculty of Medicine, Department of Medical Biology, Uşak, Türkiye
[2]Uşak University, Faculty of Medicine, Department of Biophysics, Uşak, Türkiye
[3]Uşak University, Faculty of Medicine, Department of Histology and Embryology, Uşak, Türkiye

Abstract

Aim: Sonodynamic antitumor therapy is a promising, novel method for the treatment of cancer. To determine the effects of malachite green (MG) in the presence of ultrasound (US), MG was tested in vitro on HL60 cells at different concentrations as a sonodynamic compound. We investigated cell viability, morphology, and the occurrence of endoplasmic reticulum (ER) stress after MG-mediated sonodynamic therapy (SDT) in HL60 cells.

Material and Method: Four groups were formed, including a control group, a group subjected to ultrasound (US) only, a group treated with various concentrations of MG, and a group treated with US using the same concentrations. The cells were treated with 1MHz ultrasound at 2 W/cm^2 for 3 minutes. The assessment of cell viability was conducted 24 hours post-treatment through the utilization of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Cell morphology and apoptotic index were determined using Giemsa staining, while GRP78 and PERK expressions were determined through immunocytochemistry staining.

Results: The cell cytotoxicity of HL60 cells significantly increased after MG-mediated sonodynamic therapy. After treatment, apoptotic cells with micronuclei were observed morphologically. Significant levels of GRP78 and PERK expression were observed in all groups, except for PERK expression in the US group, compared to the control group.

Conclusion: The induction of ER stress, accompanied by intense apoptosis and a marked decrease in cell viability, demonstrates the potential of MG-mediated sonodynamic therapy in cancer treatment. Investigating ER stress as a molecular target may contribute to improving the treatment method.

Keywords: HL60 cells, malachite green, sonodynamic therapy, apoptosis, ER stress

INTRODUCTION

Surgery, radiotherapy, and chemotherapy are classic treatment methods for cancer. Targeted therapy, hormonal therapy, and immunotherapy are alternative methods reported in cancer treatment. Even though these treatments had clinical success, they also have side effects and disadvantages. Chemotherapeutic drugs may cause side effects such as liver damage, gastrointestinal toxicity, and immunosuppression, while radiotherapy may have toxic effects on normal tissue. Surgical procedures may cause trauma and tumor metastasis. Therefore, non-invasive, effective, non-toxic, and reproducible cancer treatment methods are necessary (1,2).

Photodynamic therapy (PDT) has been utilized as a non-toxic and non-invasive method for tumor treatment in recent years. The mechanism of action of PDT is based on the activation of a photosensitizer that selectively accumulates in tumor tissue when exposed to light of an appropriate wavelength. This activation initiates a series of biochemical events that can potentially cause damage and lead to the death of the target tissue (3). Numerous studies have shown that PDT is an effective alternative treatment method for various cancers. Nevertheless, its effectiveness in the treatment of deep tumors is limited due to the shallow depth of penetration of light (4).

CITATION
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Corresponding Author: Sercin Ozlem Caliskan, Uşak University, Faculty of Medicine, Department of Biophysics, Uşak, Türkiye
E-mail: srn.ozlem@gmail.com

Sonodynamic therapy (SDT) emerged after Yumita and Umemura discovered in 1989 that hematoporphyrin, a photosensitizer, could be activated by ultrasound (US) and kill tumor cells (5). SDT refers to the process in which a sonosensitizer is activated by US, resulting in the production of reactive oxygen species (ROS) (6-9). The mechanism of SDT remains unclear. The mechanisms reported in the literature are the ultrasound-induced cavitation effect and the ROS formation. The predominant mechanism widely acknowledged involves the generation of ROS induced by pyrolysis or sonoluminescence. Ultrasound is a mechanical wave that has many advantages, including high-level concentrated energy, directability, and long-distance propagation. SDT has significant potential in the treatment of deep-seated tumors due to its ability to penetrate deep tissues and cause minimal damage to surrounding normal tissues (10,11).

The endoplasmic reticulum (ER) is a central organelle that is primarily responsible for protein synthesis, folding, and modification, and it controls various cellular functions, including intracellular Ca^{2+} balance and maintenance of cellular homeostasis (12). Sonodynamic treatment causes abundant ROS production, which leads to an increase in ER stress and the induction of apoptosis if this stress cannot be tolerated (13,14). The accumulation of misfolded proteins in the ER after sonodynamic treatment leads to the induction of Glucose-regulated protein 78 (GRP78), which acts as a chaperone and master regulator of the Unfolded Protein Response (UPR) (15). PERK is a transmembrane protein localized in ER. Under normal conditions, PERK is inactive and is complexed with ER chaperone immunoglobulin binding proteins. The complex is disrupted when ER stress occurs, and PERK inactivates eIF2, which is required for protein synthesis, by phosphorylating it, thereby suppressing translation and reducing ER stress (16).

The effectiveness of sonodynamic therapy largely depends on the efficacy of the sonosensitizer. Malachite green (MG) is a cationic dye with a triphenylmethane structure derived from dimethyl aniline and benzaldehyde (17). Due to the structural properties of triacyl methane dyes, their selective localization ability attracts attention as antimicrobial and anticancer agents (18). The literature lacks studies on the efficacy of sonodynamic treatment using MG, which stands out as a photosensitizer. The present study investigated the effects of MG-mediated SDT on cell viability, morphology, apoptosis, and ER stress in HL60 acute promyelocytic leukemia cells.

MATERIAL AND METHOD

HL60 Cell Culture

The human acute promyelocytic leukemia cell line HL60 was cultured in RPMI 1640 with L-glutamine medium (CEGROGEN, Biotech, Germany), supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin (Sigma, St. Louis, MO, USA).

Preparation of Stock Solutions of Malachite Green

MG stock solution (Sigma-Aldrich, USA) was prepared in PBS at a concentration of 50 µM. MG dilutions of 3.12, 1.56, 0.78, 0.39, 0.19, and 0.09 µM were prepared from the stock MB solution using RPMI 1640 medium. An equal amount of fresh medium containing $1x10^5$ cells was added to the prepared dilutions.

Experimental Groups

Group 1: (Control) The control group is composed of control untreated cells.

Group 2: (US) Only ultrasound was performed on the cells at a frequency of 1 MHz with an intensity of 2 W/cm^2 and a distance of 2 cm for 3 minutes

Group 3: (MG) Cells were exposed to MG at doses of 3.12, 1.56, 0.78, 0.39, 0.19, and 0.09 µM for one hour at 37°C, then the free MG was removed.

Group 4: (MG+SDT) Cells were incubated with MG doses of 3.12, 1.56, 0.78, 0.39, 0.19, and 0.09 µM MG for one hour at 37°C, then the free MG was removed. Finally, ultrasound was performed on the cells at a frequency of 1 MHz with an intensity of 2 W/cm^2 and at a distance of 2 cm for 3 minutes.

Determination of in Vitro Efficacy of Sonodynamic Therapy

The experimental setup for MG-mediated SDT is shown in Figure 1. HL60 cells were centrifuged at 1000 rpm for 5 min after being exposed to various doses of MB for 1 h, as in our previous studies (19). PBS was added to the precipitate. This process was repeated 3 times, and then the free MG was removed. The BTL 4710 Sono dual-frequency ultrasound therapy device (BTL, CZ) was used to apply ultrasound to cells. Cells were transferred to 1.5 mL Eppendorf tubes and exposed to ultrasound at a frequency of 1 MHz, from a distance of 2 cm, and at an intensity of 2 W/cm² for 3 minutes (20) in water. To prevent the thermal effect of ultrasound, the water was changed, and the temperature of the application environment was kept under control. Subsequent to the ultrasound treatment, fresh medium was added to the samples, and the samples were incubated at 37°C for 24 hours.

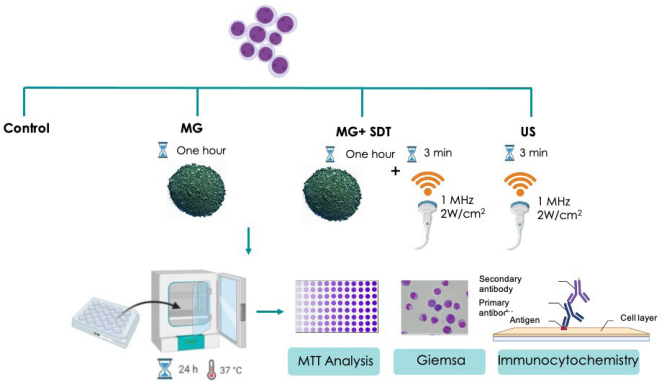

Figure 1. Experimental setup for MG-mediated SDT

MTT Analysis

Following the incubation period, cell viability was evaluated in the Control, US, MG, and MG mediated SDT groups using the MTT. The samples were incubated at 37°C and 5% CO_2 for 4 h using 10µl of MTT solution for each well in a 96-well microplate. Absorbance values were measured using a spectrophotometer at 570 nm (Multiskan Sky Microplate Spectrophotometer, Thermo Fisher Scientific).

Determination of Cell Morphology and Apoptotic Index Using Giemsa Staining

Three preparations were made for the control, US, MG, and MG mediated SDT groups. A certain amount of cells were taken from each group, spread on a slide, and allowed to dry. The dried cells were fixed by methanol. Giemsa stain, prepared in a one-to-one ratio with sterile distilled water, was dropped onto the slides with the preparations and left for 5 minutes. Subsequently, after washing off excess stain from the preparations, cell morphologies and apoptotic indices were examined under a light microscope. Cells containing condensed chromatin and micronuclei were considered apoptotic. The apoptotic index was determined by counting 100 cells in each group preparation, both apoptotic and non-apoptotic, at a magnification of 1000X.

Determination of ER Stress Markers GRP78 and PERK by Immunostaining

After centrifugation, the supernatant was removed, and the resulting precipitate was evenly distributed onto a slide. After the preparation dried, it was fixed with methanol. After PBS washing, it was incubated in a 3% H_2O_2 solution for 10 minutes to inhibit endogenous peroxidase activity. After washing with PBS again, normal goat serum (Invitrogen-50062Z) was applied for inhibition and left for 8 minutes. It was then incubated overnight with primary antibodies at +4°C: Anti-GRP78 BiP/HSPA5 (1:100, PB9640; Boster) and Anti-PERK (1:100, bs2469R; Bioss). After the incubation period, a rabbit anti-IgG secondary antibody (1/200, Thermo Scientific, 65-6140) was added to the preparations, and it was incubated for 30 minutes. Subsequently, the preparations were washed with PBS.

Horseradish peroxidase (HRP, 1/200, Thermo Scientific, 43-4323) was then added and incubated for 10 min. The reaction was further enhanced using the chromogen diaminobenzidine (DAB, Abcam, ab64238). After washing the preparations with distilled water, they were covered with Entellan and examined under a light microscope (Olympus BX50). Images were recorded using the attached camera. Immunocytochemical scoring was conducted by counting 100 cells in four different fields at 400X magnification. The staining intensities of these cells were scored as follows: strong (++++), moderate (+++), weak (+), and absent (+). The staining intensity score generally consists of four categories: negative (1), weak (2), moderate (3), and strong (4). The H-score, All red-score, and Immunoreactive score are regarded as the 'gold standard' in combined scoring systems for evaluating and presenting IHC data. These scoring systems employ different categories to assess the proportion of stained tissues or cells (21).

Statistical Analysis

All experiments were repeated at least three times in triplicate wells. The data were analyzed using the SPSS 25.0 software, and one-way analysis of variance (ANOVA) and Paired Sample t-test were employed for data analysis. Results with a p-value <0.05 were considered statistically significant.

RESULTS

Effect of Malachite Green and Malachite Green-Mediated SDT on HL-60 Cell Viability

The results showed that SDT significantly reduced cell viability at all concentrations of MG compared to the control group, and cell death increased as concentrations of MG increased ($p<0.001$). Cell viability percentages of the Control, US, and MG mediated SDT groups, from low to high concentration, respectively, were determined as 99.7%±0.5, 97.5%±1.35, 95.6%±4.47, 94.4%±0.86, 86.6%±0.48, 75.6%±0.47, 52.3%±1.05, and 43.9%±2.28. No significant difference was observed between the control and US groups in terms of cell viability ($p>0.05$). In the MG group, cell viability percentages were determined as 98.2%±1.7, 99%±0.41, 99.95%±0.95, 97.7%±0.47, 88.6%±0.47 and 72.5%±1.15, respectively, from low concentration to high. It was observed that concentrations lower than 1.56 µM of MG did not significantly affect the viability of HL60 cells, while a concentration of 1.56 µM MG did have a small impact on viability and resulted in the death of approximately half of the cells when combined with US (Figure 2).

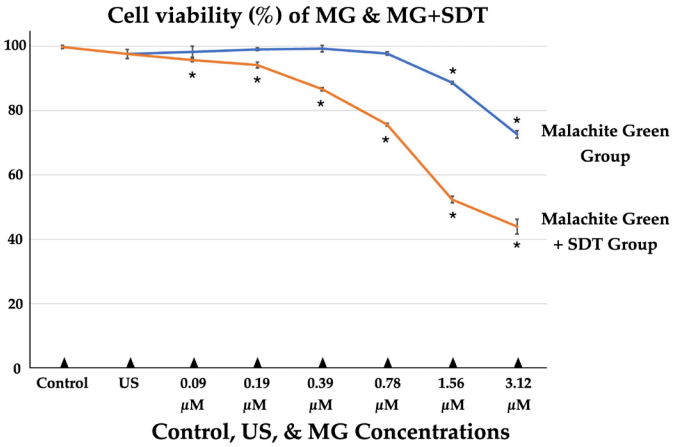

Figure 2. Evaluation of cytotoxicity after treatment with Control, Ultrasound, MG and MG-mediated SDT. The data represent the means±standard deviations (SDs) of 3 independent experiments. * indicates statistically significance compared to control group; Error bars 95% confiedence interval

Determination of the Morphology and Apoptotic Index of HL60 Cells using Giemsa Staining

Morphological changes in cells are crucial for the determination of cell apoptosis. Figure 3A-G shows the

morphological findings of the control group and the MG treatment group at various concentrations. Typical morphological characteristics of HL60 cells in the control group were observed as large, round-nucleated, and cytoplasmic (Figure 3G). An increase in apoptotic cells with micronuclei was observed in the MG-only treated groups towards low to high concentrations (Figure 3A-F).

Figure 3I-O presents microscopic images of cells treated with US and MG-mediated SDT, after Giemsa staining. MG-mediated SDT groups showed irregular changes in morphology, including cell membrane shrinkage. It was observed that the number of apoptotic cells increased significantly as the MG concentration increased, and more apoptotic bodies were formed compared to the control. Apoptotic cells with irregular cytoplasmic contours, chromatin condensation, decreased nucleocytoplasmic ratio , and micronuclei were identified, particularly at high concentrations (Figure 3I-N). In the US-only group, typical morphological features were observed, although a few cells showed apoptotic cells with micronuclei (Figure 3O).

Apoptotic index values at the end of the 24-hour experimental period were calculated as 2.11±1.05% for the control group, 5.77±0.66% for the US group, 63.11±1.16%, 40.88±0.6%, 32.11±0.92%, 24.88±0.78%, 16.88±1.36%, and 8.22±1.31% for the 3.125 µM, 1.56 µM, 0.78 µM, 0.39 µM, 0.19 µM, and 0.09 µM MG-treated experimental groups, respectively, and 72.11±1.16%, 50.88±0.78%, 42.55±1.13%, 37.66±0.7%, 29.11±1.16%, and 11.88±0.78% for the MG+SDT group in the same MG concentration order (Figure 4).

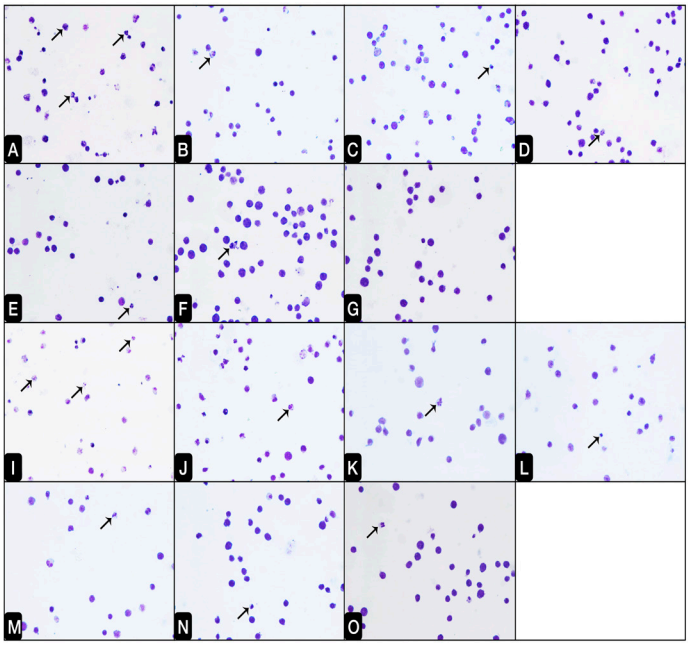

Figure 3. Morphology of the MG and MG mediated SDT group: 3.12 µM MG (**3A**), 1.56 µM MG (**3B**), 0.78 µM MG (**3C**), 0.39 µM MG (**3D**), 0.19 µM MG (**3E**), 0.09 µM MG (**3F**), Control (**3G**), 3.12 µM MG mediated SDT (**3I**), 1.56 µM MG mediated SDT (**3J**), 0.78 µM MG mediated SDT (**3K**), 0.39 µM MG mediated SDT (**3L**), 0.19 µM MG mediated SDT (**3M**), 0.09 µM MG mediated SDT (**3N**), US-Control (**3O**) Apoptotic cells with micronuclei (black arrow). Magnification X400

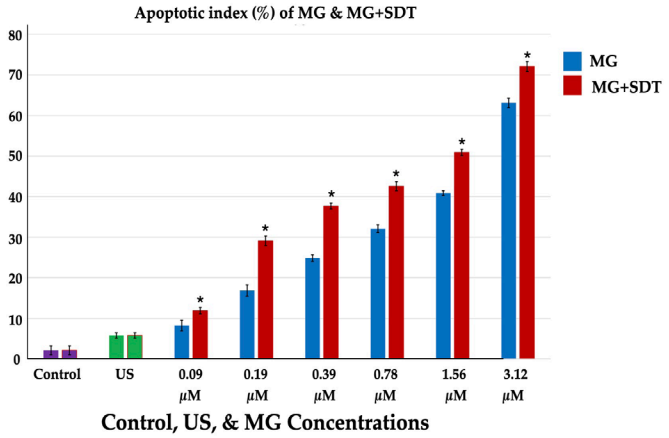

Figure 4. Apoptosis indices of control, US, MG and MG SDT groups.The data represent the means±standard deviations (SDs) of 3 independent experiments. *indicates statistically significance compared to control group; Error bars 95% confiedence interval

Determination of ER Stress Markers GRP78 and PERK Expressions by Immunocytochemistry

GRP78 and PERK expressions in all groups were analyzed by examining the changes in immunocytochemistry staining. Compared to the control group, significant levels of GRP78 and PERK expression were observed in all groups ($p<0.001$) (Figure 5-7), except for PERK expression in the US group (Figure 6G). The expression of both proteins increased in both the MG group (Figure 5A-F and Figure 6A-F) and the MG-mediated SDT group (Figure 5I-N and Figure 6I-N) as the MG concentration increased.

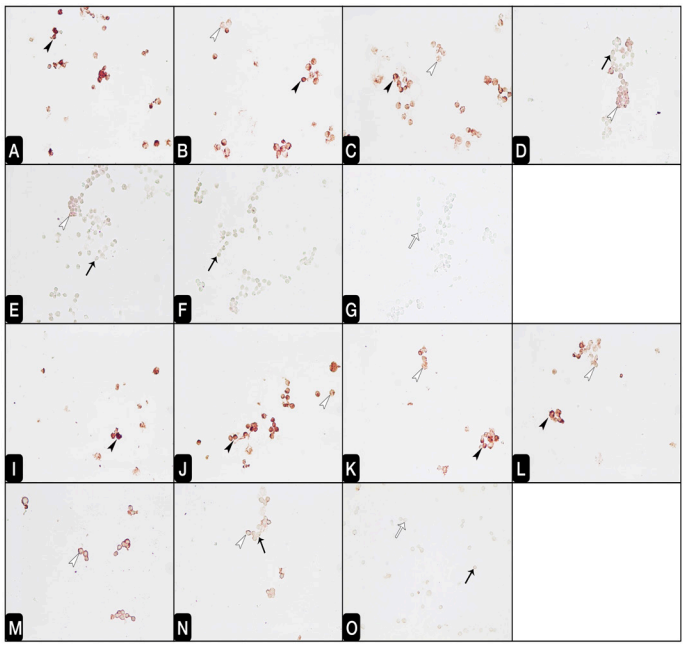

Figure 5. GRP78 immunostaining of MG and MG mediated SDT groups. 3.12 µM MG (**5A**), 1.56 µM MG (**5B**), 0.78 µM MG (**5C**), 0.39 µM MG (**5D**), 0.19 µM MG (**5E**), 0.09 µM MG (**5F**), Control (**5G**). 3.12 µM MG mediated SDT (**5I**), 1.56 µM MG mediated SDT (**5J**), 0.78 µM MG mediated SDT (**5K**), 0.39 µM MG mediated SDT (**5L**), 0.19 µM MG mediated SDT (**5M**), 0.09 µM MG mediated SDT (**5N**), US-Control (**5O**). Strong staining (black arrowhead), medium staining (white arrowhead), weak staining (black arrow), absent staining (white arrow). All photos imagination X400

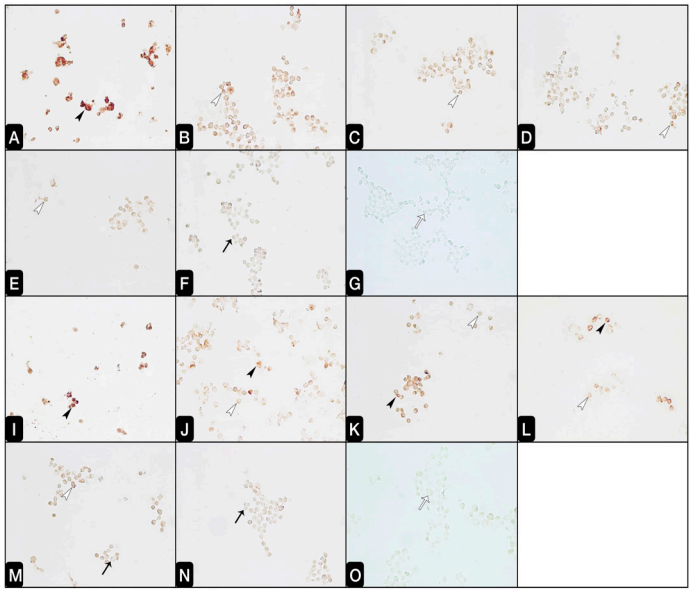

Figure 6. PERK immunostaining of MG and MG mediated SDT groups. 3.12 µM MG (**6A**), 1.56 µM MG (**6B**), 0.78 µM MG (**6C**), 0.39 µM MG (**6D**), 0.19 µM MG (**6E**), 0.09 µM MG (**6F**), US (**6G**). 3.12 µM MG mediated SDT (**6I**), 1.56 µM MG mediated SDT (**6J**), 0.78 µM MG mediated SDT (**6K**), 0.39 µM MG mediated SDT (**6L**), 0.19 µM MG mediated SDT (**6M**), 0.09 µM MG mediated SDT (**6N**), US-Control (**6O**). Strong staining (black arrowhead), medium staining (white arrowhead), weak staining (black arrow), absent staining (white arrow). All photos imagination X400

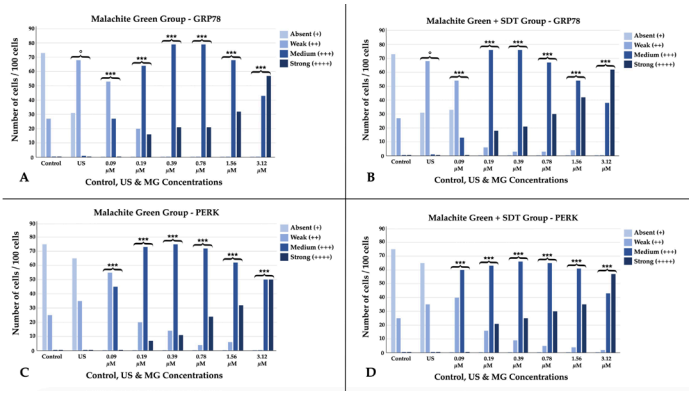

Figure 7. Statistical analysis of GRP78 MG Group (**7A**), GRP78 MG mediated SDT Group (**7B**), Statistical analysis of PERK MG groups (**7C**), PERK MG mediated SDT Group (**7D**). *** & °Significance according to the control groups

DISCUSSION

The combination of sensitizers and ultrasound has been reported to decrease cell viability and induce apoptosis in numerous studies in the literature. Li et al. reported that PpIX-mediated ultrasound treatment in the human leukemia cell line U937 significantly decreased cell viability, caused severe damage in cell morphology, DNA, and mitochondria. Furthermore, they noted that intracellular ROS were also involved in this process (22). In another study, Li et al. demonstrated that Ce6-mediated SDT suppressed the growth of K562 cells, increased intracellular ROS production, and triggered mitochondria and caspase-dependent apoptosis (23). Yumita et al. reported that the ATX-70 sonosensitizer does not cause cell damage when administered alone on HL60 cells, whereas cell damage occurred when activated with ultrasound (24). They showed in another study that apoptosis was induced by cell shrinkage, DNA fragmentation, and caspase 3 activation following fullerene-mediated SDT in HL60 cells (25). In their study on HL60 cells, Su et al. reported that protoporphyrin IX-mediated SDT increased the cytotoxicity of cells, HL-60 cell apoptosis was significantly induced, and intracellular ROS production increased significantly after SDT (26). In another study on U937 cells, they showed that Ce6-mediated SDT decreased cell viability, whereas treatment with Ce6 and US-only did not have an effect on cell viability, and that U937 cell apoptosis significantly increased after Ce6-mediated SDT (27). Trendowski et al. hypothesized that Cytochalasin B, a cytokinesis inhibitor that selectively enlarges and multinucleates malignant cells in U937 cells, may have significant therapeutic potential when combined with SDT (28).

According to our morphological results, the morphological features observed in the groups subjected to MG-mediated SDT include chromatin condensation, apoptotic cells with micronuclei, and cell shrinkage. Although a few cells with micronucleated apoptotic features were observed in groups treated only with MG and US, typical morphological characteristics were evident. These findings were consistent with apoptotic index results. In groups subjected to MG-mediated SDT, the apoptotic index ratio was found to be higher compared to the control, US, and MG-only groups.

Our results indicate that MG caused a significant decrease in cell viability starting at a concentration of 1.56 uM. However, when combined with ultrasound at this concentration, it resulted in the death of approximately half of the cells. At concentrations equal to or below this concentration, SDT is expected to have a beneficial effect. We observed that ultrasound alone did not affect cell viability, yet it caused a significant increase in GRP78 expression. We suggest that GRP78 (15), which plays a crucial role in the initial response to the increase in unfolded proteins and the triggering of the UPR, increases its expression in response to ultrasound-induced stress, This stress is tolerated by the cell without causing cell death, and viability remains unaffected. We conclude that PERK expression increases at advanced stages when ER stress becomes excessive, and naturally, no significant change occurs in its expression when exposed to the tolerable stress caused by ultrasound treatment.

CONCLUSION

All of our results indicate that MG-mediated SDT has a significant cytotoxic effect on cancer cells, accompanied or caused by significant ER stress when administered at the appropriate concentration. Further studies to elucidate the molecular mechanisms underlying this effect would contribute to the establishment of targeted therapies and the development of this alternative treatment more effectively. Our future perspective is to focus on the potential molecular mechanisms of this treatment and to investigate its similar or superior effects in other types of cancer.

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Conflict of interest: *The authors have no conflicts of interest to declare.*

Ethical approval: *Since the methodological structure of the study is a "cell culture study", it does not require ethics committee approval in accordance with the World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research on Humans".*

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MEDICAL RECORDS-International Medical Journal

Research Article

Improving Phlebotomy Practices Through Small-Volume Blood Tubes: A Survey-Based Study

Fatma Demet Arslan[1], Tugba Oncel Van[2], Canan Alpsen[3], Sevgi Cinar[4], Mehmet Koseoglu[1]

[1]İzmir Bakırçay University, Faculty of Medicine, Department of Medical Biochemistry, İzmir,Türkiye
[2]Akçakale Public Hospital, Department of Medical Biochemistry, Şanlıurfa, Türkiye
[3]Çiğli Training and Research Hospital, Quality Directorate, İzmir, Türkiye
[4]Çiğli Training and Research Hospital, Health Care Services, İzmir, Türkiye

Abstract

Aim: The blood collection for laboratory tests has been frequently performed due to evidence-based medicine. We aimed to conduct a survey on phlebotomy among phlebotomists and patients and to reduce unnecessary blood loss by using small-volume blood collection tubes.

Material and Method: A survey among phlebotomists and patients was conducted to gather their opinions. Phlebotomists received training on the importance of the preanalytical process. The blood volume required for laboratory tests was reduced by 33.3%-50.0% in children and adults, and 63.0%-84.0% in newborns. Following this intervention, we investigated its effects on the blood transfusion ratio in the neonatal and adult intensive care unit (NICU and ICU) and the amount of laboratory medical waste generated.

Results: A majority of phlebotomists (91.8%) reported difficulties in drawing blood from newborns, pediatric, oncology, hematology, and geriatric patients. Additionally, 68.9% of phlebotomists and 57.1% of patients expressed an opinion for reduced blood volume. Despite an increase in the number of laboratory tests (28.4%) and samples (15.7%), we observed a 17.8% reduction in the amount of laboratory medical waste. Although the number of patients in NICU increased statistically significant, the increase in transfusion rates was not significant. Although the number of patients in ICU increased, transfusion rates decreased, but neither was found to be statistically significant.

Conclusion: Post-graduation, phlebotomists should be educated regularly about the preanalytical process. Based on the opinions of both phlebotomists and patients, using small-volume tubes in patients with difficult blood collection may increase their satisfaction. Generally, laboratory medical waste may be reduced.

Keywords: Laboratory testing, blood collection tube, iatrogenic anemia, patient satisfaction, personnel satisfaction, laboratory medical waste

INTRODUCTION

Patient Blood Management (PCM) is a multidisciplinary diagnostic and therapeutic approach that focuses on the rational use of blood components, reducing unnecessary blood loss. Minimizing iatrogenic blood loss is an important part of PBM (1). The most common cause of iatrogenic anemia is recurrent blood draws for laboratory tests (2,3). Iatrogenic anemia is frequently seen in intensive care unit (ICU) patients (4). On the second day of admission to the ICU, more than 70% of adults have anemia and more than half of them require a blood transfusion (5). In the neonatal intensive care unit (NICU), the low body weight of patients exacerbates iatrogenic anemia. It is estimated that 15-30% of blood volumes are lost due to blood draw during the first six weeks of an infant's life in the NICU (6).

Minimum pipetted serum/dead volume should be 1-35 µL/100-500 µL, plasma/dead volume 10-50 µL/200 µL or whole blood/dead volume 40-80 µL/200 µL for each test in our laboratory. According to these data, it is thought that blood is drawn more than the required blood volumes to be analyzed. It has been reported that only 9% of blood in standard blood collection tubes is used for laboratory tests (7). Laboratories accredited by the College of American Pathologists recorded blood collection tube size/volume and analytic/discard volume for complete blood cell count and electrolyte panel in ICU patients (8). The 2.76 mL of blood was more than the analytic volume of complete blood cell counts, and 1.75 mL of blood was

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Corresponding Author: Tugba Oncel Van, Akçakale Public Hospital, Department of Medical Biochemistry, Şanlıurfa, Türkiye
E-mail: tubaoncel@hotmail.com

more than the analytic volume of routine electrolytes. The discard volume was 2.84 mL per tube for complete blood cell counts and 2.02 mL per tube for electrolyte panels. Specimen collection tube size was directly associated with overcollections and discard volumes (8).

Recently, microtubes (small-volume tubes) pre-assembled with carrier tubes (13x75 mm) can be included in the autoanalyzer without manual process, and thus laboratory worker safety can be increased.

For this purpose, the phlebotomist was trained on the importance of phlebotomy in preanalytical processes and its contribution to PBM in order to reduce unnecessary blood loss and to popularize small-volume blood collection tubes. A survey of the employees and patients was conducted regarding their experiences and opinions regarding phlebotomy. In accordance with their opinions, the blood collection volume for laboratory tests was reduced in our hospital. The effects of this intervention on the blood transfusion ratio in ICU/NICU and the amount of laboratory medical waste were evaluated.

MATERIAL AND METHOD

Local ethical approval was obtained (the date/number of decision, 29.12.2021/468) and subjects gave informed consent.

Training and Survey of Phlebotomists

In January 2022, phlebotomists (n=388) who draw blood in our hospital were trained by a biochemistry specialist on preanalytical processes, especially blood collection tube volume and filling ratio, and a survey was conducted. The personnel's experience with phlebotomy in the 1st and 2nd questions and the knowledge on phlebotomy in the 3rd and 4th questions were evaluated. In the 5th question, the opinion of the personnel on the blood collection volume was asked.

Survey of Patients

In January 2022, adult and pediatric patients were surveyed on phlebotomy in outpatient and inpatient clinics. The survey was conducted with the help of parents or relatives of pediatric patients. In the 1st, 2nd and 3rd questions, the patient's experience with phlebotomy was evaluated. In the 4th and 5th questions, the opinion of the patient on the blood collection volume was asked. Blood test to be "once a week and once a month" was accepted as "often", "once or twice a year" as "sometimes" and "other" as "rarely".

Reducing the Blood Collection Volume for Phlebotomy

In January 2022, the blood volumes required to be drawn from newborn, pediatric and adult patients for laboratory tests were reduced at a certain ratio in our hospital (Table 1). Sedimentation, blood type test, crossmatch test and blood gas analysis were excluded. Venous blood samples were taken into 1.0 mL (MiniCollect® Complete, 9NC Sodium Citrate 3.2%, 13x75 mm, GBO GmbH, Austria), 2.5 and 5.0 mL tubes (Vacusera®, Serum Gel and Clot Activator, 13x75 mm, Diseara, İzmir, Türkiye). Capillary blood samples were taken into 0.8 mL (MiniCollect® Complete, CAT Serum Seperator, 13x75 mm, GBO GmbH, Austria) and 0.5 mL (MiniCollect® Complete, K2E/K2EDTA, 13x75 mm, GBO GmbH, Austria) tubes.

Table 1. Blood volumes to be drawn in neonatal, pediatric and adult patients

Laboratory tests	2021	2022	Reduced rate of blood volume (%)
Blood volumes to be drawn in pediatric and adult patients			
Biochemical tests	5.0 mL	2.5 mL	50.0
Hormonal tests	5.0 mL	2.5 mL	50.0
Coagulation tests	2.7 mL	1.8 mL	33.3
Serological tests	5.0 mL	2.5 mL	50.0
Blood volumes to be drawn in neonatal patients			
Biochemical tests	5.0 mL	0.8 mL	84.0
Hormonal tests	5.0 mL	0.8 mL	84.0
Coagulation tests	2.7 mL	1.0 mL	63.0
Serological tests	5.0 mL	0.8 mL	84.0
Complete blood count	2.0 mL	0.5 mL	75.0
Hemoglobin A1c	2.0 mL	0.5 mL	75.0

The quality indicators through twelve months in 2021 and 2022 years [the number of laboratory tests and samples, the amount of laboratory medical waste, the number of patients hospitalized in NICU/ICU, the number of erythrocyte transfusions (ET) (unit) per patient and the ratio of patients transfused in NICU/ICU] were obtained from the hospital information system. Laboratory medical wastes included blood collection tubes, gloves, cultures, urine containers and cuvettes in microbiology and biochemistry laboratory.

Statistical Analysis

Minitab software (version 15.1.20.0; Minitab, Inc., State College, PA) was used for statistical analysis. The mean and standard deviation or median and interquartile range of data for quality indicators in the years 2021 and 2022 were calculated. The percentage change between 2021 and 2022 was calculated using the formula: 100*(average of 2022 - average of 2021) / average of 2021. The difference in averages over twelve months between 2021 and 2022

years was analyzed by the Wilcoxon test. In addition, the relationship between the answers to the survey was analyzed by the Chi-Square test. p<0.05 was considered statistically significant.

RESULTS

Evaluation of the Survey Conducted to Phlebotomist

62.9% (n=244) of phlebotomists who trained participated in the survey. Demographic characteristics and questions/answers of the phlebotomist are presented in Table 2. Phlebotomists were mostly nurses (68.0%) and female (89.8%), and had a license degree (86.9%). The mean age of phlebotomists was 35.4±9.5.

91.8% of the phlebotomists had difficulty drawing blood from newborn, pediatric, oncology, hematology and geriatric patients (Question 1). Only 30.8% of personnel who had difficulty drawing blood from these patients and 45.0% of those who had no difficulty were able to fully fill the blood collection tubes (p=0.060). 70.1% of personnel who had difficulty drawing blood and 55.5% of those who had no difficulty preferred to reduce the amount or volume of blood collected (p=0.156).

88.9% of the phlebotomists draw the necessary amount of blood into blood collection tubes with gel (Question 3). In addition, 77.5% of the personnel could fill the necessary blood in the collection tubes including anticoagulants (Question 4). The personnel want to reduce the amount/volume of blood was 68.9% (Question 5).

Table 2. Demographic characteristics and questions/answers in survey of health personnel (n=244)

Demographic characteristics		
Age, mean±standard deviation	**35.4±9.5**	**n (%)**
Gender, n (%)	Female	219 (89.8)
	Male	25 (10.2)
Position, n (%)	Nurse	166 (68.0)
	Trainee Nurse	33 (13.5)
	Midwife	31 (12.7)
	Laboratory technician	14 (5.7)
Workplace, n (%)	Adult inpatient service	110 (45.1)
	Adult intensive care	54 (22.1)
	Emergency room	23 (9.4)
	Pediatric outpatient service	22 (9.0)
	Adult outpatient service	21 (8.6)
	Blood collection unit	14 (5.7)
Education, n (%)	License degree	212 (86.9)
	Associate degree	23 (9.4)
	High school	9 (3.7)
Questions	**Answers**	**n (%)**
1. Which patients do you have difficulty drawing blood?	Newborn, pediatric, oncology, haematology, or geriatric patients	224 (91.8)
	None	20 (8.2)
2. Can you fill the blood collection tubes completely if you have difficulty drawing blood?	Yes	78 (32.0)
	Partially	151 (61.9)
	No	15 (6.1)
3. Is it necessary to draw the recommended amount of blood in blood collection tubes with gel?	Yes	217 (88.9)
	No idea	20 (8.2)
	No	7 (2.9)
4. Do you pay attention to the drawn blood amount into blood tubes with anticoagulants?	I pay attention	189 (77.5)
	I ignore it if I have difficulty drawing blood in some patients	54 (22.1)
	I don't pay attention	1 (4.0)
5. Would you like the amount/volume of blood drawn into each tube to be reduced?	Yes	168 (68.9)
	It doesn't matter	46 (18.9)
	No	30 (12.3)

Evaluation of the Survey Conducted to the Patients

Demographic characteristics and questions/answers of the patients (n=140) are presented in Table 3. 34.8% of patients with chronic diseases and 13.8% of those without chronic diseases had blood tests "often" (p<0.05). 26.6% of adult patients have frequent blood testing, while only 15.8% of pediatric patients stated that they do (p<0.05) (Question 1). The ratio of difficult blood collection in adult patients was 34.1%, while this ratio (55.2%) was higher in children (p<0.05) (Question 3). The blood drawing in adult inpatients was more difficult than in outpatients (48.0% vs. 12.8%, p<0.05) and in pediatric inpatients more difficult than outpatients (16.0% vs. 53.8%, p<0.05) (Question 3). The ratio of pediatric inpatients, who stated that the blood volume is excessive, had higher compared to those of outpatients (42.3% vs. 16.0%, p<0.05). Similarly, the ratio of adult inpatients, who stated that the blood volume is excessive, had higher compared to those of outpatients (32.0 vs. 10.3%, p<0.05) (Question 4).

37.3% of the patients who do not think the drawing blood volume was excessive and 90.3% of the patients who think it was excessive, wanted to reduce blood volume (p<0.001) (Question 5).

Table 3. Demographic characteristics and survey of patients

Demographic characteristics		**n (%)**
Clinics	Pediatric blood collection unit	50 (35.7)
	Child inpatient service unit	26 (18.6)
	Adult blood collection unit	39 (27.9)
	Adult inpatient service unit	25 (17.9)
Age group	Pediatric	76 (54.3)
	Adult	64 (45.7)
Age, mean±standard deviation	Pediatric	6.6±5.2
	Adult	55.6±17.6
Gender	Female	67 (47.9)
	Male	73 (52.1)
Chronic disease	Yes	46 (32.9)
	No	94 (67.1)
Questions	**Answers**	**n (%)**
1. How often do you have blood tests?	Often	29 (20.7)
	Sometimes	83 (59.3)
	Rarely	28 (20.0)
2. How many blood collection tubes are used when you have a blood test?	1-2	55 (39.3)
	3-4	70 (50.0)
	5 or more	7 (5.0)
	I don't know	8 (5.7)
3. Has the health personnel difficulty drawn blood from you?	Yes	39 (27.9)
	Sometimes	25 (17.9)
	No	76 (54.3)
4. Do you think the amount/volume of blood drawn from you was excessive?	Yes	31 (22.1)
	Partially	26 (18.6)
	No	83 (59.3)
5. If less blood was needed for lab tests, would you like to reduce the amount/volume of blood drawn?	Yes	80 (57.1)
	Doesn't matter	35 (29.3)
	No	17 (13.6)

Effect on Quality Indicators of Reduced the Blood Collection Volume

Compared to the pre-intervention, the blood volume needed from each patient for laboratory tests was reduced by 33.3%-50.0% in pediatric and adult patients, and by 63.0%-84.0% in newborn patients.

The quality indicators of hospital for 2021 and 2022 years are shown in Table 4. Although there was an increase in the number of samples (15.7%) and tests (28.4%) in the laboratory in 2022, a decrease (17.8%) was found in the amount of laboratory medical waste. However, the cost of laboratory medical waste increased by 47.6%. While there was an increase in the number of patients hospitalized in the NICU, no significant increase was found in the ICU. No statistically significant change was observed in the amount of ET (unit) per patient and the ratio of patients transfused in the ICU/NICU.

Table 4. Quality indicators of hospital in 2021 and 2022 years

Indicators	2021 Mean±SD Median (IQ)	2022 Mean±SD Median (IQ)	Change (%)	P value
The number of laboratory tests (n)	363,354±62,036 384,200 (307,572-412,640)	466,514±60,119 456,722 (424,458-517,920)	28.4	**0.002**
The number of laboratory samples (n)	133,118±19,732 139,698 (116,440-147,124)	154,013±15,645 154,294 (143,823-162,610)	15.7	**0.004**
The amount of laboratory waste (kilogram)	1,903±164 1,933 (1,779-2,014)	1,564±284 1,531 (1,350-1,741)	-17.8	**0.005**
The cost of laboratory waste (TL)	5,899±509 5,992 (5,514-6,247)	8,710±1,773 8,647 (7,102-9,908)	47.6	**0.002**
The number of patients in ICU (n)	267±21 265 (256-274)	272±27 283 (246-292)	1.87	0.505
The number of patients in NICU (n)	49.8±10.1 45.5 (44.0-57.2)	63.3±7.4 66.0 (55.5-70.0)	27.1	**0.021**
The number of erythrocyte transfusions (ET) per patient (unit) in ICU	2.80±0.41 2.72 (2.46-3.09)	2.62±0.32 2.61 (2.36-2.87)	-7.42	0.530
The number of erythrocyte transfusions (ET) per patient (unit) in NICU	0.92±0.91 1.00 (0-1.92)	1.71±1.25 1.50 (1.00-2.36)	85.9	0.194
The ratio of patients transfused in ICU (%)	23.4±3.2 22.0 (21.3-26.5)	22.7±4.6 23.2 (19.0-26.2)	-2.99	0.754
The ratio of patients transfused in NICU (%)	2.32±2.59 1.85 (0-4.38)	3.12±1.39 3.21 (2.87-3.69)	34.5	0.388

ET: erythrocyte transfusion, ICU: adult intensive care unit, IQ: interquartile, NICU: neonatal intensive care unit, SD: standard deviation, TL: Turkish lira

DISCUSSION

To the best of our knowledge, our study is the only research in which phlebotomists and patients were surveyed about blood collection and tube volume. According to the survey of phlebotomists, it was determined that most of them had difficulty drawing blood in newborn, pediatric, oncology, hematology or geriatric patients. Based on the answers to the 3rd and 4th questions, it was concluded that the majority of personnel do not lack knowledge. However, while most phlebotomists demonstrated attention and sufficient knowledge regarding tube blood-filling volume, it doesn't necessarily guarantee their ability to consistently fill the tubes to the desired blood volume. When compared to personnel without difficulty, those who had difficulty drawing blood had a lower rate of fully filling the blood collection tubes and a higher rate of wanting a reduction in the blood volume. Therefore, small-volume tubes can enhance personnel satisfaction when working with neonatal, pediatric, oncology, hematology, or geriatric patients.

According to the survey conducted on our patients, blood draws were frequently performed in adults and patients with chronic diseases. It was stated that blood collection was more difficult in pediatric patients. Both adult and pediatric inpatients compared to outpatients stated that blood was drawn with more difficulty and excessive volume.

59.3% of the patients did not find the drawn blood volume excessive, and 54.3% of the patients did not experience difficulty during blood draws. The patients' responses made the researchers consider that these patients might not have clear information about the effects of phlebotomy. Patients readily accepted the amount of blood to be drawn for laboratory tests without a doubt. However, most patients with a high awareness of blood collection volume preferred a reduction in blood volume compared to those who were not as aware (90.3% vs. 37.3%, respectively).

We aimed to improve preanalytical processes by training personnel in our hospital and to contribute to PBM and laboratory waste management by using small-volume blood collection tubes based on the opinions of both patients and personnel. Reduced blood volumes (specific percentages mentioned) were found to be sufficient for various types of tests, and the results could be reliably produced with less blood volume without requiring changes in laboratory processes. Adequate serum and plasma levels were available for additional tests due to the request of clinicians or repeated tests due to various reasons, such as analytical errors or device malfunctions. Much of the blood drawn for laboratory tests is discarded. Sanchez-Giron et al. found that 91% of blood in standard-volume tubes remained and 74% of blood in small-volume tubes remained when the analysis was completed (7).

About 25.0% of total medical waste in hospitals (including cafeterias, operating rooms, laboratories, emergency rooms, ambulance service and facilities) and 27.8%

of total disposable cost have been produced from laboratories (9). It was stated that most of the plastic wastes in hospital laboratories were blood collection tubes (70.0%) and gloves/lab wares (20.0%). Thus, in our study, other laboratory medical wastes such as cultures, gloves, urine containers and cuvettes were regretted. Although the number of laboratory tests (28.4%) and samples (15.7%) increased, it was determined that the amount of medical waste significantly decreased (17.8%). Although the average unit cost of medical waste in our laboratory increased by 80.6%, the increase in monthly cost was less (47.6%). Additionally, the phlebotomy tubes used can cause huge environmental impacts (10). All the plastic waste from phlebotomy tubes can't be recycled and must be incinerated, causing additional emissions of greenhouse gases and pollution (11). The use of one-size smaller tubes for pediatric and adult patients may reduce the amount of hazardous waste, and simplify the purchase and storage of tubes (8).

Systematic research conducted in 2022 showed that the use of pediatric-sized blood collection tubes in adult intensive care patients can significantly reduce the daily blood drawn (12). Using small-volume tubes reduced blood loss by 73%, the risk of severe anemia (hemoglobin <7.0 g/dL) by more than half, and the unit of packed red blood cells transfused per patient by 27% (7,13,14). Foulke et al. found that blood loss was reduced by 33%, and the percentage of patients who had at least one transfusion was reduced by 10% after using small-volumes (pediatric phlebotomy tubes, reduced syringe volumes) for the laboratory tests (15). It has been stated that the daily reported losses from blood sampling may contribute to post-operative hemoglobin fall resulting in blood transfusion (16). Matzek et al. have stated that hemoglobin concentrations declined with the intensity of phlebotomy during hospitalization in the ICU and each 100 mL of phlebotomy volume during hospitalization was associated with an increase of 1.15 multiplied in red blood cell units transfused (17).

Despite a statistically significant increase in the number of patients hospitalized in the NICU, there was no statistically significant increase in the ratio of transfused patients and the number of ET (unit) per patient. Before the use of small-blood volume tubes, blood gas analysis by capillary tube or non-invasive measurements has already been preferred for parameters such as glucose, creatinine, electrolytes, bilirubin, hemoglobin, and lactate. Although the number of patients hospitalized in the ICU increased, the ratio of transfused patients and the number of ET (unit) per patient decreased. However, these changes were not statistically significant. As a limitation of our study, transfusion indications for intensive care patients were not considered. We did not have information on when the transfusions occurred, and the frequency and amount of phlebotomy on days following the patient's hospitalization. It may be possible to reveal its effect on transfusion rates with further studies that include clinical diagnosis and follow-up information of the patients.

Small-volume blood collection tubes, point-of-care tests, closed sampling systems, capillary samples, and noninvasive analysis methods can be preferred to improve preanalytical processes and reduce iatrogenic anemia caused by phlebotomy. Integrated systems that can run multiple tests on a single blood sample can be used. Training can be given to phlebotomists and clinicians on reducing test requests and on blood collection techniques that reduce blood loss. Local governments can develop hospital quality indicators and policies on practices that reduce blood loss.

CONCLUSION

As a result, to improve preanalytical processes and reduce iatrogenic anemia and the amount/cost of laboratory waste, small-volume blood collection tubes can be preferred. As an alternative to small-volume blood collection tubes, point-of-care tests closed blood sampling systems, capillary samples, noninvasive analysis methods, and integrated systems that can run multiple tests on a single blood sample can be used. Training can be given to phlebotomists and clinicians on reducing test requests and on blood collection techniques that reduce blood loss. Local governments can develop hospital quality indicators and policies on practices that reduce blood loss.

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Research Article

Quality Assessment of YouTube Videos on Avascular Necrosis of the Femoral Head: An Analysis of Content, Reliability, and Educational Value

Ali Ihsan Kilic[1], Gokhan Sayer[2], Mehmet Akdemir[3]

[1]İzmir Bakırçay University, Faculty of Medicine, Department of Orthopaedics and Traumatology, İzmir, Türkiye
[2]Bursa City Hospital, Department of Orthopaedics and Traumatology, Bursa, Türkiye
[3]Ekol Hospital, Department of Orthopaedics and Traumatology, İzmir, Türkiye

Abstract

Aim: Videos related to avascular necrosis of the femoral head are no exception, and so the aim of this study is to evaluate the educational quality of YouTube videos on this topic.

Material and Method: A standardized video search was performed on YouTube using the terms "avascular necrosis of the hip", "osteonecrosis of the hip" and "avascular necrosis of the femoral head". The top 50 videos were then analyzed, and the characteristics and content of the videos were recorded. The Journal of American Medical Association criteria, The DISCERN score, The Global Quality Score and the new YouTube 'Avascular Necrosis of the Femoral Head Score" were all used to assess the reliability and accuracy of the videos.

Results: The median video duration of the 50 videos was 10.85±19.17 minutes. The median number of views was 10,866 (range 221 to 278,174). According to the video content, 60% of the videos contained information about the disease, 10% were about patient experience, and the remaining 30% related to surgical technique or approach. Physicians were the primary uploader on YouTube for this topic. The rate of low-quality videos was determined as following according to the different evaluative systems: 68% according to the newly defined YouTube Avascular Necrosis of the Femoral Head Score, 60% according to the DISCERN score, and 56% according to The Global Quality Score. The Journal of the American Medical Association, The Global Quality Score, and DISCERN score were significantly correlated with video duration, while the New YouTube Avascular Necrosis of the Femoral Head Score was significantly correlated with video duration, time since upload, number of views, and like rate.

Conclusion: Most of the popular YouTube videos about avascular necrosis are of a low quality.

Keywords: YouTube, avascular necrosis, femoral head, social media

INTRODUCTION

Avascular necrosis (AVN) of the hip is a major orthopaedic problem which is characterized by tissue death caused by reduced or complete cessation of blood flow to the femoral head (1). This condition usually affects young and active individuals and can lead to possibly serious problems of restricted mobility, severe pain, and loss of function (1,2).

Although the pathogenesis of AVN of the hip is not fully understood, several risk factors, such as corticosteroid use, alcohol abuse, trauma, rheumatologic diseases, as well as some genetic factors, are thought to play a role in the occurrence of this condition and the development of AVN. However, in many cases, the exact cause cannot be determined and the development of AVN can be said to have a complex etiology (2,3).

Internet and social media platforms have provided access to an extensive range of information (4). One valuable source is the popular video-based platform, YouTube, which has more than one billion users, representing one third of all Internet users (5). Patients have increasingly turned to YouTube in recent years to learn about their medical conditions and treatment options (6).

However, many of the health-related posts on YouTube have not been expertly reviewed and do not contain author or source information. This means that because the platform is open access, some videos do not have a

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Corresponding Author: Ali Ihsan Kilic, İzmir Bakırçay University, Faculty of Medicine, Department of Orthopaedics and Traumatology, İzmir, Türkiye
E-mail: ali.ihsan.88@gmail.com

scientific basis or contain false information. Users can therefore experience real difficulty in accessing accurate information, especially on health-related issues (7-10).

Although there are studies in the literature which investigate the quality of videos on YouTube on topics such as Anterior cruciate ligament injury (11), hallux valgus (12), and hip arthroscopy (13), no study has been located which evaluates the quality of videos related to avascular necrosis of the femoral head. The aim of this study is therefore to fulfil this perceived need by evaluating the quality of YouTube videos on femoral head AVN. The working hypothesis is that there are low quality YouTube videos on this topic.

MATERIAL AND METHOD

Before conducting the research, approval was obtained from the local ethics committee (IRB No: 1209). On December 1, 2022, a standard video search was performed using Google Chrome, but without a personal YouTube account, using the terms "avascular necrosis of the hip", "osteonecrosis of the hip" and "avascular necrosis of the femoral head". Videos in English with a primary topic of avascular necrosis of the femoral head were included in the search, while repetitive videos that contained only audio or video, videos with a language other than English, and videos not related to AVN of the femoral head, were excluded. Only one of the repetitive videos was included in the study, and no restriction was placed on video duration. After the exclusion criteria had been applied, the 50 most popular videos were analyzed.

All of the videos in the study were listed by title, upload time, video duration, time since video upload, number of likes, number of dislikes, number of views, video source, content type, and view ratio (the number of views/times since video upload). The videos' origin and uploaders were classified into distinct categories; 1: academic (affiliated with research institutions, universities, or colleges), 2: physician, 3: other healthcare professionals (excluding licensed physicians), 4: exercise trainers, 5: medical sources (content or animations from healthcare websites), 6: patients, and 7: commercially produced videos. The content was categorized based on its focus; 1: exercise training, 2: providing information about diseases, 3: sharing patient experiences, 4: detailing surgical techniques or approaches, 5: discussing non-surgical management options, and 6: advertising.

Criteria listed in The Journal of the American Medical Association (JAMA) were used for the reliability and accuracy of the videos (14). The DISCERN score was used to assess educational quality (10,12,14), and The Global Quality Score (GQS) (15,16) was employed to obtain a newly defined YouTube femoral head avascular necrosis score (FHAVNS). This value was produced with consideration of the current YouTube scores previously available in the literature (12,15-17). In the 14-parameter scoring system, video quality was categorized as being (0-4) poor, (5-8) fair, (8-11) good, or (12-14) excellent (Table 1). The scoring systems used for video reliability and quality were evaluated by two different observers and the average was recorded.

Table 1. Femoral Head Avascular Necrosis Score (FHAVNS) parameters

Patient profile	Point
Disease description	1
Symptoms	1
Epidemiology/affected patient group	1
Risk factors	1
Pathophysiology	1
Physician assessment	
Examination findings	1
Differential diagnosis	1
X-ray findings	1
MR findings	1
Staging	1
Treatment	
Conservative treatment	1
Surgical treatment	1
Complications of surgical treatment	1
Prognosis	1
Total	14 points

Statistical Analysis

The data IBM SPSS 22 program was used for evaluation, with mean, median values and categorical data, percentage (%) and frequency values all being considered. Conformity of the numerical data to normal distribution was ensured by the Shapiro-Wilk test, while the Spearman test was used to determine correlation in the numerical data. A correlation coefficient ranges from -1 to 1, with -1 suggesting the strongest opposite relationship, 0 indicating no relationship, and 1 signifying the strongest direct relationship.

The 'interclass correlation coefficient' (ICC) value for agreement between two observers was used to assess video quality (95% CI (confident interval)). The strength of an ICC is typically measured on a scale of 0 to 1, where 0 indicates no agreement and 1 indicates perfect agreement.

RESULTS

The mean video duration was 10.85±19.17 minutes (range, 0.32-99.52 minutes). The median number of views was 10,866 (range 221-278,174), the median view rate was 7.81 (range 0.10-1618.55), the median time since upload was 1141 (range 20-4123) days, the median number of likes was 78.50 (range 1-1300), the median number of dislikes was 3 (range 0-54) and the median like rate was 96.80 (range 87.17-100). The JAMA median value was 2 (range 1-2.5), the DISCERN median value was 40.5 (range 16-73.5), the GQS median value was 2.25 (range 1-4.5) and the FBAVNS median value was 6 (range 1-13.5).

When classified according to video sources, 3 (6%) academic, 23 (46%) physician, 22 (44%) medical, 1 (2%) patient, 1 (2%) commercial videos were determined. When classified according to video content, there were 30 (60%) information about the disease, 5 (10%) about patient experience, and 15 (30%) about surgical technique or

approach videos.

Inter-observer agreement was assessed as being good for JAMA and excellent for GQS, DISCERN score and FHAVNS (Table 2). According to FHAVNS, 14 (28%) of the videos were very poor, 20 (40%) were fair, 9 (18%) were good, and 7 (14%) were excellent; according to DISCERN score 11(22%) were very poor, 19 (38%) were poor, 13 (26%) were fair, 6 (12%) were good, and 2 (4%) were excellent. According to GQS, 56% of the videos scored 2 or less and were evaluated as being low quality. There were significant correlations between GQS and JAMA, DISCERN and JAMA, DISCERN and GQS, FHAVNS and JAMA, FHAVNS and DISCERN, and FHAVNS and GQS (Table 3).

Scores for JAMA, GQS, and DISCERN criteria were significantly correlated with the duration of the videos. FHAVNS score, on the other hand, exhibited significant correlations with multiple factors: video duration, time since upload, rate of likes, and number of views (Table 4).

Table 2. Inter-Observer Agreement for JAMA, GQS, DISCERN Score, and FHAVNS

	Observer	Mean	SD	Median	Min.	Max.	ICC (95% CI)
JAMA	1	1.92	0.488	2.00	1	3	0.856 (0.739-0.919)
	2	1.82	0.388	2.00	1	2	
GQS	1	2.54	1.054	2.50	1	5	0.966 (0.932-0.982)
	2	2.40	0.990	2.00	1	4	
DISCERN	1	40.92	15.890	41.00	16	75	0.984 (0.877-0.995)
	2	38.96	15.191	39.50	16	72	
FHAVNS	1	6.80	3.574	6.00	1	14	0.996 (0.992-0.997)
	2	6.78	3.448	6.00	1	13	

JAMA: The Journal of the American Medical Association benchmark criteria, GQS: The Global Quality Score, FHAVNS: Femoral Head Avascular Necrosis Score, In terms of interclass correlation coefficient; an ICC (95% CI) of <0.500 is considered poor, 0.500 to 0.750 moderate, 0.750 to 0.900 good, and >0.900 as excellent

Table 3. Correlation Analysis of GQS, JAMA, DISCERN, and FHAVNS

Criteria		JAMA	GQS	DISCERN
GQS	r	0.480		
	p	**<0.001**		
DISCERN	r	0.535	0.947	
	p	**<0.001**	**<0.001**	
FHAVNS	r	0.369	0.868	0.905
	p	**0.008**	**<0.001**	**<0.001**

JAMA: The Journal of the American Medical Association benchmark criteria, GQS: The Global Quality Score, FHAVNS: Femoral Head Avascular Necrosis Score, In the Spearman correlation test, r values of 0.00-0.19 indicates very weak correlation, 0.20-0.39 weak correlation, 0.40-0.59 moderate correlation, 0.60-0.79 strong correlation, and 0.80-1.0 very strong correlation. Negative values indicate reverse correlation

Table 4. Correlation of JAMA, GQS, DISCERN scores with video duration and FHAVNS with video duration, time since upload, rate of likes, and number of views

		JAMA	GQS	DISCERN	FHAVNS
Length of video	r	0.287	0.516	0.565	0.466
	p	**0.043**	**<0.001**	**<0.001**	**0.001**
Number of views	r	0.136	-0.148	-0.147	-0.422
	p	0.345	0.305	0.308	**0.002**
Time elapsed after loading	r	-0.33	-0.233	-0.261	-0.380
	p	0.822	0.103	0.067	**0.006**
Viewing rate	r	0.171	-0.035	-0.030	-0.235
	p	0.235	0.811	0.835	0.101
Number of dislikes	r	0.198	-0.210	-0.054	-0.267
	p	0.168	0.884	0.711	0.061
Rate of likes	r	-0.101	0.134	0.160	0.293
	p	0.486	0.352	0.267	**0.039**

JAMA: The Journal of the American Medical Association benchmark criteria, GQS: The Global Quality Score, FHAVNS: Femoral Head Avascular Necrosis Score, In the Spearman correlation test, r values 0.00-0.19 indicates very weak correlation, 0.20-0.39 weak correlation, 0.40-0.59 moderate correlation, 0.60-0.79 strong correlation, and 0.80-1.0 very strong correlation correlation. Negative values indicate reverse correlation

DISCUSSION

The most important finding of this study is that videos about femoral head AVN on YouTube are, according to all of the scoring systems, of a low quality and are therefore of concern in terms of credibility. This result is consistent with other studies in the literature (14,18-20). One of the reasons for this poor result may be that only 3% of the located videos are academic. It is therefore suggested that academic publications should not only be produced as a result of meetings and congresses, but that academic staff should provide more video content on YouTube and similar platforms. This would ensure that patients were provided with better quality and safer information.

Some studies in the literature found that videos containing surgical methods are not preferred by patients and have low educational value (11,21). Yüce A. et al. observed a high rate of surgical method videos in their study and concluded that videos containing surgical methods may not be of interest to patients since the target audience of the videos is orthopedic surgeons, not patients (14). In this study, 30% of the videos described surgical method. Since such videos are difficult for patients to understand, and the content of these videos is usually concerned with only one surgical method, such videos may not provide quality information about the disease and other treatment options to patients and may therefore be considered of a low quality.

Various studies in the literature employ a wide range of scoring systems designed to evaluate video quality (11,12,22,23). The system developed and used in this study for this purpose was FHAVNS. Significant correlation with other scoring systems, and excellent agreement between observers, indicate that this scoring system is less influenced by personal interpretations in the assessment of video quality. In future studies, FHAVNS may help researchers to create their own scoring systems.

Celik H. et al. showed that video duration was positively correlated with DISCERN, JAMA score and their own scoring system RCSS (rotator cuff specific score) (19). Mert A. et al. also demonstrated a positive correlation with video duration in JAMA, DISCERN and GQS (24). In this study, in line with the literature, video duration was positively correlated with JAMA score, GQS, DISCERN and FHAVNS. In addition, FHAVNS was negatively correlated with the time since the video was uploaded and the number of views, and positively correlated with the like rate. This may be due to the fact that more recent videos contain more comprehensive information than older videos. Specific video characteristics of the nature of the video content might be more time-sensitive and experience a sharp decline in views and engagement after their initial popularity wanes. In addition, the fact that these videos have been on YouTube for a shorter time than other videos may explain the negative correlation between the number of views and their quality. It is also expected that the like rate increases with the quality of the video.

Limitations of this study include the fact that only English-language videos were evaluated and only videos available on the YouTube platform were considered. It should also be noted that the scoring systems used to evaluate the videos has a subjective component. However, this particular limitation is considered to have a minimal effect due to the high inter-observer agreement.

CONCLUSION

It was concluded that the videos on YouTube about FHAVN are of low quality. Dissemination of incomplete information via such videos may cause confusion among FHAVN patients. Moreover, the incomplete information that patients will learn from these sites may have a negative effect on the trust relationship between the patient and the physician, as well as being a possible cause of disruption in the treatment process. It may therefore be an option in the preparation of video content to obtain consultancy services from academic staff. This should lead to the creation of more comprehensive videos, instead of merely descriptions of surgical techniques. Patients should also be directed to such higher quality content.

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Research Article

Inhibition of Autophagy on Melatonin-Induced Apoptosis in MCF-7 and MDA-MB-231 Cell Lines

Gozde Ozge Onder[1,2], Adnan Bayram[3], Oguz Galip Yildiz[4], Arzu Yay[1,2]

[1]Erciyes University, Faculty of Medicine, Department of Histology and Embryology, Kayseri, Türkiye
[2]Erciyes University, Genome and Stem Cell Center, Kayseri, Türkiye
[3]Erciyes University, Faculty of Medicine, Department of Anesthesiology and Reanimation, Kayseri, Türkiye
[4]Erciyes University, Faculty of Medicine, Department of Radiation Oncology, Kayseri, Türkiye

Abstract

Aim: We looked at the connection between autophagy and apoptosis after our prior research indicated that melatonin could cause MCF-7 and MDA-MB-231 cells in the present study.
Material and Method: In order to investigate the autophagy inhibition's effect on the melatonin-induced BC cells' apoptosis, melatonin and/or 3-methyladenine (3-MA, autophagy inhibitor) have been utilized. Melatonin was applied to the cells following a 5-mM 3-MA pre-cultivation. Then, apoptosis was detected by the TUNEL method. The technique for double immunofluorescence labeling was used to identify the molecular alterations in Bax/Bcl-2 expression. To evaluate the cell viability, the MTT test was used.
Results: When an autophagy inhibitor, 3-MA, and melatonin treatment were co-administered in MCF-7 cells, apoptosis was decreased, compared to melatonin treatment alone, but it was not significant. In addition, 3-MA application downregulated Bax expression compared with melatonin alone treatment. Combined therapy markedly elevated apoptosis and significantly up-regulated Bax protein in MDA-MB-231 cells.
Conclusion: Taken together, in MCF-7 cells, autophagy's inhibition contributes to the downregulation of apoptosis, whereas increased apoptosis is seen in MDA-MB-231 cells. Inhibiting autophagy in these cells treated with melatonin could serve as a self-defense mechanism, and This might be a good strategy for breast cancer adjuvant treatment.

Keywords: Autophagy, breast cancer, melatonin, 3-MA

INTRODUCTION

Breast cancer (BC) has been categorized in two ways: as expressed carcinoma that expresses the receptor 2 for human epidermal growth factor and as BC that expresses the estrogen receptor (ER) or progesterone receptor (PR), respectively (1,2). Triple-negative BC is still challenging to treat due to its aggressive characteristics and few therapeutic choices (3). The most aggressive type of BC is called triple-negative BC, and it is more likely to spread among women and is challenging to treat. Few therapy options and a poor prognosis for triple-negative BC patients make it urgently necessary to develop novel therapeutics (4). In the search for treatments for BC, the focus is on testing curative agents for their effectiveness against cancer cell proliferation using diversified cell lines. MCF-7 is a BC cell line that is ER-positive and PR-positive (5). MDA-MB-231 cells are negative for ER, PR, and HER2, so they are called triple-negative BC cells. Both MDA-MB-231 and MCF-7 cells have epithelial characteristics. They came from carcinoma cells in the mammary gland. MCF-7 is BC's luminal type histologically, while MDA-MB-231 is a basal type. MDA-MB-231 cells are characterized by a high rate of metastasis, a poor prognosis, a propensity for relapse, and an insensitivity to therapy (6-10).

Serotonin is used for the production of melatonin, which is the pineal gland's primary hormone. Also, a number of peripheral tissues and immune cells produce melatonin (11). It possesses cytoskeleton modulatory and oncostatic capabilities, capable of reducing tumor growth and cancer cells' invasiveness (12). The mechanisms underlying melatonin's anticancer actions have been thought to be its antioxidant effects (13,14) and triggering apoptotic pathways in cancer cells. (15). The morphology

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Corresponding Author: Gozde Ozge Onder, Erciyes University, Faculty of Medicine, Department of Histology and Embryology, Kayseri, Türkiye, Erciyes University, Genome and Stem Cell Center, Kayseri, Türkiye
E-mail: gozdeozgekorkmaz@hotmail.com

of cells can be used to distinguish between two main kinds of cell death: autophagy- and apoptosis-cell death. Under normal physiological conditions, apoptosis acts as a targeted removal of undesirable or dangerous cells (16,17). Autophagy degrades damaged organelles and/or proteins and recycles them to maintain cellular components' quality. It is an evolutionarily preserved catabolic process (18). Autophagy's role in cancer is arguable because, depending on the tumor's form and stage, it may be able to operate as either a tumor promoter or a tumor suppressor (19).

3-MA is one of the autophagy inhibitors. It has been noted to suppress the event of PI3-kinase and stop autophagic vacuoles from forming (20). The studies showed that treatment with specific autophagy inhibitors, such as 3-MA, may increase the apoptotic effectiveness of chemotherapeutic drugs in lung cancer (21), prostate (22), colon (23), HeLa (24), and BC cells (25). Because of this, the therapeutic potential of possible drug candidates may be increased by identifying their apoptotic molecular targets as well as their autophagic responses. Therefore, this study's purpose was to determine how autophagy inhibition affects melatonin-induced apoptosis in both BC cell lines.

MATERIAL AND METHOD

Cell Culture

The Genome and Stem Cell Center in Kayseri is where the BC cells were bought. At 37°C in humidified air with 5% CO_2, BC cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Sigma Aldrich, USA), supplemented with 1% L-glutamine (Thermo Fisher Scientific, USA), 10% fetal bovine serum (Gibco, South America), and 1% antibiotics (penicillin/streptomycin, Capricorn Scientific, Germany).

Chemicals

To dissolve melatonin, Dimethyl sulfoxide (DMSO, Sigma Aldrich, USA) was used. 5 mM stock solution a prepared. This was then kept in the dark. The DMSO content was 1% at the end. The autophagy inhibitor used was 3-MA (Sigma Aldrich, USA). Before use, 3-MA is freshly prepared as a 50 mM stock solution in dH2O. Melatonin and 3-MA were afterwards diluted in DMEM medium to reach the appropriate concentration. Melatonin concentrations for MCF-7 and MDA-MB-231 cells were 3.5 and 4 mM, respectively, as previously determined (26).

Autophagy Inhibition

On a circular coverslip, $5x10^4$ cancer cells from each of the two BC cells were planted into 12-well plates. To induce autophagy in both BC cell lines, the media were switched out for FBS serum-free medium for 24 hours. Thus, autophagy was activated in the cells. Then, to determine 3-MA's autophagy inhibition dose, different doses of 3-MA (1, 5, and 10 mM) were applied for 24 hours to the cells. The autophagy markers Beclin-1 (Novus Biologicals, Littleton, USA), LC3 (Cell Signaling Technology, Danvers, USA) and p62 (Novus Biologicals, Littleton, USA) were examined using the immunofluorescence staining method as previously published (26). As a result, the dose at which inhibition occurred was determined.

Treatment

Using the aforementioned experiment, cells were plated. After 24 hours of incubation, drugs were added by the experimental groups. Six groups were created for the experiment: control (no drug intervention), 3-MA (5 mM 3-MA treatment alone), melatonin (melatonin treatment alone), DMSO (DMSO treatment alone), 3-MA and MEL combined (3-MA and melatonin were added simultaneously), and 3-MA and DMSO combined (3-MA and DMSO were added simultaneously). Cells were incubated at 37°C with 5% CO_2 and humidity for a full day. Then, immunocytochemical assays were carried out on BC cells to verify apoptosis and the Bax and Bcl-2 protein's expression. In addition, the MTT method was also used to assess the effect of combining 3-MA and melatonin on cell viability.

Apoptosis Detected by TUNEL

According to the six experimental groups, The twelve-well plates were seeded with $5x10^4$ cells in each well and pretreated with 3-MA, melatonin, or DMSO for 24 hours. 10% formaldehyde was used to fix the cells. Cell apoptosis was assessed using the EMD Millipore's apoptosis detection kit (Darmstadt, Germany), named ApopTag Fluorescein (26). After successfully staining with a mixture of TUNEL reactions, the cells were immediately detected using a fluorescent microscope.

Analysis of the Expression of Bax and Bcl-2

To verify Bax (Novus Biologicals, Littleton, USA) and Bcl-2 (Thermo Fisher Scientific, Rockford, USA) expression profiles, a double immunofluorescence staining method was carried out (26). For the quantification of the proteins' expression, 10 images at 400x magnification were randomly obtained from microscopic fields. The immunoreactivity intensity was calculated using the Image J software program.

Cell Viability Assay

The dose-dependent effects of 3-MA, melatonin, and DMSO on BC cells were investigated using a colorimetric MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide, Roche, Indianapolis, IN, USA). In 96-well plates, 1x104 cells were seeded of per well, and drugs were added by the experimental groups. 10 µl of MTT (5 mg/ml in PBS) was added after cells had been exposed to the relevant drug combinations and doses, and the cells were then incubated at 37°C for 4 hours in order to enable mitochondrial enzymes to convert the MTT to formazan crystals. The medium was aspirated after the allotted time had passed, and for each well 100 µl DMSO was added. An ELISA reader (Promega Glomax Multireader, Wisconsin, ABD) was used to measure 570 nm absorbance.

Statistical Analysis

The total immunoreactivity intensity of each antibody was measured using the Image J software program while analyzing the immunofluorescence staining results. For

every group, pictures were captured from ten different microscopic fields at a magnification of 400X. The data that was obtained was analyzed statistically with GraphPad Prism 8.0.2.. The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to assess how much the data conformed to a normal distribution. The values were compared using the Mann-Whitney U test based on drugs, dose groups, and percent viability. When comparing more than two groups, Kruskal-Wallis tests and one-way analysis of variance were used. The Tukey and Dunn-Bonferroni tests were applied for multiple comparisons. The significance limit of $p<0.05$ was accepted.

RESULTS

Inhibition of Autophagy

To determine the dose at which 3-MA inhibited autophagy, 1 mM, 5 mM, and 10 mM 3-MA were applied to cell lines after exposure to serum-free medium for 24 hours. After the Beclin-1, LC3, and p62 expression analysis of these cells, the inhibition dose for both was determined to be 5 mM.

Cell Death in BC Cells Induced by 3-MA and Melatonin Treatment

To examine the apoptotic effect of 3-MA and melatonin on human BC cells, TUNEL assays were used. In MCF-7 cells, MEL induces nuclear shrinkage and fragmentation. Compared to the control group, DNA fragmentation was reduced in MCF-7 cells with the addition of 3-MA and MEL (Figure 1).

In MEL-treated MDA-MB-231 cells, apoptotic cell immunoreactivity was increased. In the 3-MA+MEL group, apoptotic cell immunoreactivity was observed to increase according to the MEL group (Figure 2).

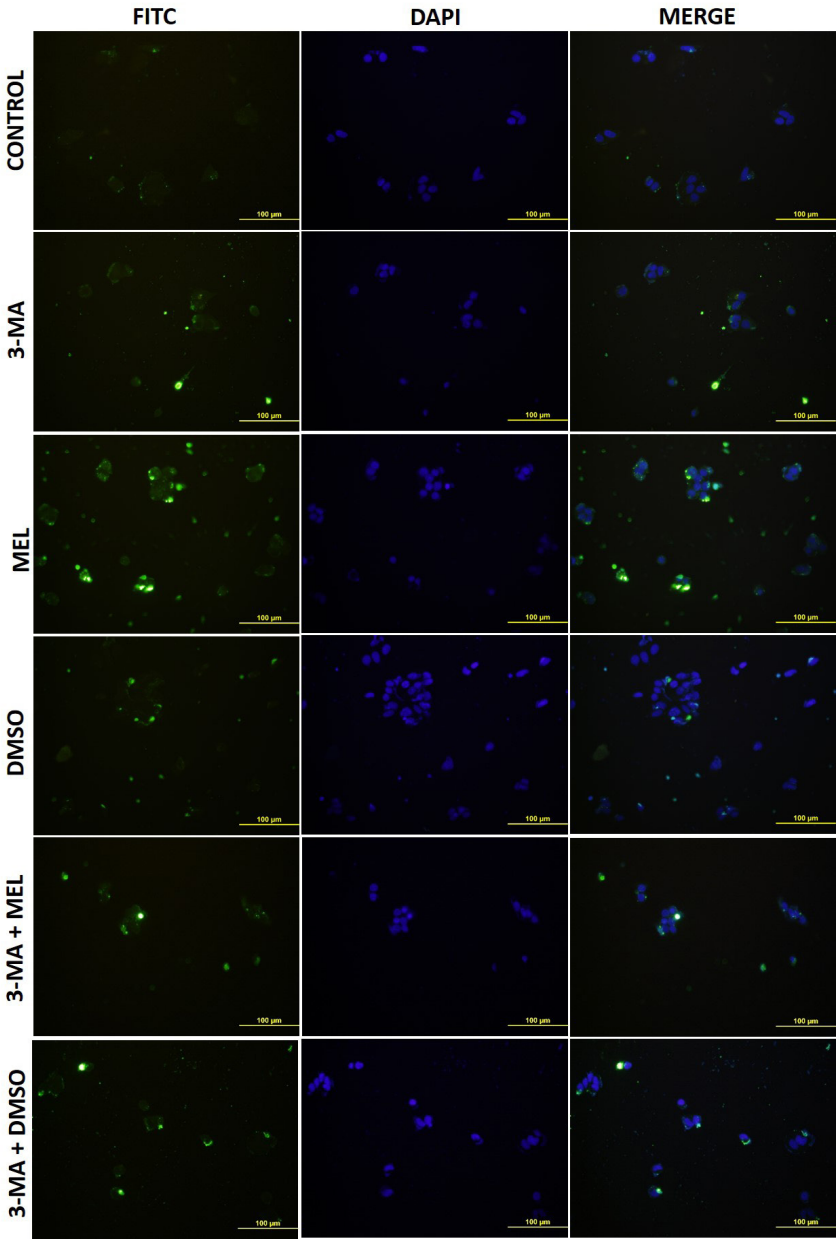

Figure 1. TUNEL images of the experimental groups using the MCF-7 cell line following the application of 3-MA and MEL. Apoptotic bodies that have formed in cells can be noticed as green fluorescent reflections under a fluorescent microscope (400X)

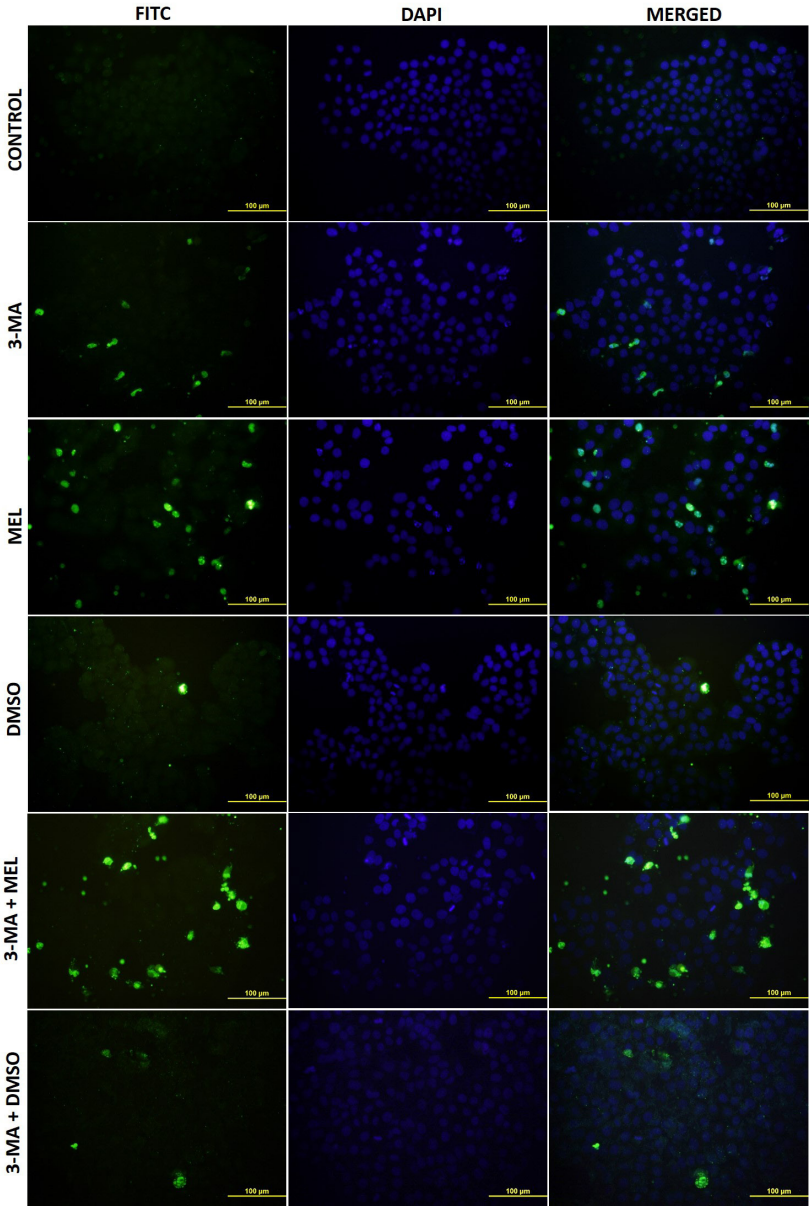

Figure 2. TUNEL images of the experimental groups using the MDA-MB-231 cell line following the application of 3-MA and MEL. Apoptotic bodies that have formed in cells can be noticed as green fluorescent reflections under a fluorescent microscope (400X)

The analysis of statistics of the immunoreactivities of apoptotic cells following 3-MA and MEL administration in the BC cell lines is shown in Table 1 according to experimental groups. It was found that the 3-MA group resembled the control group in MCF-7 cells and that between them, no statistically significant difference was seen. (>0.9999). The statistical significance of the difference between the MEL and the control group was determined (p=0.0061). TUNEL+apoptotic cell density was found to be lower in the 3-MA+MEL group than in the MEL group. In terms of statistics, there was no difference between them (>0.9999). When the experimental groups were examined, it was found that the control group's TUNEL+ cell immunoreactivity was the lowest in MDA-MB-231 BC cells. The MEL group demonstrated a statistically significant increase in comparison to the control group (p<0.0001). Comparing the 3-MA+MEL group to the MEL group, A statistically significant increase was observed (p=0.0029).

Table 1. TUNEL statistical analysis results of MCF-7 and MDA-MB-231 breast cancer cell lines treated with 3-MA and melatonin

	Groups						
	CONTROL	3-MA	MEL	DMSO	3-MA+MEL	3-MA+DMSO	p
MCF-7	(7.03±1.00)^{a}	(7.04±1.00)^{a}	(11.05±1.00)^{b}	(6.18±1.05)^{a}	(10.09±1.01)^{bc}	(7.15±1.03)^{ac}	.0003
MDA-MB-231	(4.41±0.10)^{a}	(6.8±0.10)^{b}	(11.5±0.62)^{c}	(5.56±0.15)^{ab}	(13.62±1.00)^{d}	(5.73±0.11)^{ab}	<0.0001

Data are expressed as mean±standard deviation. The same lowercase letters on the same line indicate similarity between groups, and different letters indicate difference. (3-MA: 3-methyl adenine, MEL: melatonin, DMSO: dimethyl sulfoxide)

Effects of MEL with or without 3-MA on Bax/Bcl-2 Protein Expression

The double immunofluorescence staining protocol was done to examine the effect of administering MEL with or without 3-MA on the Bax and Bcl-2 proteins' expression in both BC cell lines. Figure 3 shows images of the MCF-7 cell line, and Figure 4 exhibits images of the MDA-MB-231 cell line.

Comparing the MEL group to the control group, an important increase was observed when the Bax expression profile was compared between groups in MCF-7 BC cells ($p<0.0001$). Comparing the 3-MA+MEL group to the MEL group, it was found that there was an increase ($p=0.0002$). This rise was not statistically significant. A statistically insignificant change ($p<0.05$) was found when Bcl-2 expressions were analyzed (Table 2). In MDA-MB-231 BC cells, an increase in the MEL group relative to the control group was seen when the Bax expression profile was examined between groups. There was no statistically significant growth in this ($p=0.0708$). Comparing the 3-MA+MEL group to the MEL group, there was a statistically significant increase in Bax expression ($p=0.0034$). A statistically insignificant change ($p<0.05$) was found when Bcl-2 expressions were analyzed (Table 2).

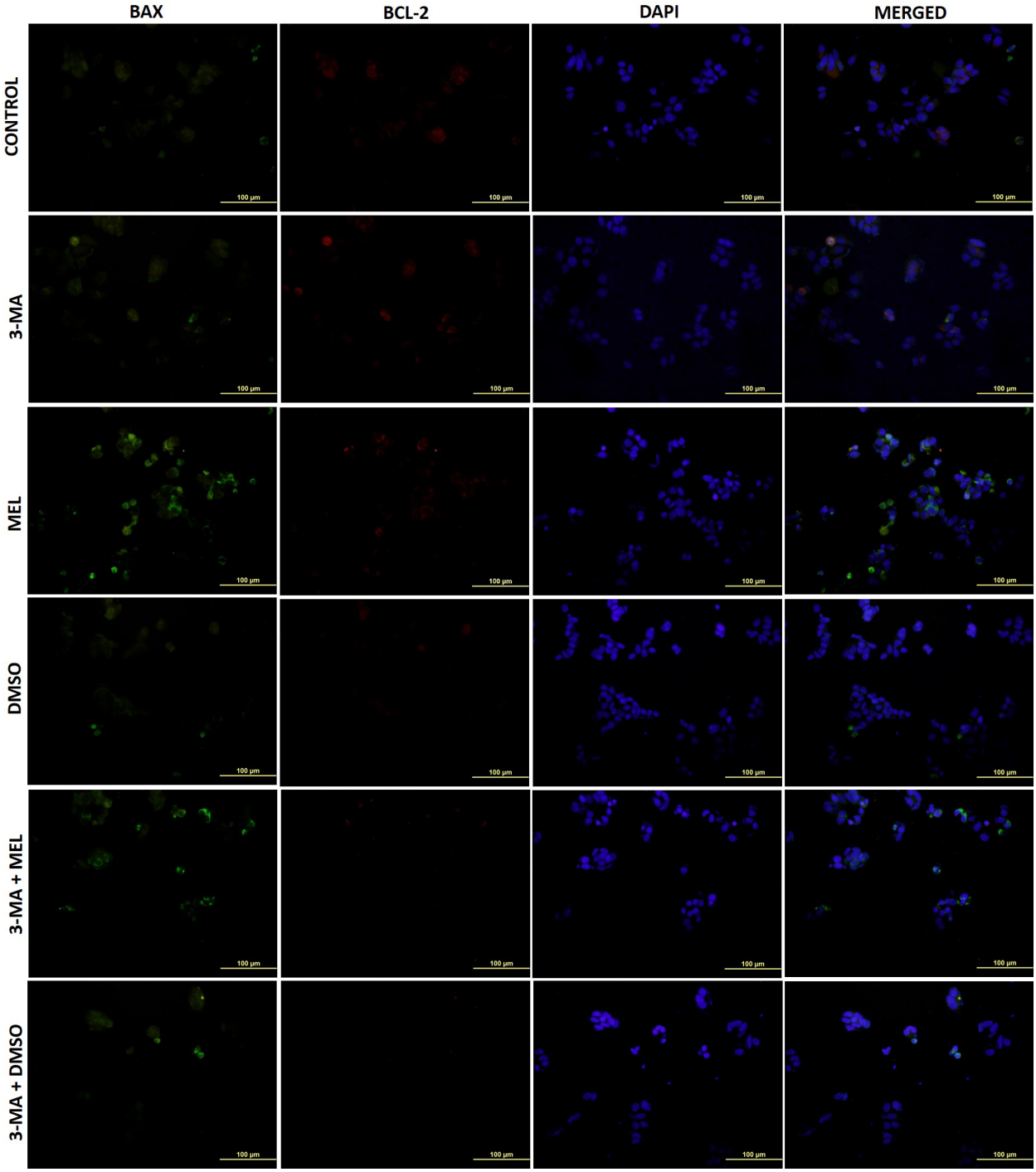

Figure 3. Expressions of Bax/Bcl-2 in the 3-MA- and melatonin-treated MCF-7 cell line Bax expression is demonstrated by bright green reflections in the first column, and Bcl-2 expression is displayed by bright red fluorescent reflections in the second column (400X)

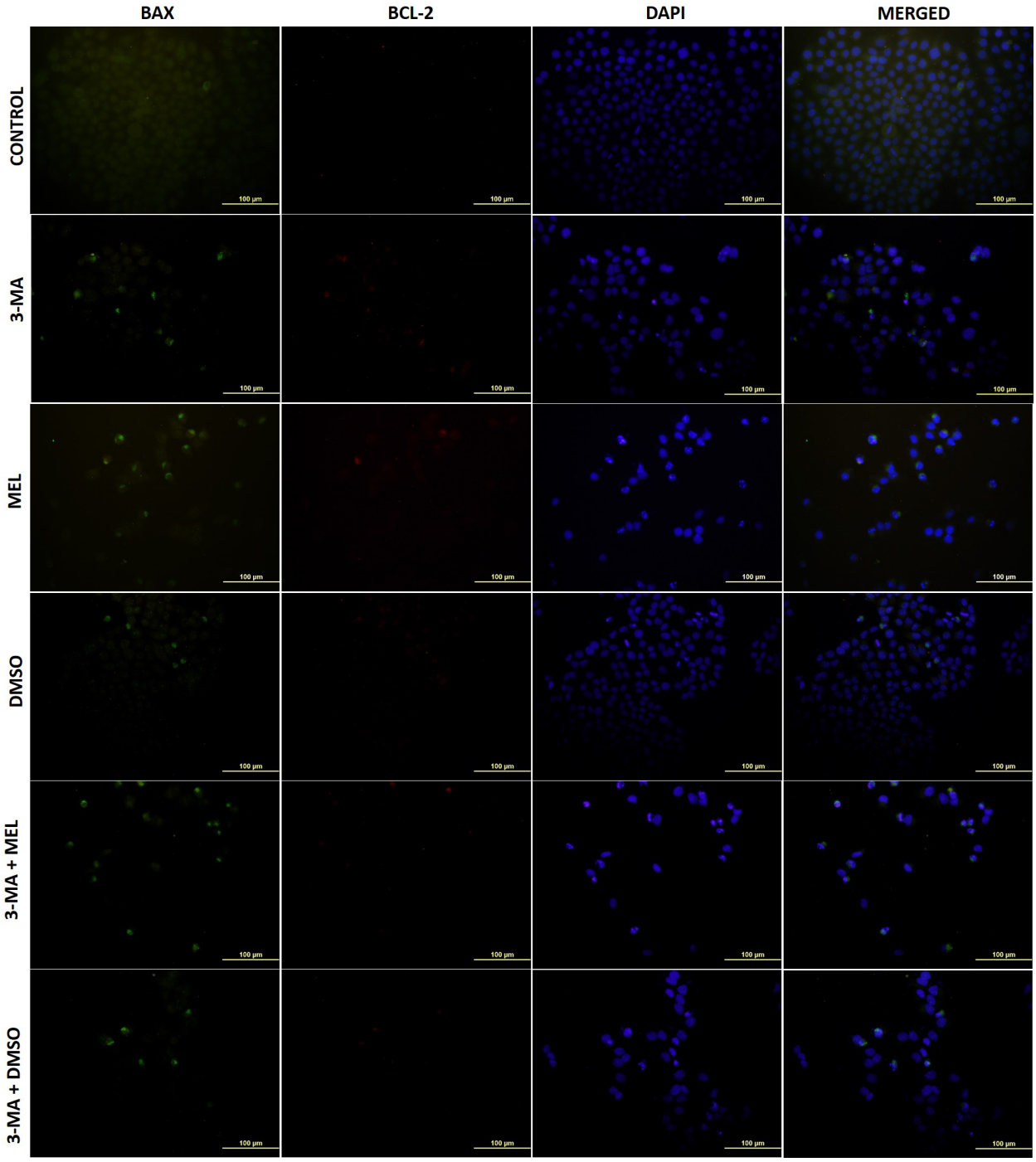

Figure 4. Expressions of Bax/Bcl-2 in the 3-MA- and melatonin-treated MDA-MB-231 cell line. Bax expression is demonstrated by bright green reflections in the first column, and Bcl-2 expression is displayed by bright red fluorescent reflections in the second column (400X)

Table 2. Statistical analysis results of Bax/Bcl-2 expression in the MCF-7 and MDA-MB-231 breast cancer cell line treated with 3-MA and melatonin

		Control	3-MA	MEL	DMSO	3-MA+MEL	3-MA+DMSO	p
MCF-7	Bax	(0.51±0.10)^{a}	(0.62±0.11)^{a}	(1.40±0.10)^{b}	(0.51±0.10)^{a}	(0.80±0.10)^{a}	(0.63±0.11)^{a}	.0001
	Bcl-2	(0.42±0.11)^{a}	(0.51±0.10)^{a}	(0.42±0.11)^{a}	(0.32±0.10)^{a}	(0.23±0.11)^{a}	(0.23±0.11)^{a}	.0355
MDA-MB-231	Bax	(0.42±0.11)^{a}	(0.62±0.10)^{a}	(0.75±0.13)^{ac}	(0.50±1.10)^{a}	(1.23±0.11)^{b}	(1.03±0.11)^{bc}	<0.0001
	Bcl-2	(0.19±0.01)^{a}	(0.30±0.10)^{a}	(0.33±0.11)^{a}	(0.16±0.05)^{a}	(0.19±0.01)^{a}	(0.18±0.02)^{a}	.0325

Data are expressed as mean±standard deviation. The same lowercase letters on the same line indicate similarity between groups, and different letters indicate difference. (3-MA: 3-methyl adenine, MEL: melatonin, DMSO: dimethyl sulfoxide)

Effects of MEL with or without 3-MA on Cell Viability

After the experimental groups were formed, the effect of the application of MEL with or without 3-MA on cell viability was demonstrated by applying MTT. The findings obtained after MTT analysis in MCF-7 cells are shown in Figure 5A. Examining the graph, it was discovered that the MEL group's cell viability was lower than the control group (p=0.0003). It was found that the cell viability in the 3-MA+MEL and MEL groups was similar (p>0.9999).

The findings were obtained after MTT analysis in MDA-MB-231 BC cells (Figure 5B). When the graph was examined, it was observed that cell viability decreased in the MEL group according to the control group. This result was statistically significant (p<0.001). Compared to the MEL group, The results showed a statistically significant decrease in cell viability in the 3-MA+MEL group (p=0.0004).

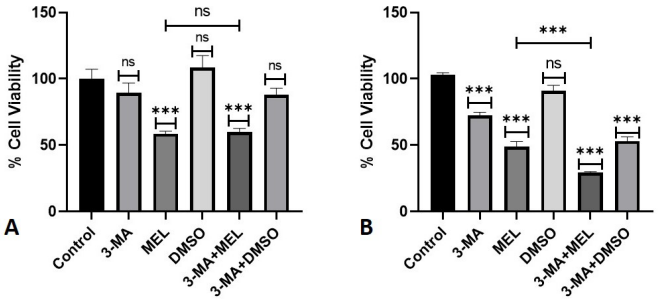

Figure 5. A. The MCF-7 cell lines cell viability after combined administration of 3-MA and MEL. **5B.** Cell viability of the MDA-MB-231 cell line after combined administration of 3-MA and MEL (ns: non-significant p>0.999, *p<0.05, **p<0.01, ***p<0.001

DISCUSSION

It is yet unknown how autophagy affects cancerous cell death or survival. According to studies, autophagy helps cancer cells survive by protecting them from hypoxia, malnutrition, and oxidative stress and by making established tumors resistant to chemotherapy (27-29). On the other hand, some studies have shown that apoptosis and autophagy can work together to induce cancer cells to die (30). However, it is currently uncertain what molecular switch or process determines whether a cell will survive through autophagy. Furthermore, the numerous processes of autophagy might be influenced by different cell types, the environment around the tumor, and the length of the period of therapy. The effectiveness of the associated drug in cancer cells, however, depends on studies into the connection between apoptosis and autophagy. In this study, the aim was to investigate the molecular pathways involved in the relationship between autophagy and apoptosis in BC cells following melatonin administration.

Suppression of autophagy by autophagy inhibitors demonstrates functional autophagy's importance in cancer cells (23,25). The effect of 5-FU and 3-MA's combination on apoptosis has been studied in a study using HT 29 and colon 26, two colon cancer cell lines. It has been showing that suppression of autophagy with 3-MA can increase the level of apoptosis caused by 5-FU. Therefore, it has been determined that autophagy occurs as a resistance mechanism to apoptosis, and when autophagy is inhibited, 5-FU increases its effectiveness on cancer cells (23). In a study with the MCF-7 cancer cell line, the effect on apoptosis and autophagy was investigated by applying oridonin treatment after autophagy inhibition with 3-MA. Consequently, it was found that autophagy contributed to the rise in apoptosis since autophagy inhibition reduced the level of apoptosis (25). Tran et al., in their study, investigated the anticarcinogenic effect of Tocomin, which contains tocopherols, on MCF-10A, MCF-7, and MDA-MB-231 cells. They stated that it potentiated apoptosis induced by Tocomin (31). In their research on BC, Chen et al. pre-treated both BC cells with wortmannin and 3-methyladenine, which are autophagy inhibitors, before anlotinib therapy to demonstrate the function of autophagy in anlotinib-induced death. Their research showed that anlotinib promoted autophagy in both cells, which at least partially, if not completely, caused apoptosis (32). Han et al. determined the anticancer specificity of myricetin in human BC SK-BR-3 cell lines. In cells treated with myricetin, 3-MA was used to assess the connection between autophagy and cell viability. The study's conclusions showed that inhibiting autophagy causes apoptosis and reduces cell viability. In addition, co-treatment with 3-MA and myricetin has been demonstrated to stimulate BC cell apoptosis (33). In the current study, we evaluated apoptosis, proapoptotic Bax, and anti-apoptotic Bcl-2 expressions after autophagy inhibition to show how the combined administration of 3-MA and melatonin affected BC cells. After all, it was shown that, when co-treatment with 3-MA, and melatonin treatment were co-administered in MCF-7 cells, there was no significant change in apoptosis compared to melatonin treatment alone. Nevertheless, it was shown that pro-apoptotic Bax expression was downregulated. The expression of Bcl-2 did not significantly differ from the other groups. On the other hand, it was determined that co-administration with 3-MA and melatonin, as opposed to melatonin therapy alone, notably enhanced the apoptotic cell numbers in MDA-MB-231 cells. Additionally, it was shown that Bax was upregulated. Bcl-2 expression among the other groups did not differ significantly.

CONCLUSION

In conclusion, according to these data, in MCF-7 cells, autophagy inhibition did not affect apoptosis triggered by melatonin, suggesting that the two processes proceed independently of each other. Also, it was determined that autophagy inhibition with 3-MA increased apoptosis in MDA-MB-231 cells; therefore, autophagy suppressed apoptosis in treatment with melatonin. As a result, it was suggested that melatonin treatment evaded MDA-MB-231 cells from cell death through autophagy.

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Conflict of interest: *The authors have no conflicts of interest to declare.*

Ethical approval*: The existence of an ethics committee is not necessary.*

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Research Article

Evaluation of Publishing Status of Doctoral Theses of Faculty of Medicine Department of Anatomy in Scientific Journals

Sibel Atesoglu Karabas[1], Selma Solgun Dag[2]

[1]Kahramanmaraş Sütçü İmam University, Faculty of Medicine, Department of Anatomy, Kahramanmaraş, Türkiye
[2]İnönü University, Faculty of Medicine, Department of Anatomy, Malatya, Türkiye

Abstract

Aim: The aim of this study was to evaluate the publication status of doctoral theses conducted by the anatomy departments of medical faculties in scientific journals.
Material and Method: In this descriptive, cross-sectional study, data were obtained retrospectively from the internet database of the National Thesis Center in the High Education Board (YÖK). A total of 148 doctoral dissertations published between 2009-2019 in the anatomy department of medical faculty were included in the study.
Results: Of the 148 theses, 82 (55.4%) were published in a journal and 66 (44.6%) were unpublished. Of the published theses, 53 (35.8%) were published in SCI/SCI-E, 3 (2%) in TÜBİTAK/Ulakbim TR index and 26 (17.6%) in other international indexes (p=.0001).
Conclusion: It was observed that the publication rate of doctoral dissertations in the anatomy department of medical faculty in Türkiye and the rate of publication as articles in journals in SCI/SCI-E were quite high.

Keywords: Anatomy, doctoral thesis, medicine of faculty

INTRODUCTION

Anatomy is the branch of science that examine the structure of the human body, organs, the location and neighborhood of these organs. The term anatomy is derived from Greek words "ana" meaning inside and "tome" meaning cutting. In Latin, it means dissection and is mostly used to mean examining a cadaver by cutting it into pieces (1). The main purpose of anatomy education is to provide the basic knowledge and skills needed (2,3). A good anatomy basis is very important for the diagnosis and treatment of diseases in the clinic. Because in order to distinguish what is pathological, it is necessary to know what is normal first. The purpose of anatomy research is to determine the standards of structures considered normal (4). Since Vesalius, who is considered the founder of anatomy, the importance of anatomy has increased and continued to develop. In parallel with the developing technology, many diversities have been provided in anatomy study areas and subjects and considerable progress has been made (5).

In addition to the contributions of these advances of science, access to education has also become easier. Postgraduate education has become widespread and has a large place in formal education (6). It is necessary to meet certain conditions to be admitted to doctoral programs, which represent the highest degree in education. Doctoral programs constitute the biggest step taken in academic terms and enable the transition to becoming a faculty member. The aim of doctoral education is to train scientists who can produce and use knowledge and solve existing problems with a productive way of thinking (3,7). Doctoral candidates are obliged to participate in a number of educational activities and write a doctoral thesis in order to achieve these determined goals. Doctoral education consists of 2 years of lesson phase and 2 years of thesis preparation. A doctoral thesis is an obligation that synthesizes information with research, interprets data, and demonstrates people's achievements (8). Thesis preparation aims to provide doctoral students with many earnings. For example, forming a hypothesis, planning a study, collecting and analyzing data, interpreting the results, and writing a scientific text are some of these purposes. It is important to convert theses into publications in terms of academic promotion and making significant contributions to

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Corresponding Author: Selma Solgun Dag, İnönü University, Faculty of Medicine, Department of Anatomy, Malatya, Türkiye
E-mail: slmslgn@hotmail.com

science (9-12). According to our literature review, we have not found any study evaluating the publication of anatomy doctoral theses in our country in scientific journals.

This study aims to evaluate the publication status of doctoral theses published in scientific journals by the Anatomy Departments of medical faculties in our country between 2009 and 2019.

MATERIAL AND METHOD

This study is a descriptive and cross-sectional study and is an original research. Ethics committee approval was received for the study from Kahramanmaraş Sütçü İmam University Medical Research Ethics Committee on 08.02.2022 (protocol number 2022/06, decision number 07). The study complied with the Declaration of Helsinki.

There are 148 articles published between 2009 and 2019 in the National Thesis Center internet database of the High Education Board (YÖK) (https://tez.yok.gov.tr/UlusalTezMerkezi/), entered into the system by the anatomy departments of all medical faculties in our country. Doctoral theses from the department of anatomy at the faculty of medicine were retrospectively examined and included. Exclusion criteria include not being able to access the full or abstract of the thesis, it being published before 2009 or after 2019, being published by an institution other than the anatomy departments of the faculty of medicine in our country, and being a master's thesis or medical specialty thesis.

The publication status of the thesis was taken from PubMED Central (PMC) (https://www.ncbi.nlm.nih.gov/pubmed) and Google academic (https://scholar.google.com.tr/) databases. The author of the thesis was determined by comparing the name-surname of the thesis advisor, the Turkish and English versions of the thesis title, the article title and the article summary with the title, subject and summary of the thesis. The journals in which the theses are published are from Science Citation Index (SCI), Science Citation Index-Expanded (SCI-E), Emerging Sources Citation Index (ESCI), other international fields (PubMED, Medline, Scopus, Index Copernicus etc.), Ulakbim TR databases. It was determined by evaluating the websites of these databases and journals in which one they were included. The study topics of theses are divided into categories as animal experiments, cadaver, anthropometry, radiology and others. The institutions where the authors of the theses currently work and their titles were determined by an internet search via Google. Apart from these, the gender of the thesis authors, the number of names in the thesis article, the academic title of the current advisor during the thesis process, whether the thesis is prospective/retrospective, the publication status of the article and the publication period if published, and the national or international indexes in which the article was scanned were evaluated.

Analysis of Data

IBM SPSS version 25 (IBM SPSS for Windows version 25, IBM Corporation, Armonk, New York, United States) software was used to evaluate the data. Mean and standard deviation were determined for numerical data, and number and percentage values were determined for categorical data. Chi-square test was used for group comparisons, and $p<0.05$ was considered significant.

RESULTS

148 medical faculty anatomy department doctoral theses recorded in the YÖK national thesis center internet database between 2009 and 2019 were included in the study. Of the thesis writers included in the study 69 (46.6%) were women and 79 (53.4%) were men. 111 (75%) of the thesis advisors are professors, 32 (21.6%) are associate professor (assoc. prof.) and 5 (3.4%) are assistant professor (asst. prof.).

82 (55.4%) of the theses were published in a journal, while 66 (44.62%) were not published. Of the published theses, 53 (35.8%) were published in SCI/SCI-E, 3 (2%) in TÜBİTAK/Ulakbim TR index and 26 (17.6%) in other international indexes (P=.0001; Table 1).

40 (48.78%) of the published studies of women, 25 are in SCI/SCI-E, 1 is in TÜBİTAK-Ulakbim TR index, and 14 are in other international indexes; It was determined that 28 of the 42 (51.22%) publications belonging to men were published in SCI/SCI-E, 2 in the TÜBİTAK-Ulakbim TR index, and 12 in other international indexes. There is no statistically significant difference between where theses written by men and women were published (p=.738). It was observed that 62 (75.60%) of the 82 studies that were published were supervised by professors, 18 (21.95%) were assoc. prof., and 2 (2.45%) were an asst prof. The advisors of the 66 studies that did not turn into publications consisted of 49 (74.24%) professors, 14 (21.21%) assoc. prof., and 3 (4.55%) an asst. prof. (p=.779; Table 1).

148 doctoral theses, 40 (27%) were retrospective and 108 (73%) were prospective (p=.0001). When the methods of these thesis studies are evaluated, 40 (27%) are experimental animal models, 33 (22.3%) are cadaver studies, 11 (7.4%) are anthropometric studies, 50 (33.8%) are radiological studies and 14 (9.5%) consisted of other studies (Table 1).

111 (75%) of the theses whose advisors were professors, 32 (28.82%) were experimental, 23 (20.75%) were cadaver, 7 (6.30%) were anthropometric, and 39 (35.13%) were radiological and 10 (9%) are other studies. 32 (21.6%) of the studies whose advisor was an assoc. prof., 6 (18.76%) were experimental, 9 (28.12%) were cadaveric, 4 (12.5%) were anthropometric, and 9 (28.12%) were radiological and 4 (12.5%) were other studies. 5 (3.4%) of the studies whose advisor was an asst. prof., 2 (40%) were experimental, 1 (20%) was cadaveric, and 2 (40%) were radiological studies (p=.781).

The average time for theses to become published was determined as 2.62±1.63 (min=1, max=7) years. Of the 82 published theses, 27 (18.2%) were published after one year, 20 (13.5%) were published after two years, 12 (8.1%) were published after three years, and 10 (6.8%) were published

after four years, 8 (5.4%) after five years, 3 (2%) after six years, and 2 (1.4%) after seven years.

The average number of authors in published articles was 5.24±1.90 (min=1, max=10). Among these, 1 (7%) with one author, 5 (3.4%) with two authors, 7 (4.7%) with three authors, 17 (11.5%) with four authors, 19 (12.8%) with five authors, 10 (6.8%) with six authors, 16 (10.8%) with seven authors, 3 (2%) with eight authors, 1 (7%) with nine authors, and 3 (2%) publications with ten authors. When we look at the author's name in the published articles, 79 (53.4%) were the first name, 2 (1.4%) were the second name, and 1 (7%) was the third name.

148 of the doctoral thesis writers, 104 (70.3%) were formerly academics, 44 (29.7%) were not academics, currently 119 (80.4%) are still academics, 29 (19.6%) of them did not continue as academics.

Considering the undergraduate graduation information of the thesis owners, 17 (11.5%) were from the physiotherapy and rehabilitation department, 17 (11.5%) were from the nursing department, 52 (35.1%) were from the faculty of medicine, 17 (11.5%) were from the veterinary medicine, 35 (23.6%) were from the biology department and 10 (6.8%) were from other departments graduated.

Table 1. Evaluation of anatomy doctoral theses

Parameters		Published theses n (%)	Unpublished theses n (%)	p
Gender	Man	42 (53.16)	37 (46.84)	.557
	Woman	40 (57.97)	29 (42.03)	
Supervisor	Professor doctor	62 (75.60)	49 (74.24)	.779
	Associate professor	18 (21.95)	14 (21.21)	
	Assistant professor	2 (2.45)	3 (4.55)	
Study topics	Cadaver	21 (63.63)	12 (36.37)	.497
	Radiology	29 (58)	21 (42)	
	Animal experiment	21 (52.5)	19 (47.5)	
	Anthropometry	6 (54.54)	5 (45.46)	
	Others	5 (35.71)	9 (64.29)	
Type of journal	SCI/SCI-E	53 (35.8)	-	**.0001**
	TÜBİTAK/Ulakbim TR Index	3 (2)	-	
	Another International Index	26 (17.6)	-	

*SCI: Science Citation Index, SCI-E: Science Citation Index-Expanded, n: number, p<0.05

DISCUSSION

A good anatomy infrastructure is the basis for patient examinations in clinics, diagnosing diseases and the most accurate treatment practices. Because detect what is pathological, it is necessary to know what is normal first. Research in the field of anatomy primarily aims to reveal normally accepted standards. Thus, it will be easier to distinguish pathological ones in clinics (4). Students studying in the field of anatomy must also complete a thesis in order to fulfill these goals and graduate successfully. This study is the first to evaluate the publication of doctoral theses in the anatomy department of the faculty of medicine published in our country in scientific journals. In our study, the publication rate of theses written between 2009 and 2019 in scientific journals was determined as 82 (55.4%), and the non-publication rate was 66 (44.6%).

When the thesis studies in different fields that have been done so far are examined, it has been determined that the results evaluating the time it takes for the theses to be published vary (9,10). On the one hand, it is stated that a period of eleven years must pass from the completion date of the thesis to the publication date. On the other hand, it has been reported that this period should not exceed five years (13). Kalcioğlu et al., they found that this period was on average three and a half years (14). When we look at the studies evaluating medical specialty theses in different branches, it takes 3.15 years in the department of ear, nose and throat (ENT), 3.20 years in the department of medical pharmacology, 3.02 years in the department of medical physiology, 2.83 years in the department of psychiatry and 3.37 years in the department of anatomy (15-19). In our study, it was determined to be 2.62 years. Our results show that anatomy department doctoral theses are published earlier than specialty theses.

When the publication rates of specialty theses in different branches are evaluated, 35.6% of 368 ENT theses in 2017, 57.3% of 87 medical physiology theses in 2019, 37.4% of 910 psychiatry theses in 2020, 10.7% of 309 medical microbiology theses, 56.5% of 108 medical pharmacology theses and 64.9% of 57 anatomy theses in 2022 were published in a journal (9,15-18). In our study, 55.4% of theses, similar to physiology, pharmacology and anatomy specialty theses, were published in a journal. It was determined that the results in our study were in the middle compared to the results determined in different areas in our country.

When we examine where the specialty thesis studies in different fields published in scientific journals are published, 35.6% ENT publications of 21.4% are in international journals, 14.1% are in national journals, 11.5%

family medicine publications of 0.8% are in SCI journals, 3.1% in SCI-E journals and 7.6% in national journals, 56.5% medical pharmacology publications of 77% are in SCI/SCI-E, 14.8% in other international directories, 4.9% in the TÜBİTAK/Ulakbim TR index, 3.3% are in national peer-reviewed journals, 57.3% physiology publications of 18.3% are in SCI journals, 20.7% are in SCI-E journals, 11% are in other international indexes, 6.1% in TÜBİTAK/Ulakbim TR index, 1.2% in national peer-reviewed journals, 54.4% of anatomy publications in SCI/SCI-E, 8.8% in TÜBİTAK-Ulakbim TR index and 1.8% in other international indexes (15-17,19,20). In our study, 35.8% of the theses were published in SCI/SCI-E, 2% in TÜBİTAK/Ulakbim TR index and 17.6% in other international indexes. In our study, it is seen that the publication status of anatomy doctoral theses in SCI/SCI-E is quite high compared to family medicine, quite low compared to medical pharmacology, and similar compared to other departments.

Due to the mobility inherent in the education process, different education models are needed to keep up with the times. Successful results of changes in the education process can only be achieved if the applied education model is correct and appropriate (21). Academic research conducted in relevant departments also changes in parallel with the changes in the educational process. In a study where 57 anatomy specialty thesis topics were evaluated, it was determined that 29.7% were radiological studies, 37.8% were cadaver studies, 27% were animal experiments studies and 5.4% were anthropometry studies (19). In our study, 27% consists of experimental animal models, 22.3% cadaver study, 7.4% anthropometric study, 33.8% radiological study and 9.5% other studies. Our results show that, unlike the study conducted, radiological studies are preferred most, followed by animal experiment studies.

Many components such as the research fields of the individual receiving training, the facilities of the institution he/she studies at, and the thesis advisor he/she works with are effective in determining a thesis topic, its quality, and its transformation into publication. When the publication status of the studies according to the thesis advisors is evaluated, in a study on psychiatry specialty theses, 40.1% of 438 theses whose advisor was a professor, 31.4% of 261 theses whose advisor was an assoc. prof., 67.2% of 122 theses whose advisor was an asst. prof. It has been observed that the works of those whose thesis advisors are asst. prof.. are more likely to be published ($p<.05$) (18). In another study conducted on anatomy specialty theses, 64.3% of the theses whose advisor was a professor, 64.3% of the theses whose advisor was an assoc. prof., and all of the theses whose advisor was an asst. prof. were published (20). In our study, 75.60% of the theses whose advisor was a professor, 21.95% of theses whose advisor was an assoc. prof., and 2.45% of theses whose advisor was an asst. prof. were converted into publications. In our study, unlike studies in other fields, the advisor of the studies that turned into publications was the professor.

CONCLUSION

As a result, it has been observed that the publication rates of doctoral theses in the anatomy department of medical faculties in our country are similar to other branches of science. Most of these studies are published in SCI/SCI-E. Radiological studies are the most preferred thesis subject.

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MEDICAL RECORDS-International Medical Journal

Research Article

Availability of Mean Platelet Volume and Neutrophil/Lymphocyte Ratio in Control of Helicobacter Pylori Eradication

Huseyin Kacmaz, Ali Riza Caliskan

Adıyaman University, Faculty of Medicine, Department of Gastroenterology, Adıyaman, Türkiye

Abstract

Aim: The most common chronic bacterial infection in humans is Helicobacter pylori (HP) and has been proven to cause gastritis, recurrent peptic ulcers, duodenal ulcers, and gastric cancer. We investigated the neutrophil-lymphocyte ratio (NLR) and the mean platelet volume (MPV) in the control of HP infection eradication success.

Material and Method: Patients with HP infection in endoscopic biopsy performed due to dyspeptic complaints were included in this retrospective analysis. Quadruple antibiotic eradication therapy was given to the patients for 14 days. Pre- and post-treatment complete blood count parameters of 217 patients whose eradication treatment was successful were evaluated.

Results: There was a statistically significant decrease in the neutrophil count after treatment compared to before treatment ($p<0.001$). NLR after treatment was significantly lower than before treatment ($p=0.002$). MPV value after treatment showed a statistically significant increase compared to before treatment ($p<0.001$).

Conclusion: In our study, a significant difference was observed between pre-/post-treatment neutrophil value, neutrophil/lymphocyte ratio, and MPV value.

Keywords: Helicobacter pylori, mean platelet volume, gastritis, neutrophil/lymphocyte ratio

INTRODUCTION

Marshall and Warren have identified the stomach bacterium in 1982 and cultured Campylobacter pyloridis, which was later reclassified as Helicobacter pylori (HP) (1). In developing countries, it is more common than in developed ones. The prevalence of HP in our country was found to be 71-80% (2,3). Low socioeconomic level, poor nutrition, poor hygiene conditions and living in crowded environments pose a risk for the disease (4,5). The prevalence of HP was found to be 82.5% with the urea breath test. Most of the cases have asymptomatic chronic inflammation (6).

During inflammation, neutrophils and lymphocytes interact with the microenvironment and tumor cells. The release of neutrophil chemotactic factors from inflammatory and malignant tissues causes the number of neutrophils to increase in peripheral blood (7-10).

The tissue damage caused by HP is originated from immune response and increased inflammatory mediators due to platelet activation. The bacterium facilitates T cell activation through antigenic molecules such as urease, lipopolysaccharide, and HP heat shock protein. The increase in T cells induces the secretion of cytokines. It causes the accumulation of platelets in the gastric mucosa (11). The HP infection alleviates the concentration of eicosanoids such as prostaglandin F and derives concomitant increase in thromboxane A2 (TXA2) with lipid peroxidation. This details the high platelet count and is also supported by increased mean platelet volume (MPV), platelet distribution width (PDW), thrombocyte (PCT) in routine laboratory examinations (12).

Vascular endothelial growth factors, tumor necrosis factor, and other cytokines that contribute to cancer progression are secreted by neutrophils. Increased neutrophils lead to lymphocytopenia or decreased lymphocyte function (13). Increased levels of cytokines and inflammatory markers were observed in gastric biopsies and serum samples of HP-positive patients (10). An increase in neutrophils and a decrease in lymphocytes occurs. Therefore, the Neutrophil/lymphocyte ratio (NLR) is a sensitive marker for inflammation (11). While the MPV provides information about platelet activity, systemic inflammation can also vary (12).

CITATION

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Corresponding Author: Ali Riza Caliskan, Adıyaman University, Faculty of Medicine, Department of Gastroenterology, Adıyaman, Türkiye

E-mail: komamir308@gmail.com

Study Hypothesis

An increase in the number of neutrophils and a decrease in the number of lymphocytes are observed in physiological stress. The ratio of these two subgroups to each other is used as a sensitive indicator of inflammation. MPV levels have been associated with chronic inflammation and higher MPV values indicate that platelets participate in proinflammatory and prothrombotic events (12,13).

Non-invasive, easily accessible and cost-effective tests are needed for the eradication control of HP due to the emerging prevalence of infection. We aimed to investigate the NLR and MPV in post-treatment eradication control of HP.

MATERIAL AND METHOD

Patients with HP infection detected in the endoscopic biopsy performed due to dyspepsia at the Gastroenterology clinic of Adıyaman Training and Research Hospital between January 2020 and June 2021 were included. The ethics committee approval has been granted on 18/05/2021 and protocol number: 2021/05-18. The study complied with the Declaration of Helsinki and informed consent has been obtained from all participants.

Database were collected from patients' medical and laboratory records. Hemogramme, coagulometric and biochemical tests were performed in all patients before endoscopy. Endoscopies were performed under general anesthesia (Propofol 1% 10 g/20 ml, Midazolam 50 mg/10 ml) after an 8-hour fast under the supervision of an anesthesiologist.

Patients who used proton pump inhibitors within one month, patients with infection, malignancy, hematological, chronic kidney disease, and patients receiving immunosuppressive therapy were excluded from the study. Patients with gastrointestinal bleeding, gastric ulcer, portal hypertensive gastropathy, on endoscopy also were excluded from the study. To detect the presence of HP, two biopsies were taken from the gastric antrum region with biopsy forceps. Mucosal samples were stained with hematoxylin and eosin. Mucosal samples were evaluated with the Sydney classification for HP positivity or negativity (13). Cases with atrophy, intestinal metaplasia, dysplasia or malignancy were not included in the study.

HP antigen in the stool was evaluated eight weeks after the end of treatment. Negative detection was considered successful eradication (7). The NLR was calculated by dividing the number of neutrophils by the number of lymphocytes. Patients who did not complete the course of antibiotics were excluded from the analysis.

Statistical Analysis

Descriptive statistics were presented by calculating frequency, percentage, mean, standard deviation, median, 25%-75% percentile (Q1-Q3) values. The assumption of normality was checked using the Shapiro-Wilk test by examining the histogram, q-q plot, skewness and kurtosis values. Wilcoxon Signed Rank Test was used to distribute the groups that did not fit the normal distribution in the comparison of two dependent measures. Results with a P value of less than 0.05 were considered statistically significant. Analyzes were made with the SPSS 23.0 package program.

RESULTS

Descriptive statistics of the patients were presented in Table 1. Almost half the (44.2%) of the subjects were male (n=96), 55.8% were female (n=121). The average age of the patients was 40.7±14.35 years. The endoscopy finding of 63.6% of the patients was antral gastritis (AG) (n=138), and 36.4% of them were pangastritis (PG) (n=79).

The pre-and post-treatment measurements of the patients were compared in Table 2. According to the Wilcoxon Peer Test results, the neutrophil value after treatment showed a statistically significant decrease compared to before treatment ($p<0.001$).

After treatment, there was a statistically significant decrease in the NLR compared to before treatment ($p=0.002$). MPV value showed a statistically significant increase after treatment compared to before ($p<0.001$).

The parameters of AG and PG groups before and after treatment were compared. While a decrease in neutrophil values was observed in the AG group after treatment ($p=0.001$), the decrease was not statistically significant in the PG group ($p=0.052$). Although the lymphocyte value did not change in the AG group ($p=0.811$), there was a decrease in the PG group ($p=0.001$). The NLR decreased in the AG group ($p=0.003$) but it did not change in the PG group. MPV value increased in both AG ($p=0.006$) and PG groups ($p<0.001$) (Table 3).

The difference values (delta) of the pre-treatment and post-treatment measurements of the groups were compared. According to the Mann Whitney U test, it was found that there was a difference between the groups only in MPV measurement, and a higher increase was observed in the PG group ($p=0.03$). In other measurements, the difference values did not change according to the groups ($p>0.05$).

Table 1. Demographic characteristics of the patients

Variable	Category	n	%	Median (SD)	Median (Q1-Q3)
Gender	Male	96	44.2		
	Female	121	55.8		
Endoscopy findings	AG	138	63.6		
	PG	79	36.4		
Age		217		40.7±14.35	41 (28-50)

Table 2. Time-bound changes

Variables	n	Median (SD)	Median (Q1-Q3)	z	p
Pre-treatment Neutrophil value	217	4.63±1.85	4.3 (3.3-5.4)	-3.80	<0.001
Post-treatment Neutrophil value	217	4.2±1.7	3.9 (3.2-4.9)		
Pre-treatment Lymphocyte value	217	2.32±0.68	2.2 (1.8-2.7)	-0.34	0.731
Post-treatment Lymphocyte value	217	2.31±0.64	2.2 (1.8-2.7)		
Pre-treatment NLR	217	2.14±1.17	1.85 (1.5-2.58)	-3.04	0.002
Post-treatment NLR	217	1.95±1.15	1.75 (1.38-2.2)		
Pre-treatment MPV	217	8.26±1.47	8.1 (7.3-8.9)	-4.73	<0.001
Post-treatment MPV	217	8.58±1.33	8.5 (7.8-9.2)		

Table 3. Antral gastritis and pangastritis parameters of groups before and after treatment were compared

	Antral gastritis				Pangastritis			
	n	Mean (SD)	Median (Q1-Q3)	Test	n	Mean (SD)	Median (Q1-Q3)	Test
Pre-treatment Neutrophil value	138	4.74±1.83	4.55 (3.4-5.7)	z=-3.23 p=0.001	79	4.45±1.88	4 (3.3-5)	z=-1.94 p=0.052
Post-treatment Neutrophil value	138	4.27±1.55	3.9 (3.2-5.1)		79	4.08±1.94	3.7 (2.9-4.5)	
Pre-treatment Lymphocyte value	138	2.35±0.64	2.2 (1.9-2.7)	z=-2.39 p=0.811	79	2.27±0.75	2.1 (1.7-2.8)	z=-0.942 p=0.001
Post-treatment Lymphocyte value	138	2.37±0.61	2.3 (1.9-2.8)		79	2.2±0.69	2.1 (1.7-2.6)	
Pre-treatment NLR	138	2.15±1.23	1.91 (1.5-2.63)	z=-2.96 p=0.003	79	2.12±1.06	1.74 (1.5-2.5)	z=-1.03 p=0.303
Post-treatment NLR	138	1.91±0.89	1.75 (1.35-2.23)		79	2.03±1.51	1.7 (1.39-2.18)	
Pre-treatment MPV	138	8.41±1.54	8.15 (7.5-9.1)	z=-2.75 p=0.006	79	8±1.31	7.9 (7.1-8.7)	z=-4.0 p<0.001
Post-treatment MPV	138	8.65±1.45	8.4 (7.7-9.4)		79	8.46±1.07	8.5 (7.8-9.1)	

DISCUSSION

NLR and platelet-lymphocyte ratio (PLR) are parameters that are cost-effective, easily obtainable, and correlate with the prognosis of systemic inflammatory diseases. MPV is an indicator of platelet functions, which play an important role in immunological and inflammatory processes (13,14).

NLR was significantly higher in the HP positive group compared to the HP negative group. In addition, post-treatment NLR in the HP positive group after successful eradication was significantly lower than pre-treatment (15). We detected a significantly higher NLR after treatment than before treatment.

NLR was found to be significantly higher in the HP-positive group than in the negative group (16). According to a research from Iran, NLR was found to be higher in HP-positive patients with peptic ulcers than in asymptomatic HP-positive patients. In the same study, NLR was found to be higher in asymptomatic HP-positive patients compared to the HP-negative control group (11). Kondo et al. reported that after a successful HP eradication therapy, a significant decrease was observed in peripheral blood leukocyte, neutrophil and monocyte values compared to pre-treatment (17). Farah et al. stated a significant increase in NLR in the HP (+) group compared to the HP (-) group. An increase in NLR was observed in proportion to HP severity (18). In addition, it was observed that NLR improved after HP eradication treatment (13).

Ümit et al. found a significantly higher MPV rate in the HP (+) group than in the HP (-) group (19). We found that the pre-treatment MPV rate was significantly higher than the post-treatment MPV rate. Sahin et al. conducted a study in a pediatric patient group of 153 HP (+) and 211 individuals. No statistically significant difference was observed between groups in terms of NLR and MPV. No significant difference was observed in terms of NLR and MPV after treatment compared to before treatment (20).

Kaplan et al. examined the relationship between PLR, NLR, PDW, PCT and have found increased values in all these parameters. They also published that alleviated platelet count due to HP infection and reduced platelets with ongoing eradication therapy espcially in women (21). On the other hand Sahwa et al. reported higher MPV, lower PDW and platelet count (22).

In this study, we have observed a significant difference between NLR and MPV before and after treatment. It is assumed that NLR reflects the balance between activation of the inflammatory pathway and anti-tumor immune function. However, there are certain downsides of NLR calculation. Any surgical operation causes certain stress thus elevating NLR and changing the course of inflammation (23). Additionally, cardiovascular diseases and infection, or medication have an important effect on NLR.

Detection of HP positivity by biopsy in the entire study population was one of the strengths of our analysis. The comparison of NLR and MPV before and after treatment was also favorable compared to previous literature. Further trials with many patients as multicenter prospective studies are needed for comprehensive data.

CONCLUSION

Regarding the results of this study one can conclude that post-treatment neutrophil lymphocyte ratio significantly decreased compared to pre-treatment period. On the contrary post-treatment MPV value showed a statistically significant increase compared to pre-treatment. Regarding the results of this study one can conclude that NLR and MPV can be utilized in the eradication success of HP infection as easy to obtain markers that are routinely obtained in daily practice.

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Ethical approval: *The ethics committee approval has been granted on 18/05/2021 and protocol number: 2021/05-18. The study complied with the Declaration of Helsinki and informed consent has been obtained from all participants.*

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MEDICAL RECORDS-International Medical Journal

Research Article

Turkish Version of Caregiver Difficulties Scale for the Children with Cerebral Palsy: A Study of Validity and Reliability

Hatice Adiguzel[1], Zekiye Ipek Katirci Kirmaci[2], Suat Erel[3], Nevin Ergun[3]

[1]Kahramanmaraş Sütçü İmam University, Faculty of Health Sciences, Department of Physiotherapy and Rehabilitation, Kahramanmaraş, Türkiye
[2]Gaziantep Islam, Science and Technology University, Faculty of Health Sciences, Department of Physiotherapy and Rehabilitation, Gaziantep, Türkiye
[3]Pamukkale University, School of Physical Therapy and Rehabilitation, Denizli, Türkiye
[4]SANKO University, Faculty of Health Sciences, Department of Physiotherapy and Rehabilitation, Gaziantep, Türkiye

Abstract

Aim: The purpose of this study is to evaluate the validity and reliability of the Turkish version of the Caregiver Difficulties Scale (CDS).
Material and Method: The CDS was translated into Turkish (TR-CDS) and 116 caregivers of children with Cerebral Palsy (CP) (mean age: 37.20±10.36 years) completed the TR-CDS. Impact on Family Scale (IPFAM), World Health Organization Quality of Life–Brief Form (WHOQOL-BREF), Fatigue Severity Scale (FSS), Nottingham Health Profile (NHP), Beck Depression Inventory-II (BDI-II), and subscales and total impact score of TR-CDS were used for the construct validity. The internal consistency was assessed using Cronbach's alpha, and the test-retest reliability was assessed using the Intraclass Correlation Coefficient (ICC).
Results: For construct validity, all subscales of TR-CDS showed negative correlation with the WHOQOL-BREF and showed positive correlation with the IPFAM, FSS, NHP, and BDI-II. ICC the results of test-retest reliability analysis were for TR-CDS (total)=0.879, impact on self (CDS-IS)=0.843, support for caregiving (CDS-SC)=0.759, social and economic difficulties (CDS-S&E)=0.827, and concern for the child (CDS-CC)=0.707. A value of 0.936 was found for internal consistency.
Conclusion: It was revealed that the TR-CDS was a valid and reliable tool for the caregivers of children with CP.

Keywords: Caregiver difficulties scale, cerebral palsy, caregivers, reliability, validity

INTRODUCTION

Cerebral Palsy (CP) is the most prevalent, non-progressive pediatric disease and a disturbance of movement, tone, and posture with a frequency of 2-3/1000 among live births (1). While motor dysfunctions differ in terms of clinical types, behavioral and sensory issues, speech impairments, and cognitive issues accompanies (1,2).

The family plays a central role in the lives of both the children and the team, contingent on the children's needs and dependency levels (1,3). The major goal of CP children's therapy is to help them become more adept at improving their adaptive abilities. Since the family plays a crucial role in ensuring that these children participate actively in life, family-centered approaches to CP rehabilitation are leading the field in this regard (4). Because of this, ensuring family engagement and identifying and addressing their needs and issues during interdisciplinary treatments is essential to the effectiveness of rehabilitation. Depending on how inadequate their physical skills are, people with CP may require assistance with Activities of Daily Living (ADL) at different degrees (2). Widespread concern, money issues, time constraints, and limitations in social and cultural activities are all faced by caregivers of children with CP (2,5,6). While some families adjust to this circumstance quite well, others find it difficult to cope and make the necessary modifications. As a result, the caregivers' quality of life and life satisfaction decline, and their general wellbeing deteriorates. Therefore, assessing caregivers with physical, psychological, or social issues, as well as in terms of a lower quality of life for individuals with CP is crucial (4). Considering important caregivers, especially mothers, is one of the best ways to manage a children with CP and their handicap (6). These children live longer, and as a result, their families face more material, spiritual, physical, and social challenges. Eventually, family members eventually have behavioral and psychological difficulties. Due to the restricted availability of basic amenities and care services for the disabled, families in

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Corresponding Author: Hatice Adiguzel, Kahramanmaraş Sütçü İmam University, Faculty of Health Sciences, Department of Physiotherapy and Rehabilitation, Kahramanmaraş, Türkiye
E-mail: fzthatis@gmail.com

developing nations are increasingly vulnerable to the detrimental effects of providing care (7).

The International Classification of Functioning, Disability and Health-Children and Youth (ICF-YH) acknowledges that the family's functioning is a fundamental environmental aspect that might impact the child's health and functionality (8). The ICF defines "participation" in life and highlights the importance of coping mechanisms for both adults and children who have incapacitating conditions (3). But caregivers are one of the most vulnerable groups in society which negatively affects caregivers and children with disabilities and makes it difficult to receive primary assistance and institutions in developing nations (9). As a result, family-centered and compassionate approaches to family members—particularly mothers—have a big influence on legislators, support organizations, campaigners, and others who support the rights of kids with disabilities. But, caregivers' accountability additionally plays a part in this role (9). In order to give caregivers access to chances and accurate support, it is required to measure the duties of care. This can only be done by employing questionnaires that assess a suitable caregiver's challenges in order to comprehend the family burden (9). It's very common practice to assess caregivers using questionnaires. The Caregiver Difficulties Scale (CDS) has gained prominence recently (10). It assesses all duties that are unique to caregivers of children with CP and covers them completely. Some of the scales like Zarit Caregiver Burden Scale (ZCBS) and Impact on Family Scale (IPFAM) can be used to evaluate the distress experienced by caregivers of children with CP (11,12). ZCBS scale was developed to assess the level of stress and impact of the disease in caregivers of elderly or dependent individuals. This scale was developed for relatives of Alzheimer's patients. The items of ZCBS generally address the social and emotional domains. The IPFAM was designed to measure the impact of pediatric chronic health conditions on family. Also it is longer than CDS, and scoring of the IPFAM is difficult. But CDS is a questionnaire designed to be completed by the principal caregiver of children with CP. Each subscale represents a major area of caregiver concerns and a high cumulative score obtained for a particular subscale is usually indicative of the area needing interventions. Sinhalese version of CDS showed that a high CDS score (above 42) is predictive of caregiver psychological problems. Therefore, CDS will also be useful as a potential screening tool for identification of caregivers who are at risk of psychological problems such as stress, depression and anxiety in Türkiye. This study aimed to develop the TR-CDS and to examine whether it is a valid and reliable tool for assessing the impact of having a child with CP.

MATERIAL AND METHOD

Study Design

This study is a methodological research.

Translation Procedures

The questionnaire was translated into Turkish and culturally adjusted as the first stage of the study. The validity and reliability analysis was the second phase. The concepts of Beaton et al. and Guillemin et al. were applied during the cultural adaptation process (7,13). Two independent physical therapists (PT) who knew English and were native Turkish speakers translated the questionnaire into Turkish. Together, these two PTs with clinical backgrounds in working with caregivers of children with CP, developed the TR-CDS. Two native English speakers who were unaware of the first form of the questionnaire translated it back into English. In a follow-up discussion, two translators and two physiotherapists decided to employ the Turkish translation in the pilot program. To that end, 15 caregivers were invited to fill out the questionnaire and explain the challenges they encountered with each item. Consequently, the questionnaire's final form was made.

Individuals

The study included the caregivers of children with CP who were enrolled in outpatient rehabilitation centers and underwent physiotherapy and rehabilitation, were 1-18 ages and have any other neurological or systemic diseases. The caregivers of children with CP were between the ages of 17 and 65 (n=116). Caregivers who were on neuroleptic and antidepressant medication, could not speak Turkish, or were caregivers with a documented or treated mental illness who were judged unable to complete the questionnaire were excluded from the study. All of the comorbidities, parental risk factors (diabetes, blood pressure, heart disease, and smoking), surgeries, and applications of botulinum toxin (Btx) of CP children were noted, along with the demographic information of the caregivers and the children (Figure 1).

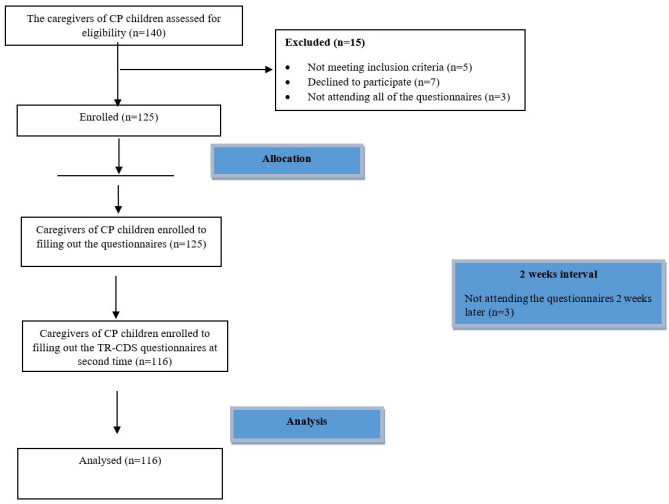

Figure 1. Flowchart of the study

Primary investigator administered the TR-CDS to caregivers of children with CP (10). The caregivers were also asked to fill in the World Health Organization Quality of Life-Brief Form (WHOQOL-BREF), Impact on Family Scale (IPFAM), Beck Depression Inventory-II (BDI-II), Nottingham Health Profile (NHP), and Fatigue Severity Scale (FSS) for the validity of TR-CDS (14-17). CDS's subscales consist of support for caregiving, impact on self, social and economic strain, and concerns for the

child. The CDS is similar to the IPFAM survey in terms of its subparameters. However, CDS is also predictive of caregiver psychological problems. In this respect, the BDI-II was used for the validity of the TR-CDS. NHP shows the quality of life in relation to their health, however the WHOQOL-BREF is as a valid and reliable alternative for the assessment of the quality of life people with long-term mental health conditions. Considering CDS questionnaire and subparameters, WHOQOL-BREF and NHP tests were used because they were thought to be related to the caregivers' quality of life. FSS was used because it shows caregivers' long-term effects on the disease in terms of fatigue levels which shows impact on self.

Two weeks later, the same caregivers were given the TR-CDS questionnaire once more for the reliability.

The caregivers were given all of the surveys to complete at the same time. All of the questionnaires took an average of forty-five minutes.

Prior to the data collection, every caregiver signed an informed consent form. The SANKO University Clinical Research Ethics Committee granted the study ethical permission (2019/01). The study registered with the clinical trial number of NCT04037137.

Measurements

Caregiver Difficulties Scale (CDS): One multifaceted instrument that can be utilized on its own is the CDS. It has 25 items (CDS-T). There are four subscales made out of the items: support for caregiving (CDS-SC) (5), impact on self (CDS-IS) (7), social and economic strain (CDS-S&E) (5), and concerns for the child (CDS-CC) (8). A total score between 0 and 100 is obtained by adding the scores of each item, which are rated on a 5-point scale (0–4) that represents the frequency and intensity of each caring encounter as reported by the caregivers. A high score indicates that the caregiver was over burden (10).

Impact on Family Scale (IPFAM): The impact of the burden on caregivers was measured using the Turkish version of the IPFAM. The 33-item IPFAM inventory takes ten to fifteen minutes to finish. A score of 4 (strongly agree) to 1 (strongly disagree) is assigned to each item. Six items assess the impact of the impaired child on siblings, while 27 items gauge the overall effect on the family. The IPFAM subscales include financial support (IPFAM-FS), coping (IPFAM-C), disruption of social relationships (IPFAM-SR), and overall impact (IPFAM-TI). Low impact is indicated by low scores (11). The validity and reliability (ICC=0.95) of this questionnaire are shown in the Turkish version (11).

Nottingham Health Profile (NHP): NHP evaluated the caregivers' quality of life in relation to their health. The NHP is a subjective measure of health that assesses suffering in the following subgroups: social isolation (5), mobility (8), pain (8), emotional reactions (9), sleep (5), and energy (3). Every subgroup has a score ranging from 0 to 100. NHP's validity and reliability (ICC=0.87) are demonstrated in the Turkish version (18,19).

Fatigue Severity Scale (FSS): Nine items make up the FSS. The individuals was asked to indicate how much they agreed with each statement by selecting between 1 and 7. One score denotes a strong disagreement, while seven denotes a strong agreement. Severe weariness is often indicated by a score of 4 or higher. Nine is the lowest score while 63 is the best. A high score denotes a high level of weariness severity. Armutlu et al. carried out the validity and reliability assessment of the scale in Türkiye. Test-retest reliability exists for patients with multiple sclerosis (ICC=0.81), fibromyalgia (ICC: 0.94) and the scale's internal consistency was determined to be good (16,20).

Beck Depression Inventory-II (BDI-II): The Turkish version of the BDI- II was used. A general depressive state's associated behavior and emotions are measured with the 21-item Beck Depression Inventory (BDI) (ICC=0.90) (21).

World Health Organization Quality of Life-Brief Form (WHOQOL-BREF): Both the short version of WHOQOL-BREF and the long version of WHOQOL were employed; the WHOQOL-BREF is particularly helpful for clinical and service evaluations. Quality of life is defined by the World Health Organization (WHO) as "individuals' perceptions of their positions in life in the context of the culture and value systems in which they live and regarding their goals, expectations, standards, and concerns." This definition forms the basis of this measurement. The WHOQOLBREF is a legitimate and trustworthy substitute for the evaluation of individuals with chronic mental illnesses. It comprises a total of 26 questions with four categories: psychological, physical, social interactions, and environment. It is rated from 1 to 5 on a Likert-type scale, where higher numbers denote a higher quality of life. Every sub-parameter has a score between 0 and 100, where 100 represents the highest quality of life and 0 the lowest. Internal consistency, reliability, and construct validity (ICC=0.34) of the Turkish version of the measure are all rather good (22).

Statistical analysis

Statistical analyses were conducted using the IBM SPSS 24 package application (SPSS Inc., Chicago, IL, USA). When post-hoc power analysis was carried out, the link between CDS and IPFAM, one of the study's data, indicated that the study's power was 0.99 (G*Power 3.1, Düsseldorf, Germany). The correlation between the TR-CDS and NHP, FSS, IPFAM, BDI-II, and WHOQOL-BREF was used to assess the construct validity. Correlation coefficient values fall between -1.0 to 1.0. A computed value that is more than 1.0 or less than -1.0 indicates that the correlation measurement had an error. The total negative correlation is indicated by a correlation of -1.0, and whereas the perfect positive correlation is shown by a correlation of 1.0. Test-retest reliability and internal consistency were used to assess the reliability of the questionnaire. The Cronbach α value was used to assess internal consistency, and the intraclass correlation coefficient (ICC) and comparison of measurement scores at various times were used to assess test-retest reliability. An ICC score of less than

0.5 indicates poor reliability, a value between 0.5 and 0.75 indicates moderate reliability, a value between 0.75 and 0.9 indicates high dependability, and a value greater than 0.90 indicates outstanding reliability. For all statistical studies, the p value was evaluated at the 0.05 significance level. Based on the knowledge that the sample size should be at least 5-10 times of the number of surveys' items in validity and reliability studies, it was aimed to reach a sample size of 125 caregivers for the current study (23,24). But some of the caregivers didn't attend the questionnaires 2 weeks later. So the test-retest part of the study completed with the 116 caregivers of children with CP.

RESULTS

In this study, caregivers (n=116) were 94 (81%) female and 22 (19%) male, with a mean age of 37.20±10.36 years. The children were 59 (50.9%) female and 57 (49.1%) male, with a mean age of 7.10±5.08 years. Table 1 shows the sociodemographic information for each caregivers (Table 1).

Table 1. Sociodemographic information of the individuals in the study

Variables			(n=116) X±SD (Min-max)
Age (year)		Parents	37.20±10.36 (17-65)
		Children	7.10±5.08 (1-18)
Weight (kg)		Parents	72.60±18.10 (4-113)
		Children	31.77±22.26 (6-82)
Height (cm)		Parents	159.98±24.41 (165-185)
		Children	118.68±34.63 (130-175)
Caregiver n (%)		Mother	90 (77.6%)
		Father	18 (15.5)
		Others	8 (6.9)
Caregivers' working status		Yes	36 (68.96)
		No	80 (31.03)
Sex	Male n (%)	Parents	22 (19)
	Female n (%)		94 (81)
	Male n (%)	Children	57 (49.1)
	Female n (%)		59 (50.9)
Education level of caregivers n (%)		Primary	52 (44.8)
		Secondary	27 (23.3%)
		High	21 (18.1)
		University	16 (13.8)
Type of CP		Hemiparetic	34 (29.31%)
		Diparetic	37 (31.89%)
		Quadriparetic	25 (21.55%)
		Dyskinetic	17 (14.65%)
		Mixt	3 (2.58%)

n:number, %: percent, X: mean, SD: standard deviation, min: minimum, max: maximum, CP: cerebral palsy, kg: kilograms, cm: centimeters

Table 2 shows the mean of the sub-parameters for all tests as well as the mean of the test-retest of the TR-CDS sub-parameters (Table 2).

Table 2. Averages of TR-CDS parameters, IPFAM, NHP, WHOQOL-BREF (SR), and BDI-II test scores

	Number of Items	Test X±SD (min-max)	Retest X±SD (min-max)
CDS (CC)	8	18.06±7.64 (2-30)	17.79±9.85 (2-79)
CDS (IS)	7	12.12±6.66 (0-27)	11.44±6.85 (0-26)
CDS (SC)	5	8.51±4.5 (0-20)	8.06±4.72 (0-20)
CDS (S&E)	5	8.49±4.19 (0-19)	8.35±4.54 (0-19)
CDS (T)	25	47.19±17.53 (3-83)	45.66±19.65 (3-129)
		Test X±SD (min-max)	
IPFAM (FS)		8.12±2.61 (3-12)	
IPFAM (GI)		25.5 5±6.52 (7-47)	
IPFAM (SR)		22.49±6.61 (9-42)	
IPFAM (C)		7.54±5.60 (3-61)	
IPFAM (TI)		48.25±12.55 (20-87)	
NHP (P)		30.44±32 (0-100)	
NHP (ER)		28.64±29.46 (0-100)	
NHP (S)		25.58±32.29 (0-100)	
NHP (SI)		20.96 ±29.22 (0-100)	
NHP (PA)		21.67±23.46 (0-88.46)	
NHP (E)		43.39±37.84 (0-100)	
NHP (T)		169.23±135 (0-510.59)	
FSS		4.15±2.39 (0-21)	
WHOQOL-BREF (GH)		50.29±21.63 (0-100)	
WHOQOL-BREF (PH)		61.54±20.85 (0-100)	
WHOQOL-BREF (PS)		61.24±18.09 (0-100)	
WHOQOL-BREF (SR)		56.64±25.74 (0-100)	
WHOQOL-BREF (E)		56.60±15.58 (9.37-106.25)	
BDI-II		15.07±10.79 (0-47)	

CDS (CC): Caregiver Diffuculties Scale (CDS)-concerns for the child, CDS (IS): CDS-Impact on self, CDS (SC): CDS-Support for caregiving, CDS (S&E): CDS-Social&Economic strain, CDS (T): CDS total, IPFAM (FS): IPFAM financial support, IPFAM (GI): IPFAM general impact, IPFAM (SR): IPFAM disorders of social relationship, IPFAM (C): IPFAM coping, IPFAM (TI): IPFAM total impact, NHP (P): Nottingham Health Profile (NHP)-pain, NHP (ER): NHP-emotional reactions, NHP (S): NHP-sleep, NHP (SI): NHP-social isolation, NHP (PA): NHP-physical activity, NHP (E): NHP-Energy, NHP (T): NHP-total, FSS: Fatigue Severity Scale, WHOQOL-BREF (GH): World Health Organization Quality of Life–Brief Form general health, WHOQOL-BREF (PH): Physical health, WHOQOL-BREF (PS): Psychological score, WHOQOL-BREF (SR): Social relationships, WHOQOL-BREF (E): Environment, BDI-II: BeckDepression Inventory- II

Strong construct validity was proven by the TR-CDS scale, as shown by the significant correlations with NHP, FSS, IPFAM, WHOQOL-BREF, and BDI-II as well as between total score (TR-CDS -T) and its subscales. There were no significant correlations found between the total score of TR-CDS and any of its subscales and IPFAM (C) (Table 3). TR-CDS (T) and IPFAM (TI) showed a moderately strong positive correlation (r=0.454, p=0.000); TR-CDS (IS) and BDI-II showed a moderately significant positive correlation (r=0.555, p=0.000); CDS (S&E) and IPFAM (FS) and IPFAM (SR) showed a moderately strong positive correlation (r=0.534, p=0.000); and NHP (SI) showed a significant

and good correlation (r=0.308, p=0.001). TR-CDS (T) and TR-CDS (IS) showed a moderate, significant, negative correlation (r=-0.390 p=0.001), as did WHOQOL-BREF (GH) (r=-0.467 p=0.000). And moderate strong positive correlation (r=0.481 p=0.000) was found between NHP (ER) and TR-CDS (T). Also moderate strong positive correlation (r=0.483 p=0.000) between TR-CDS (IS) and NHP (ER). Strong moderate correlation was found between TR-CDS (T) and NHP (T) (r=0.588, p=0.000) (Table 3).

Cronbach's alpha for the TR-CDS total impact score was determined to be 0,936 (Table 4). The good test-retest reliability for the CDS subscales (ICC=0.707-0.879) were shown in Table 4.

Table 3. Spearman Correlation Coefficients Between TR-CDS and IPFAM, NHP, FSS, WHOQOL-BREF, BDI-II in caregivers of children with CP (n=116)

	CDS (CC)	CDS (IS)	CDS (SC)	CDS (S&E)	CDS (T)
	p (r)	p (r)	p (r)	p (r)	p (r)
IPFAM (FS)	p<0.001* (0.433)	p<0.001* (0.391)	0.058 (0.177)	p<0.001* (0.534)	p<0.001* (0.511)
IPFAM (GI)	p<0.001* (0.325)	p<0.001* (0.382)	0.041* (0.190)	p<0.001* (0.496)	p<0.001* (0.454)
IPFAM (SR)	p<0.001* (0.426)	p<0.001* (0.452)	0.125 (0.143)	p<0.001* (0.550)	p<0.001* (0.526)
IPFAM (C)	0.721 (0.034)	0.970 (-0.004)	0.5 (-0.063)	0.608 (0.048)	0.928 (0.009)
IPFAM (TI)	p<0.001* (0.337)	p<0.001* (0.399)	0.158 (0.132)	p<0.001* (0.507)	p<0.001* (0.454)
NHP (P)	p<0.001* (0.320)	p<0.001* (0.457)	0.006* (0.255)	p<0.001* (0.396)	p<0.001* (0.474)
NHP (ER)	0.001* (0.313)	p<0.001* (0.483)	0.011* (0.235)	p<0.001* (0.422)	p<0.001* (0.481)
NHP (S)	p<0.001* (0.378)	p<0.001* (0.438)	0.3 (0.097)	p<0.001* (0.448)	p<0.001* (0.463)
NHP (SI)	0.124 (0.144)	0.001* (0.308)	p<0.001* (0.371)	0.001* (0.292)	p<0.001* (0.345)
NHP (PA)	0.019* (0.217)	0.001* (0.311)	0.110 (0.149)	0.001* (0.306)	p<0.001* (0.325)
NHP (E)	p<0.001* (0.325)	p<0.001* (0.446)	0.013* (0.230)	p<0.001* (0.404)	p<0.001* (0.467)
NHP (T)	p<0.001* (0.394)	p<0.001* (0.556)	0.001* (0.308)	p<0.001* (0.526)	p<0.001* (0.588)
FSS	0.005* (0.262)	0.001* (0.293)	0.347 (0.088)	0.079 (0.164)	0.002* (0.287)
WHOQOL-BREF (GH)	p<0.001* (-0.325)	p<0.001* (-0.390)	0.001* (-0.300)	p<0.001* (-0.423)	p<0.001* (-0.467)
WHOQOL-BREF (PH)	0.010* (-0.240)	p<0.001* (-0.326)	0.481* (-0.066)	0.019* (-0.218)	0.001* (-0.297)
WHOQOL-BREF (PS)	0.020* (0.216)	p<0.001* (-0.450)	0.004* (-0.268)	0.007* (-0.250)	p<0.001* (-0.393)
WHOQOL-BREF (SR)	0.036* (-0.196)	0.001* (-0.238)	p<0.001* (-0.382)	p<0.001* (-0.354)	p<0.001* (-0.358)
WHOQOL-BREF (E)	p<0.001* (-0.349)	p<0.001* (-0.349)	p<0.001* (-0.411)	p<0.001* (-0.410)	p<0.001* (-0.488)
BDI-II	p<0.001* (0.356)	p<0.001* (0.555)	p<0.001* (0.322)	p<0.001* (0.447)	p<0.001* (0.554)

Pearson correlation, *p<0,05, r: correlation coefficient, CDS (CC): Caregiver Diffuculties Scale (CDS)-concerns for the child, CDS (IS): CDS-Impact on self, CDS (SC): CDS-Support for caregiving, CDS (S&E): CDS-Social&Economic strain, CDS (T): CDS total, IPFAM (F): IPFAM financial support, IPFAM (GI): IPFAM general impact, IPFAM (SR): IPFAM disorders of social relationship, IPFAM (C): IPFAM coping, IPFAM (TI): IPFAM total impact, NHP (P): Nottingham Health Profile (NHP)-pain, NHP (ER): NHP-emotional reactions, NHP (S): NHP-sleep, NHP (SI): NHP-social isolation, NHP (PA): NHP-physical activity, NHP (E): NHP-Energy, NHP (T): NHP-total, FSS: Fatigue Severity Scale, WHOQOL-BREF (GH): World Health Organization Quality of Life–Brief Form general health, WHOQOL-BREF (PH): Physical health, WHOQOL-BREF (PS): Psychological score, WHOQOL-BREF (SR): Social relationships, WHOQOL-BREF (E): Environment, BDI-II: Beck Depression Inventory-II

Table 4. Internal consistency and test-retest reliability results of the TR-CDS

n=116	2 assessments on 2 sessions with 15day interval			Caregivers	
		Number of Items	Cronbach's Alpha	ICC*	95% CI
Test-Retest Reliability	CDS1-2 (CC)	8	0.828	0.707	0.603-0.787
	CDS1-2 (IS)	7	0.915	0.843	0.781-0.889
	CDS1-2 (SC)	5	0.863	0.759	0.670-0.827
	CDS 1-2 (S&E)	5	0.905	0.827	0.759-0.877
	CDS 1-2 (T)	25	0.936	0.879	0.830-0.915

*Two-way mixed-effect model on average measures with absolute agreement definition. CI: confidence interval, ICC: intra-class correlation coefficient, CDS1 (CC): first assessment of caregiver diffuculties scale (CDS)-concerns for the child, CDS2 (CC): second assessment of the CDS-concerns for the child, CDS1 (IS): first assessment of the CDS-Impact on self, CDS2 (IS): second assessment of the CDS- Impact on self, CDS1 (SC): first assessment of the CDS-Support for caregiving, CDS2 (SC): second assessment of the CDS-Support for caregiving, CDS1 (S&E): first assessment of the CDS-Social&economic strain, CDS2 (S&E): second assessment of the CDS-Social&economic strain, CDS1 (T): first assessment of the CDS total, CDS2 (T): second assessment of the CDS total

DISCUSSION

The CDS, which assesses the challenges faced by caregivers of children with CP, was translated into Turkish in the current study, and the TR-CDS was also found to be valid and reliable in the Turkish population. Additionally, this study demonstrated the consistency and potential for measuring similar properties among all TR-CDS subscales.

Despite the fact that a lot of researches has been investigated the social, emotional, and financial implications of caring for children with CP, it was found that the majority of caregiver burden questionnaires were English. This is why the current study aimed to have the TR-CDS questionnaire first. This questionnaire was used in this study without any modifications, and it was determined that the Turkish population could easily interpret the questionnaire. Furthermore, TR-CDS is considered to be a useful screening tool for identifying caregivers who might be at risk for psychological problems such as stress, anxiety, and depression.

Establishing the validity of the Turkish version of the scale, which assesses the burden of caring for children with CP, was another important goal of the current study. Based on previous studies, factors such as a family's financial situation, educational level, the number of family members, age of the child, and the amount of time that caregivers spend with their children, raise stress levels, and increases the caregivers' burden (5,8,25). No relationship was found between the age of the children and any of the sub-parameters, specifically financial support in the current study. Nonetheless, the current study examined how the disease affected caregivers using five subscales. The construct validity of the TR-CDS was also established by using the correlation of the IPFAM total score. We can therefore state that IPFAM has good validity, with the exception of coping and total effect. The relationship found between the overall score of IPFAM and the total effect score of TR-CDS indicates that having a children with CP can serve as a good predictor of the caregiver's burden. It has also been demonstrated that when using comparable screening scales, the impact of children with CP on their caregivers can lead to parallel findings in terms of caregiver burden. The highest relationships between the IPFAM and TR-CDS subscales were seen in the IPFAM social relations, financial support, and IPFAM total effect, and TR-CDS total effect, and TR-CDS social or economic challenges. These results demonstrated the need for further in-depth research on the family's employment situation, level of financial assistance, and interpersonal relationships. There was no correlation between the TR-CDS subscales and IPFAM coping score. We think that the absence of questions about coping in the TR-CDS led to this result. CDS scale consists of the 4 subscales called concerns for the child, impact on self, Support for caregiving, social & economic strain. This result shows where CDS and IPFAM differ from each other. The IPFAM survey examines the family's ability to cope with the problems, as well as the family's impact on the child's illness. Farajzadeh et all stated that all subscales of the CDS were positively correlated with the caregiver burden scale (CBS), BDI-II and FSS and negatively correlated with the WHOQOL-BREF similar to our study (9).

The strong correlations seen between the NHP total score, the TR-CDS subscales, and the overall score point to a detrimental effect on caregivers' health condition as caregiver burden rises. Analogous research has demonstrated a decline in health-related quality of life with an increase in caregiver burden (26).

The need for psychosocial support platforms to be developed in order to evaluate these people's depression levels is indicated by the strong correlations found between the BDI-II and the individual effect score and overall score of the TR-CDS subscales. These findings were discovered to be consistent with findings from other research investigations in the literature (27-29).

Compared to healthy groups, having a dependent kid and playing the role of caregiver to children with CP puts mothers and children under stress and increases the likelihood that depression may develop. The WHOQOL-BREF and TR-CDS domains displayed moderate, strong, and negative correlationon, in line with previous research. It was discovered that these outcomes agreed with both the initial research of Carona et al. It was found to be similar to the results with this study. Carone et al. showed that quality of life was impacted by the stress of giving care in both direct and indirect ways. Caregivers experience a profound decline in their psychological and physical well-being and quality of life as the demands and challenges of providing care grow, leaving them feeling hopeless. Our findings support those of previous research, which found a connection between quality of life and caregiver burden of care (9,28,30,31). This demonstrated how caregivers' quality of life declined as their workload rose. With the exception of the psychological parameter, a negative connection was found between the overall scores for the WHOQOL-BREF domains and TR-CDS subscales. This finding demonstrated that psychological warfare and survival skills developed along with the child's caregivers' worry levels.

Cronbach's alpha values were found to be consistent and equivalent to the internal consistency values of the other versions of the CDS in terms of reliability results of the TR-CDS study (9,10). Cronbach α value for the total scale of TR-CDS was 0.911 similar to our study (0.936) (10). Cronbach α of 0.68-0.84 in all subscales indicated satisfactory internal consistency within each subscale (10). But our results in the current study had higher internal consistency levels within each subscale (0.828-0.915). Farajzadeh et al. was found the Cronbach α values of TR-CDS between 0.743-0.887 in total score and subscales (9). These results was similar to our study. It shows a quite satisfactory that these internal consistency results are higher for each subscale and total score of the

TR-CDS.

Test-retest reliability measures the questionnaire's stability over time by administering the same TR-CDS to the same subjects twice. Two-week interval was used in this research. Similar studies have noted that test-retest reliability measurements were obtained using parent interviews over a two-week interval (5,8,9,25). Test-retest reliability was assessed twice using the Intraclass Correlation Coefficient (ICC). Our study's results demonstrated excellent test-retest reliability, matching that of the CDS versions from Iran (9). ICC values was 0.743–0.848 in the Iranian version which is similar to our study (ICC=0.707-0.879). The results related to the reliability of this study were consistent with the results of the Iranian version of this scale.

For a global understanding of the measuring properties of these questions, it is imperative that the questionnaires be translated and validated into multiple languages. These investigations make it possible to safely administer the same questionnaires in cross-cultural comparison studies and in various cultural contexts (32-35). According to us, the TR-CDS can be used to evaluate the advantages and disadvantages of social services that help families transition to community life and support their children's rehabilitation process by measuring the stress experienced by caregivers of children with CP.

We think that this useful and simple-to-use questionnaire is crucial for assessing the burden that caregivers bear in relation to the anxiety of their children, their own effects, the care they receive, and social and economic challenges. It also helps to pinpoint the areas that require additional support for families.

A constraint of our research was that it was limited to four distinct Turkish cities that housed rehabilitation clinics. In this regard, it is thought that assessing the caregiver burden in cities with varying socio-cultural levels will more accurately represent Türkiye. Other cities and caregivers from different socioeconomic backgrounds could be included to help determine the caregiver burden profile and establish social support programs in Türkiye. Even though the caregivers in the rehabilitation center in this study were selected at random and satisfied the minimal requirements outlined by Fidell and Tabachnick (36), it is advised that more extensive sampling studies be carried out nationwide. Our study was further limited by the small number of fathers who provided primary care.

CONCLUSION

The present study's findings demonstrated the validity and reliability of the TR-CDS as a tool for assessing the diffuculties that families and caregivers of children with CP bear. Test-retest scores, validity assessments, and total-sub-questionnaire correlations all demonstrate the usefulness of the TR-CDS as a family effect measurement instrument.

As the first study on the burden of care among Turkish caregivers of children with CP, the current study revealed the TR-CDS scale's "good-excellent" psychometric qualities. TR-CDS can be used as a particular assessment instrument to evaluate the burden before planning some treatments to identify the requirements of caregivers of children with CP in Turkey and to minimize the caregivers burden. But further study is needed to determine the scale's applicability to other groups. Finding the responsibilities and challenges brought on by their size and care is one method to help these people live better lives. To calculate the care burden, a method that measures the dimensions of the weight of obligation is needed. Because of this, it can be a helpful tool in identifying caregiver issues due to its excellent psychometric qualities when combined with the TR-CDS 4 subscale.

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Research Article

Inflammation Severity in Radicular Cysts and Its Relationship with Age and Gender

Muruvvet Akcay Celik[1], Havva Erdem[1], Fadime Gul Salman[2], Busra Ersan Erdem[2], Hilal Balta[3], Yeliz Kasko Arici[4], Ozge Kaya Korkmaz[1]

[1]Ordu University, Faculty of Medicine, Department of Pathology, Ordu, Türkiye
[2]Ordu Training and Research Hospital, Department of Pathology, Ordu, Türkiye
[3]Fırat University, Faculty of Medicine, Department of Pathology, Ordu, Türkiye
[4]Ordu University, Faculty of Medicine, Department of Biostatistics and Medical Informatics, Ordu, Türkiye

Abstract

Aim: In this study, in radicular cyst cases in our department; It was aimed to evaluate the severity of inflammation (active and chronic inflammation) and to investigate its relationship with age and gender.
Material and Method: Radicular cyst cases between 01.01.2013 and 31.01.2022 in Ordu University, Faculty of Medicine, Department of Pathology were included in the study. Ethical approval was taken from Ordu University Medical School, Clinical Trials Ethical Committee (2022/82). Preparations of the cases were found from the archive and examined microscopically to score the severity of inflammation. To examine any relationships that existed between categorical variables, Chi-square test was used. To determine any relationships between the score variables, correlation analysis was done.
Results: A statistically significant moderate negative correlation was observed between age and chronicity in women (r=-0.410, p=0.013). A statistically significant moderate positive correlation was observed between activity and chronicity scores in men (r=0.592, p<0.001). When all patients were considered, weakly significant negative correlation was found between activity and chronicity (r=0.312, p<0.001). While one was increasing, the other was increasing.
Conclusion: In radicular cyst cases, the severity of inflammation may differ from case to case. In our study, the severity of chronic inflammation was observed as high-grade (grade 3) in most of the cases, acute inflammation was found to be mild (grade 1) in most of the cases. It is thought that determining the severity and type of inflammation in the histopathological evaluation may be beneficial for diagnosis and treatment.

Keywords: Radicular cyst, odontogenic cyst, inflammation

INTRODUCTION

Odontogenic cysts are generally divided into inflammatory and developmental types according to their etiology. Developmental cysts; includes dentigerous cyst, primordial cyst, eruption and gingival cysts. Inflammatory odontogenic cysts include lateral periodontal cysts and radicular cysts (RC) (1). Inflammatory periapical lesions constitute 63.24% of the material examined in the oral pathology department (2). Apical inflammatory lesions are generally thought to occur with the progression of dental caries causing pulp necrosis (3). Ramachandran Nair et al. detected 39 (15%) of 256 periapical lesions as apical cysts (4).

RC is a subtype of apical lesions, and its prevalence varies between various studies. In a study by Johnson et al., it was reported that RC were observed with a rate of 54.6% among odontogenic cysts (5). Cystic lesions are partially or completely (true cyst) covered by epithelial lining and these lesions are defined as pathological cavity associated with damaged tooth apex (6,7). Microscopically, the capsule consists of cystic epithelium supported by connective tissue; stratified squamous epithelial tissue consists of several layers of cells (8,9).

RC are common lesions in daily dental practice. Histologically, the lumen of the cyst is lined by stratified squamous epithelium, which originates in the epithelial remnants of Malassez. The cyst wall consists of fibrous connective tissue containing a chronic infiltrate of

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Corresponding Author: Muruvvet Akcay Celik, Ordu University, Faculty of Medicine, Department of Pathology, Ordu, Türkiye
E-mail: drmakcaycelik@gmail.com

inflammatory cells. Intense bone resorption by active osteoclasts contributes to the intraosseous expansion of the RC growing within the periapical bone tissue. However, the mechanisms related to cavity enlargement and epithelial lining formation are not fully known (10).

MATERIAL AND METHOD

Radicular cyst cases between 01.01.2013 and 31.01.2022 in ODU Faculty of Medicine, Department of Pathology were included in the study. Preparations of the cases were found from the archive. Hematoxylin&eosin (HE) stained preparations were examined microscopically to score the severity of inflammation. RC cases were divided into 3 grades according to the severity of inflammation. Each preparation is graded at 200X magnification. All samples were graded for inflammatory status in three consecutive microscopic fields. For inflammation, examination was performed starting from the epithelial-connective tissue border and extending to the lamina propria.

Grade I: Inflammatory cells, less than 1/3 per area,

Grade II: Inflammatory cells, 1/3 to 2/3 per area

Grade III: inflammatory cells scored more than 2/3 per area (10).

Data Analysis

Chi-square test was used to examine any relationships that existed between categorical variables. In the test, Likelihood ratio test statistic was used instead of Pearson's test statistic when the expected counts were <5 in the crosstabs. To determine relationships between the activity, Chronicity, and age, Spearman's rank correlation coefficient was calculated. The type I error rate was taken into account as 5%. IBM SPSS v28 (IBM, Armonk, NY, USA) was used as a statistical program.

RESULTS

Frequency analysis of the data was performed and a total of 70 patients, 51.4% female and 48.6% male, between 2013-2021 were included in the study. 36 patients were female, 34 patients were male. The mean age was found to be 36.64±15.52.

According to Table 1, RC was observed more frequently in the mandible (57.1%) as localization.

Table 1. Distribution of samples according to localizations and activity and chronicity scores

		n	%
Localization	**Maxilla anterior**	9	12.9
	Maxilla right	9	12.9
	Maxilla left	12	17.1
	Mandible anterior	5	7.1
	Mandible right	18	25.7
	Mandible left	17	24.3
Activity score	**0**	28	40.0
	1	33	47.1
	2	7	10.0
	3	2	2.9
Chronicity score	**1**	12	17.1
	2	23	32.9
	3	35	50.0

The relationships between age, activity and chronicity are shown in Table 2. The activity score did not change with increasing age in women and there was no significant relationship between them (r=-0.156, p=0.363). The chronicity score showed an inverse relationship with age, and a moderately significant negative correlation was determined between age and chronicity (r=-0.410, p=0.013). There was no significant relationship between activity and chronicity scores (r=0.312, p=0.064).

The relationship between age and activity in men was not statistically significant (r=-0.154, p=0.386). Similarly, there was no significant relationship between age and chronicity (r=-0.243, p=0.167). A moderately significant positive correlation was observed between activity and chronicity scores (r=0.592, p<0.001).

Considering all the patients, there was no statistically significant relationship between age and chronicity (r=-0.183, p=0.128), but there was a moderately significant negative correlation between age and chronicity (r=-0.354, p=0.003). A weakly significant positive correlation was also observed between activity and chronicity (r=0.312, p<0.001), while one was increasing, the other was increasing too.

Table 2. Relationship between age, activity and chronicity

			Age		Activity	
		n	r	p	r	p
Female	**Activity**	36	-0.156	0.363	0.312	0.064
	Chronicity	36	-0.410	0.013		
Male	**Activity**	34	-0.154	0.386	0.592	**<0.001**
	Chronicity	34	-0.243	0.167		
Total	**Activity**	70	-0.183	0.128	0.481	**<0.001**
	Chronicity	70	-0.354	0.003		

r: Spearman's rho correlation coefficient

According to Chi-square test, there was no significant relationship between localization and activity score (p=0.380). The activity score did not differ according to localizations (Table 3).

According to Chi-square test, there was no significant correlation between localization and chronicity score (p=0.599). The chronicity score did not differ according to localizations (Table 4).

According to the Chi-square test, the activity score did not differ significantly according to localization (p=0.207). The chronicity score also did not change according to localization (p=0.380) (Table 5).

Table 3. Relationship between localization and activity score

	Activity								Total	
	0		1		2		3			
Localization	n	%	n	%	n	%	n	%	n	%
Maxilla anterior	4	44.4	2	22.2	2	22.2	1	11.1	9	100.0
Maxilla right	2	22.2	7	77.8	0	0.0	0	0.0	9	100.0
Maxilla left	4	33.3	5	41.7	2	16.7	1	8.3	12	100.0
Mandible anterior	2	40.0	3	60.0	0	0.0	0	0.0	5	100.0
Mandible right	6	33.3	10	55.6	2	11.1	0	0.0	18	100.0
Mandible left	10	58.8	6	35.3	1	5.9	0	0.0	17	100.0
Total	28	40.0	33	47.1	7	10.0	2	2.9	70	100.0
p	0.380 (χ^2=16.037)									

χ^2: Chi-square test

Table 4: Relationship between localization and chronicity score

	Chronicity								Total	
	0		1		2		3			
Localization	n	%	n	%	n	%	n	%	n	%
Maxilla anterior	3	33.3	2	22.2	4	44.4	9	100.0	9	100.0
Maxilla right	0	0.0	4	44.4	5	55.6	9	100.0	9	100.0
Maxilla left	3	25.0	3	25.0	6	50.0	12	100.0	12	100.0
Mandible anterior	1	20.0	3	60.0	1	20.0	5	100.0	5	100.0
Mandible right	3	16.7	5	27.8	10	55.6	18	100.0	18	100.0
Mandible left	2	11.8	6	35.3	9	52.9	17	100.0	17	100.0
Total	12	17.1	23	32.9	35	50.0	70	100.0	70	100.0
p	0.599 (χ^2=8.307)									

χ^2: Chi-square test

Table 5. Relationship between localization and activity, chronicity score

		Location				Total		p
		Maxilla		Mandible				
		n	%	n	%	n	%	
Activity	0	10	33.3	18	45.0	28	40.0	0.207
	1	14	46.7	19	47.5	33	47.1	(χ^2=4.561)
	2	4	13.3	3	7.5	7	10.0	
	3	2	6.7	0	0.0	2	2.9	
	Total	30	100.0	40	100.0	70	100.0	
Chronicity	1	6	20.0	6	15.0	12	17.1	0.827
	2	9	30.0	14	35.0	23	32.9	(χ^2=0.380)
	3	15	50.0	20	50.0	35	50.0	
	Total	30	100.0	40	100.0	70	100.0	

χ^2: Chi-square test

According to Chi-square test, there was a significant correlation between activity and chronicity score (p<0.001). Activity score varied according to chronicity score (Table 6). The distributions of those with a chronicity score of 1, 2, and 3 in those with an activity score of 0 were 35.7%, 42.9%, and 21.4%, respectively. This ranking is 3.0%, 30.3% and 66.7% for those with an Activity score1, and 0.0%, 14.3% and 85.7% for those with an Activity score2, and 50.0%, 0.0%, and 50.0% in those with an Activity score3. Among those with an activity score of 0, those with chronicity 2 had the highest frequency (42.9%), those with activity scores 1 and 2 had a higher frequency of those with chronicity 3 (66.7% and 85.7%, respectively).In those with an activity score of 3, the patient with a chronicity score of 2 was never seen and half of the patients had a chronicity score of 1 (50.0%) and the other half had a chronicity score of 3 (50.0%). Chronic inflammatory cell infiltration is observed in figures 1 and 2.

Table 6. Relationship between activity and chronicity score

	Chronicity						Total	
	1		2		3			
Activity	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
0	10	35.7	12	42.9	6	21.4	28	100.0
1	1	3.0	10	30.3	22	66.7	33	100.0
2	0	0.0	1	14.3	6	85.7	7	100.0
3	1	50.0	0	0.0	1	50.0	2	100.0
Total	12	17.1	23	32.9	35	50.0	70	100.0
p	**<0.001** (χ^2=25.405)							

χ^2: Chi-square test

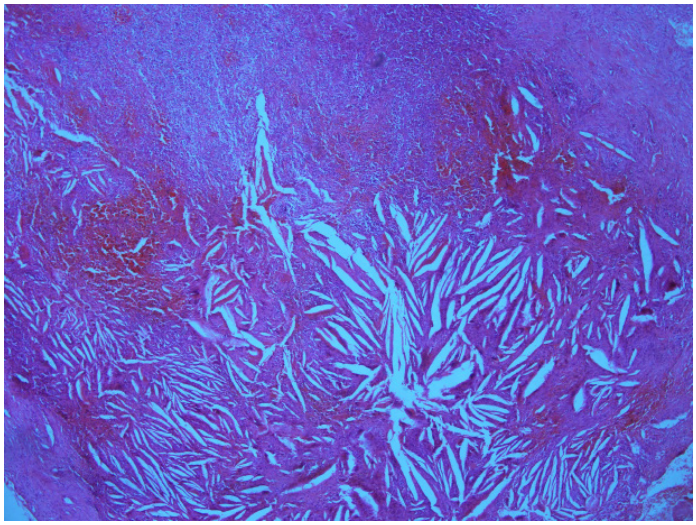

Figure 1. HEx200, Grade II score and cholesterol clefts

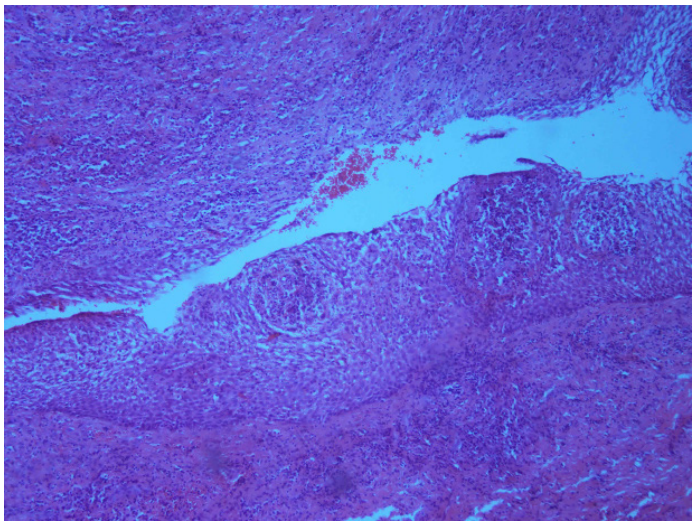

Figure 2. HEx200, Grade III score

DISCUSSION

RC's are common lesions in daily dental practice. RC's are defined as inflammatory odontogenic cysts of endodontic origin The cyst wall consists of fibrous connective tissue containing a chronic infiltrate of inflammatory cells (11).

Gutmann et al. observed a lack of standardization in naming apical lesions of endodontic origin (12). Johnson et al. found that RC are the most common odontogenic cyst, followed by dentigerous cyst and keratocystic odontogenic tumor (when classified as a cyst). They observed the rate of RC as 54.6% among odontogenic cysts (5). According to studies in the literature, RCs account for 6% to 57.69% of all periapical lesions. This variation can be attributed to differences in the definition of this cyst type (13,14).

In this study, it was aimed to evaluate the severity of inflammation in RC (active and chronic inflammation) and its relationship with age and gender. Histopathological evaluation is required to score the severity of inflammation.

According to Tsai et al., most of the inflammation in a radicular cyst is predominantly lymphocytic cell infiltration, they found in their study 16.7% grade I (mild inflammation), 30.0% grade II (moderate inflammation), and 53.3% grade III (severe inflammation) (10). According to Santos et al., chronic inflammatory infiltrate is predominantly observed in radicular cyst cases. In their study, 49.3% intense inflammation, 30.1% discrete inflammation, 20.5% moderate infiltrate are observed. Acute inflammation characterized by the presence of polymorphonuclear leukocytes (7 out of 73 cases) and mixed inflammation (2 cases) were observed at a very low rate (15). In a study in the literature, the intensity of the inflammatory infiltrate in periapical lesions was observed to be variable.

All periapical lesions examined by light microscopy displayed a large number of infiltrating inflammatory cells characteristic of the chronic granulomatous inflammatory process. Inflammatory infiltrate consisting of plasma cells, lymphocytes, macrophages and rarely polymorphonuclear (PMN) leukocyte accumulation was observed (16).

In Cohen's study, the epithelium of RC is predominantly infiltrated by neutrophilic polymorphonuclear leukocytes, also the connective tissue capsule is predominantly infiltrated by chronic inflammatory cells (17). Consistent with other studies, one study found plasma cells and lymphocytes associated with the fibrous capsule (13). Although intense inflammation was observed in the studies, the intensity of the inflammatory infiltrate showed differences. Proliferative activity in epithelial cells is thought to affect the degree of inflammation in RCs (18). According to Domingues et al., there is chronic infiltration of inflammatory cells, lymphocytes and plasma cells in RCs (8).

In our study, chronic inflammation was severe in 50.0% of the cases, moderate in 32.9%, and mild in 17.1%. Active inflammation was mild in 47.1%, moderate in 10.0%, intense in 2.0%, and active inflammation grade 0 was detected in 40.0% of the cases. These findings were evaluated in accordance with the literature. In the literature, there are generally studies on detecting the intensity of chronicity. Chen et al. found radicular cyst cases to be 57.3% in women and 42.7% in men in their study.

In our study, 51.4% of the cases were female and 48.6% were male. This finding was consistent with the literature. The incidence of RS is high among third-decade patients and male gender (19). According to Lin et al., RS is most commonly found in the third to sixth decades and, it has a slight male disposition (20). In one study, the mean age of RC was 40.5 years (cases ranged from 13 to 78 years).

Lesions were mostly detected in the 3rd and 5th decades of life (50.0% of the total cases) and were observed as 18.5% in the 4th and 14.2% in the 6th decade (21). In our study, the mean age was found to be 36.64. In the study of Lin et al., approximately 60% of RCs were found in the maxilla, which tends to the anterior maxillary teeth (20). According to Suarez et al., 60.0-80.0% of RCs are in the upper jaw and preferably include anterior teeth (22). In the study of Chen et al., 86.6% of the cases were found in the maxilla and 13.4% in the mandible (21).

Anatomically, apical cysts are seen in all tooth-bearing sites of the jaw, but they are more common in maxillary teeth than mandibular teeth (19). In our study, the majority of the cases were located in the mandible, 30 of them were located in the maxilla and 40 of them were located in the mandible. This finding was not consistent with the literature.

CONCLUSION

In conclusion, radicular cysts are usually detected incidentally on routine radiographs. They may not cause symptoms and clinical signs unless they are infected. Therefore, they are an entity that should be considered in the clinic. RCs may show different histopathological findings in the same lesion, suggesting morphological variations of RCs.

Variations in cyst diagnosis can be attributed to differences in the definition of this cyst type. In RC cases, the severity of inflammation may differ from case to case.

In our study, the severity of chronic inflammation was observed as high-grade (grade 3) in most of the cases, acute inflammation was found to be mild (grade 1) in most of the cases. It is thought that determining the severity and type of inflammation in the histopathological evaluation may be beneficial for diagnosis and treatment.

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MEDICAL RECORDS-International Medical Journal

Research Article

The Effect of Bilateral Intravitreal Ranibizumab Administration on Pain in Diabetic Retinopathy

Mehmet Coskun

Karabük University, Faculty of Medicine, Department of Ophthalmology, Karabük, Türkiye

Abstract

Aim: To investigate the effect of bilateral intravitreal ranibizumab (IVR) on pain in diabetic patients.
Material and Method: Of the 42 patients who underwent bilateral IVR for diabetic retinopathy and macular edema, 42 eyes injected first were considered as group 1 and 42 eyes injected second were considered as group 2. During the injection, pain was assessed using a numerical scale (NS) and a verbal category scale (VCS) .
Results: The mean age of 20 male (47.7%) patients in the groups was 59.90±6.03 years, and the mean age of 22 female (52.3%) patients was 60.72±3.88 years (p=0.52). In Group 1, the NS was 3.78±1.11, while in Group 2 it was 4.14±1.37, the difference was statistically significant (p=0.01). In group 1, VCS was 2.30±0.71, while in group 2, VCS was 2.73±0.93, the difference was statistically significant (p=0.01).
Conclusion: In diabetic patients who underwent bilateral IVR in the same session, pain sensation in the first injected eye was found to be less. This should be taken into consideration in bilateral IVR application.

Keywords: Ranibizumab, pain, intravitreal injection

INTRODUCTION

Diabetes mellitus (DM) is a disease that causes serious morbidity and mortality, affecting approximately 246 million people worldwide according to the International Diabetes Federation. The prevalence of DM among all age groups was approximately 2.8% as of 2000, and this rate is expected to increase to 4.4% in 2030. Diabetic retinopathy (DR) and cataract are complications of DM that affect vision. DR is a frightening disease as it leads to irreversible loss of vision (1).

Ranibizumab (Lucentis; Genentech, South San Francisco, CA/Roche, Basel, Switzerland, introduced in 2006) is an officially licensed agent containing the Fab fragment of a monoclonal antibody effective on all VEGF-A subtypes, with increased affinity, prepared for intravitreal injection (2).

According to the International Organisation for the Study of Pain, pain is an unpleasant sensory and emotional experience accompanying or identifiable with existing or potential tissue damage (3). One-dimensional scales used in pain assessment are directly aimed at measuring pain intensity and the patient makes the assessment himself/herself. They are used in the evaluation of acute pain. One-dimensional scales include verbal category, numerical and visual comparison scale. The numerical scale (NS) is a method for determining the intensity of pain and aims to explain the patient's pain with numbers. Numerical scales start with the absence of pain (0) and reach up to the level of unbearable pain (10). The verbal category scale (VCS) is also called simple descriptive scale and is based on the patient's selection of the most appropriate word to describe the pain condition. Pain intensity is ranked from mild to unbearable (4,5).

In bilateral intravitreal ranibizumab (IVR) application, between the first injected eye and the second injected eye The pain sensation may be different, this should not be ignored during the injection.

MATERIAL AND METHOD

Eighty-four eyes of 42 patients who underwent bilateral IVR for DR and macular oedema in the ophthalmology

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Corresponding Author: Mehmet Coskun, Karabük University, Faculty of Medicine, Department of Ophthalmology, Karabük, Türkiye
E-mail: mdmehmetcoskun@gmail.com

department of Karabük University Medical Faculty Training and Research Hospital were evaluated with the approval of the ethics committee. Forty-two eyes of the patients who were injected first were considered as group 1 and 42 eyes who were injected afterwards were considered as group 2. Patients who underwent previous intravitreal injection, underwent any ocular surgery and were not able to co-operate were excluded from the study. During the injection, pain was assessed using a NS (Figure 1) and a VCS (Figure 2).

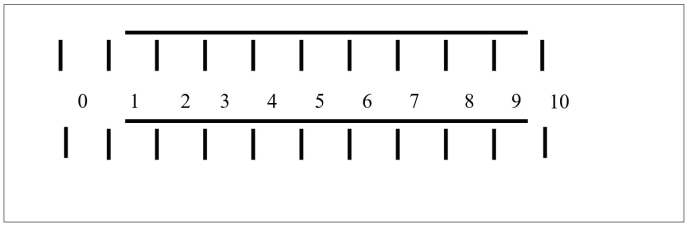

Figure 1. Numerical Scale (Black ve Matassarin 1993)

Figure 2. Verbal Category Scale (Melzack a Katz 1992)

All injections were performed by the same surgeon using the same type of blepharostat. Following topical anaesthesia, ocular surface cleaning was performed with 10% povidone iodine. Following the application of sterile drape and blepharostat, IVR 0.5 mg/0.05 ml was injected using a 30 G syringe tip, marking 3.5 mm from the limbus, without dripping topical anaesthetic again. There was no loss of light sensation in any patient. Following the injection, the entry site was massaged with ear cotton. Patients were asked to evaluate the pain using NS and VCS.

Ethics committee approval was received with the decision of Karabük University Non-Interventional Clinical Research Ethics Committee No. 2020/155.

Statistical Analyses

Statistical analyses were performed using SPSS version 16.0 (SPSS Inc, Chicago, Illinois, USA). p values below 0.05 were considered statistically significant. In the normal distribution test (Kolmogorov Smirnov test) performed before the analysis, it was seen that the variables fit the normal distribution. Mean and standard deviation values of the groups were calculated. Paired t test was used to compare the numerical variables of two groups.

RESULTS

The mean age of 20 male (47.7%) patients in the groups was 59.90±6.03 years, and the mean age of 22 female (52.3%) patients was 60.72±3.88 years (p=0.52). In Group 1, the NS was 3.78±1.11, while in Group 2 it was 4.14±1.37, the difference was statistically significant (p=0.01). In group 1, VCS was 2.30±0.71, while in group 2, VCS was 2.73±0.93, the difference was statistically significant (p=0.01) (Table 1).

Table 1. NS ve VCS values in groups

	Group 1	Group 2	P value
NS	3.78±1.11	4.14±1.37	0.01
VCS	2.30±0.71	2.73±0.93	0.01

DISCUSSION

Since intravitreal injections require repeated doses, it is important that the procedure is painless and easy in terms of patient compliance. If there is severe pain, subsequent injections may not be desired by the patients. Although intravitreal drug administration is one of the most common intraocular procedures, there is no consensus on which anaesthesia technique should be used for the procedure (6). There are anaesthesia studies for intravitreal injections with needles of different diameters (27-30 G) (7). It has been reported that pain will decrease as the needle tip width decreases (7). In all patients in our study, a 30 G needle was used and pain between the two eyes was evaluated independently of the needle diameter.

The ideal intravitreal injection should be fast, effective, safe, easy, cost effective and as painless as possible. There is a consensus on what should be done to reduce the risk of infection in intravitreal injections, but there is no consensus on which anaesthetic technique should be chosen to reduce the pain felt during injection. Studies have suggested that topical anaesthesia is preferable for intravitreal injections because it is fast, inexpensive and easy to administer (7). In our study, we applied topical anaesthesia to all patients because of these advantages.

One-dimensional scales are self-assessment methods that directly measure the severity of pain. In our study, bilateral IVR was performed in the same session. We asked the patient to compare the procedures performed on both eyes using the numerical scale and verbal categorisation scale, which are one-dimensional scales.

In the literature, 162 patients were compared with visual analogue scale (VAS) and no difference was found between aflibercept ranibizumab and dexamethasone implant in terms of pain (8). Pain was found to be less in cases of advanced age, male gender and pseudophakic (8). Patients undergoing eye surgery were not included in our study and we aimed to eliminate the effect of age and gender on pain assessment by comparing two eyes of the same patient in the same session.

In previous studies, most patients who underwent repeated injections reported that they felt that the pain was similar to the pain experienced during the previous injection or that it was reduced. However, increased waiting time may also be associated with increased discomfort in repeated injections (9,10). We excluded patients with previous injections from our study and tried to reduce the factors that may have an effect on pain as much as possible by performing bilateral injections in the same session.

CONCLUSION

In conclusion, in our study, pain in the later eye was found

to be higher in patients who received injection in two eyes in the same session. We believe that within the same individual, during a single session, assessing the pain in both eyes before and after injection will make a valuable contribution to the existing literature. Although the pain is slightly higher, we think that injection in two eyes in the same session should not be avoided.

Although only two eyes were compared, the limitations of our study are that we did not evaluate parameters such as gender and age and the number of patients was not larger.

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Research Article

The Evaluation of Gastric Polyps Detected During Upper Gastrointestinal Endoscopy

Ali Riza Caliskan, Huseyin Kacmaz

Adıyaman University, Faculty of Medicine, Department of Gastroenterology, Adıyaman, Türkiye

Abstract

Aim: Management of gastric polyps depends on the clinical condition of the patient and the malignant potential of the detected polyps. This study aimed to evaluate the frequency of polyps detected during the gastroscopy procedure, the demographic characteristics of patients diagnosed with gastric polyp, the endoscopic and histopathological characteristics of polyps, other endoscopic findings accompanying polyps, and treatment methods.

Material and Method: A total of 177 patients diagnosed with gastric polyp via endoscopy were enrolled in this retrospective analysis. Patients' age, gender, upper gastrointestinal endoscopy, and epicrisis reports were obtained from the hospital's electronic database. The histopathological characteristics of gastric polyps detected during endoscopy and polypectomy were evaluated.

Results: There was no statistically significant difference in the number of polyps and polyp size according to the gender of the patients. It was observed that there was no statistically significant relationship in terms of the anatomical localization of the polyp and the polyp pathology groups. No statistically significant difference was found in the number of polyps according to the age groups of the patients. Still, a statistically significant difference was observed in the size of the polyps ($p<0.05$). As a result of the Mann Whitney U test with Bonferroni correction, which was performed to determine which age group this difference originates from, it was determined that the difference arises from the 18-50 age group and the over 65 age group. It was observed that there was no statistically significant relationship between the anatomical localization of the polyp and the pathology of the polyp according to age groups.

Conclusion: Due to the prevalence of nonspecific findings, upper endoscopic examination should be performed in patients with these complaints, especially those over fifty. When detected, excision or follow-up with a biopsy of the polyp and surrounding tissue is required if this is not possible.

Keywords: Gastric polyps, endoscopy, gastroscopy, malignancy, polypectomy

INTRODUCTION

A gastric polyp (GP) is a sessile or stalked lesion from the mucosa or submucosa that protrudes into the lumen. GP is detected in 0.3%-6% of all upper gastrointestinal endoscopy patients. Nowadays, this rate is increasing with the widespread use of endoscopic examination. Although GPs are usually detected incidentally during an endoscopy performed for another reason, they may also present with gastric bleeding, pyloric stenosis, iron deficiency anemia, and abdominal pain (1).

GPs are most commonly located in the antrum and corpus and are generally divided into two groups: epithelial and non-mucosal intramural polyps. While the majority are hyperplastic (HP) and located in the fundus (FGP), a small portion consists of adenomatous polyps (AP) and other polyps. Although most GPs are non-neoplastic, the fact that some have the potential for malignant transformation increases the importance of diagnosing and treating GPs (2). GPs are usually detected incidentally, and their frequency and histopathological types may vary depending on the population examined. Gastric polyps were reported to be more common in women. However, this is a controversial issue (3).

Although most GPs are single in the literature, multiple GPs have been reported between 8.2% and 58.7%. Additionally, 64.1%-87% of GP sizes are below 10 mm in the literature. Studies defining the location of GPs elaborated on various locations. While some studies reported that GPs were

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Corresponding Author: Ali Riza Caliskan, Adıyaman University, Faculty of Medicine, Department of Gastroenterology, Adıyaman, Türkiye

E-mail: komamir308@gmail.com

most frequently localized in the antrum (40.7%-51%), others said the most common localization was in the corpus (36.4%-64%). FGP and HP are the most common gastric polyps. HPs have been reported between 36.2% and 88% and are thought to trigger chronic inflammation (2-4).

Management of gastric polyps depends on the clinical condition of the patient and the malignant potential of the detected polyps. If possible and safe, all detected polyps should be removed endoscopically (5). This study aimed to evaluate the frequency of polyps detected during the gastroscopy procedure, the demographic characteristics of patients diagnosed with GP, the endoscopic and histopathological characteristics of polyps, other endoscopic findings accompanying polyps, and treatment methods.

MATERIAL AND METHOD

Patients who underwent endoscopic polypectomy in our clinic between January 2016 and October 2023 were included in the study. A total of 177 patients diagnosed with gastric polyp via endoscopy were enrolled in this retrospective analysis. Patients whose all-stomach segments were evaluated and whose polypectomy material was evaluated by pathology were included in the study. Endoscopy procedures performed for emergencies and patients whose polypectomy material was not evaluated by pathology were excluded from the study. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Ethics committee approval has been granted from our institution with protocol number 2023/3-9, and informed consent has been obtained from all participants.

Patients' age, gender, upper gastrointestinal endoscopy, and epicrisis reports were obtained from the hospital's electronic database. The histopathological characteristics of gastric polyps detected during endoscopy and polypectomy were evaluated.

Statistical Analysis

Patient data collected within the scope of the study were analyzed with the IBM Statistical Package for the Social Sciences (SPSS) for Windows 26.0 (IBM Corp., Armonk, NY) package program. Frequency and percentage for categorical data and mean and standard deviation for continuous data were given as descriptive values. For comparisons between groups, the "Independent Sample T–test" was used for two groups, and the "Pearson Chi-Square Test" was used to compare categorical variables. The results were considered statistically significant when the p-value was less than 0.05.

RESULTS

Within the scope of the study, 177 patients aged between 18 and 99 were evaluated. Regarding gender, 36.2% (n=64) of the patients were male, and 63.8% (n=113) were female. The mean age of the patients was 59. The baseline demographic and clinical findings are denoted in Table 1.

Table 1. Distribution of demographic and clinical findings of the patients

Variable (N=177)	n (%)	Mean±SD	Median (Min-Max)
Age (years)		59±15.3	59 (18-99)
18-50 years old	48 (27.1)		
50-65 years old	66 (37.3)		
Over 65 years old	63 (35.6)		
Gender			
Male	64 (36.2)		
Woman	113 (63.8)		
Anatomical localization of the polyp			
Antrum	65 (36.7)		
Fundus	35 (19.8)		
Cardia	26 (14.7)		
Corpus	51 (28.8)		
Size of polyp (mm)		6.2±5.1	5 (1-30)
Number of polyps		1.5±1.1	1 (1-8)
Pathology of the polyp			
Fundic gland polyp	38 (21.5)		
Hyperplastic	129 (72.9)		
Neuroendocrine cell hyperplasia	7 (4.0)		
Neuroendocrine tumor	3 (1.7)		
HP status	n=141		
Negative	83 (58.9)		
Positive	58 (41.1)		

The number and size of polyps, anatomical localization, and their distribution in terms of pathology according to gender are elaborated in Table 2. When the table was examined, there was no statistically significant difference in the number of polyps and polyp size according to the gender of the patients. It was observed that there was no statistically significant relationship in terms of the anatomical localization of the polyp and the polyp pathology groups.

Table 2. The characteristics of gastric polyps with respect to gender

Variables (N=177)	Gender		
	Male (n=64)	Female (n=113)	p-value
Number of polyps			0.958
Mean SD	1.5±0.9	1.5±1.2	
Median (Min-Max)	1 (1-4)	1 (1-8)	
Polyp size (mm)			0.854
Mean SD	6.3±5.2	6.2±5.1	
Median (Min-Max)	5 (1-30)	5 (1-30)	
Anatomical localization of the polyp, n (%)			0.331
Antrum	29 (45.3)	36 (31.9)	
Fundus	10 (15.6)	25 (22.1)	
Cardia	9 (14.1)	17 (15)	
Corpus	16 (25)	35 (31)	
Pathology of the polyp, n (%)			0.066
Fundic gland polyp	7 (10.9)	31 (27.4)	
Hyperplastic	54 (84.4)	75 (66.4)	
Neuroendocrine cell hyperplasia	2 (3.1)	5 (4.4)	
Neuroendocrine tumor	1 (1.6)	2 (1.8)	

The number and size of polyps, anatomical localization, and their distribution in terms of pathology according to the age groups are shown in Table 3. When the table was examined, no statistically significant difference was found in the number of polyps according to the age groups of the patients. Still, a statistically significant difference was observed in the size of the polyps ($p<0.05$).

As a result of the Mann Whitney U test with Bonferroni correction, which was performed to determine which age group this difference originates from, it was determined that the difference arises from the 18-50 age group and the over 65 age group. It was observed that there was no statistically significant relationship between the anatomical localization of the polyp and the pathology of the polyp according to age groups.

Table 3. The characteristics of gastric polyps with respect to age groups

Variable (N=177)	Age group				
	18-50 age[1] (n=48)	50-65 age[2] (n=66)	65≤ age[3] (n=63)	p-value	Difference*
Number of polyps				0.663	
Mean SD	1.6±1.2	1.6±1.2	1.4±0.7		
Median (Min-Max)	1 (1-6)	1 (1-8)	1 (1-4)		
Polyp size (mm)				**<0.001**	**1-3**
Mean SD	**4.3±3.5**	**6±4.5**	**8±6.2**		
Median (Min-Max)	**3 (1-20)**	**5 (2-20)**	**6 (2-30)**		
Anatomical localization of the polyp, n (%)				0.533	
Antrum	15 (31.3)	23 (34.8)	27 (42.9)		
Fundus	11 (22.9)	14 (21.2)	10 (15.9)		
Cardia	5 (10.4)	9 (13.6)	12 (19)		
Corpus	17 (35.4)	20 (30.3)	14 (22.2)		
Pathology of the polyp, n (%)				0.567	
Fundic gland polyp	10 (20.8)	15 (22.7)	13 (20.6)		
Hyperplastic	33 (68.8)	47 (71.2)	49 (77.8)		
Neuroendocrine cell hyperplasia	3 (6.3)	3 (4.5)	1 (1.6)		
Neuroendocrine tumor	2 (4.2)	1 (1.5)	0 (0)		

*Mann Whitney U test with Bonferroni correction

DISCUSSION

The most important clinical features of polyps are that they show malignant transformation, ulcerated polyps cause anemia, and can cause intermittent obstruction at the gastric outlet. Therefore, diagnosis and treatment are essential. Histopathological evaluation is required to confirm the diagnosis of gastric polyp. WHO previously divided polypoid lesions into neoplastic and non-neoplastic lesions. However, some classifications are more practical today and cover all subtypes. Upper gastrointestinal tract polyps are usually detected incidentally. This is because there are no apparent symptoms of polypoid lesions (6,7).

There is no standard approach yet regarding the approach to polyps detected during upper endoscopy. In the algorithm of the British Association of Gastroenterology, it is recommended to take a biopsy from all polyps to prove the diagnosis and the presence of dysplasia (8). In addition, excision of the adenomatous polyp should be done after 6 months when the polyp cannot be removed entirely because it contains dysplastic changes. It has been reported that 1–5% of benign hyperplastic and adenomatous polyps show malignant transformation. For this reason, Vallot put forward a different opinion, stating that biopsy cannot detect malignant changes in hyperplastic and adenomatous polyps larger than 5 mm (9). On the other hand, Han et al. (10) found approximately 5.3 times more neoplastic transformation in hyperplastic polyps larger than 1 cm than in smaller polyps, despite some studies reporting that this risk is valid for polyps larger than 2 cm.

Previous studies have suggested that patients with hyperplastic and adenomatous polyps have a risk of synchronous neoplasia in the nonpolypoid gastric mucosa. Abraham et al. (11) stated that the risk of developing adenocarcinoma around the polyp was higher than the polyp (6% and 0.6%, respectively).

Archimandritis et al. (12) observed 258 gastric polyps in 157 patients in their retrospective study and found 67.5% of them to be 60 or older. 75.6% of these polyps are hyperplastic, and 43.8% are most frequently reported in the antrum location. Morais et al. (13) reported the most common hyperplastic polyp in a study including 26.000 cases. In our study, 14.7% of the polyps in the stomach were in the cardia, 19.8% in the fundus, 28.8% in the corpus, and 36.7% in the antrum. In our study, the mean age of the patients was 59 years. In parallel with other studies, lesions were most frequently observed in the antrum, and histopathologically, the most common hyperplastic polyp was reported. In our study, no statistically significant difference was observed in the number of polyps and polyp size according to the gender of the patients. There was no statistically significant relationship between the anatomical localization of the polyp and the polyp pathology groups. Additionally, no statistically significant difference was found in the number of polyps according to the age groups of the patients.

Fundic gland polyps differ from other polyps in terms of location and endoscopic appearance. It is common in patients who use proton pump inhibitors (PPIs) extensively and in conditions characterized by hypergastrinemia, such as Zollinger-Ellison syndrome. Although it is mainly considered benign, it should be kept in mind that it may accompany patients with familial adenomatous polyposis (FAP) syndrome. FAP screening should be performed in more than 20 fundic gland polyps located in the antrum and detected in patients under 40 years of age. The risk of fundic gland polyp increased 3.8 times in 5 years of PPI use (14). If these polyps are larger than 1 cm, the risk of malignancy is considered 1% on average (15). If they are localized in an ulcer or antrum, the risk of malignancy increases. In our study, no statistically significant difference was found in the number of polyps according to the age groups of the patients, but, a statistically significant difference was observed in the size of the polyps.

Adenomatous polyps constitute 6-10% of stomach polyps. Unlike the other two types of polyps, Adenomatous polyps carry the risk of malignancy. Serial endoscopy follow-up has shown that 11% progress to dysplasia or carcinoma in situ within four years (16). They usually develop in the mucosa, showing chronic gastritis and intestinal metaplasia. They are often single and large, and may be sessile or stalked. According to their histopathological features, they are called tubular, villous, and tubulovillous. Approximately 90% of adenomatous polyps are tubular adenomas, 5-10% are tubulovillous, and 1-2% are villous type. While the risk of malignancy development is lower in tubular adenomas, this risk is reported to be 33% in villous and tubulovillous adenomas (17).

Hyperplastic polyps constitute 75-90% of stomach polyps. These non-neoplastic polyps are common in the elderly and peak in the 6th and 7th decades. Their incidence does not change depending on gender. Hyperplastic polyps occur due to excessive epithelium regeneration due to chronic inflammatory stimulation. They are usually benign and rarely have the potential to become malignant. The risk of malignancy is related to whether the polyp histopathology focuses on intestinal metaplasia or dysplasia. Focal dysplastic foci are observed in 1-20% of patients (18,19).

FGP and HP are the most common gastric polyps. HPs have been reported between 36.2% and 88% and are thought to trigger chronic inflammation. HPs are the most common type in countries where H. pylori infection is common. It is generally solitary and localized in the antrum. Although neoplastic transformation in HPs is reported to be between 1.5% and 2.1%, since it can usually develop in polyps of 10 mm and above, polypectomy should be performed, and H. pylori eradication treatment should be given to these polyps (20). In some studies, the most common polyp type is FGP, and the incidence of FGP

has been reported to be between 16% and 51% (21).

CONCLUSION

As a result, gastric polyps are incidental formations with no prominent symptomatic features. They are essential because of their malignant transformation. The limiting features of our study are that it is single-center and retrospective. Due to the prevalence of nonspecific findings, upper endoscopic examination should be performed in patients with these complaints, especially those over fifty. When detected, excision or follow-up with a biopsy of the polyp and surrounding tissue is required if this is not possible.

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