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Protective effects of glutathione administration on ischemia-reperfusion injury in rat ovaries

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Abstract

We aimed to examine the biochemical and histopathological potential beneficial effects of glutathione administration on the ovarian ischemia/reperfusion injury (IRI) model. Thirty Wistar Albino female rats were used in this experimental study and were divided into five groups. Group 1 (sham) underwent observational laparotomy. Group 2 (torsion) had their left ovaries torsioned. Group 3 (torsion + detorsion) was detorsioned after torsion. Groups 4 and 5 received the same procedure as group 3. 0,2 ml glutathione was applied to the left ovaries of group 4 (torsion + detorsion + intraovarian glutathione injection) after detorsion. Group 5 (torsion + detorsion + intraperitoneal glutathione injection) was administered 1 ml glutathione intraperitoneally five times. Fifteenth-day blood samples were taken to examine total antioxidant status, total oxidant status, oxidative stress index, anti-mullerian hormone (AMH), and malondialdehyde (MDA) values. Besides, the left ovaries were resected for histopathological examination. Total antioxidant status was significantly higher in the intraperitoneal injection group ($p<0.05$). The AMH values of the sham and intraovarian groups were similar ($p>0.05$). MDA value did not differ significantly between the sham, intraovarian, and intraperitoneal injection groups ($p>0.05$). In histopathological examination, no significant benefit of glutathione application on follicle numbers was shown. The main limitations of our study were the relatively small size of our series, the absence of serial blood measurement, the absence of a group in which intraovarian and intraperitoneal injections were administered together, and the absence of a sham + drug group. Glutathione administration reduces the detrimental effects of ovarian IRI.

Keywords: anti-mullerian hormone, glutathione, ıschemia reperfusion ınjury, ovarian torsion

1. Introduction

Ovarian torsion is a gynecological emergency that causes reduction or cessation of blood flow due to the partial or complete rotation of the ovaries around their ligament supports. Even though ovarian torsion is most common in women between 20 and 30, it can be seen at any age, from prepubertal to postmenopausal period (1,2).

The venous return becomes interrupted since venous pressure is lower after ovarian torsion. Edema develops in the ovarian tissue due to the continuation of the arterial blood flow. This edema causes ischemic damage by slowing down and stopping the blood flow (3). Early diagnosis and appropriate treatment should be made quickly to prevent possible ovarian necrosis, decrease in fertility, and life-threatening complications. In the case of ovarian torsion, the first therapeutic option is surgical detorsion as soon as possible because delay in diagnosis and treatment is associated with decreased ovarian reserve and infertility (4).

Abundant oxygen uptake occurs after ovarian detorsion, and the resulting free oxygen radicals increase tissue damage. Following detorsion, the return of blood flow increases, and accordingly, the production of toxic reactive oxygen species (ROS) increases (5,6). By providing oxygen during reperfusion following detorsion, xanthine oxidase converts the accumulated hypoxanthine to xanthine. Thus, an excessive amount of free oxygen radicals is formed, which causes a deterioration in the antioxidant mechanism of the body (7,8). Overproduction of ROS causes a significant increase in lipid peroxidation, creating malondialdehyde (MDA), which can destroy the human body's antioxidant defense systems (9,10).

Glutathione is a tripeptide (cysteine, glycine, and glutamic acid) molecule found in high concentrations in tissues. It plays an essential role in pathophysiological processes such as reducing oxidative stress, increasing metabolic detoxification, and regulating immune system functions (11,12).

Many studies have investigated the effects of glutathione on female fertility. For example, while intracellular glutathione levels were found to be higher in young women, glutathione levels were found to be low in cases of premature ovarian

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insufficiency and ovarian cancer (13,14). In addition, glutathione protects follicles from damage caused by oxidative stress during folliculogenesis. Thus, oocytes with high intracellular glutathione levels produce healthier and stronger embryos (15).

In experimental animal models, the protective effect of glutathione administration has been shown in liver damage, liver transplantation, neurotoxicity, and testis torsion situations (16-19). Although many studies demonstrate the protective effect of glutathione administration against ischemia-reperfusion injury (IRI) in various tissues, no studies have been performed on ovarian IRI.

Glutathione is the cell's primary antioxidant and can reduce oxidative stress by preventing the formation of harmful free radicals in the reproductive system (20). Based on the known positive effects of glutathione on the ovaries and follicles, we hypothesized that glutathione administration could have histopathologically and biochemically protective effects in a rat model ovarian IRI.

2. Materials and Methods

This study was approved by the Institutional Animal Use and Care Committee and performed in accordance with the Helsinki Declaration of World Medical Association recommendations on animal studies. The rats included in the study were female Wistar Albino type, 12 weeks and weighing 187-220 grams. Rats were placed in individual cages and fed ad libitum in an environment at a 20-22 ° temperature, 50%-55% humidity, and 12-hour light/dark cycles. Before the study, the rats were given ten days to get used to the environment. The rats were randomly divided into five groups. All surgical interventions were performed under general anesthesia and sterile conditions, with a midline (2.0 cm) incision in the lower abdomen of the rat.

2.1. Group I (Sham group, n: 6)

The ovaries and tubes were imaged. After the evaluation, the incision was closed with 4.0 polyglactin 910 sutures.

2.2. Group II (Torsion group, n: 6)

Adnexal torsion was performed by fixing the left adnexa containing the ovarian vessels and tubes to the abdominal wall by rotating it in a clockwise direction by 720 °. After the procedure, the incision was closed.

2.3. Group III (Torsion - Detorsion Group, n: 6)

After the torsion process, 3 hours were spent waiting to complete the ischemic process, and laparotomy was performed again. A Detorsion procedure was applied to the left ovary. The incision was closed.

2.4. Group IV (Torsion - Detorsion + Intraovarian Glutathione Injection Group, n: 6)

After the torsion and detorsion procedure, 0.2 ml of glutathione (Tationil 600 mg / 4 Ml) was applied to the left ovarian tissue, and the abdominal wall was closed.

2.5. Group V (Torsion - Detorsion + Intraperitoneal Glutathione Injection Group, n: 6)

After the torsion and detorsion procedure, five times intraperitoneal glutathione (Tationil 600 mg / 4 Ml) application was performed (on the day of the operation, on the 3rd day after the operation, on the 6th day, on the 9th day, and the 12th day). In each administration, 0.2 ml glutathione was injected intraperitoneally.

The rats were taken into cages in all groups for 15 days following the first laparotomy. At the end of the fifteenth day, following anesthesia, intracardiac blood samples were taken from all rats, and their left ovaries were resected. Euthanasia was applied after the procedure. The rats were anesthetized with xylazine hydrochloride (Rompun, Bayer, Germany) and ketamine hydrochloride (Ketalar, Eczacıbası, Turkey).

2.6. Histopathologic Evaluation

Ovarian samples were fixed in 10% formalin for 48 h, dehydrated in ethanol series, cleaned, and embedded in paraffin. The paraffin blocks were sectioned at a thickness of 5 mm using a sliding microtome (Leica RM2125RTS Nussloch Germany). Sections were stained with eosin and haematoxylin and analyzed using a light microscope (Nikon Eclipse E600 microscope) by an experienced pathologist. Hemorrhage, edema, and inflammation (neutrophil infiltration) were scored from 0 to +3 for ovarian injury as follows: None, Mild, Moderate, Severe

Follicles were counted in the largest section of the ovary to evaluate the ovarian reserve. Total scores were calculated. Follicles were defined as primordial follicle, primary follicle, preantral (secondary) follicle, and antral (tertiary) follicle (21):

2.7. Biochemical Evaluation

Blood samples were centrifuged at 3000 g for 10 minutes to obtain serum. Serum samples were stored at -80°C until measurement. In the blood samples, AMH (Elabscience, USA), Inhibin B (Elabscience, USA), Malondialdehyde (Bioassay Technology Laboratory, China), Total Antioxidant Status (TAS) (Bioassay Technology Laboratory, China) and Total Oxidant Status (TOS) (Bioassay Technology Laboratory, China) were measured by Enzyme-linked Immunosorbent Assay. Readings were done by a microplate reader (Biotek Synergy Reader).

2.8. Statistical Analysis

Mean, standard deviation, median, minimum, maximum value frequency, and percentage were used for descriptive statistics. The distribution of variables was checked with the Kolmogorov-Smirnov test. Kruskal-Wallis and Mann-Whitney U tests were used to compare quantitative data. SPSS 27.0 was used for statistical analysis.

3. Results

The weights of the rats in the groups were found to be similar ($p>0.05$). TOS value in the detorsion group, intraovarian injection group, and intraperitoneal injection group was significantly higher than the torsion group ($p<0.05$). TOS value

did not differ significantly between the detorsion group, intraovarian injection group, and intraperitoneal injection group (p>0.05). The TAS value of the intraperitoneal injection group was significantly higher than the sham group, torsion group, and detorsion group (p<0.05) (Table 1). The oxidative stress index (OSI) value of the detorsion group was significantly higher (p<0.05) than the other groups.

MDA values were similar (p>0.05) between the sham group, intraovarian injection group, and intraperitoneal injection groups (Table 1). AMH values of the torsion, detorsion, and intraperitoneal injection groups were significantly lower than the intraovarian and sham groups (p<0.05). AMH values were similar (p>0.05) between the sham and intraovarian groups (Table 1).

Table 1. Comparison of biochemical and histopathologic features between groups

		Control Group[1]	Torsion Group[2]	Detorsion[3]	Intra[4]	Intra[5]	p		1,2	1,3	1,4	1,5	2,3	2,4	2,5	3,4	3,5	4,5
Weight	Mean±sd	204.3±10.2	200.8±8.4	202.3±11.7	203.0±7.9	202.5±8.4	0.975	K	0.522	0.631	0.936	0.748	0.936	0.629	0.630	0.873	0.873	0.936
	Median	206.0	200.5	202.5	202.5	200.0												
TOS	Mean±sd	7.01±0.39	7.75±0.39	8.42±0.61	8.58±0.98	9.00±0.90	***0.001***	K	***0.016***	***0.006***	***0.004***	***0.004***	***0.045***	***0.048***	***0.005***	1.000	0.229	0.261
	Median	6.97[2345]	7.79[3345]	8.40	8.20	8.80												
TAS	Mean±sd	2.64±0.38	2.39±0.40	1.94±0.46	3.46±1.38	3.48±0.44	***0.003***	K	0.199	***0.037***	0.261	***0.010***	0.065	0.078	***0.010***	***0.016***	***0.006***	0.522
	Median	2.61[5]	2.41[5]	1.74[1345]	3.03	3.62												
OSI	Mean±sd	2.71±0.46	3.33±0.61	4.49±0.82	2.80±1.03	2.63±0.46	***0.010***	K	0.109	***0.006***	0.873	0.873	***0.025***	0.423	0.052	***0.025***	***0.006***	0.522
	Median	2.73[3]	3.28[3]	4.65	3.03[3]	2.41[3]												
MDA	Mean±sd	0.68±0.07	0.88±0.13	1.17±0.26	0.68±0.09	0.76±0.09	***0.001***	K	***0.006***	***0.004***	0.936	0.078	***0.045***	***0.010***	0.149	***0.004***	***0.006***	0.173
	Median	0.67[23]	0.87[3]	1.15	0.68[23]	0.79[3]												
AMH	Mean±sd	3.89±0.65	2.02±0.75	2.79±0.22	3.72±0.66	2.89±0.79	***0.001***	K	***0.004***	***0.004***	0.873	***0.037***	0.065	***0.004***	0.055	***0.010***	1.000	***0.048***
	Median	3.63[235]	2.14	2.74	3.91[235]	2.76												
Hemorrhage Score	Mean±sd	0.00±0.00	3.00±0.00	1.83±0.75	1.33±0.52	1.67±0.82	***0.000***	K	***0.001***	***0.002***	***0.002***	***0.002***	***0.007***	***0.002***	***0.007***	0.212	0.665	0.465
	Median	0.00[2345]	3.00	2.00[2]	1.00[2]	1.50[2]												
Edema Score	Mean±sd	0.00±0.00	3.00±0.00	1.83±0.75	1.33±0.52	2.00±1.10	***0.000***	K	***0.001***	***0.002***	***0.002***	***0.007***	***0.007***	***0.002***	***0.021***	0.212	0.546	0.127
	Median	0.00[2345]	3.00	2.00[2]	1.00[2]	2.00[2]												
Inflammation Score	Mean±sd	0.00±0.00	3.00±0.00	2.00±0.89	1.33±0.52	1.33±0.82	***0.000***	K	***0.001***	***0.002***	***0.002***	***0.001***	***0.022***	***0.002***	***0.005***	0.162	0.149	0.673
	Median	0.00[2345]	3.00	2.00[2]	1.00[2]	1.00[2]												
Primordial Follicle Count	Mean±sd	6.33±1.03	0.17±0.41	4.17±1.17	3.00±1.10	2.50±1.05	***0.000***	K	***0.003***	***0.014***	***0.003***	***0.004***	***0.003***	***0.003***	***0.003***	0.128	***0.032***	0.356
	Median	6.00[2345]	0.00	4.00[25]	3.00[2]	2.50[2]												
Primary Follicle Count	Mean±sd	7.00±2.37	1.00±0.63	4.33±1.03	3.50±1.05	2.67±0.52	***0.000***	K	***0.003***	***0.041***	***0.012***	***0.003***	***0.003***	***0.004***	***0.004***	0.209	***0.007***	0.120
	Median	7.00[2345]	1.00	4.00[25]	3.50[2]	3.00[2]												
Preantral Follicle Count	Mean±sd	6.83±1.83	1.50±0.55	4.00±0.63	2.67±1.03	2.67±1.03	***0.000***	K	***0.003***	***0.005***	***0.004***	***0.004***	***0.003***	***0.045***	***0.045***	***0.022***	***0.022***	1.000
	Median	6.50[2345]	1.50	4.00[245]	3.00[2]	3.00[2]												
Antral Follicle Count	Mean±sd	5.50±1.38	1.50±0.55	3.83±1.47	3.83±1.17	3.17±1.17	***0.002***	K	***0.003***	***0.045***	***0.045***	***0.014***	***0.007***	***0.007***	***0.012***	0.935	0.410	0.323
	Median	5.00[2345]	1.50	3.50[2]	4.00[2]	3.00[2]												

[K] Kruskal-wallis test (Mann-whitney u test) [1] Difference with Control Group [2] Difference with Torsiyon Group / [3] Difference with Intraperitoneal Group

[4] Difference with Intraovaryen Group / [5] Difference with Detorsiyon Group

Hemorrhage, edema, and inflammation scores were significantly higher (p<0.05) in the torsion group, detorsion group, intraovarian injection group, and intraperitoneal injection group, respectively, compared to the sham group. The scores in the torsion group were significantly higher (p<0.05) than the detorsion group, intraovarian injection group, and intraperitoneal injection group (Fig. 1).

While the number of primordial and primary follicle numbers were significantly lower (p<0.05) in the intraperitoneal injection group than in the detorsion group, there was no significant difference (p>0.05) between the detorsion group and intraovarian injection groups. The preantral follicle count was significantly lower than the detorsion group in the intraperitoneal injection group and intraovarian injection group (p<0.05). Follicle numbers were not significantly different between the detorsion group, intraovarian injection group, and intraperitoneal injection groups (p>0.05) (Table 1).

4. Discussion

We evaluated the difference between biochemical markers TOS, TAS, OSI, AMH, and MDA values as a primary outcome. In addition, we compared the change in follicle numbers by making a histopathological evaluation as a secondary outcome.

TAS was measured to determine the general antioxidant status, and TOS was measured to determine the oxidant status (22,23). OSI is calculated as the ratio of TOS to TAS. OSI is considered an indicator of oxidative stress in the tissue (24). We found that glutathione administration increased the TAS value, especially with intraperitoneal administration. It was

found that OSI value increased mostly in the detorsion group. With the application of glutathione, we could keep OSI at a lower level than detorsion. Thus, similar to the publications showing that glutathione reduces oxidative stress damage in the female reproductive system, we revealed that glutathione protects the ovaries from the effects of free oxygen radicals in cases of ovarian IRI (20, 25).

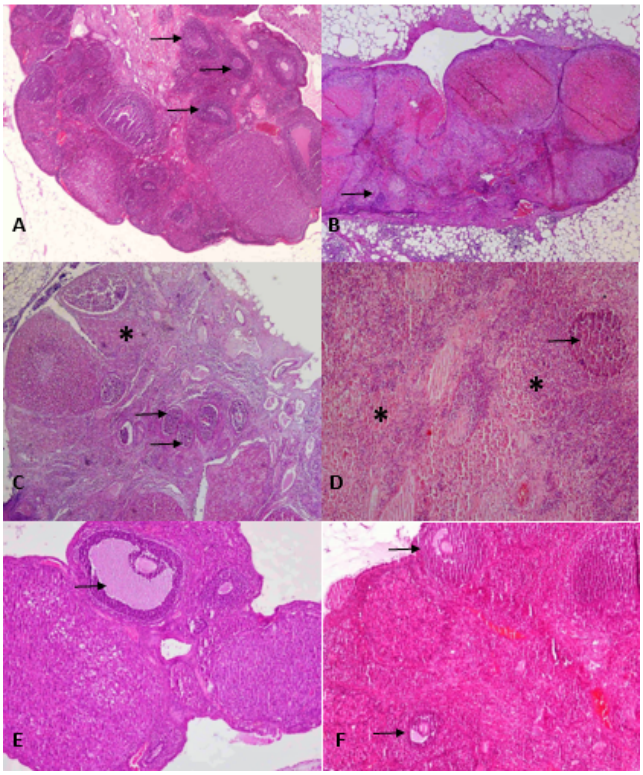

Fig. 1 - Histopathologic Evaluation

A. Sham group with a normal appereance of ovary (4X magnification)
B. Torsion and detorsion group with increased inflamation, hemorage and edema (4X magnification)
C. Torsion group with similar morphologic features to the B.
D. Intense polymorphonuclear cell infiltration and intercellular edema are seen in torsion group. Note the seperation and degenerative changes in follicles (20X magnification)
E. Torsion,detorsion and intraovarian glutathion injection group with decreased inflammation, hemorage and edema (10X magnification)
F. Torsion, detorsion and intraperitoneal glutathion injection group has similar morphology.
Ovarian follicles are marked with black arrows, dense inflammation is marked with *

The most harmful effect of free oxygen radicals in the cell is lipid peroxidation, and its' end product is MDA. Lipid peroxidation causes loss of cell membrane integrity, increased permeability to ions, and cell damage. Therefore, inhibiting or reducing the lipid peroxidation process may help prevent tissue damage (26, 27). Our study found that MDA levels increased in torsion and torsion-detorsion groups compared to the sham group. This result supports that ischemia and IRI increased tissue damage. We also noticed that the MDA value did not differ significantly between the sham group and intraperitoneal, intraovarian injection groups ($p>0.05$). Thus, we showed that intraperitoneal and also intraovarian administration of glutathione has a reducing effect on tissue damage by inhibiting lipid peroxidation.

Anti-mullerian hormone (AMH) is produced by granulosa cells. It is used as an indicator of ovarian follicle reserve and can be measured in serum. Decreased AMH values are generally associated with decreased ovarian reserve (28). AMH value was significantly higher in the intraovarian injection group than in the torsion group, detorsion group, and intraperitoneal injection group. AMH values did not differ significantly ($p>0.05$) between sham and intraovarian injection groups. We determined the protective effect of ovarian reserve based on the positive effect of intraovarian injection of glutathione on AMH value.

While primordial and primary follicle numbers were not significantly different between the detorsion group and intraovarian injection group ($p>0.05$), they were significantly lower in the intraperitoneal injection group ($p<0.05$). Preantral follicle number was significantly lower in the intraperitoneal injection group and intraovarian injection group than in the detorsion group ($p<0.05$). Antral follicle numbers were similar between the detorsion, intraovarian injection, and intraperitoneal injection groups ($p>0.05$). Some researchers stated that follicle numbers might not give accurate results as an indicator of ovarian reserve in such studies. They argued that it is not a definitive indicator of the viability of damaged follicles that are not included in the count because they are damaged (29). We attributed the difference in terms of follicle numbers in our study to this reason.

The main limitation of our study was the relatively small size of our series and the absence of serial blood measurement in this experimental model. In addition, we identified the absence of a group in which intraovarian and intraperitoneal injections were administered together and the absence of a sham + drug group as another limitation of our study.

The results of the study showed that exogenous glutathione administration might be effective in preventing tissue damage, oxidative stress, and loss of ovarian reserve caused by IRI in rat ovaries. In order to provide the most effective therapeutic benefit in clinical practice, further studies are needed to define dosage setting, administration method, and frequency of administration.

Conflict of interest

None for each author

Funding

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Maltepe University Animal Ethics Committee Approval Number: 2019/09/04

Authors' contributions

Concept: A.G., B.O.I., Design: A.G., B.O.I., Data Collection

or Processing: A.G., E.D.T., K.A., Analysis or Interpretation: A.G., B.O.I., A.G.S., M.E.S., Literature Search: A.G., Writing: A.G., K.A.

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The protective impact of glutamine on anti-tuberculosis drug-induced nephrotoxicity in Wistar rats

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Abstract

This study assessed the protective effect of glutamine (GTN) against rifampicin/isoniazid/pyrazinamide/ethambutol (RIPE)-induced nephrotoxicity in rats. Thirty adult Wistar rats (200±20 g) of both sexes were grouped into 6 of 5 rats/group. The rats were treated daily for 30 days as follows: Group 1 (Vehicle control [normal saline 0.2mL]), group 2 (GTN 200 mg/kg), group 3 (RIPE 150, 75, 400 and 275 mg/kg in vehicle), group 4 (GTN 50 mg/kg +RIPE), group 5 (GTN 100 mg/kg +RIPE) and group 6 (GTN 200 mg/kg +RIPE). After treatment, blood samples were obtained and assessed for serum renal biomarkers. Kidneys were harvested, weighed and assessed for oxidative stress markers and histology. RIPE significantly ($p<0.01$) decreased body weight and significantly ($p<0.01$) increased kidney weight when compared to the control. Serum urea, creatinine, uric acid levels and kidney malondialdehyde levels were significantly ($p<0.001$) increased in RIPE-treated rats when compared to the control. Serum total protein, albumin, kidney glutathione, catalase, superoxide dismutase and glutathione peroxidase levels were significantly decreased ($p<0.001$) in RIPE-treated rats when compared to the control. RIPE caused tubular necrosis and collapsed glomeruli in the kidneys of rats. However, body and liver weights were significantly restored in GTN 100 mg/kg +RIPE and GTN 200 mg/kg +RIPE-treated rats at $p<0.05$ and $p<0.01$, respectively when compared to RIPE. Serum and kidney oxidative stress markers were restored in GTN 50 mg/kg +RIPE, GTN 100 mg/kg +RIPE and GTN 200 mg/kg +RIPE-treated rats at $p<0.05$, $p<0.01$ and $p<0.001$ respectively, when compared to RIPE. GTN restored kidney histology. GTN protects against RIPE-induced nephrotoxicity in a dose-related fashion.

Keywords: anti-tuberculosis drug, kidney, toxicity, glutamine, rat

1. Introduction

The kidneys, which receive 20 - 25% of cardiac output are the primary organs for maintaining homoeostasis of extracellular fluid volumes. The kidneys perform two major functions; excretion of the end-products of metabolism and the control of the concentration of body fluids. The kidneys are also involved in drugs and metabolites excretion through glomerular filtration and tubular secretion (1). The regulatory effect of the kidneys on drugs and their metabolites predisposes them to nephrotoxicity. Drug-induced nephrotoxicity is recognized as a contributor to acute and chronic kidney diseases. Nephrotoxicity reflects damage to different sections of the nephron, which may results to renal failure (2).

Rifampicin/isoniazid/pyrazinamide/ethambutol (RIPE) is a frequently used anti-tuberculosis regimen. It is used for treating tuberculosis for two months followed by isoniazid/rifampicin for eight months (3). One of the primary complications of using RIPE is the development of nephrotoxicity, which may be acute or chronic (4-6). Rifampin has the highest propensity for nephrotoxicity, which can be augmented by partner anti-tuberculosis drugs (7). In animal studies, RIPE has been associated with impaired renal biomarkers, kidney inflammatory reactions and oxidative stress marked by lipid peroxidation (3,8). RIPE may cause kidney morphological changes such as tubular necrosis (9,10).

Glutamine (GTN) is one of the most abundant amino acid in human body. It is involved in the regulation of protein synthesis, acid–base balance, immune function and adaptation to stress (11,12). It is as essential chemical substance for glutathione, which is an important antioxidant in the body (13). Glutathione is a potent ubiquitous antioxidant that is important for many drugs and endogenous substance metabolisms (14, 15). GTN has potent antiulcer, antioxidant, antibacterial, cardioprotective, anticancer, and anti-apoptotic potential (16). Moreover, its nephroprotective effect has been reported in animal studies (17). It decreased renal cell apoptosis and inflammatory markers in cisplatin-induced nephrotoxicity in rats (18). It stabilized antioxidant status and reduced renal lipid peroxidation in cisplatin- treated rats (19). GTN decreased tubular necrosis, stabilized renal biochemical markers and decreased oxidative stress in gentamycin –treated rats (20). The current study evaluated the protective effect of GTN on RIPE-induced nephrotoxicity in Wistar rats.

2. Materials and Methods

2.1. Drugs/chemicals and animals

Glutamine (GTN) (Qualikems Fine Chemical Private Limited

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Nandesari, Vadodara, Gujarat, India) and Rifampicin, isoniazid/ pyrazinamide/ethambutol (RIPE) (Lupin Limited Chikalthana, Aurangabad India) were used. Approval was obtained from the Research Ethics Committee (NDU/PHARM/PCO/AEC/068) of the Department of Pharmacology/Toxicology, Faculty of Pharmacy, Niger Delta University, Nigeria. Thirty Wistar rats of both sexes (200±20g) were used. The rats were supplied by the animal handling unit of the Department of Pharmacology/Toxicology, Faculty of Pharmacy, Niger Delta University, Nigeria. The rats were randomized into six groups of 5 rats /group and were housed under standard laboratory conditions (12 h light: 12 h dark cycle at 25±2°C). The rats were orally treated daily for 30 days as follows: Group 1 (Vehicle control [normal saline 0.2mL]), group 2 (GTN 200 mg/kg) (20), group 3 (RIPE 150, 75, 400 and 275 mg/kg in vehicle) (3), group 4 (GTN 50 mg/kg +RIPE), group 5 (GTN 100 mg/kg +RIPE) and group 6 (GTN 200 mg/kg +RIPE). The rats were weighed and exposed to anesthesia at the end of treatment. Blood samples were collected from the heart and analyzed for serum renal biomarkers. The rats were dissected and kidneys were harvested, weighed and assayed for oxidative stress markers and histology. The kidneys assayed for oxidative stress markers were rinsed in cold saline and homogenized in chilled phosphate buffer (0.1 M, pH 7.4) containing KCl (1.17%). The homogenates were centrifuged (15000 rpm for 30 min at 4 ^{O}C) and the supernatants were collected and assayed for oxidative stress markers.

2.2. Evaluation of renal biomarkers

Serum creatinine, urea, uric acid, total protein and albumin concentrations were measured using an auto analyzer.

2.3. Oxidative stress marker assay

Malondialdehyde (MDA) was estimated using an established method described by Buege and Aust (21). Super oxide dismutase (SOD) was assessed using the procedure described by Sun and Zigman (22). Glutathione (GSH) was assayed according to the method reported by Sedlak and Lindsay (23). Glutathione peroxidase (GPx) was measured using the procedure described by Rotruck *et al*. (24). The method reported by Aebi (25) was used for the estimation of catalase (CAT).

2.4. Histology of the kidney

Kidney samples were cleaned and stored in a solution of formalin saline (10%) for 24hr. Thereafter, the kidney samples were dehydrated in graded concentration of alcohol solutions, processed and embedded in paraffin wax. Kidney tissues were sectioned (3µm thickness) and stained with Haematoxylin and Eosin on slides. The slides were examined using a light microscope.

3. Results

3.1. Effects of glutamine on body and kidney weights of antituberculosis drug-treated rats

GTN had no effects (p>0.05) on the body and kidney weights when compared to the control (Table 1). On the other hand, RIPE significantly (p<0.01) decreased body weight and significantly (p<0.01) increased kidney weight when compared to the control (Table 1). However, treatment with GTN 100 mg/kg +RIPE, and GTN 200 mg/kg +RIPE significantly restored the body and kidney weights at p<0.05 and p<0.01, respectively when compared to RIPE (Table 1).

Table 1. Effects of glutamine on body and kidney weights of anti-tuberculosis drug-treated rats

Treatment (mg/kg)	FBW(g)	AKW(g)	RKW (%)
Control	210.8±16.7	0.75±0.07	0.36±0.07
GTN 200	220.6±14.9	0.73±0.03	0.33±0.05
RIPE	120.7± 16.6#	1.88±0.04#	1.56±0.08#
GTN 50 + RIPE	140.1±15.5	1.70±0.01	1.21±0.06
GTN 100+RIPE	175.0±15.2*	1.10±0.06*	0.63±0.04*
GTN 200+RIPE	200.2±17.5**	0.78±0.09**	0.39±0.02**

Data as mean ± SEM (Standard error of mean), n=5, GTN: Glutamine, RIPE: Rifampicin/isoniazid/pyrazinamide/ethambutol, FBW: Final body weight, AKW: Absolute kidney weight, RKW: Relative kidney weight, # p<0.01 differ significantly when compared to the control, *p<0.05 and **p<0.01 differ significantly when compared to RIPE

3.2. Effects of glutamine on renal biomarkers of antituberculosis drug-treated rats

GTN had no significant (p>0.05) effects on serum renal biomarkers when compared to the control. In contrast, RIPE significantly (p<0.001) increased serum creatinine, urea and uric acid levels when compared to the control (Fig. 1a-1c). On the other hand, treatment with GTN 50 mg/kg +RIPE, GTN 100 mg/kg +RIPE and GTN 200 mg/kg +RIPE significantly decreased serum creatinine, urea and uric acid levels at p<0.05, p<0.01 and p<0.001, respectively when compared to RIPE (Fig. 1a-1c). RIPE significantly (p<0.001) decreased serum total protein and albumin levels when compared to the control (Fig. 1d and 1e). However, treatment with GTN 50 mg/kg +RIPE, GTN 100 mg/kg +RIPE and GTN 200 mg/kg +RIPE significantly increased serum total protein and albumin levels at p<0.05 and p<0.01 and p<0.001, respectively when compared to RIPE (Fig. 1d and 1e).

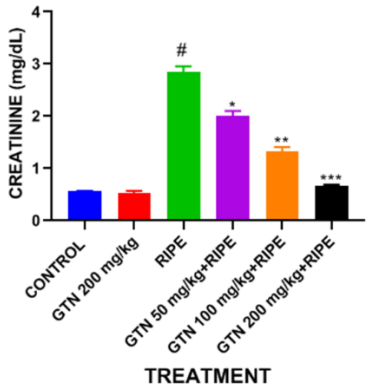

Fig. 1a. Effect of glutamine on serum creatinine of anti-tuberculosis drug-treated rats. Data as mean ± SEM (Standard error of mean),n=5, GTN:Glutamine,RIPE:Rifampicin/isoniazid/pyrazinamide/ethambutol, #p<0.001 differ significantly when compared to the control, *p<0.05, **p<0.01, and ***p<0.001 differ significantly when compared to RIPE

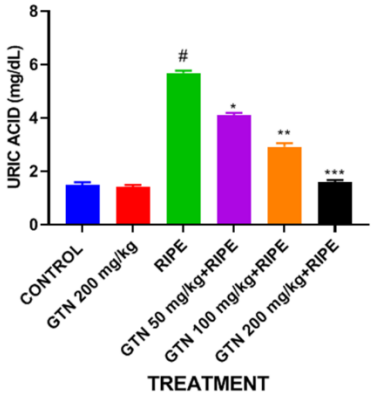

Fig. 1b. Effect of glutamine on serum uric acid of anti-tuberculosis drug-treated ratsData as mean ± SEM (Standard error of mean), n: 5, GTN:Glutamine,RIPE:Rifampicin/isoniazid/pyrazinamide/ethambutol, #p<0.001 differ significantly when compared to the control, *p<0.05, **p<0.01, and ***p<0.001 differ significantly when compared to RIPE

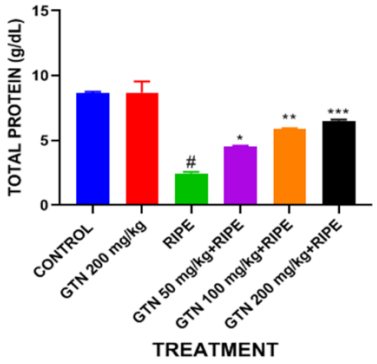

Fig. 1d. Effect of glutamine on serum total protein of anti-tuberculosis drug-treated ratsData as mean ± SEM (Standard error of mean), n: 5, GTN:Glutamine,RIPE:Rifampicin/isoniazid/pyrazinamide/ethambutol, #p<0.001 differ significantly when compared to the control, *p<0.05, **p<0.01, ***p<0.001 differ significantly when compared to RIPE

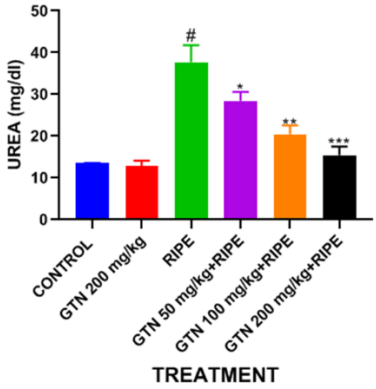

Fig.1c. Effect of glutamine on serum urea of anti-tuberculosis drug-treated rats.Data as mean ± SEM (Standard error of mean), n: 5, GTN: Glutamine, RIPE: Rifampicin/isoniazide/pyrazinamide/ethambutol, #p<0.001 differ significantly when compared to the control, *p<0.05, **p<0.01, and ***p<0.001 differ significantly when compared to RIPE

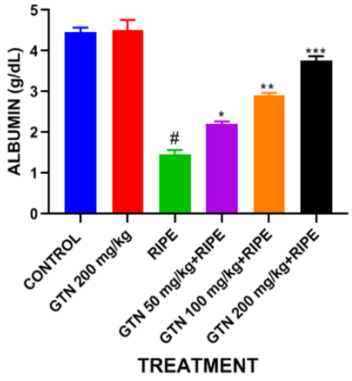

Fig. 1e. Effect of glutamine on serum albumin of anti-tuberculosis drug-treated rats. Data as mean ± SEM (Standard error of mean), n: 5, GTN:Glutamine,RIPE:Rifampicin/isoniazid/pyrazinamide/ethambutol, #p<0.001 differ significantly when compared to the control, *p<0.05, **p<0.01, and ***p<0.001 differ significantly when compared to RIPE

3.3. Effect of glutamine on kidney oxidative stress markers of anti-tuberculosis drug-treated rats

GTN didn't produce significant (p>0.05) effects on kidney SOD, CAT, GPx, GSH and MDA levels when compared to the control. RIPE significantly (p<0.001) decreased kidney SOD, CAT, GPx, and GSH levels when compared to the control (Table 2). However, treatment with GTN 50 mg/kg +RIPE, GTN 100 mg/kg +RIPE and GTN 200 mg/kg +RIPE significantly increased kidney SOD, CAT, GPx, and GSH levels at p<0.05 and p<0.01 and p<0.001, respectively when compared to RIPE (Table 2). RIPE produced significant (p<0.001) decrease in kidney MDA level when compared to the control (Table 2). Interestingly, GTN 50 mg/kg +RIPE, GTN 100 mg/kg +RIPE and GTN 200 mg/kg +RIPE significantly decreased kidney MDA levels at p<0.05, p<0.01, and p<0.001, respectively when compared to RIPE (Table 2).

Table 2. Effect of glutamine on kidney oxidative stress indices of anti-tuberculosis drug-treated rats

Treatment (mg/kg)	GSH µmole/mg protein	GPx U/mg protein	SOD U/mg protein	CAT U/mg protein	MDA nmole/mg protein
Control	12.80±1.07	25.40±2.14	30.50±3.10	25.46±3.11	0.23±0.01
GTN 200	13.31±1.11	25.51±3.32	31.00±3.21	25.67±3.33	0.22±0.07
RIPE	5.52±0.28#	10.62±0.38#	13.48±1.14#	8.48±0.10#	1.44±0.09#
GTN 50+RIPE	7.67±0.18*	14.80±1.10*	18.52±1.19*	12.56±1.39*	1.00±0.07*
GTN 100+RIPE	9.72±0.29**	19.91±1.07**	22.54±2.09**	17.72±1.19**	0.60±0.06**
GTN 200+RIPE	11.75±1.07***	24.60±2.13***	28.66±2.10***	23.52±2.29***	0.33±0.01***

Data as mean ± SEM (Standard error of mean), n=5, GTN: Glutamine, RIPE: Rifampicin/isoniazid/pyrazinamide/ethambutol, GSH: Glutathione, GPx: Glutathione peroxidase, SOD, Superoxide dismutase, MDA: Malondialdehyde. #p<0.001 differ significantly when compared to the control, *p<0.05, **p<0.01, and ***p<0.001 differ significantly when compared to RIPE.

3.4. Effect of glutamine on kidney histology of anti-tuberculosis drug-treated rats

The kidneys of the control (Fig. 6A) and GTN (200 mg/kg) treated rats (Fig. 6B) showed normal renal tubules and glomeruli. On the other hand, the kidney of RIPE-treated rats showed tubular necrosis, lipid accumulation, collapsed glomeruli and widened Bowman's space (Fig. 6C). The kidney of GTN 50 mg/kg+ RIPE-treated rats showed tubular necrosis, collapsed glomeruli, and widened Bowman's space (Fig. 6D). The kidneys of GTN 100 mg/kg+ RIPE-treated rats (Fig. 6E) and GTN 200 mg/kg+ RIPE –treated rats (Fig. 6F) rats showed normal tubules and normal glomeruli.

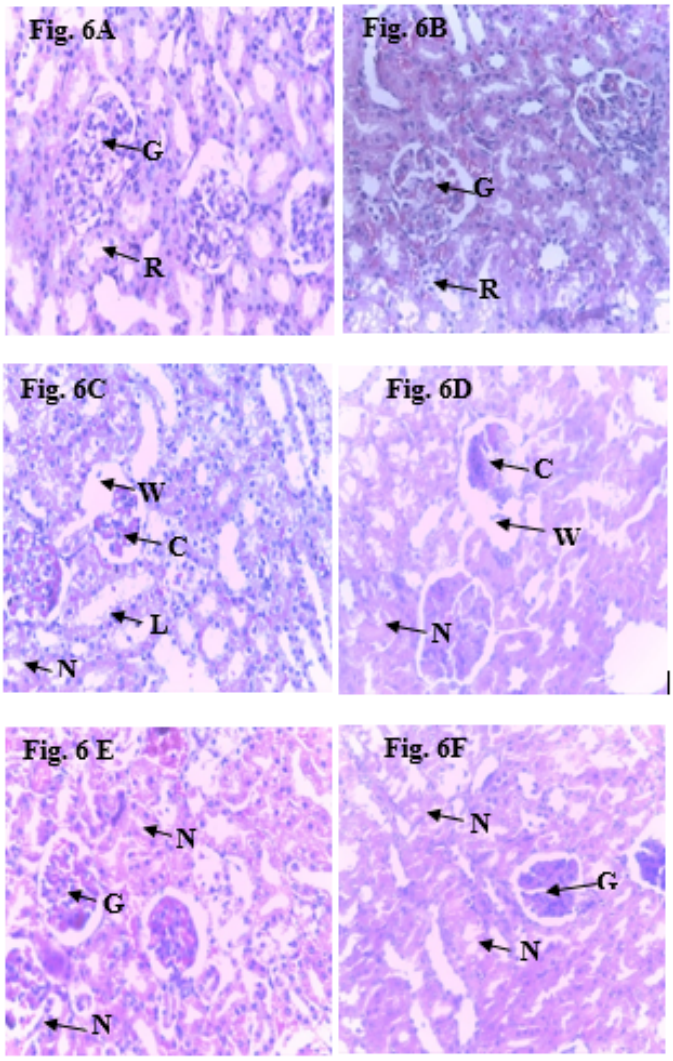

Fig 6A-F. are kidney micrographs of the control and experimental rats. 6A: Control, 6B: Treatment with GTN (200mg/kg), C: Treatment with RIPE, D: Treatment with GTN (50mg/kg) + RIPE, E: Treatment with GTN (100mg/kg) + RIPE. Treatment with GM (200mg/kg) + RIPE. R: Normal renal tubule, G: Normal glomerulus, N: Tubular necrosis, LP: Lipid accumulation, C: Collapsed glomerulus, W: Widened Bowman's space. x 400

4. Discussion

Nephrotoxicity is one of the common challenges associated with the use of RIPE (26). GTN may play an important role in protecting cells from chemical-induced damage (18). The current study assessed the protective effect of GTN on RIPE-induced nephrotoxicity in rats. The assessments of body and organ weights are essential in toxicity studies (27). In this study, RIPE decreased body weight and increased kidney weight in treated rats. This is consistent with decreased body weight and increased kidney weight in isoniazid-rifampicin-treated rats reported by Prince *et al.* (26). The observed decrease in body weight might have been caused by the appetite suppressive effect of RIPE. On the other hand, increase in the kidney weight might be a consequence of RIPE-induced renal inflammation. Studies showed that the induction of renal inflammation might be one of the mechanisms by which RIPE perturbs renal function (26). But GTN supplementation restored body and kidney weights in a dose–related fashion. GTN supplementation might have stimulated appetite and down-regulated the induction of renal inflammation by RIPE. Over the years, the assessments of biochemical markers have been used to diagnose and establish the types and magnitude of perturbations caused by underlying ailments and drugs (28). This study assessed creatinine, urea, uric acid, total protein and albumin levels to ascertain the toxic impact of RIPE on the renal status of rats (28). RIPE remarkably increased serum creatinine, urea, uric acid levels and decreased serum total protein and albumin levels in rats. These are signs of renal dysfunction caused by RIPE. Similarly, Martins and Sabina (29) reported altered levels of the aforementioned parameters in rats treated with anti-tuberculosis drugs. But GTN Supplementation restored renal biomarkers in RIPE-treated rats in a dose-related fashion.

Reactive oxygen species (ROS) are produced due to exposure to many exogenous chemicals or plenty of endogenous metabolic processes including redox enzymes and electron transport chain (30). Excess ROS are usually neutralized by endogenous antioxidants such as CAT, SOD, GPx and GSH. The inability of antioxidants to neutralize ROS can cause oxidative stress leading to cellular damage (30). In this study, RIPE caused notable decreases in kidney SOD, CAT, GSH and GPx levels. This is consistent with depleted kidney antioxidants caused by antituberculosis drugs in rats reported by Sharma *et al.* (31). The depleted kidney antioxidants in RIPE-treated rats may be due to excess ROS production leading to oxidative stress (31). Oxidative damage has been speculated to be involved in the etiology of many chronic diseases and drug-induce toxicities (32). However, in a dose-related fashion, kidney antioxidants were restored in GTN supplemented rats. Studies have shown that GTN can increase the antioxidant capacity of tissues, by enhancing CAT, SOD, GSH, and GPx activities (33, 34). GTN might have inhibited the production of ROS by RIPE thereby incapacitating its capacity to deplete kidney antioxidants. Lipid peroxidation (LPO) is a free radical oxidation of poly unsaturated fatty acids that has been associated with some diseases. It is a self-sustaining process that is capable of causing extensive tissue damage. Many toxic aspects of LPO are attributed to reactive aldehydes produced from oxidized lipids, which react with cellular nucleophiles such as nucleic

acids, proteins and GSH forming a variety of adducts. The formation of adducts has been associated with a number of disease conditions (35). MDA, one of the reactive aldehydes has been used as a yardstick for LPO (36). In this study, RIPE increased kidney MDA levels in treated rats. This is in agreement with increased MDA concentration in antituberculosis drug-treated rats reported by Hussein *et al*. (37). The elevated levels of kidney MDA may be due to the oxidation of poly unsaturated fatty acids caused by RIPE. However, GTN supplementation decreased kidney MDA levels in a dose-related fashion. This may be due to the ability of GTN to inhibit the lipid peroxidative activity of RIPE in the kidneys of treated rats.

Histology is necessarily a largely descriptive and interpretive science. It gives structural manifestations of diseases at the light-microscopic level (38). In this study, the histological assessment of the kidneys of RIPE-treated rats showed tubular necrosis, lipid accumulation, collapsed glomerulus and widened Bowman's space, which correlate with observed changes in evaluated biomarkers. Adikwu *et al.* (28) reported similar kidney histological changes in anti-tuberculosis drug-treated rats. This observation may be due to the induction of oxidative stress by RIPE through ROS production causing structural and functional damage to kidney biomolecules (DNA, lipids, and proteins) (28). However, supplementation with GTN restored kidney histology. GTN might have restored kidney histology by safeguarding kidney biomolecules from the menace of oxidative stress induced by RIPE.

GTN supplementation protects against RIPE-induced nephrotoxicity in a dose-related fashion. It may have clinical use for RIPE associated nephrotoxicity.

Ethical statement

Approval was obtained from the Research Ethics Committee (NDU/PHARM/PCO/AEC/068) of the Department of Pharmacology/Toxicology, Faculty of Pharmacy, Niger Delta University, Nigeria.

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Conflict of interest

The authors declare there was no conflict of interest

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Authors' contributions

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

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Knowledge of health care providers about Covid-19 disease in pregnancy and lactation in Ardabil

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Abstract

Healthcare workers and healthcare providers need sufficient knowledge and awareness about disease to provide high quality health services to pregnant and breastfeeding women during the Covid 19 pandemic. This study aims to investigate the knowledge of healthcare providers about Covid-19 disease in pregnancy and lactation in Ardabil.This descriptive study was conducted in 2020-2021. The study's statistical population consisted of all healthcare workers working in health centers in Ardabil who care for pregnant and lactating mothers. The census calculated the sample size. Data were collected using a self-administered questionnaire to assess health care providers' knowledge, which was designed using a review of texts, related articles, and instructions of the Ministry of Health, and the validity and reliability of the questionnaire have been confirmed. Data were analyzed using SPSS20 by descriptive statistics, chi-square, and ANOVA statistical tests. Mean age of the participants was 35.51±5.74. In this study, most participants scored more than 33 and had a good level of knowledge about Covid 19 disease in pregnancy and lactation. The study's results showed that participants had higher knowledge about Covid 19 during pregnancy and lactation. The knowledge gained about Covid 19 during pregnancy and lactation is not absolute and may get updated with the reporting of newer studies. Healthcare providers must be trained in person and online during the Covid 19 virus epidemic to receive up-to-date information regularly from the healthcare system.

Keywords: knowledge, Covid-19, health care providers, pregnancy and lactation

1. Introduction

The coronavirus disease started in 2019 in Wuhan, China, and affected the country's large population (1, 2). This virus is a new form of the known Coronavirus, and the resulting disease was called Covid-19 (3). On January 30, 2020, the World Health Organization declared Covid-19 a public health emergency, and later on March 11, 2020, reported it as an outbreak pandemic (4, 5).

On February 20, 2020, two death cases from the disease were reported in Iran for the first time. The number of infected people raised increased in Iran (6). Early scientific reports suggested that Covid-19 may be transmitted from animal to human, but subsequent studies have shown that respiratory droplets cause direct human-to-human transmission (7, 8).

The virus can infect humans in different severities, from upper respiratory tract infections such as colds to lower respiratory tract infections such as bronchitis, pneumonia, and even acute respiratory syndrome (9). The first symptoms seen in these patients included fever, dry cough, shortness of breath, chest pain or pressure, fatigue, muscle aches, dizziness, and leukopenia. Patients with mild symptoms require supportive care and patients with more severe symptoms require oxygen therapy and hospitalization (7, 10, 11).

The covid-19 disease affects various groups, including pregnant women (12). Due to the weakened immune system and physiological changes in the cardiovascular system during pregnancy, pregnant mothers are vulnerable to infections and viruses, and therefore the Covid epidemic can have severe consequences during pregnancy (13, 14).

Studies have shown that emerging infections significantly impact pregnancy and the fetus, for instance, maternal and embryonic complications due to the H1N1 Flue pandemic in 2009 (15, 16). Pregnant women are worried about giving birth (13), and despite programs of social distancing and fear of developing Covid-19, they tend to reduce the number of visits to health centers. Further, lack of care for pregnant women causes harmful maternal and embryonic complications (17, 18).

As Covid-19 is an emerging disease, people have limited knowledge about it, and healthcare professionals need to know about Covid-19. Healthcare providers are at the forefront of caring for pregnant women and require an understanding of the disease to improve the quality of care for pregnant mothers by providing the correct information to mothers and the proper counseling. Midwives and healthcare providers are responsible for promoting the health of mothers and infants, and their knowledge can affect the community's health (12). Misinformation directly increases the risk of disease (19).

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There was an urgent need in the community to establish a plan for the deadly Covid-19 and provide community members with guidelines, especially health care providers, to control the disease, promote maternal health and reduce maternal mortality and neonatal complications. Healthcare providers have an essential and decisive role in combating this disease. Familiarity of midwives with different aspects of the disease is fundamental, and they can provide better and more efficient services to pregnant women by gaining the necessary knowledge in this field. The Covid-19 epidemic offers a unique opportunity to assess the level of knowledge of healthcare providers. Thus, the present study evaluated healthcare providers' knowledge about Covid-19 disease during pregnancy and lactation in Ardabil.

2. Materials and Methods

The present study is a descriptive study (2020) that aims at assessing the knowledge of healthcare providers who care for pregnant and lactating mothers against Covid-19. The study's statistical population includes all 180 healthcare providers of pregnant and lactating mothers working in health centers in Ardabil included in the study by the census. The list of active healthcare workers and their places of employment was received from Ardabil Health Center. Inclusion criteria were, midwifery and family health graduates with an associate's and bachelor's degree with at least six months of experience as health care providers in health centers willing to participate in the study. Exclusion criteria were the presence of health care providers on maternity and sick leave or incomplete completion of questionnaires.

The data collection tool was a researcher-made questionnaire to determine healthcare providers' knowledge about Covid-19 in pregnancy and lactation. This tool has two sections: demographic-social characteristics and measurement of healthcare providers' knowledge about Covid-19 during pregnancy and lactation. The questionnaire of demographic-social characteristics provides information about age, marital status, number of children, level of education, the field of study, service history, employment status, place of work, medical history, setting of obtaining information about Covid-19, having previous information about the coronavirus outbreak, and the second section measures the knowledge of health care providers with 50 questions about the Covid-19 virus during pregnancy and lactation.

Each question is scaled from 0 to 1, and in case of correct answers, 50 points are awarded. In terms of grading, the knowledge of health care providers was assessed at three levels: poor (0-16), moderate (17-33), and good (34-50). This questionnaire was designed by studying texts, articles, and instructions from the Ministry of Health. Face validity and content validity were used to evaluate the instrument's validity. Face validity is the most convenient way to check the validity of a tool, that is, whether the instrument's appearance correctly measures the variable under study.

In face validity, the tool's structure is examined from the point of view of the target group. In this step, to check the face validity and reliability, the questionnaire was handed out to 14 Faculty members, including midwives, reproductive health professionals, and nurses at Ardebil University of Medical sciences. In the next step, content validity was performed quantitatively using the content validity ratio (CVR) and content validity index (CVI). According to the CVR decision table, the ten experts' minimum acceptable validity value is 0/62. The resulting number for our study tool was 0/8. The content validity was confirmed. Experts were asked to rate each question on a scale of 1 to 4.

The four-item scales included "fully relevant", "relevant", "semi-relevant" and "irrelevant". CVI is obtained by dividing the total number of positive scores for each item of the questionnaire (obtained 3 and 4 scores that are; "relevant but requires precision" and "fully relevant") by the number of experts. So, 0.8 was obtained for tools, and the study's validity was confirmed. Furthermore, the internal reliability of the questionnaire was obtained using Cronbach's alpha coefficient of 0.87.

After getting approval from the ethics committee of Ardabil University of Medical Sciences (ethics code IR-ARUMS.REC.1399.549), the researchers attended the research site permitted by the permission of the health deputy and the director of Ardabil health center and distributed questionnaires among them. Participants were allowed to leave the study if they wanted to refuse. Finally, 150 questionnaires were returned to the researchers.

The present study analyzed data using SPSS 20 and descriptive statistics (frequency and percentage, mean and standard deviation), and inferential statistics. In inferential statistics, one-way ANOVA and chi-square tests were used. The ethics committee has approved the proposal of this study of Ardabil University of Medical Sciences.

3. Results

A total of 150 people participated in the study. The mean age of participants was 35.51± 5.74 years. 85.3% participants were married (n=139), 45.3% had one child, 82.7% had a bachelor's degree, 8.7% had an associate's degree, and 8.7% had a master's degree. The majority of healthcare providers included midwives (74.7%), who majored in family health studies (18%), and who studied in other fields (7.3%) (Table 1).

The majority of participants, 128 (85.3%), had no history of the disease. Most participants (84.7%) knew nothing about Coronavirus before the outbreak; 110 (73.3%) of the participants had obtained their information about Covid-19 from the Ministry of Health or medical universities. In the present study, 109 people (72.7%) scored more than 33, which means a good level, 38 people (25.3%) had a moderate level of knowledge, and 3 people (2%) had poor knowledge about Covid-19 disease in pregnancy and lactation.

Table 1. Percentage and frequency of demographic characteristics of research samples (n=150)

Variables		No.(%)	Variables		No.(%)
	Single	26 (14.7)		No child	40 (26.7)
Marital status	Married	139 (85.3)	**Number of children**	1 child	68 (45.3)
				2 children	38 (25.3)
				3 children	4 (2.7)
Field of major	Midwifery	112 (74.7)	**Education level**		
	Family Health	27(18)		Associate degree	13 (8.7)
	Others	11 (7.3)		Bachelor degree	124(82.7)
Experience (years)				Masters	13 (8.7)
	1-5	66(44)	**Employment state**	Official	40(26.7)
	6-10	41(27.3)		Temporary	12 (8)
	11-15	13 (8.7)		Internship	4(2.7)
	16-20	11 (7.3)		Contract	94(62.7)
	21-25	18 (12)			
	26-30	1(0.7)			
Setting	Health center	29(19.3)	**Disease history**	No history	128 (85.3)
	Base	121(80.7)		Respiratory	2 (1.3)
				Cardiovascular	7(4.7)
				Gastrointestinal	1(0.7)
				Glandular disease	8(5.3)
				Renal disease	2(1.3)
				Dermal disease	2(1.3)
Data resources	Websites and statements	47(31.3)	**Knowing Coronavirus before the pandemic**	Yes	23(15.3)
	WHO			No	121(84.7)
	Social networks	49(32.7)			
	National Media	53(35.3)			
	Ministry of Health and University of Medical Sciences	110(73.3)			
	Colleagues	31(20.7)			
	Others	8(5.3)			

In the present study, the Chi-square test showed no statistically significant relationship between the age of health care providers and the knowledge variable (P = 0.8) and no significant difference between the level of knowledge in married and single groups (P= 0.13).

The study of the relationship between education level and knowledge of health care providers showed no statistically significant difference between the three groups with associate degrees, bachelor's degrees, and master's degrees (P = 0.97). Moreover, there was no statistically significant difference between the field of study, the experience of healthcare providers, and their level of knowledge (P = 0.5, P = 0.33). There was no statistically significant difference between having previous knowledge about the prevalence of Coronavirus and their level of knowledge about Covid-19 disease during pregnancy and lactation (P = 0.8).

4. Discussion

The present study entitled "Knowledge level of health care providers about Covid-19 disease during pregnancy and lactation in Ardabil," was conducted in Iran in the middle of the Covid-19 outbreak in 2020. Covid-19 has been classified as a high-risk infection since it is newly emerged and has spread worldwide, and people, especially pregnant and lactating mothers, are at risk.

The pandemic resulting from the Coronavirus is currently severe and contagious, affecting the world's population (20-22). Therefore, the knowledge level of healthcare providers about Covid-19 disease, who care for pregnant mothers in health centers, is essential, and it is essential to inform them of the severity of the disease to develop preventive measures to reduce and control this infection, especially in pregnant and lactating women. Health policymakers in the Ministry of Health and Medical Education can make the right plans. According to our survey, this is the first descriptive study since the outbreak of Covid-19.

According to the present study results, most healthcare providers had a good level of knowledge about Covid-19 disease during pregnancy and lactation. In the study of Taghrir et al. (2020), the level of knowledge of medical students about Covid-19 in Iran has been reported at a high level (23). In the study by Zhang et al. in Hanan, China, 89% of health workers had sufficient knowledge about Covid-19; physicians had higher knowledge scores than nurses and other health workers (24). A study by Ping et al. in China showed that most

undergraduate students had a good knowledge of Covid-19 (25), consistent with the present study results. A study by Bhagavathula et al. (2020) shows that health workers did not have sufficient knowledge about Covid 19, which is inconsistent with the results of our study (26). In the present study, only healthcare providers who cared for pregnant and lactating mothers were studied.

The present study showed that most study participants (73.3%) obtained their information through the Ministry of Health or medical universities. Accordingly, the study of Bhagavathula et al. showed that the primary source of information for the research participants was official government sites (26).

In contrast, in the Kushalkumar study in India on medical students, most students (65.17%) acquired knowledge about Covid 19 from social media (27), which was the reason for the difference in research samples; students tend to read all news and social sites. These students, the main body of the health care system in the future, should obtain valid information on some of the above websites due to the existence of unverified information and should refer to reputable scientific and official government websites.

It should be noted that healthcare providers should ensure the health of mothers by providing primary and correct care during Covid 19 disease, which is why it is necessary to increase the knowledge and awareness of healthcare providers. Studies show that infection of the pregnant mother with Covid 19 leads to pregnancy complications such as miscarriage, premature rupture, fetal growth restriction, fetal distress, and preterm delivery (28-34). Therefore, prenatal care is essential in this regard. During pregnancy, especially in the third trimester, fetal health care is provided mainly for mothers infected with Covid 19 (15, 28, 35).

The present study results can be used as a pilot study by health policymakers for educational programs to prevent Covid19 and reduce the complications of the disease in pregnant and lactating mothers. One of the limitations of this study is that this study was conducted in only one city and the generalization of the results to other healthcare providers in Iran requires further studies. The result of the study depends on the participants' honesty in answering the questions in the questionnaire.

Our healthcare providers have excellent or high levels of Covid-19-related knowledge in pregnancy and lactation. As pregnant mothers undergo changes in physiology and immune system during pregnancy and need high-quality care, healthcare providers must receive up-to-date information regularly from the healthcare system in person and online.

Conflict of interest

There is no conflict of interest in the present study

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Authors' contributions

Concept: R.K., P.A.K., Design: R.K., P.A.K., Data Collection or Processing: R.K., L.R., Analysis or Interpretation: S.D.F., L.R., Literature Search: P.A.K., L.R., Writing: R.K., S.D.F.

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Comparison of anxiety, depression, and quality of life of husbands of women with and without breast cancer

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Abstract

The aim of this study was to compare the anxiety, depression, and quality of life of husbands of women with breast cancer referred to the chemotherapy clinic with husbands of non-affected wives. This was a case-control study, with purposive-convenience sampling method. 190 husbands of patients with or without breast cancer participate in this study. Data collection tools included a four-part questionnaire, consisted of demographic characteristics, shortened form of quality of life questionnaire, Beck depression inventory, and Beck anxiety inventory. In the areas of social relations and living environment (QolD3, QolD4), a statistically significant difference was found between the two groups in quality of life (p-value < 0.001). Pearson correlation coefficient showed a statistically significant difference in the case group between the history of chemotherapy and mean anxiety score (p-value < 0.001), and quality of life with number of children (p-value < 0.002). The findings of this study showed that in addition to giving attention to the patients, attention to health needs and quality of life of their spouses is also a priority.

Keywords: anxiety, breast cancer, depression, quality of life

1. Introduction

Cancer is one the most common health problems worldwide. (1) It is the third leading cause of death in Iran after cardiovascular diseases and accidents. Breast Cancer is the most common cause of cancer death in women. (2) It is one of the most common malignancies in the world and unfortunately has been increasing in Iranian women. (3, 4) Diagnosis and treatment of breast cancer have a significant impact on the lives of patients and their families. Women with breast cancer experience a lot of psychological disorder . Psychologic distress affects various aspects of the quality of life of women with breast cancer, especially anxiety and self-image (5). The spouses of such woman become more influential because of their active role in choosing treatments and providing their wives with comprehensive support (6). Studies have shown that spouses who care for their partner with cancer are subject to a wide range of physical, psychological, and social attenuation (7). Couples have an interactive effect on each other's quality of life, mental health, and well-being (6). All members of the family are affected by the illness of a family member, but the supportive role of spouses is much more important than the supportive role of friends or other family members. (5, 6) Feeling good about one spouse affects another. Family members, especially spouses who are the main caregivers to the patient with cancer are directly and indirectly affected by changes in the patients' condition. (7) Qualitative studies on husbands of women with breast cancer described their major experiences as unpleasant experiences of cancer and changes in their mutual connections. The spouses of such patients share that "the world on which we had built our lives and our regular daily lives were destroyed when cancer was diagnosed (8-9) They were trying to do something to support their spouses. (8) Studies have shown that husbands of women with breast cancer experience a lot of stress and are unprepared for the challenges of caring for their spouses. (9) Husbands have increased stress and distress, affecting their well-being and decreasing their quality of life (10). Symptoms of distress, such as anxiety and depression, have a negative effect on the quality of life of such women and their husbands (13,14) Thus, this study was designed to compare anxiety, depression, and quality of life of husbands of women with and without breast cancer referred to the chemotherapy clinic.

2. Materials and Methods

2.1. Research design and sample

This case-control study was performed on married men referred to Shahid Motahari Clinic, affiliated to Shiraz University of Medical Sciences. This clinic is a central clinic, located in Shiraz, southwestern Iran, and has several sections in 6 floors and is referred for clients with different kinds of health problems or diseases from all levels of the community. According to similar studies[4,9], and considering α=0.05 and 90% power (β=1.0) the sample size by using MedCalc

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software was estimated as 190 subjects (each group 95).

$$n=\frac{z_{1-\alpha/2}+z_{1-\beta})^2[p_1(1-p_1)+p_2(1-p_2)]}{d^{*2}}$$

Purposive-convenience sampling method was used and 95 husbands of women with breast cancer who referred for chemotherapy were included in the case group, and 95 husbands of healthy women who referred to the clinic for other reasons were included in the control group. Inclusion criteria for the control group were the presence of husbands and in both groups were the willingness to complete the questionnaire and lack of any physical or mental illness to interfere with their ability to answer the questionnaire. The exclusion criterion was reluctance to complete the questionnaire.

2.2. Data collection and instruments

Data collection tool was a four-part questionnaire. In the first part of the questionnaire, the demographic characteristics of the women's and their spouses, and the status of the women's disease were asked. The second part was the shortened form of the Quality-of-Life Questionnaire. The third part was the Beck Depression Inventory II, and the fourth part was the Beck Anxiety Inventory. The World Health Organization Short Form Quality of Life Questionnaire is a short form of the World Health Organization's 100-question Quality of Life Questionnaire, which measures the quality of life in four health-related domains: physical health, psychological, social relations, and living environment domains. This questionnaire contains 26 questions, which the first and second questions examine the quality of life and health status, respectively. The next 24 questions assess the quality of life in the four areas mentioned above. This questionnaire has been validated for the Iranian population with a validity of 0.7 to 0.9 (15,16). The Beck Depression Inventory II includes 21 questions. These questions vary with the Likert scale from 0, which indicates the absence of the symptoms to 3, which indicates the severe symptoms. The final score of this questionnaire varies from 0 to 63. The scores of 0-15 indicate normal state, 16-31 mild depression, 32-47 moderate depression, and 48-63 severe depression. (11, 12)

The Beck Anxiety Inventory has 21 items on a 4-point scale from 0 to 3. The score varies from 0 to 63, with scores of 0-7 indicating low or no anxiety, scores of 8-15 mild anxiety, scores of 16-25 moderate anxiety, and scores of 26-63 with severe anxiety. The validity and reliability of Beck Anxiety Inventory and Beck Depression Inventory II have been reported and used in many studies in Iran (16-18) Beck Anxiety Inventory with 0.72 validity and 0.83 reliability, and Beck Depression Inventory with 0.93 validity and 0.89 reliability have been confirmed (18).

The questionnaire was completed by husbands who met the inclusion criteria and were assigned to either the case or control group according to whether their spouses had breast cancer or was healthy. Data were analyzed using SPSS version 22.0 software, and the descriptive statistics including mean and standard deviation were utilized. Analytical tests including chi-square for comparison two group, Pearson correlation coefficient was used to investigate the relationship between variables, and ANOVA to evaluate the relationship between independent variables (breast cancer) and dependent variables (quality of life, anxiety, and depression).

3. Results

All the participants, including the case and control groups, lived with their spouses at the time of the study. Duration of chemotherapy in spouses of case group varied from 3 months to 144 months. The mean age of the participants in the study was 41.2±10.2, with most common level of education of bachelor's or higher 79 (41.8%), 85 (45%) with freelance jobs, and the average of having two children. 31 (16.4%) of the spouses participating in the study were government employees. The highest level of education was high school diploma in 68 (36.0%) subjects. Demographic variables differed only in age between the two groups, and the mean age of the case group was higher, and for the other variables the two groups were matched. Table 1 shows the demographic characteristics and variables of the two groups.

Table 1. Comparison of demographic characteristics of case and control groups

Group		p
Job	**Case**	0.72
	Control	
Education	**Case**	0.27
	Control	
Marital status	**Case**	0.45
	Control	
Child	**Case**	0.31
	Control	
Wife job	**Case**	0.24
	Control	
Wife education	**Case**	0.51
	Control	

Overall mean score for quality of life in the case group was 84.5%±14.1 and in the control group was 87.2%±12.6, which did not show a significant difference. In the areas of social relations and living environment (QolD3, QolD4), there was a statistically significant difference between the two groups. Table 2 compares the scores of different domains of quality of life and its overall score in the case and control groups.

Anxiety was higher at all levels in the case group. In the case group 16.8% (n=17) had severe depression but in the control group only 9.6% (n=10) had severe depression. Table 3 compares the anxiety and depression scores in the two groups. One-way analysis of variance (ANOVA) showed that there was no significant difference between total score of quality of life and level of anxiety, and between total score of quality of life and depression in case and control groups. Table 4 shows the comparison of the mean scores of anxiety and depression in the case and control groups.

Table 2. Comparison of scores of Qualities of Life Questionnaire in case and control groups

Variable	Group	Mean	S.D.	p-value
Quality of life dimention 1	**Case**	26.0737	4.26575	0.815
	Control	27.8191	4.11648	
Quality of life dimention 2	**Case**	21.4000	3.87408	0.574
	Control	22.1596	3.66120	
Quality of life dimention 3	**Case**	25.6842	7.13288	0.000
	Control	11.2553	1.93405	
Quality of life dimention 4	**Case**	11.3158	4.11087	0.000
	Control	26.1702	5.21297	
Quality of life total score	**Case**	84.5789	14.18763	0.172
	Control	87.2872	12.68275	

Table 3. Comparison of the mean frequency distribution of anxiety in the case and control groups

Anxiety					
Group	**Low No (%)**	**Weak No (%)**	**Moderate No (%)**	**Severe No (%)**	**Total No (%)**
Case	51 (53.7)	31 (32.6)	11 (11.6)	2 (2.1)	95 (100)
Control	64 (68.1)	11 (11.7)	11 (11.7)	9 (8.5)	95 (100)

Table 4. Comparison of the frequency distribution of depression in case and control groups

Depression					
Group	**Low No (%)**	**Weak No (%)**	**Moderate No (%)**	**Severe No (%)**	**Total No (%)**
Case	30 (32.6)	18 (38.3)	30 (31.6)	17 (16.8)	95 (100)
Control	34 (36.2)	29 (30.9)	22 (23.4)	10 (9.6)	95 (100)

One-way ANOVA showed that there was no significant difference between total score of quality of life and level of anxiety and between total score of quality of life and depression in case and control groups. Table 5 shows the comparison of the mean scores of anxiety and depression in the case and control groups.

In regards to relationship between quality of life, anxiety score, and depression with demographic variables, Pearson correlation coefficient showed that in the case group, there was a statistically significant difference between the history of chemotherapy and mean anxiety score (p-value < 0.001), and quality of life with number of children (p-value < 0.002). There was no statistically significant difference between demographic variables, anxiety, depression, and quality of life in the control group.

Table 5. Comparison of anxiety and depression in two case and control groups

	Case Mean ± S.D.	Control Mean ± S.D.	p-value
Anxiety	8.50± 6.81	7.63± 8.36	0.123
Depression	9.4± 8.09	7.2± 6.76	0.0495

4. Discussion

Based on the results of this study, the diagnosis and treatments of breast cancer in women influence the mental health and quality of life of their spouses. A study by Götze et al. found that sexual partners of patient with cancer in regards to the physical dimension of quality of life, had no statistically significant difference with the rest of society, but there was a significant difference in mental and social dimensions (22).

The distress created for couples as a patient and caregiver, indicates the need for psychological and supportive interventions (8). Longitudinal studies of the effects of cancer on the quality of life and mood of couples whose one of their partners has cancer diagnosis, have contradictory findings (20,21).

In this study, the level of anxiety in the case group was higher than the control group. Symptoms of depression in caregivers of patients in terminal stages of the disease should not be considered as normal, but require attention and intervention. (13)

Reducing the level of anxiety and depression improves the quality of life in sexual partners of patients with cancer. A study aimed to assessing distress and quality of life in patients with cancer and their caregivers during home care showed that 33% of the caregivers of patients with cancer experience high levels of anxiety and 28% experience depression. Compared with other caregivers, spouses of patients requiring palliative care experience more distress (19). Results of a study by Bigatti and co-workers with title of "Depression in Husbands of Breast Cancer Patients: Relationships to Coping and Social Support" showed that the rate of depression in husbands of patients with breast cancer was higher than control group (23). A review study by Li et al. analyzed 25 articles published from 2000 to 2012 with the aim of examining spouses' experience of caring for patients with cancer. According to the results of that study, the spouses of patients with cancer experienced more negative emotions, such as decreased mental health and quality of life (24). Diagnosis of cancer and the death caused by it have a

negative impact on different aspects of caregivers' lives, especially their spouses. Most partners experienced severe symptoms of depression and poorer health soon before and right after their spouse's death (25). Women diagnosed with breast cancer and their spouses experience specific issues regarding illness, treatment, response, and coping. The disease affects the whole family. Among these experiences are adaptation to changes in the role and negative emotions such as decreased quality of life, depression, and anxiety associated with the patient and treatment of the disease (26).

In the case group, there was a statistically significant difference between the history of chemotherapy and the mean score of anxiety and quality of life with the number of children. A study by Sun et al. aimed to investigate the effectiveness of interdisciplinary palliative care intervention for home caregivers in patients with lung cancer showed that there was no statistically significant difference between caregivers' demographic characteristics and other variables including quality of life (27). The results of another study examining the quality of life of home caregivers of patients with cancer in Korea and Albania did not show a statistically significant difference between the demographic characteristics of the subjects and their quality of life (14). Other studies of patients with cancer in other parts of the world also confirm these results and are different from the results of this study. (15-17) This difference could be resulted from cultural differences and the burden of childcare.

Describing health problems and their effects on different aspects of health and quality of life is the first step toward considering the dimensions and solutions of the problem. In this descriptive study, the effects of breast cancer treatment on quality of life, anxiety, and depression of spouses were investigated. The impact of diagnosis and treatment of different types of cancer on the dimensions of family life, especially spouses, is a complex issue with hidden angles and other socio-cultural factors need to be examined for a better understanding. In addition, future evidence-based studies of this group's specific needs and appropriate methods of training and support should be examined and tested.

Findings of this study comparing the effects of diagnosis and treatment of cancer on quality of life and distress of spouses of women with and without breast cancer showed that besides patient, attention to health needs and quality of life of their spouses is also a priority, because they have the key role in supporting their sick wives. Nurses should enhance their ability to design and implement programs and be able to empower patients and their spouses to reduce stress or anxiety and possible depression. There seems to be a need for an appropriate framework to work with such patients and their spouses. The creation of strong social networks and support groups have been mentioned in some studies.

Ethical statement

Ethical approval will be sought in accordance with Vice-Chancellor for Research Affairs of Shiraz University of Medical Sciences and all participant completed the informed consent form (IF.SUMS.NUMIMG.REC.1400.007.2021.06.19).

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: F.W., F.D., Design: F.W., F.D., Data Collection or Processing: M.M., Analysis or Interpretation: M.M., F.D., Literature Search: F.W., M.M., Writing: M.M., F.D.

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The association between KRAS gene expression and breast tumors in a sample of Iraqi women

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Abstract

In Iraq, breast cancer is considered one of the most widespread cancer types causing death in women due to changes in the genes that control cell growth and proliferation. KRAS gene plays a role in 30% of human cancers. This study aims to evaluate the expression of the KRAS gene and its role in women with breast tumours. A total of 120 women (60 women with breast tumours and 60 samples from apparently healthy women) were enrolled in this study to determine the expression of the KRAS gene. The RT-qPCR was used for this purpose. Fibroadenoma (FA) was the most common tumours, accounting for 53% of cases, followed by fibrocystic change (26%). Other benign tumours accounted for 20% of the cases; however, IDC is the most common type of invasive breast cancer, accounting for 70% of all breast cancers. Moreover, KRAS gene expression in women with breast tumours (benign and malignant) was not- significantly different from that seen in normal healthy women.

Keywords: breast tumors, KRAS, gene expression, Iraqi women

1. Introduction

Breast cancer (BC) is one of the most common cancers that cause death in women, and its prevalence is increasing in Asia. Invasive BCs are a diverse group of tumours that vary in clinical presentation, behavior, and morphology (1, 2). In Iraq, from 2000 to 2019, BC accounted for roughly one-third of all cancer cases recorded in the country (3).

In the progression of breast cancer, multiple genetic events can activate dominant-acting oncogenes and disrupt the function of specific tumour suppressor genes (4). In addition, the *KRAS* gene is a proto-oncogene located on chromosome 12p12.1 that codes for a protein termed small GTPase transductor (5, 6). KRAS protein plays an important role in the epidermal growth factor receptor (EGFR) signalling pathway, and oncogenic mutations in protein can drive downstream activation of this pathway. *KRAS* 3'-untranslated regions (UTR) of humans contain several putative tumour suppressor lethal-7 (*let-7*) complementary sites (*LCS*) (7). *KRAS* gene is controlled at the post-transcriptional level by a highly complicated interplay of *cis-acting* elements inside its 3′ UTR due to its infrequently long 3′ UTR length (8). Therefore, genetic variants of *KRAS* 3'-UTR may prevent *let-7* miRNA from binding to *KRAS* and control the activity of *KRAS* to regulate the expression of its protein. A number of studies have found that the SNPs in the *KRAS* 3'-UTR might cause high levels of the KRAS oncogenic protein and lower levels of the *let-7* miRNA (7, 9,10).

Moreover, MicroRNAs (MiRNAs) are small non-coding RNAs that regulate gene expression at the post-transcriptional level by binding to complementary sites in the 3′ UTR of target messenger RNAs (mRNAs) (11,12). This case-control study aims to determine the role of *KRAS* gene expression and its association with the incidence of breast tumours.

2. Materials and Methods

2.1. Subjects

A total of 120 women participated in this study. Sixty women (who attended the Oncology Teaching Hospital in Baghdad) were diagnosed with breast tumours (both malignant and benign), with 60 appearing to be healthy controls. Details of clinical data and demographic characteristics for patients were collected from medical reports. The women with breast tumours were early-diagnosed cases before surgery or treatment.

2.2. RNA Isolation

RNA was extracted from whole blood samples using the Trizol TM Reagent (Promega, USA) in accordance with the manufacturer's protocol. The RNA concentration was determined using a Quantus fluorometer (Promega, USA). Aliquots of 199µl of diluted QuantiFluor Dye were mixed with 1 µl of RNA after 5 minutes of incubation at room temperature, RNA concentration was measured and stored in an -80 °C deep freeze until use.

2.3. Real-time PCR and cDNA synthesis

RNA was reversed to cDNA using one-step *RT-qPCR* (Promega, USA). The expression level of *KRAS* and *GAPDH* as reference genes was also detected by using the *RT-qPCR* SYBR Green assay. Primer sequences for KRAS gene Forward

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(5′- TGAGGACTGGGGAGGGCTTT 3′) Reverse (5′- AGGCATCATCAACACCCTGTCT 3′) (13) and for *GAPDH* Forward (5′- AGAAGGCTG GGGCTCATT TG 3′) Reverse (5′- AGG GGCCAT CCACAG TCTTC 3′). The mRNA expression level was normalized by using Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) as a housekeeping gene (HKG). *RT-qPCR* was performed using a total of 10 µl PCR reaction mixture that contains 1 µl of RNA, 0.25 µl of reverse transcriptase, 0.5 µl of primers for both forward and reverse primers, 0.25 µl of MgCl2, 2.5 µl of nuclease-free water, and 5 µl of *GoTaq 1-Step RT-qPCR* Master Mix (Promega, USA). *RT-qPCR* program was carried out under thermal-cycling conditions involving reverse transcriptase enzyme activation step at 37 °C for 15 min (one cycle), initial denaturation step at 95 °C for 10 min, followed by 40 cycles for 95 °C for 15s as denaturation, annealing step at 57 °C for 20 s, extension step at 72 °C for 7 min.

The *KRAS* gene expression was determined by the ΔCt method, the 2^−ΔΔCt equation (14), and the relative quantitative method using the comparative Ct formula:

Folding = 2^−ΔΔCT where:
ΔCT = CT (target gene) − CT (reference gene).
ΔΔCT = ΔCT (Patients) − ΔCT (Control).

2.4. Statistical analysis

Data were represented as mean ± S.D. SPSS software (Armonk, NY: IBM Corp) was used for the statistical analysis. The least significant difference –the LSD test (ANOVA) was used to compare between means. The value of $P<0.05$ was considered to be statistically significant.

3. Results

The age of benign breast tumour cases varies from 17-53 years old, with a mean age and median of 32±2.3 and 34, respectively. Results showed that fibroadenoma was the most prevalent type of benign breast tumour, representing 53.3% of the total samples of benign breast tumours; fibrocystic changes were 26.6%, breast cyst was 11%, and intra-ductal papilloma was 9% as shown in Fig. 1.

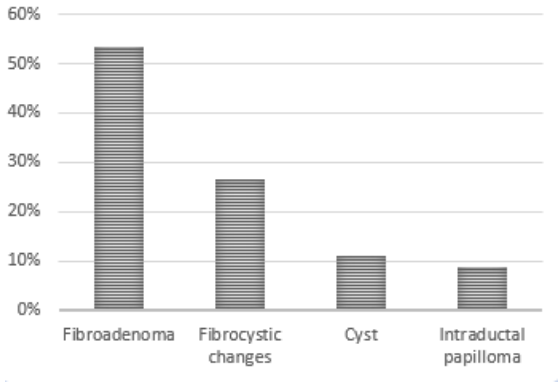

Fig. 1. Frequencies of histopathological for breast benign tumors in Iraqi women

While the age of Iraqi women with malignant breast tumours varied from 35-70 years old, with a mean age of 51±1.2 years and a median of 52. There are three Histopathological types characterized in this study. The first was invasive ductal carcinoma (IDC), invasive lobular carcinoma (ILC), and ductal carcinoma in situ (DCIS), with frequencies of 78%, 15%, and 7%, respectively, as shown in Fig. 2.

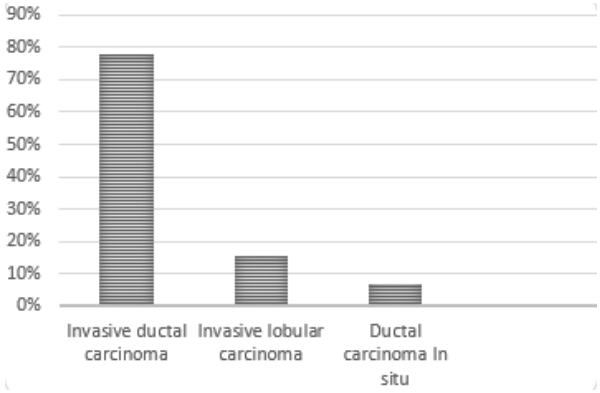

Fig. 2. Frequencies of histopathological for malignant breast tumours in Iraqi women.

QRT-PCR was used to determine the level of *KRAS* mRNA in the blood sample of case-control subjects (women with malignant breast tumours, women with benign breast tumours and healthy control women).

Results showed that the fluorescence measured during each PCR cycle was proportional to the number of PCR products. The actual C_t value was inversely proportional to the total RNA concentration in blood samples.

The melting curve revealed the specificity of the primers designed for *KRAS* and *GAPDH* indicated by a specific single peak in the melting curve graph. No primer dimer was seen in PCR amplification (Fig. 3 and 4).

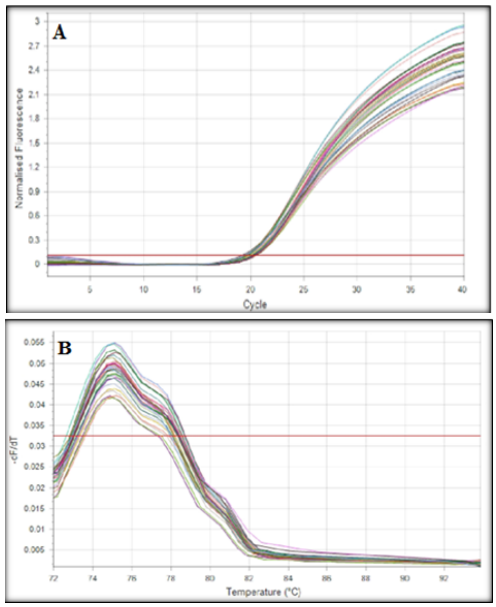

Fig. 3. Melting curve analysis of the real-time polymerase chain reaction (RT-PCR) for *KRAS* expression. A: Ct values the photograph was taken directly from Mic-qPCR. B: The dissociation stage was performed.

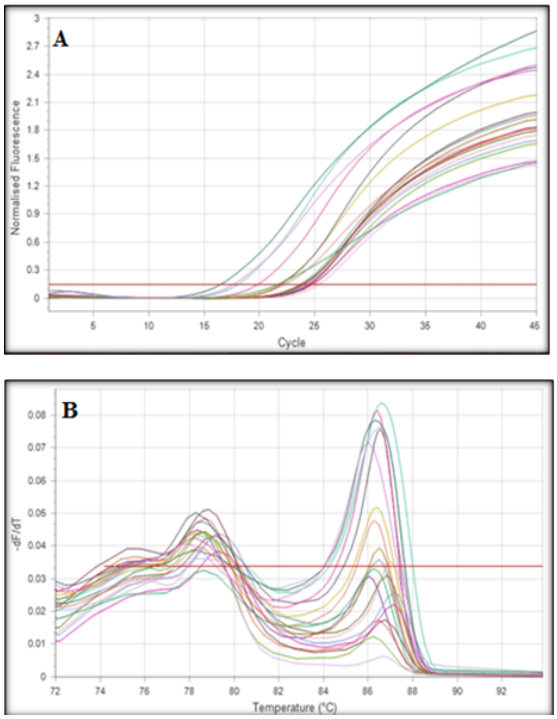

Fig. 4. Melting curve analysis of the real-time polymerase chain reaction (RT-PCR) for glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*). A: Ct values ranged from 17 to 24. B: The dissociation stage was performed

The *KRAS* gene expression in women with breast tumours (benign and malignant) was non-significantly different from that seen in apparently normal healthy women (Fig. 5)

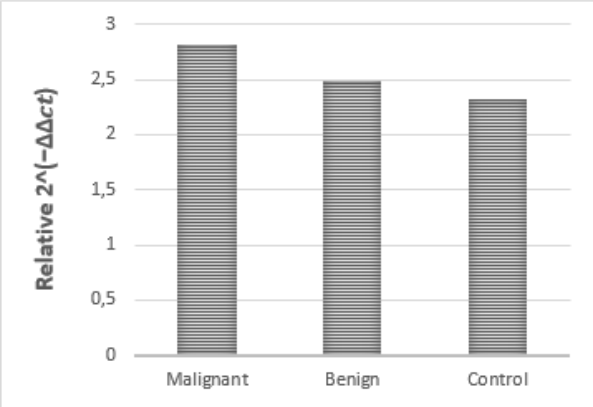

Fig. 5. Expression of *KRAS* mRNA in women with breast tumors (malignant and benign) compared to apparently healthy women

4. Discussion

In humans, *KRAS* proto-oncogene contains six exons, and it encodes guanosine triphosphatase (GTPase) KRAS isoform, a single amino acid substitution in *KRAS* is responsible for an activating mutation. In addition, the *KRAS* gene is known to be associated with human malignancies (15). SNPs in 3′-UTRs alter microRNA target recognition by disrupting sequence complementarity; some polymorphisms interfere with miRNA function and affect the expression of miRNA targets (16). According to the results of chin et al. (17), the prognostic role of LCS6 variants in KRAS 3'-UTR causes an increase in the expression of KRAS. In addition, (18) stated that LCS6 variants of the KRAS gene were statistically non-significant in women with breast tumours compared with healthy control. Therefore, there are no comparatively different levels of KRAS mRNA, which may also be due to the human mRNA being mostly targeted by several miRNAs at different target sites, which may enable recompensing of the loss of a single binding site (19).

Furthermore, miRNAs have been involved in regulating various cellular processes, including tumour development and progression, indicating that they can act as either oncogenes or tumour suppressors. The irregular expression of miRNAs plays a significant role in the progression of cancer; therefore, reducing the expression of these miRNAs may cause negative regulation of *KRAS*. In addition, the molecular mechanism of miRNA mediated in the control of KRAS would be useful for developing therapy (20, 21). Several miRNAs were acting as indirect targets to inhibit or increase KRAS-driven tumorigenesis; for instance: in NSCLC, miR-21 promotes *KRAS*-driven tumorigenesis by affecting negative regulators of the Ras/MAPK pathway (22). While miR-96, miR-30c and miR-181a, miR-143, and miR-145 have been shown to control KRAS in several cancers (23, 24). Also, another study found that miR-16 inhibits the expression and tumorigenesis of KRAS, which directly targets the 3′-UTR of KRAS mRNA (25). This paper shown that fibroadenoma occurs at an early age; however, invasive ductal carcinoma is the most prevalent type of malignant breast tumour. The results of this study indicate that the expression of the *KRAS* gene, which is conducted by *RT-qPCR*, has no correlation with breast tumours compared to healthy women, so the *KRAS* gene has not been represented as a risk factor in breast tumours.

Ethical statement

The study was ethically approved by the Iraqi Ministry of Health (decree order 18815 on 23/5/2018) and the scientific committee of Al-Nahrain University's College of Biotechnology.

Conflict of interest

The authors have no conflict of interest

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None.

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Authors' contributions

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

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HOMA-IR level in obese type 2 diabetic rat model treated by Sleeve gastrectomy and pancreatic omentoplasty

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Abstract

Obesity is a health problem that occurs due to the wrong lifestyle, such as lack of physical activity and the wrong diet. Accumulation of adipose tissue in obesity may increases pro-inflammatory cytokines, particularly in pancreatic beta cells leading to type 2 diabetes mellitus (T2DM). Sleeve gastrectomy (SG) is an alternative therapy for T2DM in obese patients by losing about 40-60% of body weight and increasing GLP-1 secretion which stimulates increased insulin secretion. However, the SG procedure cannot reduce pro-inflammatory cytokines which promote damaged pancreatic cells. Pancreatic omentoplasty can suppress pro-inflammatory cytokines and promote the regeneration of damaged pancreatic cells. The goal of this study is to investigate HOMA-IR in obese rats with diabetes mellitus who underwent SG and pancreatic omentoplasty procedures. An experimental using pre and post-test control group design were done in this study. Eighteen Diabetes Mellitus rats were divided into 3 groups: K1 (SG), K2 (SG + Omentoplasty), and K3 (control). Blood glucose and insulin level were measured using a glucoDR Glucometer Bio-sensor kit and ELISA, respectively before and 10 days after the procedure. HOMA-IR measurement was calculated based on insulin and blood glucose level. A significant decrease of fasting blood gluocese levels were shown in all treatment groups of this study after day 10^{th}. There was a significant increase in the insulin levels after day 10^{th}. Highest decrease of the blood glucose levels and increase of the insulin levels were shown in group K2. Furthermore, a significant decrease of HOMA-IR was shown in the K2 on day 10^{th}. From this study, we may conclude that SG and Pancreas Omentoplasty may significantly reduce the HOMA-IR value in obese rats with T2DM.

Keywords: obesity, diabetes mellitus, gastrectomy, omentum, insulin resistance

1. Introduction

In both developing and developed countries, obesity-related problems are still a challenge for public health. Obesity can occur due to unhealthy lifestyle factors such as lack of physical activity, unbalanced diet, and hereditary factors may be responsible in obesity (1). The accumulation of adipose tissue can increase the secretion of pro-inflammatory cytokines that cause impaired secretion of hormones such as insulin, resulting in insulin resistance. To reduce the risk of diabetes mellitus type 2 (T2DM), a weight loss program can be carried out, this program can also be carried out to improve insulin resistance. However, carrying out this program requires a long time and if patients do not have compliance to run this program, the results obtained will not be optimal (2).

Bariatric surgery such as sleeve gastrectomy (SG) could be an alternative therapy for T2DM in obese patients. This procedure results in weight loss of about 40-60% in almost 75% of patients and complete remission. However, this bariatric procedure is not accompanied by a decrease in pro-inflammatory cytokines so additional procedures are needed to reduce pro-inflammatory cytokines (3). Omentum is adipose tissue that can act as a defense agent to suppress inflammation and increase cell regeneration (4). This tissue can be used as an adjuvant in bariatric procedures to obtain optimal results. The omentum has a role in regulating T cells or Visceral Adipose Tissue (VAT), thereby increasing the expression of chemokine receptors, such as CCR1 and CCR2, and anti-inflammatory cytokine, including IL-10 leading to local immune responses regulation (5).

Measurement of insulin resistance plays an important role in the development of basic science and clinical practice. The gold standard for measuring insulin resistance is the euglycemic hyperinsulinemia clamp, however, it has a complicated procedure that makes it difficult to apply to large-scale tests. Another indicator for determining insulin resistance is the homeostasis model assessment-insulin resistance (HOMA-IR).

This study performed an animal model in obese rats with T2DM that underwent SG and omentoplasty procedures as surgical therapy for diabetes mellitus in obese patients to improve insulin resistance.

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2. Materials And Methods

2.1. Animals

Eighteen male Sprague-Dawley rats aged 6-8 weeks with a body weight of 170-200 grams, (6) were adapted for 1 week using a standard diet at the Integrated Research and Testing Laboratory (LPPT), Gadjah Mada University, Yogyakarta.

2.2. Animal adaptation

The rats were treated and kept clean from pathogens every day and the substrate for the rats, namely sawdust, was replaced twice a week. Rats were kept at 24±4°C room temperature, 50% relative humidity, and 12:12-hour light-dark cycle according to laboratory standards for animal models (Fig. 1). Nutritional content was adjusted using Teklad Global 14% Protein Rodent Maintenance Diet 2014S feed from HarlanTM Laboratories (2014). Drinking was given ad libitum. The time for adaptation was carried out for 7 days, then fattening with High-fat feed was carried out for 4 weeks and weighed and then assessed by the Lee Index. Rats were obese if the Lee index was more than 300.

Fig. 1. Mouse adaptation

2.3. Diabetes induction

Nicotinamide (NA) at a dose of 230 mg/kg was injected intraperitoneally. A single dose of 65 mg/kg of Streptozotocin (STZ) was administered intraperitoneally 15 minutes after the NA injection. Diabetes mellitus status was measured using the glucoDR Glucometer Bio-sensor kit. Measurements were made by fasting for 4-6 hours. Rats were declared diabetic if their fasting blood glucose was more than 126 mg/dL (7).

2.4. Experimental design

After confirming the status of diabetes and obesity, the mice were randomly divided into 3 groups. Rats underwent Gastrectomy (K1), Gastrectomy, Pancreatic Omentoplasty (K2), and control (K3) procedures. Body weight, fasting blood glucose, and fasting insulin was measured a day before surgery and 10 days after the procedure.

2.5. Sleeve gastrectomy procedure

Before surgery, mice were fasted for about 10 hours. Injection of ketamine 20 mg/kg BW was done intramuscularly. The fur on the abdomen was washed using a hair clipper until the skin of the rat is visible. Asepsis and antisepsis were performed in the operating area. A left subcostal transverse incision was made starting from the xiphoid process to the lateral abdomen. The incision was deepened from the cutis, subcutis, and muscle to the peritoneum and intraperitoneal cavity. Gastric identification was performed (Fig. 2), followed by partial gastrectomy along the major curvature with clamps first to minimize bleeding. Gastric suturing was performed with polyglycolic acid 5.0 (Fig. 3)

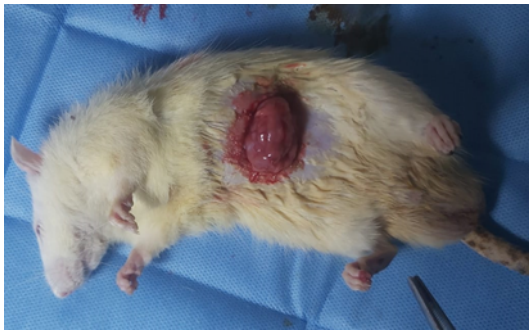

Fig. 2. Gastric identification

Fig 3A. Gastric clamping

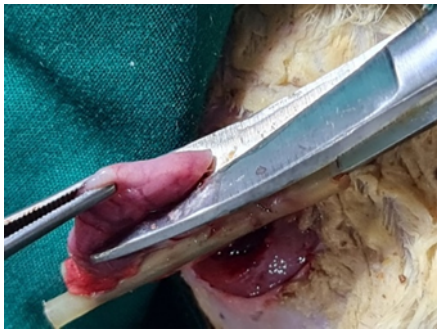

Fig 3B. Gastric cutting

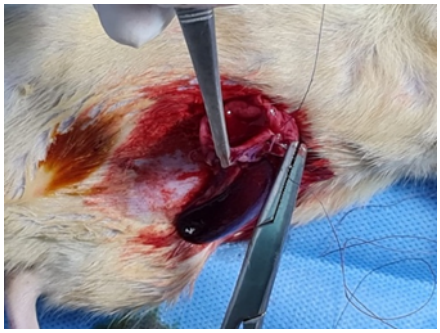

Fig 3C. Gastric suturing

2.6. Pancreatic omentoplasty procedure

The free omentum was sutured 1 piece of a suture in the body of the pancreas so that the omentum was attached to the pancreas with PGA 5 sutures (Fig 4). Bleeding was controlled with pressure gauze or sutures. The surgical wound was

sutured with PGA 5.0 thread. The wound was cleaned with 0.9% NaCl and smeared with Povidone Iodine (Fig 5).

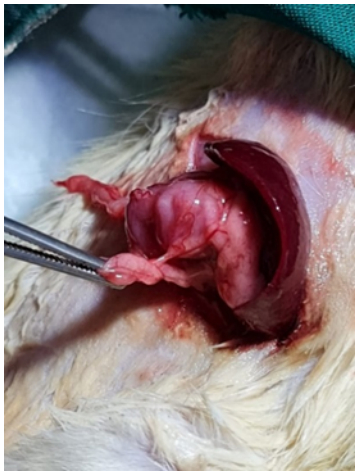

Fig. 4. Omentoplasty

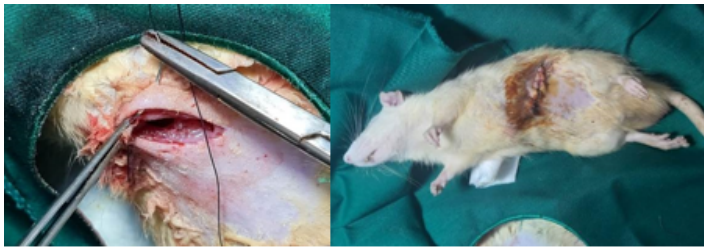

Fig. 5. Abdominal closure

2.7. HOMA-IR measurement

Insulin level were measured from blood serum samples collected at day 10 using ELISA kit. The HOMA-IR value was obtained from the analysis of insulin and blood glucose levels. HOMA-IR measured by using formula={fasting glucose (mmol/L) x fasting insulin (µU/L)}/22.5.

2.8. Statistical analysis

Body weight, fasting glucose levels, and fasting insulin were shown in the form of mean±SD. Shapiro-Wilk was used for the normality test. The HOMA-IR value was hypothesized using repeated ANOVA. Difference between groups were analyzed using Bonferonni post-hoc test. A P<0.05 was considered statistically significant.

3. Result

3.1. Animal experimental models

Eighteen diabetic and obesity rats were successfully made after induced with NA and divided into three groups evenly. After surgical treatment, body weight in treatment groups was decreased on day 10. The K2 group has an optimum decreased body weight level (Table 1). The K2 groups showed earlier effect after treatment than another group.

Table 1. Description of weight data (grams)

Group	N	Mean±SD	
		Pre	Post
K1	6	248.50±1.67	205.83±1.90
K2	6	250.83±2.02	194.50±1.96
K3	6	244.65±2.82	239.17±2.93

3.2. Fasting blood glucose

Fasting blood glucose were measured using ELISA to investigate the suppressing capacity of SG and omentoplasty after 10 days treatment. All intervention groups showed a significant reduction in blood glucose levels compared to the control group (p<0.05; Table 2). The K2 group showed the highest reduction in fasting blood glucose levels.

Table 2. Description of fasting blood glucose data (mg/dL)

Group	N	Mean±SD		p
		Pre	Post	
K1	6	265.34±2.37	188.50±2.28	0.000*
K2	6	260.94±0.72	145.29±1.44	
K3	6	265.48±0.82	268.98±0.86	

* p: repeated ANOVA followed by Bonferroni's post hoc test

3.3. Fasting insulin

To investigate the capacity of SG and omentoplasty in enhancing fasting insulin, the level of insulin in serum was analyzed using ELISA in day 10th. All intervention groups showed a significant increase in insulin levels (p<0.05; Table 3). The K2 group showed the optimum increase in insulin levels.

Table 3. Description of fasting insulin data (µU/L)

Group	N	Mean±SD		p
		Pre	Post	
K1	6	421.09±2.37	458.28±3.20	0.000*
K2	6	412.79±0.79	467.57±2.53	
K3	6	408.77±1.26	405.15±0.89	

* p: repeated ANOVA followed by Bonferroni's post hoc test

3.4. HOMA-IR value

HOMA-IR value were measured in SG and omentoplasty group. A significant decrease in all treatment groups were shown in this study (p<0.05; Table 4). While the K2 group showed the maximum reduction in the level of HOMA-IR.

Table 4. Description of HOMA-IR value data

Group	N	Mean±SD		p
		Pre	Post	
K1	6	5.86±0.06	4.54±0.05	0.000*
K2	6	5.66±0.01	3.57±0.02	
K3	6	5.86±0.06	5.73±0.02	

* p: repeated ANOVA followed by Bonferroni's post hoc test

4. Discussion

STZ induction can lead to pancreatic beta cell damage by mimicking a glucose analog and entering pancreatic cells via the subtype 2 glucose transporter (GLUT-2) which induces toxicity by producing DNA alkylation. Nicotinamide (NA) was given to prevent damage and reduce the destruction of pancreatic beta cells. Administration of a high-fat diet and induction of STZ-NA in a mouse model of T2DM could form obesity and insulin resistance (8, 9).

Based on this study, there was a decrease in blood glucose levels in the K1 and K2 groups. The sleeve gastrectomy procedure affects hormone metabolism in the body, one of which is Glucagon Peptide-1 GLP-1. GLP-1 is a peptide hormone secreted by the intestine, ileum, colon, and certain neurons in the CNS nucleus via enteroendocrine L cells. This hormone could play a role in the metabolism of vital hormones in the body and affect appetite through anorexigenic processes, delaying gastric emptying, and reducing food intake. GLP-1

could decrease blood sugar levels and improve glucose tolerance by increasing insulin secretion by inhibiting pancreatic cells and stimulating pancreatic cells, thereby causing a decrease in postprandial hyperglycemic status and insulin resistance. Increased glucose metabolism by SG was associated with increased GLP-1 secretion (10).

This study also showed that sleeve gastrectomy and pancreatic omentoplasty performed an optimum decrease in blood sugar levels, increasing insulin secretion, and reducing insulin resistance as assessed by the HOMA-IR. The K2 has optimum HOMA-IR decrease. This result showed that pancreatic omentoplasty can provide an optimal role in improving insulin resistance conditions. The function of the omentum itself is as a growth factor, neurotrophic and hemostatic factor, and an inflammatory mediator. Pluripotent stem cells are found in the omentum which can differentiate into various types of cells. Recent studies have shown that in the omentum there are multipotent-mesenchymal stem cells (MSCs) and myeloid-derived suppressor cells (MDSCs) which act as anti-inflammatory agents and immunomodulators. In addition, omentum could improve the wound healing process through the process of tissue regeneration. Several authors have described that MSCs in the omentum also differentiate into endodermal and ectodermal cells which include pancreatic islets (4).

There are several limitations to this study. In this study, inflammatory cytokines such as IL-6, IL-10, and TNFα were not analyzed to investigate the exact mechanism of insulin resistance improvement. Relevant serum hormone levels like GLP-1 were also not performed in this study, which may give a better understanding of insulin resistance enhancement. More futur studies may be needed to assess the combination of its surgical procedure to continue to the clinical study.

SG and Pancreatic Omentoplasty could reduce insulin resistance in obese T2DM rats. SG and Pancreatic Omentoplasty could reduce blood glucose levels, increases insulin secretion, and reduce insulin resistance as seen from the HOMA-IR value.

Ethical statement

All laboratory and animal procedure were done under the standard guideline for animal experimental studies. This study was approved by the Ethics Committee of Health Studies, Faculty of Medicine, Universitas Diponegoro (Ethical clearence number: 51/EC/H/FK-UNDIP/V/2021).

Conflict of interest

The authors declare no competing interest in this study.

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Authors' contributions

Concept: A.M., V.M.E., Data Collection or Processing: R.T.S, A.T.P, Analysis or Interpretation: I.P., D.E., Literature Search: A.M., V.M.E., I.P., Writing: A.M., V.M.E.

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The effect of chamomile on nausea and vomiting after laparoscopic cholecystectomy: A triple-blind randomized clinical trial

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Abstract
The present study aimed to evaluate the effect of Chamomilla Recutita on nausea and vomiting after laparoscopic cholecystectomy. Chamomilla Recutita (L.) fell into either chamomile or placebo groups randomly. The intervention was performed one hour before the operation. The severity of nausea and the frequency of vomiting was measured using a visual analog scale. Both groups were studied in three stages before the operation, after the operation in recovery, and 2 hours after the operation. Data were analyzed using descriptive and analytical statistics (SPSS). The mean severity of nausea increased significantly over time in both groups; however, this increase was significantly slighter in the chamomile group than that in the placebo group. The frequency of preoperative nausea in the chamomile and placebo groups was 6.2% and 25%, respectively, before being discharged from the recovery. Two hours later, in the surgical ward, this frequency was estimated to be 31.2% in the chamomile group and 75% in the placebo group ($P < 0.05$). The frequency of vomiting in the surgical ward was 15.6% and 56.2% in the chamomile and placebo groups, respectively. Thus, this frequency in the chamomile group was significantly lower than in the placebo group ($P<0.001$). It seems that in laparoscopic surgeries, the use of chamomile drops as a preventive drug reduces postoperative nausea and vomiting.

Keywords: Chamomilla Recutita, nausea, vomiting, laparoscopy, cholecystectomy

1. Introduction

Postoperative nausea and vomiting (PONV) is a common complication found in 20 -30% of cases (1, 2), and its prevalence in laparoscopic surgery (up to 80%) is caused by carbon dioxide gas used for pneumoperitoneum (1, 3, 4). Therefore, laparoscopic surgery is the only risk factor for PONV (5). One of these surgeries is laparoscopic cholecystectomy which is a standard surgical procedure for patients with symptomatic gallstones(6). The treatment of complications following laparoscopic cholecystectomy is complex and significantly affects the patient's quality of life (7). Inadequately controlled pain can cause PONV as well (4). Nausea and vomiting can cause spasms, pulmonary aspiration, electrolyte imbalance, bleeding, and abdominal pain. If continued, it reduces blood pressure, increases intracranial pressure (ICP) and intraocular pressure (IOP), and delays discharge from the recovery ward. Furthermore, it burdens costs, so it is necessary to eliminate it (1, 3-5, 8, 9).

Several drugs (such as Metoclopramide and Ondansetron) commonly used to treat PONV can lead to headaches, gastrointestinal disorders, hypotension, and extrapyramidal complications (2, 10). These complications may continue even with the widespread use of chemical drugs such as serotonin and neurokinin receptor antagonists.

To date, no single strategy has conclusively demonstrated to prevent PONV (4, 11). For this reason, further studies for novel treatments are required due to the ineffectiveness of chemical drugs and their side effects, the public's tendency to use herbal medicines, the variety of herbal medicines in Iran, and their minor side effects (8, 12, 13).

The global trend towards complementary medicine entices researchers to study the sources of traditional medicine (13) . The World Health Organization has considered developing traditional medicine to fulfill the slogan of "Health for All" (14).

One of the most prominent medicinal plants is Chamomilla Recutita, which stands out in traditional Iranian medicine and is used to treat disorders related to the nervous, gastrointestinal, and respiratory systems (15). Chamomile, an anti-inflammatory, anti-spasm, anti-flatulence drug, is used to treat stomach ulcers, eliminate digestive disorders, and relieve pain and fever (16-21).

Chamomile has minor side effects and occasionally leads to minor allergic reactions (12, 17). There is no adequate information on the toxicity of chamomile Recutita (species: *M. chamomilla*) (17).

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Sanaati et al. (2016) performed studies on the effect of chamomile and ginger on nausea and vomiting in patients undergoing chemotherapy and showed that both plants affected patients' vomiting but had no effect on nausea (19). Conversely, Borhan et al.'s study (2017) on patients undergoing chemotherapy showed that the consumption of chamomile extract reduces nausea caused by chemotherapy; but does not mitigate vomiting (21). Pakniat et al.'s (2018) study on the effect of chamomile, ginger, and vitamin B6 on the treatment of nausea and vomiting during pregnancy showed that the use of all three drugs effectively reduces nausea and vomiting during pregnancy (22). A study performed by Zargaran et al. (2018) on patients with migraines showed that pain, nausea, and vomiting were significantly reduced by topical application of chamomile Oleogel (23). Johnson et al. (1988) investigated the effect of chamomile oil extract in the treatment of migraines. Patients receiving placebo had severe headaches, nausea, and vomiting (24). The study of Putri et al. (2019) on 30 patients with cervical cancer undergoing chemotherapy showed that chamomile aromatherapy effectively reduces nausea after chemotherapy (25).

Since the technician plays a vital role in providing patient care during, before, and after surgery (26), it is essential to eliminate postoperative complications and ensure patients' convenience. As nausea and vomiting are the most common complications after the surgery and general anesthesias and drugs used to prevent these complications can cause side effects, the present study aimed to determine the effect of chamomile on nausea and vomiting after laparoscopic cholecystectomy surgery. The hypothesis of the study focuses on the mean score of severity of nausea, frequency of vomiting, and nausea in the control group and the intervention group when coming to the operation room, before discharge from recovery, and 2 hours later in the ward.

2. Materials and Methods

The study population comprises all patients referred to the operating room of selected training hospitals affiliated with Isfahan University of Medical Sciences for cholecystectomy in June, July, and August 2020. In the study, the following formula with 95% confidence interval and 80% test power, the number of samples for each group (n=32) was used:

$$n = \frac{(Z1 + Z2)2(2S)2}{d\,2}$$

The patients were selected by the convenience sampling method and divided into chamomile (n= 32) and placebo groups (n= 32) randomly.

Inclusion criteria included being on the list of laparoscopic cholecystectomy surgery, insensitivity to herbal medicine, the age range of 18 to 65 years, BMI range of 18 to 28, consciousness or the lack of psychotic symptoms, not being pregnant, not having vestibular symptoms, gastrointestinal diseases, lack of addiction to drugs and benzodiazepines and willingness to participate in the study.

Exclusion criteria included the inability to continue cooperation for any reason, a transformation of the anesthesia method, transfusion of blood during the operation, and receiving any anti-nausea drug as prophylaxis.

After obtaining permission from the educational supervisor and the head nurse of the operating room and explaining the study's aims to the patients, the researcher obtained their written consent. One hour before the surgery and on arrival in the operating room, the intervention group received two dosages of 20 drops each of a standardized chamomile water extract containing 17% Chamazulenein and 45% Bizabolol solution in 20 cc water (DER=80kg/0.4kg=200) with a glass of distilled water and the placebo group received only distilled water. The study population was randomly divided into control and intervention groups using a table of random numbers. All patients were unaware of the type of substance and its effects. We did not need to show both drugs (water and chamomile) to the patient at the same time to notice their color differences. Patients' demographic information and clinical and therapeutic conditions were obtained through clinical histories and interviews.

At first, the severity of nausea and vomiting was determined and measured by the VAS scale before the intervention (According to the duration of patient evaluation, the VAS scale was the best choice). This tool consists of a 10 cm line (0-10). Zero shows no nausea, and the number 10 is equal to severe nausea. The score 1 to 3 in this tool indicates the severity of mild nausea, the score 4 to 7 indicates the severity of moderate nausea, and score 8 to 10 indicates the severity of severe nausea. The researcher instructed all patients to rate their severe nausea based on the visual scale criteria. The frequency of vomiting was recorded based on the researcher's observation and patients' self-declaration.

To prevent the psychological effect of the type of intervention on the results, patients undergoing laparoscopic cholecystectomy were asked to drink a harmless substance to ensure they were safe. However, the type of substance and its effect on the patient were not described (single-blind). The severity of nausea and vomiting was recorded in 3 stages: before the intervention, before being discharged from the recovery ward, and two hours after the surgery based on the patient's self-declaration. The researcher recorded all preoperative information. On the other hand, he provided all postoperative information (severity and frequency of nausea, frequency of vomiting) in questionnaires handed out in the recovery room and two hours after the operation (Double_blind).

After the patients entered the recovery room and the surgical ward, the severity of nausea was measured using the VAS scale. According to the anesthesiologist's instructions, medication would be routinely started for them if patients gave

severe nausea with a score of 4 or higher. Data were obtained by a statistical consultant who did not know the groups. (triple_blind; analytical statistics).

Mean, standard deviation, and frequency indices were used to report the descriptive statistical part of the results. The necessary statistical tests include a t-test (to compare age, BMI, and surgical indicators), analysis of variance with repeated measurement (to compare the severity of nausea), Fisher's exact test, and Chi-square (to compare the frequency of nausea and vomiting) were used.

The article was prepared based on the consort checklist.

The research flowchart is shown in Fig. 1.

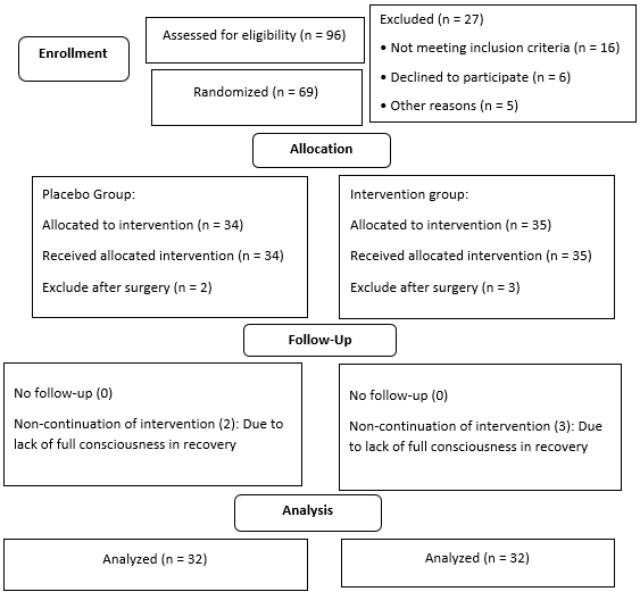

Fig. 1. Consort statement

3. Results

Five of the total 69 samples in this study were excluded from the study due to the lack of full unconsciousness in the Recovery Unit. Thus, 32 cases aged 23 to 65 years in the chamomile group and 32 cases aged 19 to 65 years in the placebo group (with a BMI range of 18 to 28) were studied. The results of the independent t-test showed that the mean age and body mass index were not significantly different between the chamomile and placebo groups (25.05 ± 2.28 vs. 25.55 ± 2.22) (P> 0.05) (Table 1).

Moreover, there was no significant difference between both groups regarding the frequency distribution of sex (X^2=1/04 and P> 0.05). In the chamomile group, 53.1% of patients were male, and 46.9% were female; and in the placebo group, 65.6% were male, and 34.4% were female. The results of the Mann-Whitney test showed no significant difference between both groups in terms of the level of education (P> 0.05).

Analysis of variance with repeated observations of the significant effect of time on nausea severity score (P <0.001). Therefore, the mean severity of nausea increased significantly over time in both groups. However, this increase in the chamomile group was significantly slighter than that in the placebo group (Table 2).

Table 1. Mean age, weight, height, and body mass index in the intervention and placebo groups

Variable	Chamomile group		Placebo group		independent t-test		
	Mean	SD	Mean	SD	t	df	P
Age (year)	43.66	13.70	44.37	12.43	0.22	62	0.83
Weight (kg)	69.89	11.79	70.56	10.47	0.24	62	0.81
Height (cm)	166.37	9.42	165.81	8.27	0.25	62	0.80
Body Mass Index (BMI)	25.05	2.28	25.55	2.22	0.89	62	0.38

Table 2. The mean of nausea severity in different periods in the intervention and placebo groups

Time	Chamomile group		Placebo group		P^1(effect of time)	P^2 (effect of group)
	Mean	SD	Mean	SD		
Before surgery	0.66	0.15	0.66	0.14	<0.001	<0.001
Before leaving recovery	0.37	0.14	1.47	0.22		
At ward	1.19	0.32	5.28	0.68		

Fisher's exact test showed that the frequency of nausea before the operation and on arrival in the recovery room was not significantly different between the two groups (P> 0.05). The Chi-square test showed that the frequency of nausea before leaving the recovery room and in the ward in the chamomile group was significantly less than that in the placebo group (P <0.05) (Table 3).

Table 3. the frequency distribution of nausea in different periods in the intervention and placebo groups

Time	Chamomile group		Placebo group		Chi-square test		
	No.	%	No.	%	χ^2	df	P
Before the operation	3	9.4	4	12.5	-	-	0.50
Before leaving recovery	2	6.2	8	25	4.27	1	0.040
At ward	10	31.2	24	75	12.30	1	<0.001

Vomiting was not observed in either group before the operation and on arrival in the recovery room. Fisher's exact test showed that the frequency of vomiting before leaving the recovery room was not significantly different between the two groups (P> 0.05). The Chi-square test showed that the frequency of vomiting in the surgical ward was 15.6% and 56.2% in the chamomile and placebo groups, respectively. These results indicate that the frequency of vomiting in the ward in the chamomile group was significantly lower than that in the placebo group (P<0.001) (Table 4).

Table 4. The frequency distribution of vomiting in different periods in the intervention and placebo groups

Time	Chamomile group		Placebo group		Chi-square test		
	No.	%	No.	%	χ^2	df	P
Before the operation	0	0	0	0	-	-	1
Before leaving recovery	0	0	2	6.2	-	-	0.25
At ward	5	15.6	18	56.2	11.47	1	0.001

The Chi-square test showed that the frequency of ondansetron use was 0% (in the chamomile group) and 15.6% (in the placebo group) in the recovery room, 25% in the surgical ward (in the chamomile group), and 62.5% (in the placebo group). On the other hand, the frequency of ondansetron use in the recovery room and the ward in the chamomile group was significantly less than that in the placebo group ($P < 0.05$). The independent t-test showed that the mean amount of Morphine administered, gas volume, and gas pressure was not significantly different between both groups. Also, the duration of operation was not significantly different between both groups. To control this confounding variable, random allocation of samples was used between the intervention and control groups. Different surgical operations in terms of operation duration were randomly assigned to each of the two intervention and control groups, and in this way, the confounding effect of the duration of the operation was removed. ($p > 0.05$). These results show that anesthesia and surgery were the same in both groups (Table 5). Complications such as gallbladder perforation, intra-peitoneal drain and etc. were not observed in any of both groups. Nasogastric tube was not placed in any of both groups because all patients were NPO.

Table 5. A dose of anesthesia drugs, duration of operation, gas volume, and gas pressure in the intervention and placebo groups

Variable	Chamomile group		Placebo group		Independent t-test		
	Mean	SD	Mean	SD	t	df	P
The dose of received fentanyl	119.37	15.76	132.81	18.54	1.03	62	0.31
The dose of received Morphine	9.50	1.83	9.84	1.55	0.81	62	0.42
Operation period (min)	82/8	0.49	81	0.45	0.25	62	0.80
Gas volume	97.59	10.75	93.83	8.70	0.27	62	0.79
Gas pressure	14.12	1.07	14.47	0.88	1.40	62	0.16

4. Discussion

The results of the present study showed that the scores of severe nausea and frequency of vomiting before the intervention in both groups were not statistically significant; however, the mean severity of nausea increased significantly over time in both groups, but the severity of increase in chamomile group was significantly lower than that in the placebo group. Furthermore, the frequency of vomiting in the surgical ward, before leaving the recovery room, and two hours after the operation were significantly lower in the chamomile group than that in the placebo group.

Factors playing a role in the development of PONV included patient-related factors (old age, female gender, history of movement disease), factors related to anesthesia and operation techniques (anesthesia drugs, general anesthesia, longer duration of operation, intra-abdominal surgery including gynecological and laparoscopic surgery), and postoperative factors (use of opioid drugs to control pain) were noted (9, 27, 28) in the present study. The study groups were homogeneous for the prevention of the effect of the above factors.

In a study on 105 pregnant women comparing the effects of ginger and chamomile on reducing nausea and vomiting during pregnancy, Modarres et al. (2011) showed that oral chamomile capsules reduce nausea and vomiting symptoms during pregnancy (12). These results are consistent with those of the present study and emphasize the effect of chamomile in reducing nausea. Although the samples differ in terms of gender in both studies, the results are consistent.

The results of Zargaran et al.'s study (2018) on 100 patients with migraines without aura support the effectiveness of chamomile Oleogel as a pain reliever and reduce nausea and vomiting in this type of migraine. The results of this study are consistent with those of the present study (23). Johnson et al. (1988) investigated the effect of chamomile oil extract in treating migraines. Based on their results, patients receiving a placebo had significantly higher frequency and severity of headache, nausea, and vomiting (24). The results of this study are consistent with those of the present study. In this study, patients were evaluated shortly after the intervention (30 minutes), which is consistent with the present study and emphasizes the immediate effect of chamomile. In addition to these studies, Pakniat et al. (2018) investigated the effect of three drugs, including chamomile, ginger, and vitamin B on the treatment of nausea and vomiting in 105 pregnant women with nausea and vomiting. The results showed that all three drugs reduce nausea and vomiting during pregnancy, although there were no significant therapeutic benefits from these three drugs (22). This study's results are consistent with those of the present study and emphasize the effect of chamomile on reducing nausea and vomiting.

In the present study, the mean score of nausea in patients in the surgical ward was 1.19 (in the chamomile group) and 5.28 (in the placebo group); the score difference of the nausea severity between both groups was 4.09. In a study on 30 patients with cervical cancer undergoing chemotherapy, Putri et al. (2019) showed that chamomile aromatherapy can reduce nausea after chemotherapy. According to this study, the mean

score of nausea was 7.33 and 2.87 in the control and intervention groups, respectively. On the other hand, the score difference for nausea intensity was 4.46 in both groups (25). Although chamomile was used by inhalation in this study, overall results confirm those of the present study.

Matthews et al. (2015) conducted a review study of various interventions on nausea and vomiting in the early stages of pregnancy. In this study, 37 clinical trials were performed on 5049 women in the early stages of pregnancy. These studies concluded that ginger and vitamin B6 could reduce nausea effectively and mentioned chamomile as one of these interventions; However, due to the lack of objective evidence, they could not recommend any of these interventions (29). The results of this study are consistent with those of the present study, although the present study concluded significant results from the use of chamomile.

Sanaati et al. (2016) showed that ginger and chamomile had no effect on the severity of nausea in cancer patients undergoing chemotherapy and were effective only in the frequency of vomiting (19). These results differ from those of the present study regarding the efficiency of chamomile on the severity of nausea, which may be due to the research methods or differences in sex and type of disease. Of course, the role of gender in this study may not be considered a reason for differences in the results because, in the study of mentioned researchers (Modares et al. (2011), Matthews et al. (29) and Pakniat et al.) (22), the gender (all were female) was different from those of the present study. However, the results are consistent with those of the present study and emphasize the effect of chamomile on reducing the severity of nausea.

Conversely, in a study on 60 patients undergoing chemotherapy, Borhan et al. (2017) showed that consumption of chamomile extract reduced nausea caused by chemotherapy; but did not reduce vomiting (21). This study differs from the present study as chamomile was administered orally and once. However, the results of this study are different from those of the present study regarding the effectiveness of chamomile on the frequency of vomiting in patients. This difference results from differences in the subjects or dosage of chamomile. Another reason for the difference is that samples were evaluated two hours after the operation in the present study but 12 hours in Borhan's study.

Research limitations include:

Prolonged sampling, the reduced number of hospitalized patients due to the prevalence of coronary heart disease,

Short-term evaluation of patients only up to two hours after surgery,

Lack of cooperation of some anesthesiologists to give chamomile drops to patients before surgery,

Making a mistake in diagnosing the severity of nausea as it was self-declaration,

Due to the importance of controlling nausea and vomiting in patients following laparoscopic surgery, the present study seems to be the first study done in this field after laparoscopic surgery. It seems that chamomile drops, as a preventive drug, effectively reduce postoperative nausea and vomiting in laparoscopic surgeries. However, further studies with a longer-term evaluation to investigate the effectiveness of chamomile in reducing nausea and vomiting will be done.

Ethical statement

This triple_blind randomized clinical trial study was registered in the Iranian clinical trial database with the code IRCT20200612047737N1 and in the ethics committee of Isfahan University of Medical Sciences (IR.MUI.RESEARCH.REC.1398.745).

Conflict of interest

The authors declared no conflict of interest.

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Acknowledgments

The study complies with the guidelines for human studies and is performed ethically based on the World Medical Association Declaration of Helsinki. Subjects have given their written informed consent. The Ethics Committee of Isfahan University of Medical Sciences approved the study (IR.MUI.RESEARCH.REC.1398.745).

This triple-blind randomized clinical trial was registered in the Iranian clinical trial database with the code IRCT20200612047737N1.

Authors' contributions

Concept: S.B., R.S.Z. Design:S.B.,R.S.Z. Data Collection or Processing: R.S.Z., S.K.,G.K. Analysis or Interpretation: R.S.Z.,S.B.,G.K. Literature Search: R.S.Z.,S.B.,G.K., Writing: R.S.Z.,S.B.,G.K.

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Change of complete blood count parameters according to blood type and smoking

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Abstract

In this study, it was aimed to investigate the change in the parameters of complete blood count depending on blood type and smoking status. The individuals were grouped according to their blood group type and status of cigarette use, and the statistical differences in their levels of Neutrophil/Lymphocyte ratio (NLR), Platelet/Lymphocyte ratio (PLR), and Mean Platelet Volume/Lymphocyte ratio (MPVLR) were evaluated.3119 individuals participated in the study. 26.67% of them were smokers. According to the ABO blood group system, 44.47% of the participants had type A, 32.48% had type O, 16.09% had type B, and 6.96% had type AB. 88.81% of the participants were Rh(+) and 11.19% of them were Rh(-). The NLR, PLR, and MPVLR of the smokers were higher than those of the non-smokers. The participants' NLR, PLR, and MPVLR levels showed statistically significant differences depending on ABO and Rh blood groups. This difference was seen to arise from ARh (+) individuals. The NLR, PLR, and MPVLR levels were the highest in ARh (+) individuals and the lowest in the ABRh (-) individuals. The NLR, PLR, and MPVLR values were the highest in the ARh (+) smokers and the lowest in ABRh (-) non-smokers. It was found that there were significant differences in the participants' blood count parameters depending on blood type and smoking status. This may imply that ARh (+) individuals who smoke have higher parameters than those who have other blood groups and do not smoke, which may be beneficial in predicting the risk of chronic inflammation-related diseases.

Keywords: ABO blood group, healthy individuals, Rh factor, smoking

1. Introduction

Various blood group systems have been identified based on different blood group antigens. Clinically valid system is ABO and Rhesus (Rh). The ABO blood group system consists of four basic groups, that is, A, B, AB, and O, which change depending on the presence of A and B antigens. These antigens are controlled by three alleles (A, B, and O genes) on the long arm of chromosome 9 (1).The Rh blood group system plays an important role in blood transfusion. In Rh system, blood groups are classified as Rh (-) and Rh (+) (2). Population studies have shown that the frequencies of ABO group phenotypes vary widely between ethnicities (3). While the distribution of ABO blood groups is O> A> B> AB across the world; in Turkey, it is A> O> B> AB and Rh (+)> Rh (-) (4).Although ABO blood group antigens are mainly expressed by erythrocytes, they are also expressed by different cells in various human tissues, including epithelial cells, vascular endothelial cells, and neurons. Therefore, studies have focused on the role of ABO antigens in the pathogenesis of various systemic diseases (5).Studies have shown that ABO and Rh blood groups are associated with cancer, cardiovascular diseases, infections, thyroid diseases, diabetes mellitus, chronic kidney failure, postpartum depression, and rheumatological diseases (6-8).

Exposure to tobacco smoke has been linked to the development of chronic diseases, including pulmonary diseases, heart infections, and cancer (9). Smoking causes many pathological processes to start and progress. Negative effects of smoking on the hematopoietic system have been shown in previous studies. Smoking causes an increase in the number of leukocytes and platelets in the blood (10). It has been reported that smoking increases systemic inflammation (11).

Neutrophils and leukocytes play an important role in inflammatory processes. Neutrophil/Lymphocyte ratio (NLR), which is calculated by dividing the absolute neutrophil count by the absolute lymphocyte count, is considered as a new marker that can be used to evaluate the systemic inflammatory response (12).

While high platelet counts are associated with poor cardiovascular outcomes, low lymphocyte counts are associated with increased cardiovascular morbidity and mortality. The increase of Platelet/Lymphocyte ratio (PLR), which is a combination of both parameters, is a predictor of poor prognosis in cardiovascular diseases and malignant conditions (13). The increased NLR, PLR, and Mean platelet volume/Lymphocyte ratio (MPVLR) levels were found to be associated with inflammation and poor prognosis (14,15). PLR is seen as a predictor of mortality in heart, lung diseases and some malignant diseases (16).

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Although high MPV values pose an increased risk of cardiovascular disease, they have been considered as one of the markers of systemic inflammatory response in recent years (17). MPVLR, a marker that can be easily detected with complete blood count (CBC), has been shown to increase in the presence of inflammation (14,15). NLR, PLR, and MPVLR levels can be evaluated quickly, cheaply, and easily by clinicians. Shown to be the new markers in the evaluation of systemic inflammatory response; NLR, PLR, and MPVLR have been being used to monitor the prognosis, morbidity, and mortality of many diseases (10).

There are many studies in the literature investigating the effects of ABO and Rh blood groups on chronic diseases (6-8).However, we could not find any study in the literature investigating how CBC parameters and NLR, PLR and MPVLR levels are related to blood types and smoking status.

The purpose of our study was to investigate the effects of ABO and Rh blood groups and smoking status on CBC parameters and NLR, PLR and MPVLR levels.

2. Materials and Methods

The individuals who applied to local University Faculty of Medicine Family Medicine outpatient clinic between January 2018 and January 2020 were included in the cross-sectional study. Sociodemographic data such as age, gender, smoking, and blood group, and complete blood count (CBC) test results were scanned from the hospital automation system. In our hospital, blood samples taken for CBC are collected in K3EDTA tubes and analyzed with the automated hematology analyzer Mindray BC-6000. NLR, PLR, and MPVLR levels were calculated. It was evaluated whether there was any change in the CBC parameters depending on blood groups. Again, it was evaluated whether there was a difference in the CBC parameters between the smokers and non-smokers depending on blood type.

The individuals without any disease were included in the study. These people applied to the polyclinic for reasons such as student registration, check-up, driver's license report, etc. The individuals with acute or chronic diseases, any systemic disease that will affect the results of CBC (cardiovascular diseases, diabetes mellitus, hypertension, cancer, digestive system diseases, respiratory system diseases, psychosis, musculoskeletal system problems, hormonal diseases, kidney failure, obesity, vitamin and mineral deficiencies), drug users, individuals under 18 and over 65, and pregnant individuals were excluded from the study. In addition, subjects with abnormally high and low complete blood counts and other laboratory values (anaemia, leukocytosis, leukopenia, or other hematological, biochemical, or serological abnormalities) were excluded from the study (Fig. 1).

This study was approved by the clinical studies ethics committee of local University (Decision Number: 2020/240).

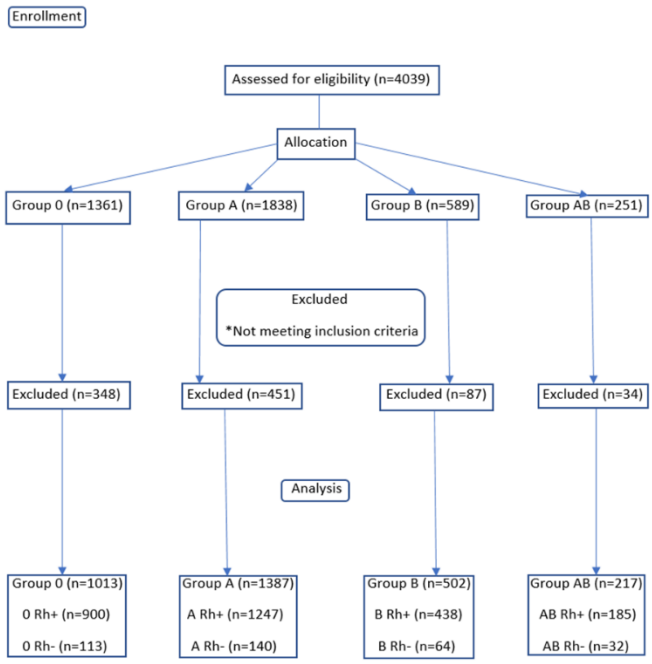

Fig. 1. Flow diagram of the study

2.1. Statistical Analyses

IBM SPSS software package (v.22.0) was used in the statistical data analysis. Descriptive statistics were expressed in number, percentage, and mean ± standard deviation. One Way ANOVA test was used for the parametric data and Kruskal-Wallis test for the non-parametric data. The categorical data were compared with chi-square test. The statistical significance was set at $p<0.05$.

3. Results

Data of 4039 people were scanned for the study, but 920 people who did not meet the inclusion criteria were excluded (Figure 1). Of the 3119 individuals included in the study, 1542 (49.43%) were male and 1577 (50.57%) were female. The average age of the individuals was 34.07±12.52. The rate of smoking in the women was 13.63% (n = 215), and this rate was 40.01% (n = 617) in men. 832 (26.67%) individuals were smokers in the whole group (Table 1).

Table 1. Sociodemographic data of patients

Variables	Smoker	Non-smoker	Total
Age, mean±SD	36.26±11.44	33.08±13.15	34.07±12.52
Male, n (%)	617 (40.01)	925 (59.99)	1542 (49.43)
Female, n (%)	215 (13.63)	1362 (86.37)	1577 (50.57)
Total, n (%)	832 (26.67)	2287 (73.33)	3119 (100)

Table 2. Blood group distributions of patients

Blood Group	Rh (+), n (%)	Rh (-), n (%)	Total, n (%)
O	900 (28.93)	113 (3.55)	1013 (32.48)
A	1247 (40.07)	140 (4.40)	1387 (44.47)
B	438 (14.06)	64 (2.03)	502 (16.09)
AB	185 (5.94)	32 (1.02)	217 (6.96)
Total	2770 (88.81)	349 (11.19)	3119 (100)

The population consisted of 1387 (44.47%) individuals with blood group A, 1013 (32.48%) with blood group O, 502 (16.09%) with blood group B, and 217 (6.96%) with blood group AB. The number of the A Rh (+) individuals, the most crowded group, was 1247 (40.07%), while the number of the AB Rh (-) individuals, the least crowded group, was 32 (1.02%) (Table 2).

The NLR ($p<0.001$), PLR ($p<0.001$), and MPVLR ($p<0.001$) levels of the smokers were statistically significantly higher than those of the non-smokers (Table 3).

Table 3. Changes in hemogram test results and systemic inflammatory parameters according to smoking

Parameters	n(%)	Neutrophil (10^3 /mm^3) mean±SD	Lymphocyte (10^3 /mm^3) mean±SD	Platelets (10^3 /mm^3) mean±SD	MPV (fl) mean±SD	NLR mean±SD	PLR mean±SD	MPVLR mean±SD
Smoker	832 (26.67)	4.56±1.45	2.28±0.69	255.37±58.02	9.99±1.14	2.14±0.91	119.77±39.55	4.74±1.50
Nonsmoker	2287 (73.23	4.29±1.34	2.46±0.89	258.41±62.55	9.98±1.14	1.87±0.62	116.34±41.48	4.50±1.54
Total	3119 (100)	4.36±1.37	2.41±0.85	257.60±61.38	9.98±1.14	1.94±0.71	116.34±41.48	4.56±1.53
p		<0.001	<0.001	0.222	0.926	<0.001	0.005	<0.001

p value, ANOVA test; n, number; SD, Standard Deviation; MPV, Mean Platelet Volume; NLR, Neutrophil / Lymphocyte ratio; PLR, Platelet / Lymphocyte ratio; MPVLR, MPV / Lymphocyte ratio.

There were significant differences between ABO and Rh blood groups in terms of NLR ($p<0.001$), PLR ($p<0.001$), and MPVLR ($p<0.001$) levels. The NLR, PLR, and MPVLR levels were found to be the lowest in the AB Rh (-) patients (Table 4).

Table 4. Changes in hemogram test results and systemic inflammatory parameters according to ABO and Rh blood groups

Blood Groups	n(%)	MPV(fl) mean±sd	NLR mean±sd	PLR mean±sd	MPVLR mean±sd
AB Rh (-)	32 (1.02)	9.80±2.00	0.57±0.34	69.12±34.03	2.69±1.19
AB Rh (+)	185 (5.88)	9.86±1.13	0.85±0.14	74.23±25.52	2.87±0.75
A Rh (-)	140 (4.48)	9.89±1.24	2.01±0.02	116.96±43.97	4.76±1.85
A Rh (+)	1247 (39.82)	10.00±1.17	2.61±0.58	136.35±42.68	5.38±1.48
B Rh (-)	64 (2.05)	9.68±1.56	1.43±0.01	89.09±22.12	3.23±0.74
B Rh (+)	438 (14.05)	9.99±1.06	1.59±0.08	106.58±32.41	4.15±1.21
O Rh (-)	113 (3.62)	9.90±1.07	1.38±0.31	99.17±32.24	3.65±1.13
O Rh (+)	900 (28.78)	10.04±1.05	1.54±0.29	107.70±32.95	4.22±1.20
Total	3119 (100)	9.98±1.14	1.94±0.71	116.34±41.48	4.56±1.53
p	-	0.126	<0.001	<0.001	<0.001

p value, ANOVA test; Rh, Rhesus; n, number; SD, Standard Deviation; MPV, Mean Platelet Volume; NLR, Neutrophil / Lymphocyte ratio; PLR, Platelet / Lymphocyte ratio; MPVLR, MPV / Lymphocyte ratio.

In the statistical analysis carried out considering the ABO and Rh blood groups and smoking status, a significant difference was found in terms of NLR ($p<0.001$), PLR ($p<0.001$), and MPVLR ($p<0.001$) levels. The NLR, PLR, and MPVLR levels were found to be the lowest in the non-smoker AB Rh (-) individuals (Table 5).

Table 5. Changes in hemogram test results and systemic inflammatory parameters according to ABO and Rh blood groups and smoking

Parameters		n (%)	MPV(fl) mean±sd	NLR mean±sd	PLR mean±sd	MPVLR mean±sd
AB Rh (-)	Smoker	12 (0.38)	10.03±0.98	0.92±0.22	76.74±39.75	1.32±0.24
	Non-smoker	20 (0.52)	9.66±2.43	0.36±0.19	64.55±30.27	2.50±1.09
AB Rh (+)	Smoker	30 (0.94)	9.67±0.67	0.92±0.16	83.91±25.14	3.09±0.86
	Non-smoker	155 (4.86)	9.90±1.17	0.85±0.13	73.43±25.98	2.87±0.75
A Rh (-)	Smoker	42 (1.34)	10.14±1.11	2.02±0.02	113.65±35.71	5.12±2.21
	Non-smoker	98 (3.14)	9.79±1.29	2.00±0.01	118.37±47.17	4.60±1.67
A Rh (+)	Smoker	339 (10.86)	10.00±1.29	2.96±0.87	136.00±41.86	5.43±1.41
	Non-smoker	908 (29.16)	9.99±1.12	2.48±0.34	136.49±43.01	5.37±1.50
B Rh (-)	Smoker	18 (0.51)	9.95±0.59	1.43±0.01	93.88±17.26	3.48±0.33
	Non-smoker	46 (1.36)	9.57±1.80	1.43±0.01	87.22±23.66	3.13±0.83
B Rh (+)	Smoker	98 (3.14)	9.98±1.12	1.65±0.07	107.71±31.82	4.26±1.27
	Non-smoker	340 (10.87)	9.99±1.04	1.57±0.07	106.26±32.62	4.12±1.19
O Rh (-)	Smoker	35 (1.15)	10.03±0.97	1.54±0.28	106.07±29.59	3.76±0.88
	Non-smoker	78 (2.57)	9.85±1.11	1.31±0.30	96.07±33.07	3.60±1.22
O Rh (+)	Smoker	258 (8.24)	9.98±1.03	1.59±0.27	113.85±33.76	4.46±1.24
	Non-smoker	642 (20.58)	10.07±1.06	1.53±0.29	105.23±32.32	4.13±1.18
Total		3119 (100)	9.98±1.14	1.94±0.71	116.34±41.48	4.56±1.53
p		-	0.220	<0.001	<0.001	<0.001

p value, ANOVA test; Rh, Rhesus; n, number; SD, Standard Deviation; MPV, Mean Platelet Volume; NLR, Neutrophil / Lymphocyte ratio; PLR, Platelet / Lymphocyte ratio; MPVLR, MPV / Lymphocyte ratio

4. Discussion

In our study, we examined the future health risks as a result of the reflection of blood Type and smoking on hematological parameters. We investigated the effects of blood types and smoking on NLR, PLR and MPVLR values in healthy individuals. In our study, we found that complete blood count parameters changed depending on blood type and smoking status.

In our study, 832 (26.67%) of the individuals included in the study were smokers. The rate of smoking in women was 13.63% (n = 215), and this rate was 40.01% (n = 617) in men. According to the 2012 data of Government Statistical Institute, the rate of smoking in adults was 27% in Turkey. This rate was 41.4% for men and 13.1% for women (18), which is in line with the data in our study. In our study, the frequency of blood groups was as follows: A> O> B> AB. These data were in line with the data in Turkey.

In our study, the NLR, PLR, and MPVLR levels of the smokers were found to be higher than those of the non-smokers. The analysis carried out considering the ABO, Rh blood groups and smoking status showed statistical differences in terms of NLR, PLR, and MPVLR levels. In the post hoc analysis, this difference was observed to be caused by the A Rh (+) individuals. The NLR, PLR, and MPVLR levels were found to be the highest in the A Rh (+) individuals and the lowest in the AB Rh (-) individuals. The NLR, PLR, and MPVLR levels were found to be the highest in the A Rh (+) smokers and the lowest in the non-AB Rh (-) smokers.

Behçet's disease, spondyloarthropathy, vasculitis, and rheumatoid arthritis were reported to be more common in the patients with group A blood. Familial Mediterranean fever, systemic lupus erythematosus, systemic sclerosis, and Sjögren's syndrome have been reported to be more common in the patients with group O blood. inflammatory diseases were found to be the lowest in people with group AB blood (4). In another study, blood type A was found to be a risk factor for myocardial infarction. It was reported that there was a link between the distribution of ABO blood group antigens and the risk of developing specific types of cancer (5). Cancer can cause pathological conditions in many different systems in patients (19). Various studies have suggested that there is a relationship between ABO blood group and tumor behavior and later clinical outcome in patients with various malignancies in lung, kidney, ovary, colorectum, and pancreas. Observational studies have recently shown a relationship between the blood group O and the low risk of exocrine pancreatic tumors (20). In another study, it was shown that while the blood group A increased the risk in gastric cancers, the blood group O was protective (3). Another study reported that individuals with group A had a higher risk of developing glioblastoma, while individuals with group O had a lower risk (5). In their study, Franchini et al. found a positive relationship between A blood group and plasma lipid levels and clinically observed increased susceptibility to cardiovascular disease in individuals with non-O blood types (21).

In a meta-analysis, the frequencies of the blood groups A, B, O, and AB in the individuals infected with COVID-19 were reported as 36.22%, 24.99%, 29.67%, and 9.29%, respectively. The frequencies of the blood groups A, B, O, and AB in cases of death due to COVID-19 infection were calculated as 40%, 23%, 29%, and 8%, respectively. It was shown that the individuals with blood group A were at a higher risk for COVID-19 infection, while those with blood group O were at a lower risk (22). COVID-19 can cause diseases in many systems (23).

In the literature, it has been reported that the individuals with blood group O have a lower risk of cardiovascular disease, high total cholesterol levels, and type 2 diabetes (2). In another study, the probability of developing type 2 diabetes was found to be higher in the individuals with blood group A than in those with other blood groups. It was suggested that blood type A be considered as a risk factor for type 2 diabetes screening (24).

Lung cancer has been reported to be more common in Rh (+) people in the literatüre (25). In another study, Rh factor positivity was found to be higher in rheumatologic patient groups (4). In the study of Tulgar et al., white blood cell, neutrophil, basophil and eosinophil counts; mean corpuscular volume, red cell distribution width and NLR were significantly higher in smokers when compared to non-smokers ($p<0.05$) (26). In our study, the NLR, PLR, and MPVLR levels were found to be high in the A Rh (+) individuals and the smokers. When the groups were combined, the NLR, PLR, and MPVLR levels were found to be at the highest level in the A Rh (+) smoker patients. It may be thought that A Rh (+) individuals have a higher risk for inflammatory diseases, and smoking increases this risk.

The limitation of our study is that it is single-centered and retrospective. Examination of people's smoking addiction level and pack/year status is one of the limitations of the study. One of the limitations of the study is that other laboratory values of the patients (such as white blood cell, hemoglobin, B12, lipid profile, liver function tests, urea and creatinine for kidney function) were not examined. Multicenter studies are needed in which the relationship between the prospective and other systemic inflammatory markers and the parameters such as C-Reactive Protein and sedimentation is examined.

Although there are many studies in the literature on blood groups and chronic diseases, there is no study investigating the effect of blood groups and smoking on NLR, PLR, and MPVLR values in healthy individuals. In this regard, we think that our study will contribute to future studies.

In this study, we found that the parameters of complete blood count changed depending on blood group and smoking status. It was observed that the A Rh (+) individuals who smoke had higher levels of total blood count parameters. This

result may be helpful in predicting the possibility of developing chronic inflammation-related diseases based on blood groups and smoking status.

Ethical Statement

This study was approved by the clinical studies ethics committee of local University (Decision Number: 2020/240).

Conflict of interest

The authors declare no conflicts of interest.

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None to declare.

Authors' contributions

Concept: H.I., Design: H.I., Data Collection or Processing: H.I., Analysis or Interpretation: H.I., Literature Search: H.I., Writing: H.I.

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Research Article

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Role of transvaginal ultrasonography in determining endometrium cancer risk in asymptomatic postmenopausal women with thickened endometrium

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Abstract

The aim of this study is to determine the cut-off value for the histopathological evaluation of premalignant-malignant endometrial pathologies from benign pathologies in postmenopausal asymptomatic patients with increased endometrial thickness. This cross-sectional study included a population that included asymptomatic 481 postmenopausal women with an endometrial thickness of more than 5mm in TVU who underwent diagnostic/ operative hysteroscopy and full curettage between January 2015 and January 2018. Demographic characteristics TVU, hysteroscopy findings of patients were recorded. As a result, in the histopathological outcome, 154(3%) women were evaluated as having normal endometrium, 189(39.3%) women as having endometrial polyps, 93(19.3%) women as having endometrial atrophy, and 22(4.6%) women as having endometrial simple hyperplasia, 5(1%) women as having endometrial atypical hyperplasia, 17(3.5%) women as having endometrial atrophy and only one (0.2%) woman as having fibroids. In the 187 postmenopausal women with normal diagnostic hysteroscopic evaluation, histopathological findings were: 13(7%) endometrial hyperplasia, 2(1.1%) atypical endometrial hyperplasia, 27(14.4%) endometrial polyps, 4 2.1%) endometrial atrophy, and 2(1.1%) endometrial carcinoma. The endometrial thickness was analyzed with the ROC curve for cutoff value differentiating atypical endometrial hyperplasia/endometrial carcinoma from benign lesions and 10.5 mm was found with 90% sensitivity and 63% specificity. In conclusion, hysteroscopy is highly effective for identifying the endometrium and focal intracavitary pathologies such as polyps, myomas and foreign bodies in women with abnormal uterine bleeding. However, for the diagnosis of endometrial hyperplasia and cancer, hysteroscopic-guided biopsy with uterine curettage seems to be the best method.

Keywords: endometrial thickness, hysteroscopy, uterine intracavitary pathology, postmenopausal women, transvaginal ultrasonography

1. Introduction

In developed countries, endometrial cancer (EC) is the most frequent gynecological cancer of the female genital tract (1). Obesity rates are rising worldwide, while fertility rates are falling, indicating that the number of postmenopausal women with diagnosed endometrial cancer will continue to increase and endometrial cancer will be a significant public health concern (2, 3). There is no agreed-upon protocol for screening EC, and in this regard, endometrial thickness (ET) measurement by transvaginal ultrasonography (TVU) was discussed previously (4).

TVU is a common examination tool for patient evaluation in gynecology. Women suffering from postmenopausal bleeding who have an ET of less than 5 mm on TVU have a higher risk of EC (5, 6). Although vaginal bleeding is recognized as the most frequently observed symptom of EC, up to 20% of patients diagnosed with EC are asymptomatic (7 An endometrial sample is required to rule out malignancy in these women with postmenopausal bleeding. However, studies have found that malignancy rates range from 0% to 3% in asymptomatic postmenopausal women with thicker endometrium (traditionally classified as 5 mm) (8, 9). There is no agreement on the endometrial thickness threshold that distinguishes normal from malignant pathology in asymptomatic postmenopausal women. Clinical care of postmenopausal women with thicker endometrium discovered by chance is challenging for clinicians and has not been standardized or established yet (10).

In the diagnosis of endometrial pathologies, dilatation/curettage and hysteroscopy with histopathologic evaluation are well-known methods and can be added to TVU

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(11). Considering the invasive profile and cost of these methods, it may not be necessary to perform these procedures on every patient with a thick endometrium (4).

The purpose of this research is to assess the relationship between the endometrial thickness of benign and malignant endometrial pathologies, and to contribute to an approach for postmenopausal women with asymptomatic thick endometrium.

2. Materials and Methods

This research was carried out as a prospective study, and observations were conducted on a group of postmenopausal women admitted to the outpatient clinic of the tertiary hospital between January 2015 and January 2018. All patients confirmed written informed consent. The study was ethically conducted in compliance with the Helsinki Declaration of 1964 and its amendments. The Committee of Ethics of the Scientific Board of the Ministry of Health of Republic of Turkey and the administrative board of the hospital gave their approval to this study (registration no:1828-903).

2.1. Patients

The study population included asymptomatic postmenopausal women whose observed endometrial thickness was greater than 5 mm in transvaginal ultrasonography (TVU) and who had a hysteroscopy and complete curettage as a diagnostic or operative procedure. All materials were evaluated histopathologicaly. Twelve months of amenorrhea was defined as menopause. The demographic characteristics TVU, hysteroscopy findings of patients were recorded. The histopathology findings of patients were obtained from medical records.

Patients with chronic disease (diabetes mellitus, hypertension, hypothyroidism), use of hormone therapy, progesterone treatment and tamoxifen, as well as patients being monitored for endometrial polyps, endometrial hyperplasia, endometrial cancer were excluded.

2.2. Transvaginal ultrasonography

Endometrial thickness was measured by the TVU using a Siemens Acuson x 300 device and a 7 MHz vaginal probe at the sagittal plane of the uterus from anterior to posterior at its thickest point and perpendicular to the outer edge of the endometrium. If presence of fluid was observed in endometrial cavity, the thickness of endometrium was measured by subtracting the fluid intervals from all endometrial thickness. All TVU examinations were performed by experienced gynecologists of the same clinic.

2.3. Hysteroscopy

Under general anesthesia, diagnostic hysteroscopy was performed using a 5 mm and 30° continuous-flow hysteroscope (Karl Storz, Germany) with saline solution (0.9% sodium chloride) as a distension medium. A 10 mm and 12° resectoscope using a continuous-flow hysteroscope (Karl Storz, Germany) with saline solution (0.9% sodium chloride) as a distension medium was used to remove endometrial lesions. All hysteroscopic evaluations, removal of lesions and full curettage of patients were performed by experienced gynecologists of the same clinic. All histopathological materials were evaluated in the same pathology department.

2.4. Primary outcome

The primary outcome of the study was to determine the cutoff value for histopathological evaluation of premalign-malign endometrial pathologies from benign pathologies in postmenopausal asymptomatic patients with increased endometrial thickness.

2.5. Statistics

For data analysis, SPSS version 22.0 (IBM Corp., Armonk, NY, USA) was used. The normality of the demographic data was assessed via The Shapiro-Wilk test. The interquartile range and median were considered for the case of non-normally distributed data, while the standard deviation and mean value were considered for data following a normal distribution. For analyzing continuous data, The Mann-Whitney U test was used and for analyzing categorical variables, Fisher's exact test or the Chi-Square test was employed. Significant was defined as a p-value less than 0.05. Cutoff values for the detection of endometrial cancer or atypical endometrial hyperplasia were designated using the receiver operator characteristic (ROC) curve (AEH).

3. Results

A total of 500 postmenopausal asymptomatic women whose endometrial thickness was over 5 mm on TVS were recruited for the study. Thirteen women were excluded due to refusing further examination, and six of them were excluded due to technical problems encountered in storing their ultrasound images. Consequently, the total number of patients included in the final analysis is 481.

In Table 1, the demographic and clinical characteristics of postmenopausal women are summarized.

Table 1. Demographic and clinical characteristics of postmenopausal women

Characteristics	Values (median) (IQR)	Min-max
Age year	54.0 (8.0) (41-80)	(41-80)
Age at menopause year	46.0 (5.0) (30-55)	30-55)
Duration of menopause year	6.0 (7.0) (1-30)	(1-30)
BMI (kg/m^2)	27.5 (2.7) (20.9-37.2)	(20.9-37.2)
Endometrial stripe thickness (mm)	10 (7) 11.9 (5.6) [a] (5-32)	(5-32)
Parity	2.0 (2) (0-10)	(0-10)

BMI: Body mass index, IQR: Interquartile Range, SD: standard deviation
[a]mean (SD)

In the diagnostic hysteroscopic procedure, 187 (38,9%) women were evaluated as having normal endometrium, 156 (32.4%) women as having endometrial polyps, 118 (24.5%) women as having endometrial atrophy, and 20 (4.2%) women as having endometrial hyperplasia. In the 187 postmenopausal women with normal diagnostic hysteroscopic evaluation, histopathological findings were: 13 (7%) endometrial

hyperplasia, 2 (1.1%) atypical endometrial hyperplasia, 27 (14.4%) endometrial polyps, 4 (2.1%) endometrial atrophy, and 2 (1.1%) endometrial carcinoma.

Histopathological evaluation revealed the most common intrauterine lesion detected in the study population was endometrial polyps diagnosed in 189 (39.3%) cases, and other histopathologic findings of the patients are shown in Fig. 1. Five endometrial carcinomas and one endometrial hyperplasia were identified based on the presence of endometrial polyps.

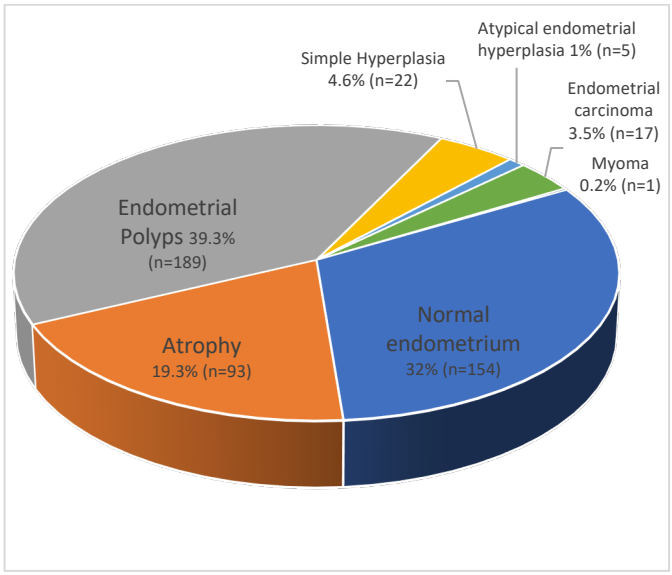

Fig. 1. The histopathologic outcome in 481 menopausal asymptomatic women with endometrial thickness ≥5 mm

Clinical and demographic characteristics of patients with benign and atypical endometrial hyperplasia/ endometrial carcinoma are presented in Table 2.

Table 2. Demographic and clinical characteristics of patients with benign and atypical endometrial hyperplasia/ endometrial carcinoma

	Benign (n=459) (Median) (IQR)	AEH and Endometrial carcinoma (n=22)	*P*
Age year	53.0 (8.0) (41-80)	55.0 (7.0) (46-72)	.252
Age at menopause year	46 (5) (30-55)	47.5 (7) (33-54)	.288
Duration of menopause year	6 (7) (1-30)	6.5 (8.0) (2-24)	.604
BMI (kg/m²)	27.3 (2.8) (20.9-37.2)	28.2 (3.0) (23.9-33.3)	.122
Endometrial thickness (mm)	10 (7) (5-30)	18.5 (15) (5-32)	
	Mean (SD)	**Mean (SD)**	.000
Endometrial thickness (mm)	11.5 (5.2) (5-30)	18.8 (7.9) (5-32)	

BMI: Body mass index, AEH= Atypical endometrial hyperplasia, IQR: Interquartile Range, SD: standard deviation

The endometrial thickness was analyzed with the ROC curve for cutoff value differentiating AEH/endometrial carcinoma from benign lesions and 10.5 mm was found with 90% sensitivity and 63% specificity (area under curve = 0.783; P<.0001; 95% confidence interval) (Fig. 2). At this threshold value, the rate of endometrial cancer was 0.4% (n=2) and the endometrial thicknesses of the cases were 6 mm and 7 mm. The positive predictive value of 10.5 mm endometrial thickness was % 8.66 and the negative predictive value was % 99.2.

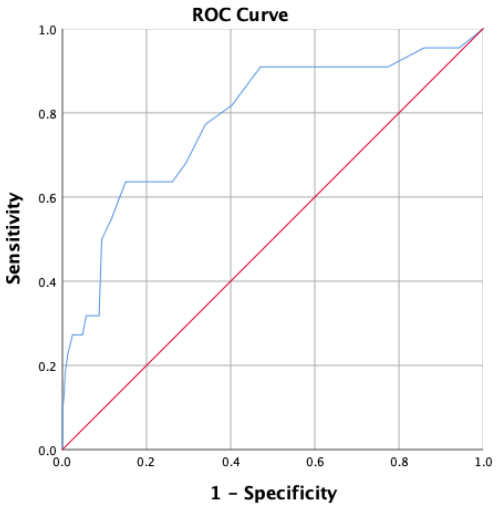

Fig. 2. ROC curve associated with the risk-scoring model. The area under the curve was 0.783 (95% CI 0.674 to 0.893; P<.0001)

4. Discussion

The findings of this study show that the cut-off value we determined for the diagnosis of endometrial cancer and atypical hyperplasia is 10.5mm. 20 women with endometrium cancer and atypical hyperplasia were seen above our cut-off value, and the negative predictive value was calculated as 99.2%. While endometrial cancer was observed in only 2 cases below the cut-off value, no cases with atypical hyperplasia were observed by hysteroscopy, one of these two cases was diagnosed as atrophy, and the other as an endometrial polyp.

Since benign lesions are more common in women with increased endometrial thickness, the positive predictive value was calculated as 8.66%. In recent years, TVU has been the method of choice for endometrial examination of premenopausal and postmenopausal women due to its high accuracy and lack of invasiveness (12). Asymptomatic submucosal fibroids and endometrial polyps are benign lesions, they usually do not require any treatment.10 In asymptomatic postmenopausal women, endometrial polyps were the most commonly observed localized intrauterine lesions (13). In our study, endometrial polyps were detected within 189 (39.3%) patients. The incidence of endometrial cancer based on the presence of polyps was 10% in postmenopausal women with symptoms and whose endometrial thickness was measured as greater than 5 mm on TVU, it was reported 0.9% in asymptomatic postmenopausal women, which is a very low percentage when compared (14). Similarly, in our study, the rate of cancer development in the presence of polyps was found to be 1% (n=5) in asymptomatic postmenopausal women.

Endometrial cancer can develop in postmenopausal women without causing bleeding, therefore, a thicker endometrium

discovered by chance may necessitate an endometrial assessment. However, for endometrial cancers and other pathologies there is no standardized screening procedure or cut-off value. The current guidelines suggest that only women with Lynch syndrome which is a genetic condition associated with risk of developing endometrial cancer, be screened for endometrial cancer (15).

The UK Collaborative Ovarian Cancer Screening Study (UKCTOCS) presented a sensitivity of 80.5% and a specificity of 85.7% when the cut-off value is applied as 5 mm for the diagnosis of endometrial cancer or atypical hyperplasia (16). In another study, an endometrial thickness limit of 11 mm was suggested for an incidentally measured increase in endometrial thickness of a patient with no symptoms (6). In our study, the optimal cut-off value for premalignant and malignant lesions was 10.5 mm with 91% sensitivity and 63% specificity. Moreover, the malignancy or atypical hyperplasia risk for patients having endometrial thickness measurements under the threshold value is extremely low (0.4%, n=2), and the proportion of women with malignancy and atypical hyperplasia above the threshold is 4.2% (n=20). Even if endometrial curettage is not performed in asymptomatic patients above the threshold value, these women may require follow-up.

Endometrial polyp (74.3%) was determined to be the most common pathology in hysteroscopy in asymptomatic women with endometrial thickening on transvaginal ultrasound in a previous study (17). Similarly, in our study, the most common pathology observed in hysteroscopy was endometrial polyp. Hysteroscopy is an easy and safe technique for detecting intrauterine lesions (polyps, fibroids), but it is not sufficient for the diagnosis of endometrial hyperplasia and endometrial cancer. Hysteroscopic appearance, along with histopathological evaluation, may be the gold standard for endometrial evaluation (18).

The limitation of this study was that since we excluded women with risk factors of endometrial carcinoma, there was no evaluation based on the patient's specific characteristics. In conclusion, TVU may be useful in the evaluation of endometrial pathology and in the diagnosis of early endometrial cancer. In this study, 2 cases of endometrial cancer were detected in women with endometrial thickness less than 10.5 mm, negative predictive value was very high. Above the cut-off value, about 9 times of endometrial cancer cases were diagnosed Because of their low positive predictive value, endometrial thickness measurements with TVU should not be used alone to make a decision about further evaluation. These findings, however, can be used to aid clinical decisions.

Ethical statement

The Committee of Ethics of the Scientific Board of the Ministry of Health of Republic of Turkey and the administrative board of the hospital gave their approval to this study (registration no:1828-903).

Conflict of interest

The authors declare no potential conflict of interests for this work.

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None to declare.

Authors' contributions

Concept: O.S.G., Design: O.S.G., A.B., Data Collection or Processing: M.T., A.B.T., Analysis or Interpretation: Z.G.O., U.K., Literature Search: U.K., M.T., Writing: N.T., A.B.T.

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Research Article

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Comparison of depression and anxiety in inflammatory bowel patients treated with anti-TNF or immune modulators

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Abstract

The aim of this study was to investigate patients diagnosed with inflammatory bowel disease (IBD), the effects of drug groups used in the treatment of inflammatory bowel disease on depression and anxiety. This study was a single-center prospective study involving 94 patients with inflammatory bowel disease, 51 of whom had ulcerative colitis (UC) and 43 had Crohn's disease (CD). Harvey-Bradshaw activity scores for CD and Mayo Clinic activation scores for UC were calculated. Depression and anxiety data were collected with the Beck Depression Inventory and Beck Anxiety Inventory. The mean age for UC and CD were 40.25 ± 14 and 38.9 ± 13.8 years, respectively. There was a positive correlation between disease activation and depression and anxiety levels in both IBD subgroups ($p<0.05$). All patients included in the study were compared in terms of depression and anxiety levels before and after treatment, and a statistically significant improvement was found with the remission of both ulcerative colitis and Crohn's disease ($p<0.001$). There was a significant difference in depression and anxiety levels before and after treatment according to treatment options ($p<0.001$). There is a positive relationship between the disease activation score and the level of depression and anxiety in IBD. Immunosuppressive and immunomodulatory drugs used in the treatment of IBD may also improve the parameters of depression and anxiety in this disease.

Keywords: ulcerative colitis, Crohn disease, depression, anxiety, disease activation

1. Introduction

Both Crohn's disease (CD) and ulcerative colitis (UC) are chronic inflammatory diseases that affect the gastrointestinal system. They impair quality of life and are characterized by periods of relapse and remission. In the general population, the prevalence of depression and anxiety is higher in patients with chronic diseases (1).

Studies have shown that the frequency of depression and anxiety is higher in patients with inflammatory bowel disease (IBD) compared to the general population (2). While depressive symptoms can affect the activity of the disease, higher rates of depression and anxiety have been reported in the active phase of the disease compared to the remission period. Anxiety and depression rates are between 29-35% in the remission period; at the time of relapse, this rate goes up to 80% for anxiety and 60% for depression (2).

Improvement in IBD activity has also been linked to a reduction in the severity of depressive and anxiety symptoms, according to research. Several studies suggest that anti-tumor necrosis factor alpha (anti-TNF), an agent used in the treatment of IBD, may reduce depressive symptoms and improve quality of life. In addition, there are data showing that immunomodulatory treatment types such as azathioprine and methotrexate, which are widely used in the treatment of IBD, also benefit depressive symptoms.

In this study, it was aimed to evaluate the effect of any newly initiated immunosuppressive therapy (anti-TNF therapy or immunomodulatory therapy) on anxiety and depression symptoms in the active phase of inflammatory bowel disease. We aimed to evaluate whether the regression in disease activity is associated with improvement in depressive symptoms and whether it differs in the level of depression according to the treatment options they receive.

2. Materials and methods

2.1. Study design

This study was conducted between June 2018 and January 2019. Patients with active UC and CD who applied to the gastroenterology outpatient clinic or followed up in the gastroenterology service were included. Depression and anxiety data of the patients were obtained with the Beck Depression Inventory (BDI) and the Beck Anxiety Inventory (BAI), and the relationship between the treatment they received and depression and anxiety were evaluated.

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The patients were informed about the study, and a written consent form was obtained from the patients before starting the study.

Inclusion Criteria

- To be diagnosed with IBD clinically, endoscopically and pathologically
- Being in active disease

Exclusion Criteria

- Refusing to volunteer and sign the consent form
- The inability to read or write.
- Presence of substance abuse, psychiatric illness or any neurological disease
- Using psychotropic medication in the last 1 year

Workflow

After clinical, laboratory, endoscopic and histological evaluation, patients diagnosed with active IBD according to the current ECCO guideline and followed up in the Gastroenterology outpatient clinic and service were asked whether they would like to be included in the study. The study was explained in detail to the patients who stated that they wanted to participate, and their written consent was obtained. All patient groups were evaluated twice with the Beck Depression Inventory (BDI) and Beck Anxiety Inventory (BAI) before starting treatment and 3 months after starting inflammatory bowel disease treatment. The questionnaires were filled by the person participating in the study after being informed by the doctor. Before starting treatment, disease activity on the day of the survey was semi-quantitatively determined and recorded by the Harvey-Bradshaw Index for CD and Mayo scoring for UC. Whether the patients were in remission or not was determined according to these indices.

2.2. Data collection tools

Beck Depression Scale

It is used to have information about the risk of depression and to measure the level and severity of depressive symptoms. It was developed by Beck et al. The Turkish validity and reliability study of the BDI was conducted (3). It is a self-evaluation scale. It can be applied to both healthy individuals and psychiatric patients. It consists of a total of 21 self-evaluation sentences. Each question is evaluated on a scale of 0 to 3. It ranges from 0 to 63 in total and is classified as follows; (4).

- Normal 0-9
- Mild depression 10-18
- Moderate depression 19-29
- Severe depression 30-63

Beck Anxiety Scale

It is an inventory consisting of 21 questions developed by Beck et al (5). Each question is evaluated between 0 and 3 and categorized as follows;

- Minimum anxiety 0-7
- Mild anxiety 8-15
- Moderate anxiety 16-25
- Severe anxiety 26-63

Mayo Clinic Ulcerative Colitis Disease Activity Index

It is a scoring system that grades the severity of the disease in the follow-up of UC. Results are obtained by evaluating stool pattern, rectal bleeding severity, endoscopic findings and general well-being of the physician.

It ranges from 0 to 12 in total and there is a positive correlation between the increasing score and the severity of the anxiety clinic/disorder. Score of 2 points or less is associated with remission, 3-5 points mild disease, 6-10 points moderate disease, and 11-12 points severe disease.

Harvey-Bradshaw Index

It is used to evaluate the severity of the disease in the follow-up of CD. Scoring is done by stool pattern, mean number of abdominal pains, general well-being, systemic complications, and whether there is a mass in the abdomen. A score of less than 5 is associated with remission, a score of 5-7 is associated with mild disease, a score of 8-16 is associated with moderate disease, and a score greater than 16 is associated with severe disease.

2.3. Statistical analysis

Data analysis was performed using the Statistical Package for the Social Sciences 22 (SPSS, Inc, Chicago IL, USA). As a statistical analysis, in the descriptive findings section, categorical variables will be presented as numbers and percentages, and continuous variables will be presented as mean±standard deviation for normally distributed data and median (min, max) for non-normally distributed data, and number of cases and (%) for nominal variables. The Mann-Whitney U-test was used to compare categorical data between groups that did not show a normal distribution. The Wilcoxon test was used to compare the continuous data between groups after checking whether they fit the normal distribution. The p value < 0.05 was accepted as significant according to the 95% confidence interval.

3. Results

127 patients, including 73 UC and 54 CD, were evaluated for the study, 9 of these patients did not come for follow-up after 3 months, and 24 patients were excluded because they had one or more of the exclusion criteria. Thus, a total of 94 patients were included (39.65 ± 13.93 years), 51 patients with UC (21 female), 43 patients with CD (23 female). In the mean age was 40.25±14 in UC and 38.9±13.8 in CD. There was no significant difference between the groups in terms of age and gender.

3.1. Disease activity

The mean of Harvey-Bradshaw Index, which was an activation indicator for CD, was 8.09 ±1.9, while the lowest activity score

was 5 and the highest was 13. According to the Harvey-Bradshaw index, 46% of patients with CD had a mild disease and 53.5% had a moderate disease. For UC, the mean of Mayo Clinic UC activity index was 7.75 ±1.32 and while the lowest activity score was 4 and the highest was 11. According to the Mayo Clinic UC activity index, 9.8% of UC patients had a mild disease, 86.3% had a moderate disease, and 3.9% had severe disease.

3.2. Beck Depression Inventory and Beck Anxiety Inventory Scores

BDS ranged from 0 to 23, and the mean was 14.72±5.07 in the entire patient group before treatment. It was determined that 12.8% of the patients were normal, 63.8% mild depression, 22.3% moderate depression and 1.1% severe depression before treatment.

BAS ranged from 0 to 28, and it was 10.29±3.68 in the entire patient group before treatment. It was found that before the treatment, 23.4% of the patients had minimal anxiety, 64.9% had mild anxiety and 11.7% had moderate anxiety.

The mean BDS was 13.89±4.98 in females and 15±5.09 in males in the entire patient group before treatment. Before treatment, the mean BAS was 10±3.48 in women and 10.5±3.8 in men. There was no significant difference in depression and anxiety levels according to gender before and after treatment. (p>0.05).

It was determined that there was a change in this mean, as 5.88 ± 2.20 for BDS and 3.27 ± 2.02 for BAS, 3 months after starting IBD treatment. Similarly, a statistically significant difference was found in UC patients in terms of depression and anxiety levels before and after treatment (p<0.001) (see Table 1).

In patients with CD, the mean BDS was 15.6±5.38 and the BAS was 11±3.74 before treatment. It was detected that 3 months after starting the inflammatory bowel disease treatment, the mean BDS changed to 6.33±2.27 and the mean BAS was 3.56±2.42.

It was observed that there was a statistically significant difference between depression and anxiety levels before and after treatment in CD patients, as in patients with UC. (p<0.001) (see Table 1).

There was a significant decrease in anxiety and depression scores before and after treatment in anti-TNF and anti-integrin, 5-ASA, 5-ASA±azathioprine treatment groups (p<0.001) (see Table 2).

Table 1. Comparison of depression and anxiety levels before and after treatment in the study population

	Before treatment BDS	After treatment BDS	*p*	Before treatment BAS	After treatment BAS	*p*
CD	15,6±5,38	6,33±2,27	<0,001	11±3,74	3,56±2,42	<0,001
UC	13,96±4.97	5,88±2,20	<0,001	9,69±3,56	3,27±2,02	<0,001

Wilcoxon Test was used. UC, ulcerative colitis; CD, Crohn's disease; BDS, Beck depression scale; BAS, Beck anxiety scale

Table 2. Change in anxiety and depression levels in the study group according to the treatment they received

	Before BDS	After BDS	*p*	Before BAS	After BAS	*p*
5-ASA n=24	11.29±4.80	5±2.24	<0,001	9.04±3.82	2.58±1.99	<0,001
Azathioprine n=21	13.43±4.74	5.52±1.7	<0,001	9.38±2.71	2.86±1.79	<0,001
Anti-TNF and anti-integrin n=49	16.96±4.40	6.86±2.15	<0,001	11.29±3.75	4.04±2.30	<0,001

Wilcoxon Test was used. UC, ulcerative colitis; CD, Crohn's disease; BDS, Beck depression scale; BAS, Beck anxiety scale

A positive correlation was assigned between the level of disease activation and depression and anxiety in both UC and CD patients (see Table 3)

Table 3. Evaluation of the relationship between activation scores and Beck depression and anxiety scores in the study group

	Beck Depression score		Beck Anxiety Score	
	r	*p*	*r*	*p*
Mayo score, n=51	0.780	<0.001	0.474	0.001
Harwey-Bradshaw, n=43	0.708	<0.001	0.474	0.001

r: Spearman's Correlations Coefficient

4. Discussion

Although psychiatric diseases are a public health problem that negatively affects general health, the presence of chronic diseases is one of the stressor factors that facilitate the emergence of psychiatric diseases such as anxiety and depression or negatively affect the prognosis.

Recent studies have noted increased rates of depression and anxiety in patients with IBD (6). In studies on IBD and other chronic diseases, the prevalence of depression was found to be 6-14% (1, 7). In our study, the prevalence of depression at baseline was higher than in other studies. This can be explained by the inclusion of patients in the active disease stage in the study.

Likewise, the presence of anxiety and depression negatively affects the prognosis of chronic diseases. A prospective study reported that individuals with IBD who had higher depressive symptoms at disease onset were more likely to relapse than individuals without depression. (8).

Studies show that IBD treatment can actually affect depressive symptoms. Minderhoud et al. found a significant improvement in depressive symptoms after one or two infusions of infliximab every 14 weeks with CD (9). Similar to the previous study, improvement in depressive symptoms was observed in a small study of 15 patients who received a single infliximab infusion and were followed for 8 weeks (10). Similarly, in our study, we found a significant decrease in the number of patients with moderate and severe depression symptoms in the 3-month follow-up after starting IBD treatment.

Some small studies have found that improvement in depressive symptoms in inflammatory diseases such as CD and ankylosing spondylitis is not associated with improvement in disease activity after anti-TNF therapy (10, 11). In our study, on the contrary, significant improvement in depression and anxiety symptoms was observed in both groups receiving anti-TNF therapy and immunomodulatory therapy.

Improvement of depressive symptoms may not be due to the initiation of IBD treatment alone. On the contrary, there is important literature that also associates depression with the proinflammatory process and disease activation (12). Supporting this association, a randomized placebo-controlled trial evaluated the role of infliximab in the treatment of patients with treatment-resistant depression independent of inflammatory disease. They found no significant improvement in depressive symptoms with infliximab treatment, but improvement in depressive symptoms was found in patients with high baseline C-reactive protein (CRP) levels (hCRP>5 mg/l) (13). In the current study, high depression and anxiety scores in the active period may be associated with high CRP and active pro inflammatory processThere is a positive correlation between the decrease in CRP level after the patients enter remission and the decrease in depression and anxiety symptoms.

In the present study, depression and anxiety levels in the active disease period were found to be significantly higher than in the remission period, similar to other studies. A decrease in depression and anxiety levels may be due not only to the decrease in disease activity, but also to the improvement of multifactorial causes such as discontinuation of corticosteroids used in the active period, improvement of other medical conditions or the passage of time.

The small number of patients, lack of sociodemographic data, previous use of corticosteroids, smoking status and incomplete data on sociocultural status are the weaknesses of our study. These incomplete data make it difficult to control for confounding factors affecting depression and anxiety symptoms.

The Beck depression and anxiety questionnaire was approved for the assessment of depressive symptoms. However, it is not a definitive method to evaluate depressive disorders. Another factor limiting our study is that the depression status of the patients could only be obtained with Beck depression and anxiety scores in a short time during hospital visits.

In conclusion, the present study highlights the importance of treating all aspects of the disease, including psychological comorbidities such as depression, in patients with IBD. At the same time, it should be kept in mind that depressive and anxious symptoms may accompany the disease and even adversely affect the prognosis of the disease.

This study shows that; immunosuppressive and immunomodulatory drugs that make IBD go into remission can also improve psychological parameters in this disease.

More research is needed in this area to understand both the pathophysiology of IBD and the most common psychological comorbidities associated with the disease.

Ethical statement

The study was designed as a prospective study approved by the ethics committee of Gazi University Faculty of Medicine, Ankara, Turkey (Approval number: 254-2018/05, Date: 05.06.2018). The study protocol was designed in accordance with the ethical guidelines of the Declaration of Helsinki published in 1975.

Conflict of interest

The authors have no conflicts of interest to declare.

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None to declare.

Authors' contributions

Concept: E.Ç., N.E., M.C., Design: E.Ç., N.E., M.C., Data Collection or Processing: E.Ç., N.E., M.C., Analysis or Interpretation: E.Ç., N.E., M.C., Literature Search: E.Ç., N.E., M.C., Writing: E.Ç., N.E., M.C.

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The investigation of concomitant dermal and oral lichen planus in patients referred to Zahedan University centres

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Abstract

Lichen planus (LP) is a prevalent chronic immunologic inflammatory epidermal and mucocutaneous condition which involves the oral mucosa, the skin, as well as occasionally both, and the leading reason of which is still unidentified. Because of the significance and frequency, this study was performed to measure the relationship of oral and dermal lesions in LP patients. In this descriptive study, a total number of 99 patients with involvement of LP in skin or mucosa were selected from 6510 patients who referred to the Dermatology Clinic and Department of Oral and Maxillofacial Diseases, Zahedan Dental School. The selected patients were exanimated completely and interviewed, and in case of presence of suspected lesions, the histopathologic tests achieved by biopsy were used for the assessment. After physical examination and taking history, a questionnaire was filled for each case. The study showed a 38 percent relation between oral and dermal lesions in the population of the study. In comparison, individual oral lesion was observed in 60.6 percent of patients and individual dermal lesions in 39.3 percent. The most common symptom of oral lichen planus was the Oral ulcer, and the most common site of involvement was the buccal mucosa was and the erosive form showed high prevalent. The most reported symptom of dermal lichen planus in this study was Itching, as well as Simultaneous hand and foot conflict was the main site of involvement and the Papular form was highly prevalent. The high prevalence of dermal and oral lichen planus relationship suggests attention to complete systemic examinations of the patients. Since this study was performed in the dermatology clinic, it appears that other studies in dental schools and all over the public will be essential to confirm the results. According to this study, no significant statistical relationship was determined between LP and other variables.

Keywords: oral lichen planus, dermal lichen planus, association, concomitant

1. Introduction

The oral lichen planus is a cutaneous and mucosal disease that can affect the skin of the oral mucosa and other mucous membranes. The etiology of (lichen planus) LP, an inflammatory disease, is unknown. Still, it has been attributed to an immune response (often related to CD_4^+ lymphocyte cells) to keratocystic antigens (1), which involves an IV hypersensitivity reaction to a variety of antigens in the mucosa and skin lining (2).

In 1866, Erasmus Wilson, an English physician, described the pathology of the lesion. He suggested that stress could be the cause of the lesion (3). Lowiswickhem was then able to describe the lesion as the Lichen planus. Then, the grey lines and spots on the lesion were called Wickham striae (1, 2).

OLP usually is present in the mouth for years and has periods of healing or deterioration. In the worsening phase of the lesion, pain, erythema, or ulcer areas are seen in the lesion. Patients feel relief after intense itching of the lesions. Trauma may exacerbate a disease known as the Koebner phenomenon. Other factors that can cause it to include mechanical trauma caused by dental processes, irritation and friction caused by sharp objects, rough dental restorations, heat and temperature stimulation by smoking, and oral habits such as chewing gum (1).

Clinical manifestations can range from painless keratotic lesions to painful wounds. The most commonly involved lesion site is the buccal mucosa, where the lesions appear bilaterally. According to Andreasen's division, lichen planus lesions appear in six different views:

1-Reticular, 2-Papular, 3-Plaque-like, 4-Erosive, 5-Atrophic, 6-Bullous (1).

Different types of dermal LP include hypertrophic lichen planus (LP), follicular LP, linear LP, actinic LP, pigmented LP, annular (ring-like) LP, atrophic LP, guttate LP, acute and subacute LP, and LP on hands and feet (4).

The demographic and clinical data of patients with oral

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lichen planus were not evaluated in this geographical area. Many discussions are found on the tendency of the disease to become malignant. In this regard, the present study aimed to assess patients' demographic and clinical characteristics with oral lichen planus.

2. Materials and Methods

A descriptive cross-sectional study was designed to evaluate the demographic and clinical characteristics of lichen planus patients referred to Zahedan Dental School and Dermatology Clinic. The study is approved by ethics committee of Zahedan University of Medical Sciences (IR.ZAUMS.REC.1398.370, 2019.11.17).

In this regard, a form consisting of personal information such as age, sex, and types of LP (oral or dermal) was designed based on the clinical examinations, histopathology examination, and histopathologic manifestation of OLP. OLP is diagnosed through clinical findings as well as pathological appearance. Clinical signs of OLP are including 2 sections: red and white; the different surface features of the lesion contribute to the classification of OLP. The white and red components of the lesion may have the following surface features: network (reticular), plaque-like, papules, erythematous, bullous, and wounded. To differentiate the OLP, the reticular and papules components should be existing. The histopathologic features of OLP are containing the zones of hyperparakeratosis or hyperorthokeratosis characteristically with a thickening of the granular cell layer besides a saw-toothed appearance to the rete pegs; liquefaction degeneration or the basal cell layer necrosis; as well as an eosinophilic band that is below the basement membrane which is named eosinophilic coagulum. The patients were diagnosed according to the distinctive clinical and histological characteristics via a dental specialist. Therefore, the routine assessment of these cases is not useful in OLP diagnosis.

In papule biopsy, irregular acanthosis of the epidermis with compressed hypergranulosis is seen in the center. Besides, the granularized layer of cell infiltration thickens, which corresponds to the appearance of Wickham striae (2).

Reticular Oral Lichen Planus does not need any pathological examination; thus, patients with oral lichen planus do not undergo reticular biopsy. Further, the pathological diagnosis of dermal lichen planus was done in some cases with no clinical manifestation of typical lesions [EE3], then completed after taking a history, required examination, and checklist. The lesion site, the lesions' clinical form, the lesions' duration, and the patient's symptoms at the time of diagnosis are classified based on the table of variables; then, the extracted information is recorded in a checklist designed for this purpose.

The data were described after reviewing their quality in SPSS 24. For this purpose, tables of frequency distribution and statistical graphs such as bar graphs, box pie charts, and histograms were applied. Distance estimates for the proportion of lesions in each subgroup were also reported. The Kappa coefficient was used to evaluate the association between lesions; α equaled 5% in all analyses.

3. Results

The present study aimed to investigate the concomitant dermal lichen planus and oral lesions in patients referred to Zahedan University Centres during 2009-2010. So, 99 patients with dermal and oral lichen planus were included in the study, of which 60 patients had oral lichen planus, and 39 had dermal lichen planus. The value of the kappa coefficient was 0.35, which was statistically significant. Patients are divided into oral lichen planus (n=60, 60.6%) and dermal lichen planus groups (n=39, 39.4%). The mean age of patients with oral lichen planus was 84/8± 53/42; most patients with oral lichen planus were females (n=40, 66.7%).

Most patients with oral lichen planus had erosive lesions (n=49, 81.7%) (Table 1).

Table 1. The Frequency distribution of a clinical form of oral lesions in patients with oral lichen planus

The clinical form of lesions	Frequency	%
Erosive	49	81.7
Non-erosive	3	5.0
Concurrent erosive and non-erosive	8	13.3
Total patients	60	100

The mean duration of lesions in patients with oral lichen planus was 10.98±9.64 months (Table 2).

Table 2. The Frequency distribution of the presentation duration of lesions in a patient with oral lichen planus

Descriptive index	Mean	Standard deviation	Max.	Min.
Duration presence of lesion	10.98	9.64	36	1

The most common symptoms of patients with oral lichen planus were wounds (n=31, 51.7%) (Table 3).

Table 3. The frequency distribution of patients' symptoms at the diagnosis of a patient with oral lichen planus

Patients' symptoms	Frequency	%
Ulcer	31	51.7
Burning	6	10
Concomitant ulcer and burning	23	38.3
Total patient	60	100

The highest involvement of patients with oral lichen planus was related to cheek mucosal involvement [n=40 patients (66.7%)] (Table 4).

Table 4. The Frequency distribution location of oral lesions in a patient with oral lichen planus

Location of oral lesions	Frequency	%
Buccal mucosa	40	66.7
Dorsal of the tongue	7	11.7
Lateral of the tongue	13	21.7
Total patient	60	100

The mean age of dermal lichen planus involvement patients was 39±14.16 years (Table 5).

Table 5. The frequency of age distribution in a patient with Dermal lichen planus

Descriptive index	Mean	Standard deviation	Max.	Min.
Age	39.36±39	14.16	80	22

Most of the patients with dermal lichen planus were females (n= 33, 84.6%) (Table 6).

Table 6. The frequency of sex distribution in a patient with dermal lichen planus

Sex of patients	Frequency	%
Female	33	84.6
Male	6	15.4
Total patients	39	100

Based on the clinical form of lesion of patients with dermal lichen planus, the highest number of involvements was in patients with papules (n=16, 41%) (Table 7).

Table 7. The frequency of clinical forms of a skin lesion in a patient with dermal lichen planus

Clinical forms of skin lesions	Frequency	%
Papule	16	41
Plaque	10	25.6
Hyperpigmentation	1	2.6
Simultaneous conflict	12	30.8
Total patients	39	100

The mean period of lesions in patients with skin lichen planus was 23 ± 58.55 months.

The symptom of most patients with skin lichen planus involvement was itching (n=37, 94.9) (Table 8).

Table 8. The frequency distribution of patients' symptoms at the diagnosis of a patient with dermal lichen planus

Patients Symptoms	Frequency	%
Itching	37	94.9
Hair Loss	1	2.6
Itching and Hair Loss	1	2.6
Total patient	39	100

Most patients with dermal lichen planus had comorbidity lesions (n=22, 56.4%) (Table 9).

Table 9. The frequency distribution location of oral lesions in a patient with dermal lichen planus

Location of oral lesions	Frequency	%
Hand	2	5.1
Foot	13	33.3
Neck	2	5.1
Simultaneous conflict	22	56.4
Total patient	39	100

The kappa coefficient was equal to 0.35, which was statistically significant (P <0.001) (Table 10).

Table 10. Determine the amount of association between dermal and oral lichen planus

Type of lichen planus	Frequency	%
Oral lichen planus	60	60.6
Dermal lichen planus	39	39.3
Oral lichen planus and dermal lichen planus	38	38.3
Total patient	99	100

4. Discussion

LP is a chronic dermal mucosal disease with unknown etiology. The mean age of patients with this lesion is about 55 years, and its prevalence is higher in women (5). However, oral LP is white, bilateral, and sometimes ulcerative lesions with various clinical forms, including reticular, papular, plaque-like, bullous, erosive, and ulcerative (6).

Oral Lichen planus lesions take two forms: reticular and erosive. The reticular type is more common and asymptomatic. Malignant changes are a more common erosive type, especially if the lesion is on the tongue, palate, and floor of the mouth (7). Due to malignancy and the risks of these changes, the oral Lichen planus becomes important in oral diseases (8).

The study on the frequency and epidemiological changes of oral lichen planus lesion in Iran has been minimal, including the study of Pakfetrat et al. (9) in Mashhad (2008), in which the mean age of patients was 16.41 years, and 9 / 64% of them were female. Further, in 2.85% of patients, the cheeks were the most commonly involved area. The study of Esmaili et al. (10) in Tehran (2003) showed that oral LP is more common in men in their fourth decade. Furthermore, the study of Khalili et al. reported that the age of incidence of the lesion was 42 years, in the range of 5-83 years.

The clinical characteristics of patients in current study demonstrate many similarities and dissimilarities with previously reported studies. We detected that the males outnumbered the females (ratio M: F=1.61:1), which disagree with many other studies (11-13). In most studies, female predominance is reported. OLP is more predominant in the third decade of life according to this study (mean age of 36.9 years), which is lesser than the mean age stated in the central China [50.4 years], the Spain [56.4 years], the United Kingdom [52.0 years], and the Italy [56.7 years] (13-16). This was perhaps because of our cohorts' ethnic population as well as the geographic difference in comparison to the preceding reports. OLP in minor juveniles or children is rare, and in this study childhood form of the OLP was not detected. Though childhood OLP is very infrequent, early recognition is vital to make suitable treatment and relieve signs in little children (17, 18).

LP is a relatively common disorder that affects about 0.5-2% of the population. Besides the higher prevalence of OLP than the dermal type, it is more resistant to treatment.

In the present study, the demographic pattern and clinical profiles of patients with OLP were recorded. In this study, the incidence of OLP was higher in women than men, which is consistent with the results of some researchers [DroreEisen (12), Silverman (3), Bermejo-Fenoll (16), Mankapur (2), and Mozaffari (19)] but inconsistent with the Munde's (20) study on India's rural populations. According to the results of this study and most similar articles, OLP is more common in females. The inconsistency of Munde's study results (20) may

be due to the clinical features and lifestyle prevailing in that area.

Accordingly, the mean age of people with OLP was 42.53.

It was consistent with Mozaffari's study (19), in which the mean age of patients was 42.13, and was closely correlated with Mankapure's study (2), in which the mean age of patients was 45.4.

Based on the present study results and similar articles, we conclude that OLP occurs in the 5th and 6th decades of human life.

Based on the results of this study and all similar papers, the buccal mucosa is the most common site of OLP lesions, followed by the prevalence of OLP in the gums and tongue with a much lower percentage. On the other hand, relating to the clinical form of OLP lesions, it can be stated that in the present study, similar to that of Bermejo-Fenoll's (16), the erosive form of lesions with 49% OLP has the highest clinical form. This difference between the results of both studies is due to the lifestyle of patients living in Zahedan.

Patients with OLP have a variety of symptoms. Based on the present study results, the ulcer is one of the most common symptoms in 51.7% of patients, which is consistent with Mankapure's study (2).

Regarding the duration of OLP lesions, patients reported that lesions stay for 10.98. 9.64 months.

Among 39 patients with dermal lichen planus, 33 (846.3%) were female, indicating a higher disease prevalence in women, which is consistent with Boyd's theory that women are more likely to develop lichen planus than men (21).

In this study, the mean age of patients was 39± 14.16 years, consistent with that of Black's study on 200 patients with lichen planus, with a mean age of 4 decades (22).

The most common involved site reported in the present study was concomitant involvement of the hands and feet, accounting for 56.4% of all patients, and consistent with Black's study stated that the anterior wrist, lumbar region, and around the ankle (22) are the most commonly involved sites.

The most common clinical form of the present study was the papular pattern (41%) which was observed on the face, neck, and hands, confirming the ROOK theory stating that the maximum prevalence of actinic lichen planus is found in the Middle East, East Africa and India (23). In the present study, six men (1.1%) had genital zone involvement, lower than the 25% reported in Arndt's study (24).

It may be due to the small sample size of the present study or the impossibility of a full-body examination due to cultural problems. But the Annular (ring-like) lichen planus is found in only ten patients (2.9%), which is less than the 10% mentioned in the sources (5).

According to Boyd, 38 (38.3%) patients had concomitant dermal and oral involvement.

Merely about three patients developed an oral carcinoma and from them 2 were smokers and the other one was smok¬ers and drinker, which is lower than some other reports from malignant transformation rates (25, 26). Studies of the malignant potential of oral lichen planus (OLP) have been hampered by contradictions in the diagnostic criteria used for OLP (27), nevertheless, since most patients will have long-standing OLP, and possibly a risk of malignant transformation, it is crucial that such patients be wisely monitored through a well-experienced clinician in long term (28-30).

oral lichen planus is prone to the risk of malignancy (0.4-4.3%), and WHO considers it a pre-malignant lesion, it is essential to consider the follow-up of patients, the concomitant dermal and oral involvement and timely referral of patients with oral lesions to a dermatologist and those with dermal lesions to an oral and maxillofacial specialist.

There was concomitant dermal and oral lichen planus in patients referred to Zahedan University Centres. It is recommended to conduct a study with a larger sample size considering different age groups and sex in various types of mucosal LP and referral of patients with oral lesions to dermatologists and those with dermal lesions to oral and maxillofacial specialists.

Ethical statement

The study is approved by ethics committee of Zahedan University of Medical Sciences (IR.ZAUMS.REC.1398.370, 2019.11.17).

Conflict of interest

None to declare.

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Authors' contributions

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

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Efficacy of kinesio taping versus extracorporeal shock wave therapy in the short term for the calcaneal spur

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Abstract

The calcaneal spur is a common cause of heel pain, and there are various approaches to its treatment. Our aim in this study is to investigate the short-term effectiveness of kinesio taping against Extracorporeal Shock Wave Therapy (ESWT) in the treatment of calcaneal spur. This study was designed as an experimental, prospective and single-center clinical trial. A total of 93 patients with calcaneal spur (18 men, 75 women) were included. Patients were divided into two groups (based on gender, age, BMI, and pain duration) by stratified randomization (kinesio tape group=46, ESWT group=47 patients). 3 sessions of ESWT were applied to the heels of the patients in the 1st group with an interval of 1 week. Kinesio taping was applied to the 2nd group. It was applied once a week, for 3 weeks. Visual Analogue Scale (VAS) and Foot Function Index (FFI) scores of the patients in both groups before the treatment and 1 week after the end of the treatment were recorded. When we evaluated the pre-treatment and post-treatment parameters, we found a significant decrease in VAS, FFI-pain, FFI-disability and FFI-activity restriction scores in both groups (p=0.0001). We also found that kinesio taping was more effective than ESWT in reducing FFI-pain (for after treatment FFI-pain inter group p=0.005, for FFI-pain difference inter group p=0.002) and FFI-disability (for after treatment FFI-disability inter group p=0.023, for FFI-disability difference inter group p=0.0001) scores. To conclude, although both kinesio taping and ESWT treatments reduced patients' pain, disability and activity limitation, kinesio taping was found to be more effective in reducing pain and disability in the short term. Studies with longer follow-up are needed to clarify our results.

Keywords: calcaneal spur, extracorporeal shock wave therapy, kinesio tape, heel pain

1. Introduction

Plantar heel pain is one of the most common feet pains in adults (1). Heel pain has many causes, one of which is a calcaneal spur (2). Studies have shown that the rate of calcaneal spur in the cause of heel pain was found between 30 and 89% (3). Clinical symptoms are inflammation, swelling of the feet, pain and limitations of movement. Treatment options are; nonsteroidal anti-inflammatory drugs, local steroid injections, stretching exercises, acupuncture, iontophoresis, extracorporeal shock wave therapy (ESWT), low-intensity laser therapy, silicone heel pad, medial arc support, night splint, physical therapy modalities, kinesio taping, cold application and rest (4).

Kinesio taping acts by supporting damaged joints and muscles, lifting the skin, increasing lymph and blood circulation, accelerating the removal of inflammatory mediators from the damaged area, and reducing clinical pain. In a recent study investigating efficacy of kinesio taping for plantar fasciitis, Tezel et al. (5) showed that both kinesiotaping and ESWT treatments improved pain levels and quality of life in patients with plantar fasciitis, but kinesio taping also improved functionality. In another study Ercan et al. (6) evaluated the effect of kinesio tape application in addition to extracorporeal shock wave therapy for plantar fasciitis. It was stated that extracorporeal shock wave therapy reduced the pain scores of plantar fasciitis patients in three weeks, but additional kinesio tape application to extracorporeal shock wave therapy did not provide any additional benefit. In the literature, we could not find any studies investigating the effectiveness of kinesio taping in the treatment of calcaneal spur.

Our aim in this study is to investigate the effectiveness of kinesio taping against ESWT, which is a frequently used method in the treatment of calcaneal spur, and to discuss our results in the light of the literature. Our study will be the first study in the literature with this aspect.

2. Materials and Methods

We designed our study as an experimental, prospective and single-center clinical study. Local ethics committee approval was obtained. It has been determined that the effect size obtained in the reference study is strong (d=0.95) (5). As a

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result of the power analysis, it was calculated that if at least 90 people were included in the study, 80% power could be obtained at the 95% confidence level. Thus, a total of 93 patients (46 patients in kinesio tape group, 47 patients in ESWT group) were included in the study. This study was approved by Pamukkale University Non-Interventional Clinical Research Ethics Committee (Date: 13/01/2022, Approval number: E.155512). Participants were informed about the study, and their written informed consent was obtained. The protocol was performed per the ethical standards in the 1964 Helsinki declaration.

2.1. Participants

Patients with a diagnosis of calcaneal spur who applied to the Physical Medicine and Rehabilitation outpatient clinic of Denizli State Hospital between February 2022 and July 2022 were included in the study. Inclusion criteria: to be 18-70 years old, heel pain persisting for at least 4 weeks, presence of radiologically detected calcaneal spur, medical treatment, cold application and stretching exercises have been applied but have not benefited. Exclusion criteria: history of acute trauma to the foot, history of injection in the heel (steroid, PRP (platellet rich plasma), etc.), history of foot surgery, inflammatory rheumatic diseases, neurological diseases (polyneuropathy, radiculopathy, etc.), pregnancy, malignancy.

2.2. Method

Age, gender, body mass index, and duration of heel pain of the patients included in the study were noted. The patients were allocated to both groups by stratified randomization (according to gender, age, BMI, and duration of pain). In terms of variables, our groups were as follows;

1- female, male,

2- age (18-45 years, 46-65 years, 66-70 years),

3- BMI (<18.5, 18.5-24.9, 25-30, >30 kg/m2),

4- duration of pain (4-12 weeks, >12 weeks).

Patients were requested not to take any analgesics and/or NSAIDs during the course of the study. 3 sessions of ESWT were applied to the heels of the patients in the 1st group with an interval of 1 week (with Chattanooga Intelect RPW device, 10 Hz, 3.2 bar, 2000 beats to the heel). Kinesio taping was applied to the 2nd group (Tmax Kinesiology Tape cotton, standard 5.0 cm tape). It was applied once a week, for 3 weeks. During the taping, the patient was in a prone position with the knee joints at 90° of flexion and the ankle joints at a neutral position. The tape was cut longitudinally into four slices of equal width. It was applied to the forefoot by stretching it by 25% (7). Visual Analogue Scale (VAS) and Foot Function Index (FFI) scores of the patients in both groups before the treatment and 1 week after the end of the treatment were recorded (8, 9). Foot function index consists of 23 items with 3 subgroups; pain, disability, and limitation of activity. Nine item-containing pain subscale measures the level of foot pain in a variety of situations. With the disability subscale containing 9 items, the foot depending on the problems, the degree of difficulty in performing various functional activities is determined. Containing five items with activity limitation subscale; due to foot problems activity limitations are evaluated.

2.3. Statistical analysis

All statistical analyses were performed using SPSS 25.0 (IBM SPSS Statistics 25 software (Armonk, NY: IBM Corp.)) software. Continuous variables were defined by the mean ± standard deviation, median, interquartile range (25.-75. percentiles), minimum- maximum values and categorical variables were defined by number and percent. In examining the differences between the groups, when the parametric test assumptions were met, Independent samples t test was used. When the parametric test assumptions were not met, Mann Whitney U test was used. In examining dependent group differences, when the parametric test assumptions were met, Paired samples t test was used. When the parametric test assumptions were not met, Wilcoxon Signed Rank test was used. Differences between categorical variables were analyzed by Chi-square analysis. Statistical significance was determined as $p<0.05$.

3. Results

A total of 93 patients (18 men, 75 women) were included in this study. The mean age of the patients was 47.48 years (min 23- max 70). The mean body mass index (BMI) was 30.77 kg/cm2. The number of patients with pain duration between 4-12 weeks was 43, and the number of patients with pain longer than 12 weeks was 50.

Table 1 shows that there is no statistically significant difference between the two groups in terms of age, BMI, gender and pain duration.

Table 1. Demographic characteristics of groups

		Kinesio tape (n=46)			ESWT (n=47)			Inter group p
		Mean±S.D	Med (IQR)	Min.-Max.	Mean±S.D	Med (IQR)	Min.-Max.	
Age		48.67±10	48.5(42.75-56.25)	26-69	46.32±8.96	46 (40- 53)	23- 70	0.235 (t=1.196)
BMI		31.03±4.42	30 (27.7-34.03)	24.6-44	30.5±4.14	30 (27.6- 32.8)	21- 43.8	0.721 (z=-0.358)
Gender (n%)	**Male**	6 (13%)			12 (25.5%)			0.127 (cs=2.323)
	Female	40 (87%)			35 (74.5%)			
Duration (n%)	**>12 weeks**	26 (56.5%)			24 (51.1%)			0.598 (cs=0.279)
	4-12 weeks	20 (43.5%)			23 (48.9%)			

ESWT: Extracorporeal shock wave therapy, BMI: Body mass index, S.D: Standard deviation, Med (IQR): Median (25.–75. Percentiles); t: Independent samples t test; cs: Chi Square test

When we evaluated the pre-treatment and post-treatment parameters, we found a significant decrease in VAS, FFI-pain, FFI-disability and FFI-activity restriction scores in both groups (p=0.0001). We also found that kinesio taping was more effective than ESWT in reducing FFI-pain (inter group p for after treatment FFI-pain=0.005, for FFI-pain difference=0.002) and FFI-disability (inter group p for after treatment FFI-disability=0.023, for FFI-disability difference=0.0001) scores. The results of the evaluation parameters of the groups before and after the treatment and the comparison of both groups are shown in Table 2.

Table 2. The results of the evaluation parameters of the groups before and after the treatment and the comparison of both groups

	Kinesio tape (n=46)		ESWT (n=47)		
	Mean±S.D	**Min.-Max.**	**Mean±S.D**	**Min.-Max.**	**Inter group p**
b.t. VAS	8.09±1.43	4-10	7.77±1.73	3-10	0.346 (z=-0.943)
a.t. VAS	4.91±1.36	1-7	5.47±1.38	1-8	0.054 (z=-1.929)
Intra Group p		**0.0001*** (z=-5.908)		**0.0001*** (z=-5.712)	
VAS difference	3.17±1.39	-1-6	2.3±1.55	-1-5	**0.004*** (z=-2.858)
b.t. FFI1	70.34±11.16	47.1-92.8	68.88±16.08	32.8-100	0.612 (t=0.509)
a.t. FFI1	45.02±10.99	21.4-67.1	52.74±4.38	21.4-82.2	**0.005*** (t=-2.904)
Intra Group p		**0.0001*** (t=13.896)		**0.0001*** (t=7.104)	
FFI1 difference	25.32±12.36	-8.6-50	16.14±15.58	-10.9-51.4	**0.002*** (t=3.143)
b.t. FFI2	67.75±15.26	22.9- 90	63.03±16.51	18.8-91.1	0.124 (z=-1.538)
a.t. FFI2	43.4±12.93	12.2- 76.6	50.24±15.39	8.8-78.8	**0.023*** (t=-2.318)
Intra Group p		**0.0001*** (z=-5.512)		**0.0001*** (t=6.744)	
FFI2 difference	24.34±15.55	-26.7-50	12.79±13	-20- 45.6	**0.0001*** (z=-4.005)
b.t. FFI3	38.66±10.05	23.3-66.6	32.52±14.04	3.3-66.6	**0.014*** (z=-2.464)
a.t. FFI3	23±11.47	10-73.3	24.79±9.45	3.3-46.6	0.14 (z=-1.477)
Intra Group p		**0.0001*** (t=11.157)		**0.0001*** (z=-4.515)	
FFI3 difference	15.65±9.52	-13.3- 36.7	7.73±9.58	-6.7-33.3	**0.0001*** (z=-4.02)

*p<0.05 statistically significant; For Inter group p t: Independent samples t test; z: Mann Whitney U test; For Intra group p t: Paired samples t test; z: Wilcoxon Signed Rank test, ESWT: Extracorporeal shock wave therapy, b.t.: before treatment, a.t.: after treatment, VAS:visual analogue scale, FFI: foot function index, FFI1: pain FFI2: disability, FFI3: limitation of activity

4. Discussion

In this study, we investigated the short-term effectiveness of kinesio taping against ESWT in the treatment of calcaneal spur and found that there was a statistically significant reduction in pain, disability and activity limitation with both treatment methods. In addition, we found that kinesio taping was more effective than ESWT in reducing pain and disability in the short term.

On the other hand, 75 (80.6 %) of the patients included in the study were women and the mean BMI of the patients was 30.77 kg/cm2. There are also studies in the literature showing that female gender and obesity are risk factors in the formation of calcaneal spurs (10). Our results also support the literature on this subject.

In the treatment of calcaneal spur, ESWT has been used for a long time and its effectiveness has been shown (11–15). Although it has some mild side effects (pain, minor hematoma, vs.), it is generally a safe and beneficial treatment option (16). Therefore, it is often compared to ESWT when a new treatment option is explored (5, 17). The short-term effectiveness of kinesio taping against ESWT has been demonstrated in our study, which will make it more preferable as a treatment option. When we offer ESWT as an option in the treatment of calcaneal spur, we find that most patients have concerns that the procedure may be painful. In this respect, kinesio taping treatment comes to the fore a little more. Because there is no pain during or after the procedure.

Kinesio taping is a taping method that has been used in sports injuries and musculoskeletal pain since the 1970s. Kuyucu et al. (18) investigated the effect of kinesiotaping in calcaneal apophysitis seen in male athletes and found that it was effective in restoring foot function but had a limited effect in reducing pain. A meta-analysis of 84 studies concluded that available evidence does not support the use of Kinesio taping applied to the ankle for improvements in functional performance, regardless of population (19). In another study, it was stated that the use of kinesio taping as an alternative to medical treatment or in combination with medical treatment would be beneficial (20). We see that there are opposite views in the literature about kinesio taping. A few studies have emphasized the need for high-quality, placebo-group studies (21, 22).

In this study, we came to a conclusion by evaluating the scales of the patients before and 1 week after the treatment. In this way, we have seen that kinesio taping is effective in a short time. However, we believe that the results may be different in longer follow-ups. The fact that our total follow-up period was 4 weeks can be considered as a limitation of the study. We think that studies with longer follow-up periods are needed to clarify our results. Another limitation of our study is the absence of a control group.

In conclusion, our study is the first to investigate the effectiveness of kinesio taping in the treatment of calcaneal spur, and our results will contribute to the literature. Kinesio

tape is a good and effective treatment option in the treatment of calcaneal spur due to its ease of application, cost effectiveness and tolerability, and the absence of side effects such as pain or hematoma.

Ethical statement

This study was approved by Pamukkale University Non-Interventional Clinical Research Ethics Committee (Date: 13/01/2022, Approval number: E.155512).

Conflict of interest

None to declare.

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Authors' contributions

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

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Research Article

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Lumbosacral transitional vertebrae: An overlooked cause of back pain on MRI

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Abstract

To evaluate the frequency of lumbosacral transitional vertebrae (LSTV) on MRI in patients with backpain and clinically suspected of sacroiliitis. Sacroiliac MRI of patients who had backpain and were clinically suspicious for sacroiliitis between November 2021-March 2022 were retrospectively analyzed from the hospital database by two different radiologists. LSTV cases were identified and subgrouped according to Castellvi classification. Presence of sacroiliitis, degeneration and /or herniation of cranial segment intervertebral disc, facet joint hypertrophy, coxarthrosis and psoas atrophy were recorded. In cases where radiologists were in conflict, consensus was made. Between November 2021-March 2022, 614 sacroiliac MRIs were obtained and 81 (13%) had LSTV. Fifty-nine patients were female (72.8%). Mean age was 43.4. The most common identified LSTV was type 1a (n=30, 10 right-sided, 20 left-sided). Sacroiliitis was significantly more common in younger patients (p=0.04) and in males (p=0.009). Disc degeneration, disc herniation, facet joint hypertrophy and psoas atrophy increased significantly with age (p=0.007, p=0.001, p=0.002 and p=0.013 respectively). No correlation was found between gender or presence of sacroiliitis and any type of LSTV. LSTV may present with backpain and should be considered in patients where sacroiliitis is clinically suspected. MRI is a useful tool to identify other accompanying pathologies in these cases.

Keywords: back pain; sacroiliitis; lumbosacral transitional vertebra; disc degeneration; spine transitional anomalies

1. Introduction

Lumbosacral transitional vertebra (LSTV) is a common congenital anatomical variant and its prevalence varies 7 to 32% in the literature (1-4). In these cases, the transverse process of the last lumbar vertebrae is somewhat elongated and forms a connection with sacrum which may manifest on a wide spectrum from an isolated elongated transverse process to complete fusion with the sacrum. According to its relation with the sacrum, LSTV can be classified in to four types by the Castellvi classification (1). Type 1 indicates an enlarged transvers process (>19 mm) of L5. Type 2 refers to pseudo-articulation of the enlarged transvers process with sacral ala whereas Type 3 involves complete fusion of the transverse process and the ala. Type 4 are those with Type 2 on one side and Type 3 on the other side. Moreover, “a” is added for unilateral involvement and “b” is added for bilateral involvement in this classification. Additionally in 1996, O’Driscoll et al proposed another classification based on the S1 lumbarization and S1-S2 intervertebral disc morphology (5).

Lumbosacral transitional vertebrae-related back pain was first introduced in 1917 by Bertolotti(6) who attributed the pain to the arthritis at the pseudo-articulation. In these patients the pain and the limited range of motion becomes more prominent with ipsilateral bending and rotation. It’s been shown in the literature that LSTV is a protective factor for disc degeneration at the level of transition, whereas it causes increased load at the cephalad disc, making it vulnerable for degeneration (7-9). Early and more advanced disc degeneration has been reported in LSTV, particularly in young patients (2, 7, 10). Additionally, LSTV prevalence was found to be higher in patients with lumbar disc herniation compared to control group (11).

In this study we aimed to evaluate the presence of LSTV in patients who underwent MRI with a pre-diagnosis of sacroiliitis.

2. Materials and Methods

2.1. Patient selection

Sacroiliac MRIs that were obtained with a sacroiliitis pre-diagnosis between November 2021-March 2022 were retrospectively analyzed from the hospital picture archiving and communication system (PACS). The study protocol was approved by the Kayseri State Hospital Ethics Committee (21.02.2023/803). Patients with major surgery or fracture history of lumbar, hip or pelvic area were excluded. Images were evaluated by two radiologists. Patients with LSTV were recorded and grouped according to Castellvi classification.

2.2. Magnetic resonance imaging and radiological analysis

Sacroiliac MRI was performed by a 1,5 T MRI system (Magnetom Aera, Siemens, Erlangen). The MRI protocol

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included a coronal T1-weighted and T2-weighted fast spin-echo (FSE) sequence, a coronal short-tau inversion recovery sequence, and an axial T2-weighted fat-saturated FSE sequences. Intervertebral discs cephalad and at the level of transitional level were evaluated using the Pfirrmann and Modic classifications (12, 13). Presence of sacroiliitis, herniation of intervertebral discs, facet joint hypertrophy, coxarthrosis and psoas atrophy were recorded. In cases where radiologists were in conflict, consensus was made.

2.3. Statistical analysis

SPSS 22 statistical package program was used for statistical analysis. Study characteristics are stated as means, frequencies or ratios.

3. Results

Of 614 sacroiliac MRIs, 81 (13%) had LSTV. Fifty-nine patients were female (72.8%). Mean age was 43.4. Thirty patients had Type 1a (10 right-sided, 20 left-sided); 13 patients had Type 1b (Fig.1); 27 patients had Type 2a (10 right-sided, 17 left-sided) (Fig. 2); 17 patients had Type 2b; 5 patients had Type 3a (4 right-sided, 1 left sided) (Fig. 3), 1 patient had Type 3b and 3 patients had Type 4.

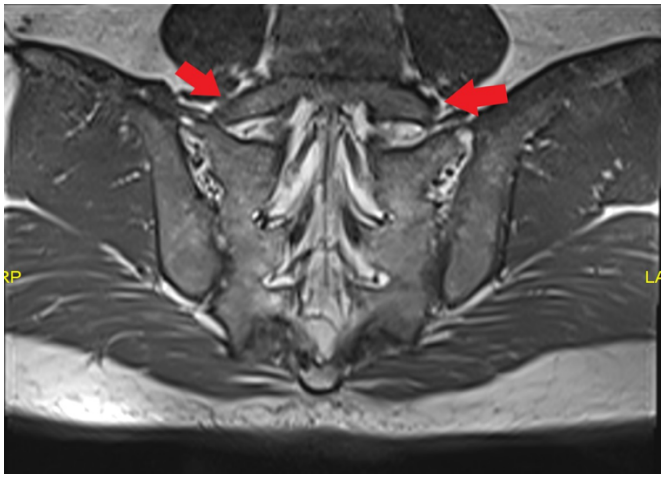

Fig. 1. Coronal T2- weighted MRI image shows bilateral enlarged transverse processes (Type 1b)

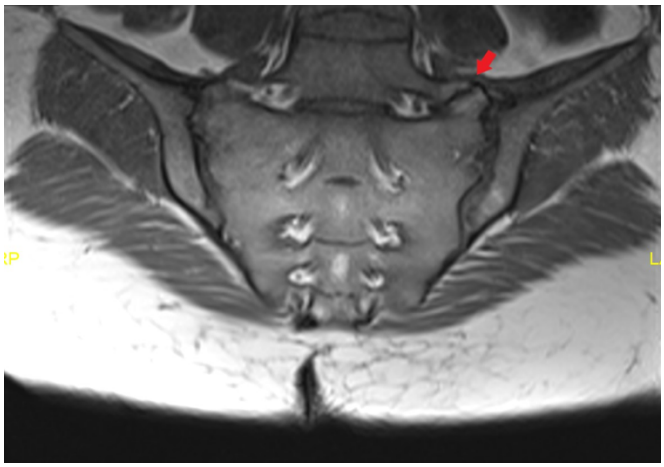

Fig. 2. Pseudo-articulation of left transvers process of L5 and sacral ala is seen on coronal T2- weighted image (Type 2a). Increased intensity is noted adjacent to the pseudo-articulation, suggesting inflammation

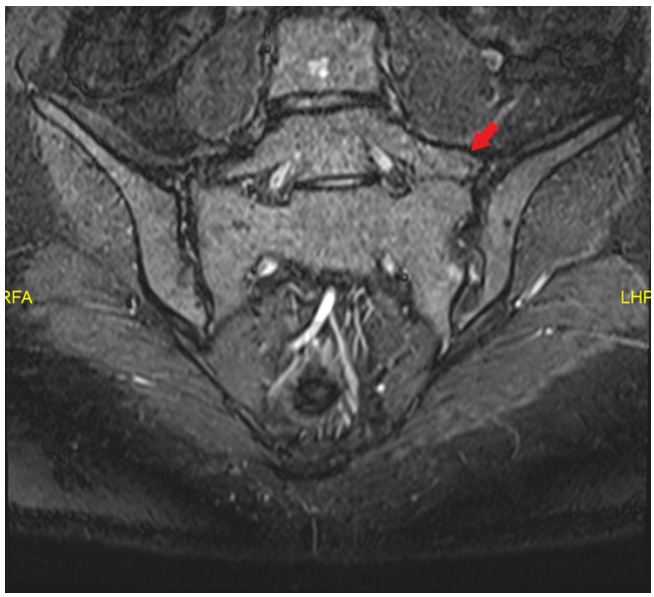

Fig. 3. Left transvers process of L5 is totally fused with sacrum on this coronal STIR image (Type 3a)

Concomitant sacroiliitis was present in 11 cases (13.5%), in 10 cases sacroiliitis was bilateral and in one case only right side was involved. Degeneration was noted in the cephalad intervertebral disc in 42 patients (52%), accompanied by disc herniation in 27 patients (33.3%). Disc was spared at the level of transition 40 of the 42 cases with cephalad disc degeneration. Facet joint hypertrophy was present in 11 patients (13.6%), coxarthrosis in two patients and psoas atrophy in 3 patients.

Sacroiliitis was significantly more frequent in younger ($p=0.04$) and in male patients ($p=0.009$). Presence of sacroiliitis was not significantly correlated with any LSTV types. Rates of disc degeneration, disc herniation, facet joint hypertrophy and psoas atrophy increased significantly with age ($p=0.007$, $p=0.001$, $p=0.002$ and $p=0.13$, respectively). LSTV type was also not correlated with gender or disco-vertebral pathologies.

4. Discussion

Lumbosacral transition is a relatively common pathology but mostly overlooked in the management of back pain. Even though x-ray is the first line for imaging in the evaluation of back pain, MRI has become increasingly more available particularly for identifying active inflammation in the sacroiliac joints and detailed visualization of disc pathologies. Reported LSTV prevalence varies among the imaging modalities used. Standard AP x-rays have 76-84% accuracy for diagnosis and 53-58% accuracy for classification of LSTV (14). Previous reports prevail a prevalence 7 to 32% with male dominance (%28.1 vs %11.1) (1-4). However, in our study, 13 percent of our patients had LSTV and majority of the cases were female. We attribute this difference to our small sample size, that our study only includes the symptomatic patients, and the more common hospital visits by the female.

Cadaveric studies indicated that SI joint width was

correlated with sacral height and sacrum AP dimension; and suggested that LSTV development may be related to load bearing capacity of normal sacrum at very early periods. When the sacrum is shorter, the SI joint surface also is smaller resulting in insufficient transfer of load to pelvis. Therefore, in these patients with small sacra with a narrow SI joint surface, sacrum may fuse with L5 to increase its load bearing capacity (15). The discrepancy of load bearing is more prominent in one-sided cases (8).

Due to the disruption of the pelvic alignment and scoliosis, degenerative changes at the intervertebral disc and the facet joint cephalad to the transition level may be seen (16). Presence of LSTV has been shown to have a protective effect on the intervertebral disc at the level of transition (7, 9, 16, 17). An increase in this protective effect was noted as the osseous bridge is more prominent (9). When compared with control group, degenerative changes were more severe and developed at a younger age (2, 7, 10). In our study disc degeneration was noted in half of the patients, and of these cases only three showed degeneration at the level of the transition. These three patients were older patients with accompanying other degenerative changes in lumber spine and hip. Other than those, disc at the level of transition was spared. Our results showed an increase of degenerative changes with age, which was expected.

In their study of 211 cases, Apazidis et al reported that Type 1a was the most common subtype, with a prevalence of 14.7% (18). However, Type 1a mostly has no clinical significance and requires no further evaluation (1). In another study prevalence of Type 1 and 2 was reported as 40% each, Type 3 was 11,5% and Type 4 was 5,25% (19). In our study Type 1 and 2 consisted of 53% and 54% of patients respectively. Type 3 was 7.5% and Type 4 was 3.7%.

Knowledge of LSTV presence has also utmost importance before surgery. In sacralized patients, pars interarticularis height and laminae width decreases, resulting in predisposition for spondylolysis and spondylolisthesis.

Pain related to LSTV still remains a controversial issue. Following the report of Bertolotti on LSTV-related back pain, some studies argued that degenerative changes at the pseudo-articulation was not related with the pain (20). On the other hand, scintigraphic activity was shown at the pseudo-articulation in symptomatic patients with no pathologies other than LSTV (21). Nerve compression by the osteophytes at the level of pseudo-articulation has been described (22). There are also case reports on compression of lumbar nerve between L5 transverse process and sacral ala, and extraforaminal nerve compression caudal to transition (23-25). In the literature, Bertolotti syndrome was found to be 4-8% in patients with back pain (4). Nardo noted that pain was more frequent in type 2 and 4 (19).

In conclusion, LSTV may manifest with backpain and should be kept in mind while evaluating MRI for back pain. MRI also enables identification other pathologies that may accompany this anatomical variant.

Ethical statement

The study protocol was approved by the Kayseri State Hospital Ethics Committee (21.02.2023/803).

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

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

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Research Article

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Evaluation of the effect of NO_2 levels on mortality in four key cities of Türkiye between 2017-2019

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Abstract

This study aimed to evaluate the NO_2 levels in four key cities of Türkiye (İstanbul, Ankara, İzmir, and Bursa) over three years and examine the effect of NO_2 on mortality and is a descriptive-ecological research. It required each city's air quality (NO_2), population and death data to cover the dates between January 1, 2017, and December 31, 2019. The study accepted 20 μg/m^3 as the annual NO_2 limit value. Numbers, percentages, mean, standard deviation, and minimum and maximum values were used in summarizing data. Calculations related to mortality attributed to NO_2-induced air pollution were made using the AirQ+ software's methodology. In 2019, 33 stations in İstanbul, four in Ankara, five in İzmir, and five in Bursa measured NO_2. In 2019, the weighted NO_2 mean was 40.61 μg/m^3 in İstanbul, 32.94 μg/m^3 in Ankara, 7.87 μg/m^3 in İzmir, and 31.68 μg/m^3 in Bursa. The estimated percentage of deaths attributed to NO_2 in 2019 was 7.95 in İstanbul, 5.07 in Ankara, and 4.58 in Bursa. No calculations were made for 2018 in Ankara and 2017, 2018, and 2019 in İzmir, as the NO_2 levels in these cities were less than 20 μg/m^3 in the years in question. The results revealed that the NO_2 levels were higher than the limit value in three of the four key cities in Türkiye and that the mortality rates attributed to NO_2 were higher compared to similar studies in the literature.

Keywords: air pollution, AirQ+, nitrogen oxide, NO_2, Türkiye

1. Introduction

Air pollution, accepted as one of the top ten risk factors threatening human health, is the most critical environmental factor threatening health (1, 2). Increasing air pollution levels can increase the burden of heart disease, stroke, lung cancer, and acute and chronic respiratory diseases. It is estimated that there were 4.2 million deaths in 2016 due to air pollution in rural and urban areas (1). 91% of the world population lives in places where the air quality limit values of the World Health Organization are exceeded (3).

According to the Global Burden of Disease study, 11.80% of all deaths and 8.40% of DALYs (Disability-adjusted life years) worldwide in 2019 are associated with air pollution (4). In the same study, air pollution in Türkiye increased by 3.5% between 2009 and 2019, making it the fifth most common cause of death and disease in 2019 (5). Reducing diseases and deaths due to air pollution, using clean energy sources in houses, and reducing the environmental burden of cities by improving air quality are among the United Nations Sustainable Development Goals (6, 7).

Nitrogen dioxide (NO_2) is one of the nitrogen oxides and a reactive gas that is an important cause of air pollution. NO2 is produced as a result of the combustion of fuels. The main source of this pollutant is exhaust emissions and power plants (8). High doses of NO2 can aggravate respiratory diseases and increase hospital admissions and emergency room visits (1, 8). It is known that long and short-term NO2 exposures cause mortality (9, 10). There are also research cohorts showing the association of NO2 with increased mortality (11, 12).

This research aimed to evaluate the NO_2 levels in the three years covering 2017-2019 and examine the effect of NO_2 on mortality in Türkiye's four most densely populated provinces (İstanbul, Ankara, İzmir, and Bursa).

2. Materials and Methods

2.1. Introduction of the Working Area

Türkiye is located in the European Region of the World Health Organization, has lands in Asia and Europe, and is in the upper-middle economic class (Fig. 1 and 2). The research was conducted in the four key cities of Türkiye. One-third of Türkiye's population lives in these four cities.

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Fig. 1. Türkiye's Location in the World

Fig. 2. The Four Key Cities of Türkiye (İstanbul, Ankara, İzmir, and Bursa) that are the subject of this research

2.2. Type of Research and Research Permits

This is a descriptive-ecological research. Ethics committee approval was obtained from Ankara University Health Sciences Ethics Committee (Number: 05-54, Date: 07.03.2022).

2.3. Research Data

This research was carried out between November 1, 2021, and June 15, .2022. This study required provincial air quality, population, and death data. The NO_2 levels, one of the air quality data, were obtained from the official website of the Turkish Republic of the Ministry of Environment and Urbanization, covering the dates between January 1, 2017, and December 31, 2019 (13), and the province-based population and death data for the years 2017, 2018 and 2019 were obtained from the Turkish Statistical Institute (14). The crude death rate per hundred thousand people was calculated by excluding those caused by poisoning and injury from the number of deaths in the relevant years. It is stated in the annual report of the Right to Clean Air Platform, created by the gathering of sixteen non-governmental organizations, that the levels of air pollutants, including NO_2, are not adequately measured throughout Türkiye (15). For this reason, the data of all air quality measurement stations that measure NO_2 value was used in the study.

2.4. Data Evaluation

The World Health Organization Regional Office for Europe has developed a software called AirQ+ to determine the effects of air quality on mortality and morbidity. AirQ+ is used to quantify air pollution parameters' effects on a specified population's health. The software is used to evaluate the impact of long- and short-term exposure to ambient air pollution and long-term exposure to household air pollution. All calculations performed with AirQ+ software are based on methodologies and concentration-response functions established by epidemiological studies. The baseline concentration-response functions are based on systematic review and meta-analysis studies (16).

For the data required by the AirQ+ software, in order to calculate the annual means of NO_2 levels, the NO_2 levels of the stations that made the NO_2 measurement in the relevant year were weighted according to the measurement frequency of the air quality station and divided by the number of stations. In addition, the population at risk in the relevant year and the crude death rate per hundred thousand people were entered the software. The relative risk value (1041, 95% CI 1019-1064) suggested by the AirQ+ software for NO_2 was used. In the study, the limit value of 20 μg/m^3, which is the annual limit value recommended in the 2013 'Health Risks of Air Pollution in Europe' (HRAPIE) project, was accepted as the NO_2 limit value (17). 10 μg/m^3, which is the current value of WHO as of September 2021, was not used as a limit value since it was determined after the date of the data within the study (1). The annual NO_2 limit value determined in Türkiye is 40 μg/m^3 (18).

Descriptive statistics from the analyzes related to the study were calculated using manual methods. Other statistics were performed in the computer environment using AirQ+ software. Numbers and percentages were used to summarize categorical data, while mean, standard deviation, and minimum and maximum values were used to summarize numerical data. Calculations related to mortality attributed to NO2-induced air pollution were made using the AirQ+ software's methodology.

3. Results

3.1. Population and Death

The population of İstanbul was 15.029.231 in 2017, 15.067.724 in 2018, and 15.519.267 in 2019. İstanbul's crude death rate per hundred thousand people was calculated as 387.744 in 2017, 398.819 in 2018, and 399.123 in 2019. Population, the number of deaths, and crude death rates per hundred thousand people in İstanbul, Ankara, İzmir, and Bursa by years are shown in Table 1.

Table 1. Population, Number of Deaths, and Crude Death Rates per Hundred Thousand Persons in İstanbul, Ankara, İzmir, and Bursa by Years

City	Year	Population	Total number of deaths	Number of deaths caused by poisoning and injury	Crude death rate per hundred thousand people
İstanbul	2017	15.029.231	59.921	1.646	387.744
	2018	15.067.724	62.547	2.454	398.819
	2019	15.519.267	63.371	1.430	399.123
Ankara	2017	5.445.026	25.413	1.111	446.316
	2018	5.503.985	26.115	1.189	452.872
	2019	5.639.076	26.405	956	451.297
İzmir	2017	4.279.677	25.756	960	579.390
	2018	4.320.519	26.471	1.101	587.198
	2019	4.367.251	27.567	1.030	607.636
Bursa	2017	2.936.803	16.309	714	531.020
	2018	2.994.521	16.636	742	530.769
	2019	3.056.120	16.697	652	525.012

3.2. NO_2 Measurements and Means

The number of stations measuring NO_2 in İstanbul was 12 in 2017 and 2018, and 33 in 2019. The weighted NO_2 mean in İstanbul was 41.39 in 2017, 34.81 μg/m^3 in 2018, and 40.61 μg/m^3 in 2019. For each city, the number of stations making measurements and the weighted NO_2 means calculated by the measurement percentages of the stations are presented in Table 2.

Table 2. Number of Stations Making Measurements in İstanbul, Ankara, İzmir, and Bursa by Years and Weighted NO_2 Means

City	Year	Number of stations	Weighted NO_2 mean (μg/m^3)
İstanbul	2017	12	41.39
	2018	12	34.81
	2019	33	40.61
Ankara	2017	4	40.88
	2018	4	15.90
	2019	4	32.94
İzmir	2017	3	13.64
	2018	10	3.74
	2019	5	7.87
Bursa	2017	5	45.28
	2018	5	27.56
	2019	5	31.68

The trend of NO_2 levels from 2017 to 2019 showed (Fig. 3) no clear increase or decrease. Some decreases and increases do not follow a certain trend from year to year and from city to city.

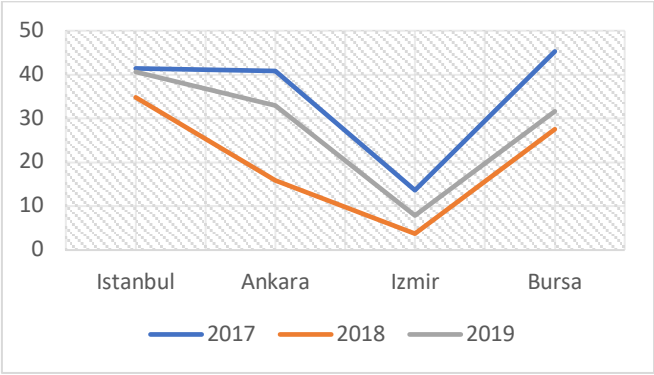

Fig. 3. Change of NO_2 by Years in İstanbul, Ankara, İzmir and Bursa

3.3. Deaths Attributed to NO_2

The number of deaths attributed to NO_2 pollution in İstanbul was 4799 (2300-7242) in 2017, 3472 (1652-5275) in 2018, and 4923 (2357-7434) in 2019. The estimated percentages of deaths attributed to NO_2 were 8.24 in 2017, 5.78 in 2018, and 7.95 in 2019 (Table 3). Calculations of 2018 for Ankara and 2017, 2018, and 2019 for İzmir were not made because NO_2 levels were less than 20 μg/m^3 in these years.

Table 3. Number of deaths attributed to NO_2 air pollution in İstanbul, Ankara, İzmir and Bursa by year

City	Year	Estimated Number of Attributed Deaths (Min-Max)	Estimated Number of Attributed Deaths Per Hundred Thousand (Min-Max)	Estimated Percentage of Attributable Deaths (Min-Max)
İstanbul	2017	4799 (2300-7242)	31.93 (15.30-48.18)	8.24 (3.95-12.43)
	2018	3472 (1652-5275)	23.04 (10.96-35.01)	5.78 (2.75-8.78)
	2019	4923 (2357-7434)	31.72 (15.19-47.90)	7.95 (3.80-12.00)
Ankara	2017	1956 (937-2952)	35.92 (17.20-54.22)	8.05 (3.85-12.15)
	2018	-	-	-
	2019	1289 (612-1963)	22.87 (10.86-34.81)	5.07 (2.41-7.71)
İzmir	2017	-	-	-
	2018	-	-	-
	2019	-	-	-
Bursa	2017	1506 (725-2264)	51.29 (24.67-77.08)	9.66 (4.65-14.51)
	2018	476 (225-728)	15.88 (7.50-24.32)	2.99 (1.41-4.58)
	2019	736 (349-1121)	24.07 (11.42-36.70)	4.58 (2.17-6.99)

4. Discussion

There is epidemiological evidence of the health effects of air pollution. The World Health Organization reported deaths attributed to ambient air pollution as age-standardized for both sexes per hundred thousand people as 46.57 (40.17-53.31) for Türkiye, 13.31 (9.51-17.78) for the USA, 46.24 (36.87-57.21) for Russia, and 66.73 (57.74-75.79) for China, 109.4 (96.73-124.2) for India, 13.75 (10.15-18.21) for UK, 6.96 (4.63-9.8) for Canada, 24.06 (19.3-30.22) for Brazil, 113.18 (9.51-17.38) for Denmark, 66.55 (54.05-79.37) for the Democratic Republic of Congo and 8.39 (5.9-11.81) for Australia in 2016 (19). Attributable to ambient air pollution DALYs (Disability-adjusted life years) for the same year for both sexes per hundred thousand were reported that 1,175 (1.041-1.320) for Türkiye, 333 (249.1-430.4) for the USA, 1,131 (953.1-1.315) for Russia, 1.475 (1.291-1.652) for China, 3.038 (2.700-3.413) for India, 300.3 (235.2-381.9) for the UK, 152.1 (109.9-200.8) for Canada, 591 (484.1-723.2) for Brazil, 271.3 (200.7-350.5) for Denmark, 2.179 (1.647-2.715) for the Democratic Republic of the Congo, and 175.2 (131.4-225.7) for Australia (20). According to the Global Burden of Disease study 2019, 11.80% of all deaths and 8.41% of all DALYs worldwide; 9.70% of all deaths and 5.72% of all DALYs in Türkiye are caused by air pollution (21, 22).

There is also evidence showing the health consequences of exposure to NO_2. NO_2 exposure results in an increased risk of lower respiratory tract infections in children (23), is associated with the development of COPD (24), causes the development of childhood asthma (25), causes asthma attacks in both children and adults (26), and may cause lung cancer (26, 27) and increases all-cause mortality (9, 28). A Danish cohort study found a relationship between NO_2 and cardiovascular mortality (29). A meta-analysis of thirteen studies found a relationship between NO_2 exposure and breast cancer (30). A systematic review study evaluating thirteen studies determined that exposure to air pollutants, including NO_2, increases the risk of dementia (31). In a systematic review including fifteen studies, NO_2 was found to be a trigger on the spread and lethality of Covid-19 (32).

In this study, NO_2 measurements in the four key cities of Türkiye were higher than the limit value, excluding every three years in İzmir and in 2018 in Ankara. The trend of NO_2 levels from 2017 to 2019 showed no clear increase or decrease. Some decreases and increases do not follow a certain trend from year to year and from city to city. A study conducted in northern China determined that NO_2 was in a decreasing trend (33). In studies conducted in Portugal, Northwest China, Spain, and India, it was found that NO_2 showed irregular decreases and increases (34-37). In addition, there are studies showing that NO_2 levels decrease during the Covid-19 pandemic period (38-40). Since the main source of NO_2 is exhaust emissions and power plants, this result can be explained by reasons such as traffic density and energy need. This may also be related to the location of the station, the number of measurements, and its accuracy.

The deaths attributed to NO_2 for 2019 in the research are 7.95% in İstanbul, 5.07% in Ankara, and 4.58% in Bursa. Since the measured and recorded NO_2 values in İzmir are below 20 $\mu g/m^3$, no deaths were attributed to NO_2. In studies conducted in other cities in Türkiye, deaths attributed to NO_2 were 9.5% for Konya in 2019, 18.9% for Erzurum in 2018, 5.57% for Kocaeli in 2018, 3.98% for Sakarya in 2018, 2.61% for Bilecik in 2018, 2.18% for Edirne in 2018 and 0.89% for Balıkesir in 2018 (41-43).

Among the studies conducted in Iran, the rate of deaths attributed to NO_2 was 1.03% for Isfahan in 2013-2014, 2.08% for Mashhad in 2015-2016, and 1.37% short-term exposure and 14.39% long-term exposure for Ahvaz in 2014-2017 (44-46). In a study conducted in North China, the rate of deaths attributed to NO_2 was 4.19%, and in a study conducted in Northwest China, it was calculated as 1.57% (33, 35). In another study in northern Italy, the rate of deaths attributed to NO_2 was 1.8% (47). There are lower percentages in the literature than the frequency of deaths attributed to NO_2 obtained in the current study. This situation may depend on the distance of the buildings to the highways, the type and amount of energy used in the houses, and the policies implemented or not implemented by the countries regarding reducing air pollution.

Our research evaluated the NO_2 values and the deaths attributed to NO_2 in the four key cities in Türkiye. To our

knowledge, this is the first study to evaluate four key cities in Türkiye. Since the research was conducted using the latest death data in Türkiye, it provides up-to-date information. These two situations are the highlights of the research.

Since the research is of ecological type, it does not evaluate the individual, but the geographical location and the society in that region. Both our study and other air quality-related studies in the literature are affected by the number of measuring stations, the location of the station (the distance to the highway, the power plant, etc.), and the number and quality of measurements. Therefore, such studies should be interpreted by taking this information into account. In cities where millions live, relatively few stations have measured and recorded NO_2. These are the limitations of the research.

As a result of the research, it is determined that the NO_2 levels were higher than the determined limit values in three of the four big cities in Türkiye, and the mortality rates attributed to NO_2 were higher compared to similar studies in the literature. It has to be considered that deaths due to air pollutants are preventable deaths. It should be ensured that measures are taken on an international, national and local basis and that air pollutant levels are measured in appropriate localizations, in sufficient numbers, and at acceptable quality. In addition, it is recommended to carry out actual research involving other cities/more cities both from Türkiye and around the world with AirQ+ software to keep the subject up to date and follow the situation.

Ethical statement

Ethics committee approval was obtained from Ankara University Health Sciences Ethics Committee (Number: 05-54, Date: 07.03.2022).

Conflict of interest

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Authors' contributions

Concept: E.N.Y.O., Design: E.N.Y.O., M.O., Data Collection or Processing: E.N.Y.O., M.O., Analysis or Interpretation: E.N.Y.O., Literature Review: E.N.Y.O., M.O., Writing: E.N.Y.O., M.O.

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What is the effect of vitamin D deficiency on Pap smear test results in the postmenopausal period?

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Abstract

This study aimed to determine the impact of vitamin D deficiency on Pap smear test results in women in the postmenopausal period. This cross-sectional study included 394 women who applied to İstanbul Medeniyet University, Göztepe Training and Research Hospital gynecology and obstetric clinic for routine Pap smear tests. This study was conducted between May 2019 and December 2020. Cases were divided into severe deficiency (<12 ng/ml), moderate deficiency (12-20 ng/ml), deficiency (21-30 ng/ml), and sufficiency (>30 ng/ml) according to the vitamin D levels measured in the postmenopausal period within the scope of check-up examinations. Pap smear test results compared with vitamin D levels. The mean age of the study group was 48.70±3.46 years. There was a statistically significant association between vitamin D values and Pap smear test results ($p<0.05$). Vitamin D greater than 20 ng/ml had a significant relationship with the normal cytology results. There was a statistically significant association between vitamin D lower than 20 ng/ml and cervicitis cytology results. The results showed that the serum vitamin D and the Pap smear test results had a significant association. It is recommended that physicians prescribe the use of vitamin D supplements in women in the high-risk group of cervical cancer.

Keywords: cervical cancer, vitamin D, Pap smear test, cervical cytology

1. Introduction

Several experts considered vitamin D a lipid-soluble hormone in steroid structure, causing different effects in different tissues (1, 2). Vitamin D has been extensively studied as a steroid in the extraskeletal system. In previous studies, vitamin D has been reported to affect several physiologic systems with various clinical influences, such as protection against degenerative disease and cancer prevention (2, 3). Since vitamin D is a naturally synthesized substance, it is an attractive material in cancer treatment and prevention studies (4). The relationship between vitamin D and the progression of cancer, known as the "Vitamin D/cancer hypothesis," has attracted the attention of researchers, particularly in the last two decades (5). The immune modulation of B and T lymphocytes is facilitated by vitamin D, playing an essential role in the natural immune system, proving them to differentiate in the adaptive immune system. This helps monocytes become macrophages and enhances their phagocytosis capacity (6).

Cervical cancer, the fourth most common cancer among women, is a global public health problem, of which the death rate is expected to reach 13.400.000 by 2070 (7, 8). Cervical cancer has quite a high incidence due to either nonexistent or poorly implemented prevention programs. Developing countries have the highest number of cases and deaths caused by cervical cancer (9). The reason is that cancer prevention and control programs are not sufficient in these countries. In developed countries, about 80% of cervical cancers have been reduced by implementing effective prevention programs and effective treatments in the early stages of cancer (10). Effective preventive health behavior in this field is to perform a Pap smear test. A Pap smear test is an easy screening method to evaluate infection and investigate the pre-cancer changes and cervical cancer (11, 12). It is an effective way to prevent cervical cancer development and a noninvasive, simple, easy, and cost-effective way to detect precancerous lesions (13). In communities with active screening programs, the death rate caused by this cancer has decreased by 40% in the past thirty years. Based on the studies, 44% of women are aware of cervical cancer screening programs and have refereed at least once for this test in Sweden (14).

Many studies have evaluated the identification of factors affecting Pap smear test results. The present study assessed the relationship between vitamin D and Pap smear results. It is essential to determine factors affecting the Pap smear test results to provide treatments to reduce costs and increase the success of the treatment. This study aims to evaluate the relationship between vitamin D and Pap smear results.

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2. Materials and Methods

The Ethics Committee of İstanbul Medeniyet University approved this prospective cross-sectional study. (Date: 13.01.2021 Decision no: 2021/0041). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. With the ethics committee's approval, the data were scanned retrospectively using the Hospital Information Management System.

We included patients who applied within the scope of the postmenopausal routine check-up program and had a Pap smear test and their vitamin D level checked. Three hundred ninety-four women participated in this study between May 2019 and December 2020.

All surgical pathology and Pap smear files were searched for "routine" Pap smears after the approval of the Institutional Review Board. We classified abnormal routine Pap smears into the following categories based on the latest Bethesda system: Atypical squamous cells of undetermined significance (ASC-US), atypical glandular cells of undetermined significance (AGUS), low-grade squamous intraepithelial lesion (LGSIL), high grade squamous intraepithelial lesion (HGSIL), and cervicitis. If available, we performed correlation with tissue biopsy from the cervix for each abnormal Pap smear. Serum vitamin D values were recorded from the hospital information management system. The scale for measuring hormones was Nanograms per milliliter (ng/ml). Patients were clustered as subjects severe deficiency (<12 ng/ml) (n=127), moderate deficiency (12-20 ng/ml) (n=70), insufficiency (21-30 ng/ml) (n=122), and sufficiency (>30ng/ml) (n=75) groups.

2.1. Statistical analysis

We used the Kolmogorov-Smirnov test to check the normality and measured Mean and standard deviations (SD) to check each continuous variable, including age, serum vitamin D, gravida, parity, abortus, body mass index (BMI), the coital debut age, and menopause.

We used the Chi-square and the Pairwise Z-Tests tests to study the difference between groups and SPSS v22 for statistical analyses. A value of $p < 0.05$ was accepted as statistically significant. To calculate the sample size with the G-Power 3.1 program, we measured two groups' total mean based on the Z test with the power of 75%, 0.5 proportion p1, 0.6 proportion p2, and 0.1 type 1 error for at least 378 women (15).

3. Results

Women's mean age and BMI were 48.70 ± 3.46 and 25.04±2.13, respectively. The frequency for the serum vitamin D<12 ng/ml interval is 127 (32.2%). The frequency for the vitamin D (12-20 ng/ml), vitamin D (21-30 ng/ml) and vitamin D (>30ng/ml) were 70 (17.8%), 122 (31%), and 75 (19%), respectively.

The mean vitamin D level was 20.05±11.82. The mean vitamin D level of four groups of women in the study, severe deficiency, moderate deficiency, insufficiency, and sufficiency were 7.09±1.50, 15.47±1.94, 25.13±2.09, and 37.98±7.18, respectively. Table 1 shows descriptive statistics of study parameters.

Table 1. Descriptive statistics of study parameters in women (n=394)

Study parameters	median (range) mean ± SD
Age (yr)	47 (41-58) 48.70±3.46
Vitamin D	20 (4.6-66) 20.05±11.82
Gravida	1 (0-2) 1.12±0.55
Parity	1 (0-1) 0.58±0.49
Abortus	0 (0-2) 0.52±0.62
BMI	25 (19-30) 25.04±2.13
The coital debut age (yr)	25 (18-31) 24.37±2.53
Menopause (yr)	3 (1-7) 3.27±1.84

SD, standard deviation.

Table 2 shows the frequency of Pap smear test results and the mean and SD of vitamin D values in each Pap test result. Women with normal Pap test results had the highest vitamin D levels (23.87±11.98). As stated in Table 2, the highest frequency of Pap smear results was normal 175 (44.4%), cervicitis 121 (30.7%), ASCUS 60 (15.2%), AGUS 9 (2.3%), LGSIL 21 (5.3%), and HGSIL 8 (2%).

Table 2. The frequency of Pap smear test results

Study parameters		n (%)	Vitamin D (mean ± SD)
Pap smear test results	Normal	175 (44.4)	23.87±11.98
	AGUS	9 (2.3)	18.98±10.45
	ASCUS	60 (15.2)	16.48±11.29
	LGSIL	21 (5.3)	17.14±10.4
	HGSIL	8 (2)	16.95±15.58
	Cervicitis	121 (30.7)	17.1±10.45

As stated in Table 3, a chi-square test found a statistically significant association between vitamin D levels and Pap smear test results ($p<0.001$). The Pairwise Z-Tests found that the percentage of women who had the normal cytology results was significantly higher for those who had vitamin D (21-30ng/ml and > 30ng/ml) than for those who had vitamin D lower than 20ng/ml.

Table 3. The relationship between Pap test results and vitamin D levels

Study parameters		<12 ng/ml n (%)	12-20 ng/ml n (%)	21-30 ng/ml n (%)	>30 ng/ml n (%)	*p*
Pap smear test results	Normal	32 (25.2)	26 (37.1)	**69 (56.6)†**	**48 (64.0)†**	<0.001*
	AGUS	3 (2.4)	2 (2.9)	3 (2.5)	1 (1.3)	
	ASCUS	30 (23.6)	8 (11.4)	14 (11.5)	8 (10.7)	
	LGSIL	9 (7.1)	5 (7.1)	4 (3.3)	3 (4.0)	
	HGSIL	4 (3.1)	2 (2.9)	1 (0.8)	1 (1.3)	
	Cervicitis	**49 (38.6)†**	**27 (38.6)†**	31 (25.4)	14 (18.7)	

*A Chi-square test. † Pairwise Z-Tests

Fig. 1 shows vitamin D levels in different Pap smear test results.

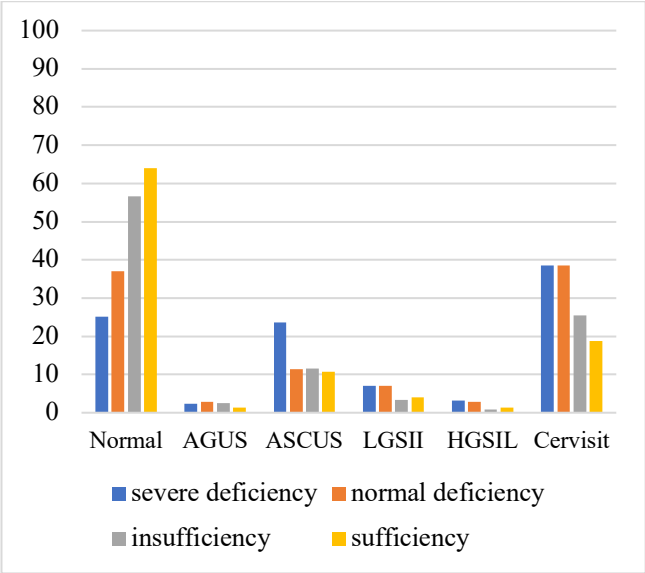

Fig. 1. Vitamin D levels in different Pap smear test results

4. Discussion

In the current study, we researched the association between Pap smear results and vitamin D levels in women. Of the 394 sexually active women, 175 (44.4%) had normal cytology results, and 219 (55.6%) women had abnormal cytology. The frequency of abnormal cytology was 2.3% (n=9) for AGUS, 15.2% (n=60) for ASCUS, 5.3% (n=21) for LGSIL, 2% (n=8) for HGSIL, and 30.7% (n=121) for cervicitis. There was a significant association between Vitamin D and Pap smear results. According to research findings, the value of vitamin D greater than 20 has a statistically significant relationship with normal cytology results. The value of vitamin D lower than 20 has a statistically significant relationship with cervicitis cytology results. By increasing vitamin D's value, the normal cytology results frequency has increased. Therefore, there is a statistically significant relationship between the normal cytology results and vitamin D.

Vitamin D, which has several vital functions, includes vitamins D1, D2, and D3 as a fat-soluble vitamin in a family of compounds. The most important functions of vitamin D for the body include: reducing the risk of multiple sclerosis (16), decreasing the chance of heart disease (17), decreasing the likelihood of severe illnesses (18), supporting weight loss (19), supporting immune health (20), and reducing the possibility of depression (21). The effect of vitamin D on women with polycystic ovary syndrome (PCOS) (22), osteoporosis in women (23), and pregnancy period was investigated in many studies (24). Our study showed that vitamin D positively affects Pap smear results. The value of 20ng/ml was an important criterion. More or less than this value will affect the Pap test results. Patients were clustered as insufficiency (21-30ng/ml) and sufficiency (>30ng/ml) groups were normal cytology results.

Sharif (25) reported that conservative sexual behavior, religious norms, and low smoking rates impact pap smear results. Sachan et al. (26) found in a study on the patients' demographic profile affecting results of pap smear tests that HGSIL and LGSIL were first seen in 41–50 year women, and a significant risk factor for cervical carcinoma is multiparity (>3). According to Gosh et al. (27), there is an association between the results of pap smear and younger age at sexual debut, a more youthful age group, and lower socioeconomic status. the positive association between the infections and young age is due to more sexual activity of younger women increasing their susceptibility to sexually transmitted infections. In several studies, socioeconomic status affecting Pap smear results was reported due to few health check-ups and poor hygiene (28-30). Studies found age and socioeconomic status to be critical factors in cervical cancer screening, affecting Pap smear test results.

The shortcoming of the study was that all samples were recovered from a single center whereas the high number of samples can be counted as its strength.

In conclusion, vitamin D may have some beneficial effects on the Pap smear test results in women. Therefore women in the high-risk group for cervical cancer may be advised to regularly check their vitamin D status. The heavy burden of cervical cancer to the health system warrants any medication which has clinically proven benefits. This study suggests that maintaining the normal levels of Vitamin D, which plays a supportive role in immune system may be an efficient yet cheap and easy accessible way to fight cervical precancerous lesions before they evolve into cervical cancer.

Ethical statement

The Ethics Committee of İstanbul Medeniyet University approved this prospective cross-sectional study. (Date: 13.01.2021 Decision no: 2021/0041).

Conflict of interest

The authors have no conflicts of interest to declare.

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None to declare.

Authors' contributions

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

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Investigation of awareness of human papilloma virus infection among female health workers at Ondokuz Mayıs University

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Abstract

Less developed areas have the highest incidence rates of cervical cancer, which vary by geographic region. If the right lifestyle changes are made, all cervical cancer risk factors can be avoided. Furthermore, vaccines have been created to guard against a number of the high-risk strains of the human papillomavirus (HPV). Not all women are aware of cervical cancer or how to prevent it, even though it is preventable. Therefore, every woman must assess level of knowledge regarding HPV prevention and cervical cancer. We applied a validated questionnaire between January and June 2021 to the female health workers at Ondokuz Mayıs University Hospital to assess their knowledge of cervical cancer and HPV protection as well as their attitudes toward it. Our findings indicate that women's knowledge on this topic is lacking, especially regarding the most recent advancements in HPV testing and vaccination-based cervical cancer prevention. Only 99% of the women who responded to the survey were actually aware that HPV testing was available. Although the incidence of cervical cancer is low, this figure is subject to change, particularly in light of the economic crisis and rising immigration rates, which could lead to higher rates of HPV infection in the population if no action is taken. Although the HPV vaccine has been available for some time, the Turkish government does not recommend it as a routine vaccine as part of the National Immunization Program. Even among the highly educated workforce in the healthcare industry, there is a lack of understanding about HPV vaccine. Healthcare professionals should be fully informed about the HPV vaccine for their own benefit so that they can discuss it with their patients. This study aimed to remove vaccination barriers and assess healthcare professionals' current knowledge of cervical cancer and HPV vaccination in a tertiary hospital.

Keywords: HPV, cervical cancer, smear, cervical pathology

1. Introduction

The fourth most common cancer in women overall is cervical cancer (1, 2). Cervical cancer is brought on by human papillomavirus (HPV) infection; HPV 16 and 18 strains, in particular, are responsible for 75% of cases (3). Having multiple sexual partners, having sex at a young age, smoking, taking oral contraceptives for an extended period of time, having more children, and giving birth at a young age are risk factors for HPV infection (4). Pregnant women and the fetus may be affected by infections like syphilis, gonorrhea, trichomoniasis, chlamydia, hepatitis B, HIV, herpes simplex viruses 1 and 2 (HSV-1-2), and human papillomavirus (HPV). (5). Although most genital HPV infections are asymptomatic, previous research has shown that high-risk HPV types are almost always the root of cervical cancer cases (6). The precursors of cervical cancer can be easily found at an early stage and effectively treated using screening methods like the Pap test, VIA (Visual Inspection with Acetic Acid), or efficient HPV-DNA detection procedures. Contrary to cancers that develop in other parts of the body, cervical cancer and its precursor lesions are treatable in the early stages of the disease and can be detected early. The majority of women do not typically exhibit symptoms until the disease has progressed, making cervical cancer screenings essential (7). Awareness of the condition, screening methods, and preventive measures are all essential to the prevention and control of cervical cancer (8). From local management to national management, the relationship between PAP smear results, HPV type distributions, colposcopic evaluations, and histopathological results is crucial in the fight against cervical cancer (9). The use of bivalent and quadrivalent HPV vaccines is authorized in Turkey (10). 9 to 12 years old is the recommended range for vaccination. Vaccination for breeding is permitted up until the age of 26 (11). Men have been tested for use of the tetravalent vaccine, which is currently licensed (12). Pregnant women are advised against receiving the vaccine (13, 14). Additionally, the vaccine is available for nursing mothers. Cervical cancer screening cannot be replaced by immunization. A further effort is being made to prevent cervical cancer in the country. Even though the HPV vaccine has been around for a while, the Turkish government still does not offer it as a routine

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vaccination as part of the National Immunization Program. Even among the highly educated population working in the healthcare sector, ignorance of its dosage, schedule, and cost exists. For their own benefit, healthcare professionals should be fully informed about the HPV vaccine so they can discuss it with their patients. In order to remove vaccination barriers and assess current knowledge of cervical cancer and HPV vaccination among healthcare professionals working in a tertiary hospital, this study was conducted.

2. Material and Method

The study was approved by the ethics committee of Ondokuz Mayıs University clinical research (OMUKAEK:2020/744). 350 female employees working at Ondokuz Mayıs University Faculty of Medicine were included in the study. Written informed consent was obtained from the participants before the questionnaire was administered. Patients were asked to fill out a questionnaire containing questions about the HPV vaccine and the HPV-cancer link. The survey consisted of two parts. The first part was about social demographics, and the second part was about self-awareness about diseases. All questions were closed-ended yes or no or multiple choice for a more accurate assessment. The questionnaire is given in Table 1.

Table 1. Questions asked to participants

No	Questions	Answers
1.	What is your age?	
2.	What is your profession?	
3.	Can cervical cancer be diagnosed early with screening?	
4.	I know that cervical cancer is caused by the HPV virus.	
5.	HPV is a sexually transmitted virus.	
6.	Can men get HPV infection?	
7.	HPV causes genital warts.	
8.	HPV is not seen in women who doesn't have sexual intercourse.	
9.	Have you heard that the state makes HPV screening test free for cervical cancer screening?	
10.	Have you ever had a cervical cancer screening test (smear or HPV)?	
11.	Can you be vaccinated to protect yourself from cervical cancer?	
12.	Who gets the HPV vaccine?	
13.	I have had the HPV vaccine.	
14.	Have any of your relatives had the HPV vaccine?	

3. Results

The age distribution of health professionals included in the study is between 20 and 55 (Fig. 1). The majority of the participants included nurses (280 women, 37.1%) and physicians (280 women, 24.6%), and the remaining health workers (Fig. 2). While 98.3% of the 290 female participants answered yes to the question of whether the early diagnosis of cervical cancer can be made by screening, the remaining 1.7% answered no (Fig. 3). When asked "I know that the HPV virus causes cervical cancer", 87.2% of participants answered "I know", and 12.8% answered "I don't know" (Fig. 4). Responding to the question of HPV is a sexually transmitted virus, a total of 86.9% of participants said "yes", 3.8% said "no" and, "9.3%" had no idea (Fig. 5). In our study, about the sexual transmission of HPV virus, 86.9% of the participants said yes, 9.3% had no idea and 3.8% answered no (Fig. 6). It was determined that the awareness of male HPV infection was high among the female health workers of Ondokuz Mayıs University. The level of awareness of the possible cause of genital warts was found to be high. To the question that HPV is not seen in women who do not have sexual intercourse, 53.4% of the participants answered "yes", while 27.6% had "no idea" (Fig. 7). The question "Are you aware that cervical cancer screening is free of charge, Have you had this test before?" reveals that the awareness of the participants is high. To the question of who is vaccinated against cervical cancer, 29.7% of the participants replied to "women", 37.2% to "both women and men", and 13.1% had "no idea". While 96.2% of the participants were not vaccinated against HPV, 3.8% were vaccinated against HPV. When asked if they have had HPV vaccine soon, 54.8% of the participants answered "no", 13.8% "yes", and 31.4% had "no idea" (Fig. 8).

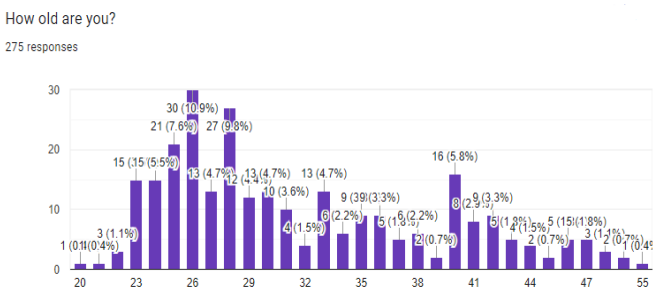

Fig. 1. Distribution of the answers given to the 1st question of the questionnaire

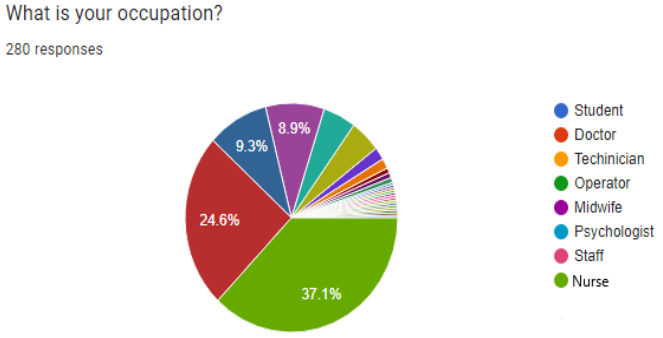

Fig. 2. Distribution of the answers given to the 2nd question of the questionnaire

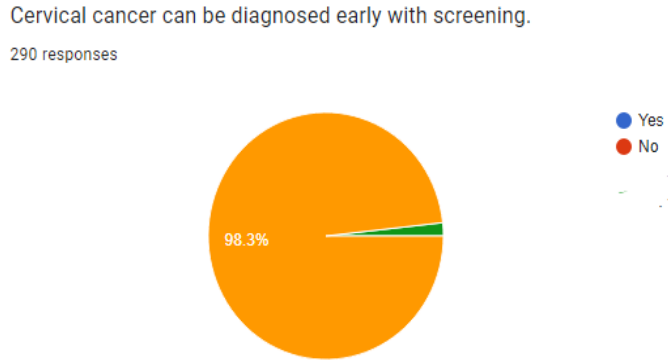

Fig. 3. Distribution of the answers given to the 3rd question of the questionnaire

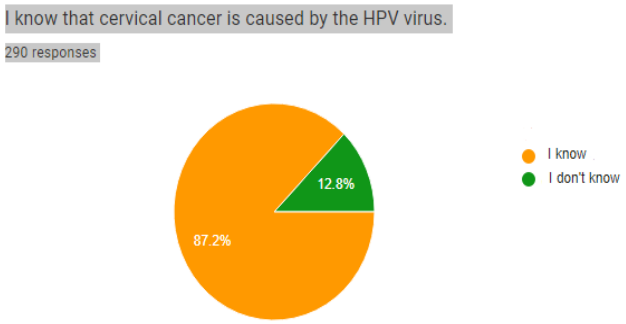

Fig. 4. Distribution of the answers given to the 4th question of the questionnaire

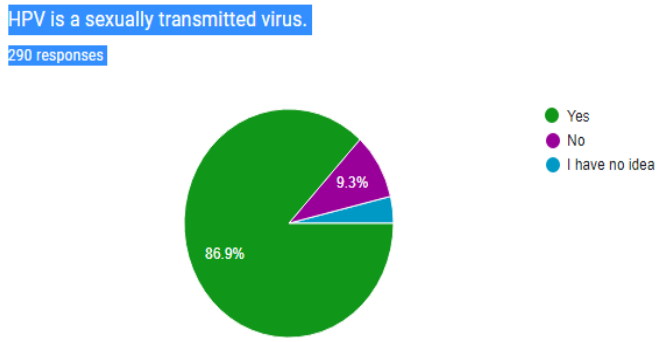

Fig. 5. Distribution of the answers given to the 5th question of the questionnaire

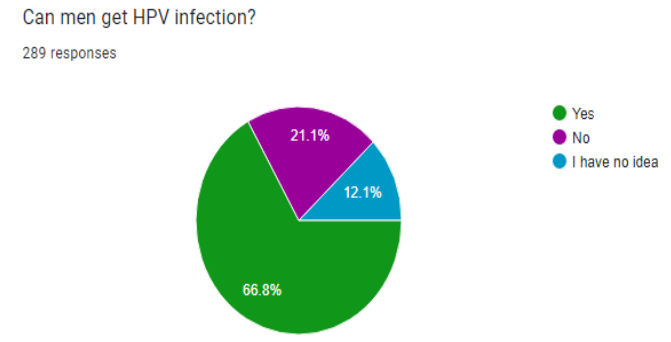

Fig. 6. Distribution of the answers given to the 6th question of the questionnaire

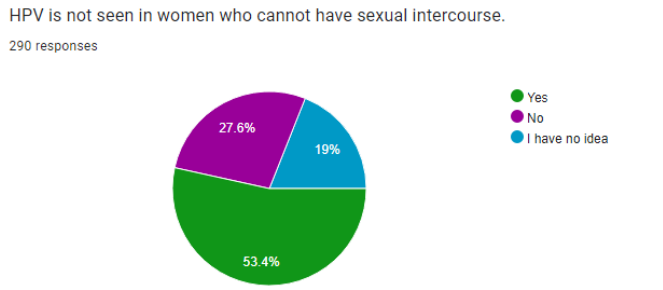

Fig. 7. Distribution of the answers given to the 8th question of the questionnaire

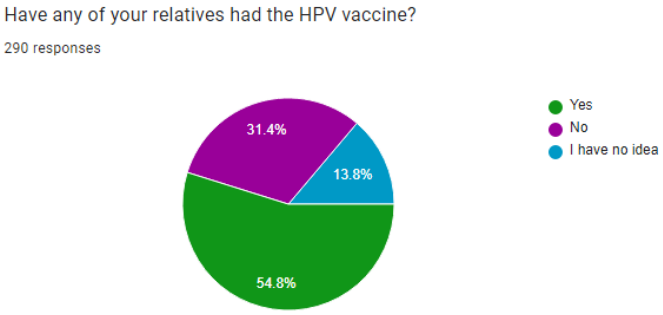

Fig. 8. Distribution of the answers given to the 14th question of the questionnaire

4. Discussion

In this study, the knowledge and attitudes of women over 20 years of age towards genital warts, cervical cancer, swab test, prevention method, HPV vaccine were evaluated. It was determined that most of the participants in the study did not know that the large HPV virus causes cervical cancer. Also, a large proportion of the participants had a high level of knowledge about the sexually transmitted disease of cervical cancer. 87.2% of them knew that HPV virus causes cervical cancer. However, 96.2% of them have not been vaccinated against HPV. We determined that most of the participants had sufficient knowledge about cervical smear test, HPV infection and male vaccines. A multicenter cross-sectional study was conducted with 1146 women and 557 undergraduate students in China and they showed that 320 (28%) of 1146 women and 66 (12%) of 557 students knew about HPV, and very few of them knew about the HPV-cancer link. (11). Even in societies where HPV vaccination is routine, awareness may be raised to a great extent, but information may still be insufficient. Bowyer et al. supported this view with the study they conducted on 1033 girls who had been vaccinated with HPV 3 years ago at 13 schools in London (12).

It is known that HPV causes cervical cancer, that vaccination is important, and that a cervical smear test is still required. In our study, 87.2% of the participants claimed to know the cause of cervical cancer, while 98.3% were aware that HPV cervical cancer could be diagnosed early with the screening method. There are other studies from Turkey about HPV infection and awareness of infection-cancer link. Ozyer et al. conducted a study with 408 participants in Turkey (8). In this study, it was shown that 41.6% of the participants were

aware of HPV, and 33% knew the link between HPV infection and cervical cancer. In the same study, only 27.9% of the participants knew about the HPV vaccine, and only 1.4% had been vaccinated. In another study, 520 nursing students working in a tertiary health institution in Ankara and a control group of 232 people were evaluated (7).

This study, which was conducted among female health workers about HPV, showed that awareness knowledge/ awareness of HPV is high among female health workers. The fact that our study was only among female health workers and in a primary health care institution may be counted among the limitations of the study. There is a need for larger-scale studies on HPV infection and vaccine to be conducted throughout Turkey.

In conclusion, we observed that the awareness rate of female health workers at Ondokuz Mayıs University was high about the connection between HPV infection and cervical cancer. We concluded that HPV infection causes cancer, vaccination prevents HPV infection to a large extent, and in-house training increases awareness about HPV infection.

Ethical statement

The study was approved by the ethics committee of Ondokuz Mayıs University clinical research (OMUKAEK:2020/744).

Conflict of interest

None to declare.

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None to declare.

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None to declare.

Authors' contributions

Concept: Y.K., İ.Y., A.B., D.G., İ.K., Design: Y.K., İ.Y., A.B., D.G., İ.K., Data Collection or Processing: Y.K., İ.Y., A.B., D.G., İ.K., Analysis or Interpretation: Y.K., İ.Y., A.B., D.G., İ.K., Literature Search: Y.K., İ.Y., A.B., D.G., İ.K., Writing: Y.K., İ.Y., A.B., D.G., İ.K.

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Evaluation of patients admitted to the emergency department after Coronavac (Sinovac) vaccination?

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Abstract

To evaluate the symptoms and laboratory parameters of patients admitted to the emergency department after CoronaVac (Sinovac) vaccination. Complaints, demographic characteristics, laboratory tests, interventions, and outcomes of patients admitted to the emergency department within seven days of receiving the CoronaVac (Sinovac) were all evaluated. Pain at the injection site, swelling, redness, and pain in the injected arm were expected side effects of the vaccination. In this study, a total of six people had syncope following the vaccination, one of whom developed an allergic reaction. The most common adverse events in non-geriatrics were fatigue, headache, fever, and abdominal pain, while in geriatric patients' fatigue, headache, chest pain, and dyspnea were most common, with fever falling in the last place. Rare symptoms following vaccination included syncope and urticaria. It is necessary to be prepared for the side effects that may occur during mass vaccination against COVID-19. In this study, the body's response to the vaccine in geriatric patients was atypical in vital signs and laboratory values.

Keywords: vaccine, side effect, COVID-19, CoronaVac, SARS-CoV-2

1. Introduction

The coronavirus disease COVID-19 started in Wuhan in December 2019. It spread rapidly around the world and was declared a pandemic. It causes severe acute respiratory syndrome in patients. The mortality of the disease varies between 0.4% and 7% (1). Vaccine studies have accelerated due to the increased mortality rates, especially with the delta variant.

On the other hand, the fact that the immunity of those who had the disease is not permanent and the reinfection rates are high revealed the importance of community immunity in order to end the pandemic (2). Vaccines are obtained using antigenic substances produced from the disease-causing agent, in order to create a defense mechanism against diseases and to produce antibodies (3). In line with the COVID-19 Vaccine Implementation Strategy (in the study's country of origin), on January 14, 2021, the vaccination of those aged 65 and over—primarily healthcare workers—began. The plan is to vaccinate all individuals by dividing them into age groups, according to the COVID-19 vaccination calendar from the Ministry of Health of Turkey. The vaccine made in the study's country of origin is the CoronaVac (Sinovac) vaccine. CoronaVac was obtained by producing and inactivating the live SARS-CoV-2 virus in a laboratory, using the traditional vaccine production method (4). The country and healthcare system aim to be prepared for the potential side effects following the COVID-19 vaccination. The most common post-vaccine side effects are reported by the World Health Organization as pain at the injection site, headache, fatigue, and myalgia (5).

As the vaccination process reaches the stage of comprehensive vaccination in the wider community, an awareness of the potential side effects makes it possible to decide where and how the vaccine will be administered, considering the gender and age of the recipient and whether they suffer from any chronic diseases (6-8).

Data on adverse events following the initial vaccination are valuable, as they can inform the healthcare system in advance of the potential side effects in preparation for mass vaccination. In turn, this allows both patients and health institutions to act more cautiously when administering the vaccine and encountering side effects, and it enables adequate preparation for the potential side effects following the COVID-19 vaccination. At the stage of comprehensive vaccination of the wider community, the location and method of vaccination can be determined, considering the gender and age of the recipient and whether they suffer from any chronic diseases.

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2. Material and Methods

The study was conducted retrospectively and included patients aged 18 and over who applied to the Emergency Department (ED) between January 11, 2021, and February 11, 2021.

Vaccination is carried out by appointment, and specific conditions must be established for patients to receive the vaccination: It is not administered to patients with a fever of 38 °C or above, those with undiagnosed acute disease, or those who are experiencing a period of acute attack from a chronic illness.

The COVID-19 disease status of the patient is checked. If they are found to have the disease, the vaccine is not administered. Being in contact with a confirmed COVID-19 case in the last ten days is checked, and if so, the vaccine is not administered. It is also checked whether the patient has received other vaccines in the month prior: Those who have received seasonal influenza, pneumococcal, meningococcal, and other inactivated vaccines must wait two weeks to make an appointment to receive the COVID-19 vaccine. Complaints, demographic characteristics, laboratory tests, and the interventions and outcomes of the patients admitted to the ED within seven days of receiving the vaccination were all evaluated. All patients admitted to the ED following the CoronaVac (Sinovac) vaccination were included in the study.

Laboratory tests, including those for white blood cells, lymphocytes, neutrophils, platelets, hemoglobin, alanine aminotransferase, aspartate aminotransferase, bilirubin, fasting blood glucose, and creatinine, are investigated to assess for any post-vaccination side effects. The data is obtained from NUCLEUS MBS—the patient clinical information system of the Medical Faculty Hospital.

2.1. Statistical analysis

The statistical analyses were performed using SPSS, version 22 (IBM SPSS Statistics for Windows, Armonk, NY; IBM Corp., 2013). First, a Kolmogorov-Smirnov test was used to determine which variables should be included in the data analysis and whether the data for the variables were normally distributed, but the data were not normally distributed. Therefore, non-parametric tests were used. The Mann-Whitney U test was used to compare continuous variables across the groups. The Wilcoxon signed Ranks Test was used to compare two dependent variables. The mean ± standard deviation, frequency, and percentage were reported as descriptive statistics. The statistical significance level was set to $p < 0.05$.

2.2. Ethics

Approval was obtained from The Bezm-i Alem Foundation University local ethics committee with dated 29.04.2021 and number 09. Due to the retrospective design of the study and its relationship with public health, consent was not obtained from the patients within the knowledge of the ethics committee.

3. Results

A total of 66 patients were admitted to the ED following CoronaVac (Sinovac) vaccination, with a mean age of 51.2±23.4 years and a median age of 47.5 years (25–90). Twenty-eight (42.4%) patients were female and 38 (57.6%) were male. The number of geriatric patients totaled 43 (65.4%). Of the 66 patients, hypertension was witnessed in 20 (30.3%), coronary artery disease in 14 (21.2%), diabetes mellitus in 12 (18.2%), chronic obstructive pulmonary disease in 12 (18.2%), and nine (21.2%) suffered from congestive heart failure. Of the 66 patients, 51 (77.3%) were discharged from the ED, 12 (18.2%) were admitted onto a ward, two (3%) were admitted to the intensive care unit, and one patient (1.5%) died (Table 1). In terms of complaints, 33 (50%) patients suffered from fatigue, 27 with headaches (40.9%), 17 with fever (25.8%), 15 with shortness of breath (22.7%), 14 with joint pain (21.2%), 14 with abdominal pain (21.2%), 11 with a cough (16.7%), 10 with nausea and/or vomiting (15.2%), eight with dizziness (12.1%), eight with chest pain (12.1%), eight with diarrhea (12.1%), six with syncope (9.1%), and two with urticaria (3%) (Table 2). Two geriatric patients experienced fever (8.7%), compared to 15 of the non-geriatric patients (34.9%) (p=0.02). Fatigue was present in nine (39.1%) of the geriatric patients, compared to 24 (55.8%) of the non-geriatric patients (p=0.196). Dyspnea was present in six (26.1%) geriatric patients, compared to nine (20.9%) of the non-geriatric patients (p=0.634). Headaches were experienced by nine (39.1%) of the geriatric patients, compared to 18 (41.9%) of the non-geriatric patients (p=0.830). Of the geriatric patients, four (17.4%) suffered from joint pain, compared to ten (23.3%) of the non-geriatric patients (p=0.755). Nausea and/or vomiting was experienced by four (17.4%) of the geriatric patients, compared to six (14%) of the non-geriatric patients (p=0.730). Cough was present in five (1.7%) of the geriatric patients, compared to six (14%) of the non-geriatric patients (p=0.495). Abdominal pain was experienced in three (13%) of the geriatric patients, compared to 11 (25.6%) of the non-geriatric patients (p=0.346). Syncope was present in four (17.4%) of the geriatric patients, compared to two (4.7%) of the non-geriatric patients (p=0.172). Of the geriatric patients, three (13%) suffered from dizziness, compared to five (11.6%) of the non-geriatric patients (p=0.867). Chest pain was experienced by seven (30.4%) of the geriatric patients but only one (2.3%) non-geriatric patient (p=0.002). Diarrhea was present in three (13%) of the geriatric patients, compared to five (11.6%) of the non-geriatric patients (p=0.867). Urticaria was only witnessed in two (8.7%) of the geriatric patients, and not at all in the non-geriatric patient sample (Table 2). Anaphylactic shock developed in one female patient, who also experienced syncope and was non-geriatric. Vital and laboratory parameters of the enrolled patients and their comparison between the geriatrics and non-geriatrics groups are presented in table 3.

Table 1. Demographics and baseline characteristics of simple

Variables		n (%)
Gender	Female	28 (42.4%)
	Male	38 (57.6%)
Age		51.2±23.4
	<65 years	43 (65.2%)
	≥65 years	23 (34.8%)
Comorbidities	Hypertension	20 (30.3%)
	Chronic obstructive pulmonary disease	12 (18.2%)
	Congestive heart failure	9 (13.6%)
	Coronary artery disease	14 (21.2%)
	Diabetes mellitus	12 (18.2%)
Outcome	Discharge	51 (77.3%)
	Service	12 (18.2%)
	Intensive care	2 (3%)
	Death	1 (1.5%)

Table 2. Symptoms of the enrolled patients and their comparison between the geriatrics and non-geriatrics groups

	Non-geriatrics	Geriatrics	p
	n (%)	n (%)	
Fever	15 (34.9%)	2 (8.7%)	**0.02**
Fatigue	24 (55.8%)	9 (39.1%)	0.19
Dyspnea	9 (20.9%)	6 (26.1%)	0.63
Headache	18 (41.9%)	9 (39.1%)	0.83
Join-muscle pain	10 (23.3%)	4 (17.4%)	0.75
Nausea-vomiting	6 (14%)	4 (17.4%)	0.73
Cough	6 (14%)	5 (21.7%)	0.49
Abdominal pain	11 (25.6%)	3 (13%)	0.34
Syncope	2 (4.7%)	4 (17.4%)	0.17
Dizziness	5 (11.6%)	3 (13%)	0.86
Chest pain	1 (2.3%)	7 (30.4%)	**0.02**
Diarrhea	5 (11.6%)	3 (13%)	0.86
Urticaria	0	2 (8.7%)	0.11

Table 3. Vital and laboratory parameters of the enrolled patients and their comparison between the geriatrics and non-geriatrics groups

		Non-geriatrics	Geriatrics	p
Vital signs	Fever	36.7±0.7	36.2±0.4	**0.01**
	Blood pressure (mm/Hg)	121±17.8	145.7±22.6	0.02
	Respiratory rate (/min)	19.5±2.1	20.8±1.7	0.23
	Heart rate (/min)	95.9±12.1	81.8±12.3	0.17
	Oxygen saturation	96.3±1.6	93.9±3.5	**<0.001**
Laboratory parameters	*White blood cell count (10^3/μL)*	6.1±1.2	8.6±3	**<0.001**
	Hemoglobin (g/dL)	13.8±1.3	11.9±2	0.16
	Platelet count (10^3/μL)	197.3±79	188±39.4	**<0.001**
	Troponin I (ng/dL)	3.9±6.4	348±1073	**<0.001**
	Prothrombin time	13.5±1.5	14.9±3.1	**<0.001**
	Partial Thromboplastin Time	27.9±7.4	32.1±7.1	0.91
	International normalized ratio	0.9±0.15	1.1±0.27	<0.001
	D-Dimer (ng/mL)	203±88.1	481±300	<0.001
	C-Reactive Protein (mg/dL)	13.3±27.4	6.5±8.2	0.05
	Lactate dehydrogenase (mg/dL)	211±71.7	380±374	**<0.001**
	Blood urea nitrogen (mg/dL)	18.9±10	32.6±14.9	0.12
	Creatinine (mg/dL)	0.8±0.2	1.6±1.4	**0.01**
	Aspartate transaminase (U/L)	31.3±23.8	64.2±144	**0.01**
	Alanine transaminase (U/L)	35.5±37.8	45.9±92	0.05
Outcome	Discharge	36 (83.7%)	15 (65.2%)	**0.08**
	Hospitalization to ward	7 (16.3%)	5 (21.7%)	
	Intensive care unit admission	0	2 (8.7%)	
	Death	0	1 (4.3%)	

4. Discussion

In the study's country of origin, health workers and patients over 65 years of age were included in the scope of the initial stage of the COVID-19 vaccination program. It aimed to vaccinate the majority of the population in order to create social immunity.

The most important risk factor for COVID-19-related mortality was advanced age. Immunization programs around the world started with health workers and the elderly, who are at risk groups (9) The goal of herd immunity is particularly important in the case of COVID-19, due to its deadly nature and limited treatment options (10). However, many people are concerned about whether vaccines protect them, whether vaccines are safe, and what the possible side effects are. In a previous study, it was shown that 29% of participants were not willing to get vaccinated, and 44% believed that COVID-19 vaccines could have serious side effects that could affect their health (11).

The most common symptoms associated with COVID-19 were fever, shortness of breath, and muscle and joint pain. Vaccination-related symptoms are similar to disease symptoms (12). Convincing people to get vaccinated can only be achieved with the help of scientific studies. Following vaccination, pain at the injection site, swelling, redness, and pain in the arm injected are all expected side effects (13). Those side effects most feared, however, are those related to allergic reactions

and the degree of involvement of vital organs (e.g., myocarditis). Mild to moderate reactions can be seen as "a sign of the immune system's response to the vaccine" (14); however, these symptoms normally only last a short time and subside on their own. In this study, a total of six patients suffered from post-vaccine syncope, and only one developed an allergic reaction. Hypotension, tachycardia, and angioedema were observed in the first five minutes following vaccination. It was discovered that this patient, whose condition improved with intervention, had a previous history of allergic reaction. It should be accepted that those with a history of allergies are potentially at risk (15). Anaphylaxis is a life-threatening allergic reaction that occurs in rare situations following vaccination, with a typical onset of minutes to several hours (16, 17). There are existing studies reporting that anaphylaxis is a risk following a COVID-19 vaccination (18); however, the prognosis of anaphylaxis is good when diagnosed and treated in a timely and correct manner (19). Vaccination centers should be equipped with the necessary materials and equipment for the treatment of anaphylaxis. In this study, the most common side effect following a COVID-19 vaccination was fatigue (50%), the second most common was a headache (40.9%), and the third most common was fever (34.9%). However, in the geriatric group, fever ranked last (8.7%), and there was a statistically significant difference between the geriatric and non-geriatric groups (p=0.02)—high fever may not always be observed in geriatric patients, even in the presence of severe infection (20). Abdominal pain was more common in those under 65 years of age (25.6%). Normal physiological changes (such as the absence of fever and leukocytosis) and physical examination can be seen in geriatric individuals, even with significant abdominal pathologies (21). While chest pain was seen at a rate of 30.4% in people over 65 years of age, it was among the rarer symptoms in the non-geriatric group. In connection with this complaint, the troponin value was found to be higher in the geriatric age group. According to these results, there is a need for further studies focusing on the relationship between vaccines and myocarditis. Complaints of chest pain in those under the age of 65 were 2.3% and troponin values were within the normal range. Common side effects listed by the World Health Organization (WHO) for the COVID-19 vaccine included fever, weakness, headache, muscle pain, and nausea (22). In this study, diarrhea, syncope, urticaria, and chest pain were added to these complaints. Post-vaccination, 77.3% of the patients admitted to the ED were discharged following clinical evaluations and only two (3%) were admitted to the ward. One patient in the geriatric age group, also suffering from additional diseases, died: They had been admitted to the ED in arrest the day following their vaccination, despite having no active complaints prior to this. The most significant laboratory parameter for this patient was a platelet count of 25,000. In a similar study, death after vaccination was associated with a low blood platelet count. In a study conducted in Norway, 23 geriatric deaths following vaccination were reported (23).

Vaccination against SARS-CoV-2, the cause of COVID-19, is the most important global strategy for controlling the pandemic. In order to achieve herd immunity, it is crucial to persuade those who are currently opposed to the vaccination; in order to successfully achieve this, scientific data should be applied. It is also particularly important to consider the potential side effects when performing mass vaccination against COVID-19. A patient who has previously experienced a severe allergic reaction should be prepared for post-vaccine anaphylactic shock. This study has also shown that post-vaccine geriatric patients may experience atypical vital signs and laboratory values, and there were statistically significant differences between the geriatric and non-geriatric groups. As a result, adverse reactions may develop in the body's response to the vaccine in geriatric patients, particularly when combined with the effects of an underlying disease.

There are several important limitations to our study. The retrospective design is the most important limitation. Secondly, to better interpret the changes in laboratory values of patients admitted to the ED post-vaccination, the same parameters should also be measured in blood samples taken prior to vaccination. The limited sample and single-center design can be considered as other limitations that could limit the generalizability of our findings.

As a conclusion, it is necessary to be prepared for the potential side effects when conducting mass vaccination against COVID-19. The results of this study can be used as evidence-based scientific data that addresses the side effects of CoronaVac (Sinovac). Thus, it can be used to increase public engagement in the vaccination program by raising awareness about CoronaVac (Sinovac).

Ethical statement

Approval was obtained from The Bezm-i Alem Foundation University local ethics committee with dated 29.04.2021 and number 09.

Conflict of interest

There are no conflicts of interest.

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None to declare.

Authors' contributions

Concept: B.T., E.S., S.Ö., Design: B.T., E.S., S.Ö., Data Collection or Processing: B.T., E.S., S.Ö., Analysis or Interpretation: B.T., E.S., S.Ö., Literature Search: B.T., E.S., S.Ö., Writing: B.T., E.S., S.Ö.

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Research Article

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Impact of an HPV diagnosis on the psychosexual sphere

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Abstract

We aimed to evaluate the psychosexual impact of being diagnosed with human papillomavirus (HPV). A cross-sectional study was conducted to assess the anxiety and depression levels and sexual functions in women with and without HPV between March and May 2021. Beck anxiety inventory, the Beck depression inventory, and the Libido scoring system scales were used as scales. A total of 575 respondents were included; 292 (50.2%) were HPV-negative, and 283 (49.8%) were HPV-positive, of whom 170 (60,1%) had high-risk HPV genotypes 16/18 and 113 (39.9%) had non-16/18 high-risk HPV-positive patients. There was no significant association between HPV-negative and HPV-positive patients in terms of sociodemographic characteristics. While 21.6% of the HPV-positive group experienced masturbation, it was 15.1% in the HPV-negative group (p=0.044). While severe depression was 4.9% in HPV-positive women, this rate was 1.4% in HPV-negative women (p=0.002). The present study showed that being diagnosed with HPV made a significant difference in sexual function, except for sexual interest, but increased anxiety and depression scores. Sexual dysfunction following an HPV diagnosis cannot be explained solely by depression and anxiety.

Keywords: anxiety, depression, human papillomavirus (HPV), sexual function

1. Introduction

Human papillomavirus (HPV) is the most common sexually transmitted infection, with approximately 80% of sexually active people having been exposed at least once in their lifetime (1). More than 120 varieties of HPV have been identified and classified as high-risk or oncogenic types and low-risk or non-oncogenic types. Although HPV infections are often asymptomatic and disappear without treatment (2), persistent HPV infections are associated with cervical intraepithelial neoplasia (CIN) and cervical cancer (3, 4). This information has led to the transformation of HPV-based cervical cancer screening programs in many countries (5).

All sexually transmitted infections, including HPV, have an adverse effect on women's psychosocial and sexual lives (6). Several studies found that positive HPV test results can lead to distress, anxiety, and depression, as well as sexual dysfunction in women (7-9), resulting in decreased quality of life (10). Given that many women experience adverse psychological and sexual consequences after being diagnosed with HPV (11), the impact of HPV on mental health and sexual function should be addressed to help alleviate the psychosexual burden.

Although there is a vast amount of literature on HPV epidemiology, screening methods, vaccines, and treatments over the past decades, yet, there have still been knowledge gaps and contradictory findings on the effects of HPV on individuals' sexual life and anxiety and depression levels. Therefore, this study aimed to assess anxiety, depression levels, and sexual functions following HPV testing.

2. Material and methods

We conducted a cross-sectional study from March 1, 2021, to May 30, 2021, to compare anxiety and depression levels and sexual functions in women with and without HPV.

We performed a sample size calculation based on a previously published study (12), and this gave a sample size of 482 women at a power of 80% for a confidence level of 95% and an alpha error of 5%. We assumed a refusal rate of 10%; the target sample was 530 women.

Women attending the outpatient gynecologic clinic were asked to participate in the study. Those who accepted to participate were informed about the study's purpose and assured of confidentiality. We included sexually active women over 18 years who received an HPV-positive test result for the first time and agreed to complete the questionnaire. Exclusion criteria were refusal to participate, having psychiatric disorders, chronic systemic diseases, sexual penetration

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disorders, pregnancy, pelvic organ prolapse, gynecological or urological cancer, premature ovarian insufficiency, skin conditions affecting the genital area, drugs that cause sexual dysfunction, and a history of pelvic pain or gynecological surgery. The Research Ethics Committee of the Zeynep Kamil Training and Research Hospital approved the study (Approval number: 21.06.2021-21/211). Written and verbal informed consent was taken from all participants.

The questionnaire contained items investigating sociodemographic factors such as age, education, marital status, number of children, work status, and income level. To assess levels of anxiety, depression, and sexual function, we used the Beck anxiety inventory (BAI), the Beck depression inventory (BDI), and the Libido scoring system scales (LSSS) as scales. We adopted the set of questions from the studies by Ulusoy et al. (1998), Hisli (1988), and Api et al. (2005) (13-15).

2.1. Beck Anxiety Inventory

The Beck anxiety inventory (BAI) is a 21-item, four-point Likert-like scale (0-3) questionnaire aimed at assessing anxiety symptoms. The total score ranges between 0 and 63 points, with higher scores indicating more severe anxiety.

2.2. Beck Depression Inventory

The Beck Depression Inventory (BDI) is a 21-item, four-point Likert-like scale (0-3) questionnaire that aims to assess the severity of depression in normal and psychiatric populations. The total score ranges between 0 and 63 points, with higher scores indicating more severe depressive symptoms.

2.3. Libido Scoring System Scale

The libido scoring system (LSS) scale, which includes four questions on four domains; orgasmic function, coital frequency, sexual desire, and self-sexual interest (masturbation), was used to assess female sexual function. A total score ranges between 0 and 12 points, with higher scores indicating better sexual functioning.

2.4. Statistical Analysis

We analyzed the data using IBM SPSS for Windows, Version 25.0 (IBM Corp., Armonk, NY, USA). Qualitative variables were expressed with frequency (n) and percentage (%) values. Quantitative variables were presented with mean and standard deviation (mean + SD) values. The Chi-Square test was used to compare categorical variables between groups. P-values less than 0.05 were considered to indicate statistically significant.

3. Results

A total of 575 women were eligible for the final analysis. The mean age of the patients was 35 years. Overall, 42.4% of the participants were university graduates, 47.0% were unemployed, 55.8% were married, 86.8% had two or fewer children, and 29.6% had a monthly income below 2000 Turkish Liras. All sociodemographic characteristics and HPV results of the study participants are presented in Table 1.

Table 1. Baseline characteristics of participants

	n (%)
Age (years)	35 (19-72)
Education	
Illiterate	10 (1.7)
Primary	132 (23)
Secondary	189 (32.9)
Tertiary (University/College)	244 (42.4)
Marital Status	
Single	178 (31)
Married	321 (55.8)
Widowed/Divorced	76 (13.2)
Number of children	
0	200 (34.8)
1	128 (22.3)
2	171 (29.7)
3	52 (9)
≥4	24 (4.2)
Employment	
Unemployed	270 (47)
Governmental employee	64 (11.1)
Laborer	67 (11.7)
Self-employed	174 (30.3)
Financial Status	
≤2000 TL	170 (29.6)
2001-3000 TL	166 (28.9)
3001-4000 TL	106 (18.4)
≥4001 TL	133 (23.1)
HPV Results	
HPV (-)	292 (50.8)
HPV (+)	283 (49.2)

Abbreviations: HPV, human papillomavirus; TL, Turkish Liras

Of the 575 women, 292 (50.8%) were HPV-negative, and 283 (49.2%) were HPV-positive. The two groups did not differ in educational level, marital status, number of children, occupation, and financial status (Table 2).

Table 2. Comparison of HPV status by sociodemographic characteristics

	HPV (-) n (%)	HPV (+) n (%)	p
Education			
Illiterate	4 (40)	6 (60)	
Primary	73 (55.3)	59 (44.7)	
Secondary	88 (46.6)	101 (53.4)	0.387
Tertiary (University/College)	127 (52)	117 (48)	
Marital status			
Single	86 (48.3)	92 (51.7)	
Married	172 (53.6)	149 (46.4)	0.279
Widowed/Divorced	34 (44.7)	42 (55.3)	
Number of children			
0	98 (49)	102 (51)	
1	64 (50)	64 (50)	
2	94 (55)	77 (45)	0.484
3	22 (42.3)	30 (57.7)	
≥4	14 (58.3)	10 (41.7)	
Employment			
Unemployed	135 (50)	135 (50)	
Governmental employee	31 (48.4)	33 (51.6)	0.223
Laborer	42 (62.7)	25 (37.3)	
Self-employed	84 (48.3)	90 (51.7)	

Table 2. Comparison of HPV status by sociodemographic characteristics (continue)

Financial Status			
≤2000 TL	84 (49.4)	86 (50.6)	0.717
2001-3000 TL	86 (51.8)	80 (48.2)	
3001-4000 TL	50 (47.2)	56 (52.8)	
≥4001 TL	72 (54.1)	61 (45.9)	

Abbreviations: HPV, human papillomavirus; TL, Turkish Liras

In comparing libido scoring system domains among women who were HPV-negative and HPV-positive, 21.6% of HPV-positive patients experienced masturbation, and 15.1% in the HPV-negative group (p=0.044). While severe depression was 4.9% in HPV-positive women, this rate was 1.4% in HPV-negative women (p=0.002). Orgasmic function, sexual intercourse frequency, and sexual desire did not differ between HPV-positive and HPV-negative groups (all p>0.05). Comparison of Libido Scoring System domains, anxiety, and depression in HPV-positive and HPV-negative women are demonstrated in Table 3.

Table 3. Comparison of Libido scoring system (LSS), anxiety and depression across groups

	HPV (-) n (%)	HPV (+) n (%)	p
Sex Frequency			
None	51 (17.5)	60 (21.2)	0.691
≤once a week	133 (45.5)	127 (44.9)	
twice a week	70 (24)	61 (21.6)	
≥ twice a week	38 (13)	35 (12.4)	
Masturbation			
Does not	248 (84.9)	222 (78.4)	**0.044 ***
Does	44 (15.1)	61 (21.6)	
Who starts the sexual activity			
Always Partner	49 (16.8)	48 (17)	0.819
Mostly Partner	208 (71.2)	195 (68.9)	
Mostly Herself	31 (10.6)	37 (13.1)	
Always Herself	4 (1.4)	3 (1.1)	
Orgasm			
Never	60 (20.5)	70 (24.7)	0.060
Sometimes	158 (54.1)	123 (43.5)	
Frequently	66 (22.6)	76 (26.9)	
Always	8 (2.7)	14 (4.9)	
Libido Scoring System			
Loss of Libido	67 (22.9)	78 (27.6)	0.077
Low Libido	157 (53.8)	122 (43.1)	
Good Libido	65 (22.3)	80 (28.3)	
High Libido	3 (1)	3 (1.1)	
Depression			
Minimal Depression	192 (65.8)	146 (51.6)	**0.002 ***
Mild Depression	57 (19.5)	73 (25.8)	
Moderate Depression	39 (13.4)	50 (17.7)	
Severe Depression	4 (1.4)	14 (4.9)	
Anxiety			
Minimal Anxiety	167 (57.2)	140 (49.5)	0.071
Mild Anxiety	65 (22.3)	67 (23.7)	
Moderate Anxiety	46 (15.8)	48 (17)	
Severe Anxiety	14 (4.8)	28 (9.9)	

Abbreviations: HPV, human papillomavirus; BAI, Beck Anxiety Inventory; BDI, Beck Depression Inventory; LSS, Libido Scoring System

4. Discussion

The present study indicated that HPV-positive women had more sexual interest than HPV-negative women. We found no association between positive HPV test results and the domains of sexual desire, frequency of sexual activity, and orgasmic experience. We found higher depression levels in HPV-positive women compared to the control group. However, we did not observe a significant difference in anxiety levels between groups.

The cervical cancer screening programs are generally population-based interventions. Globally, millions of women receive HPV-positive test results at cervical screening programs each year. World Health Organization recognizes HPV as a global burden, associated with an estimated 570,000 new cases of cervical cancer and leading to 311,000 deaths worldwide (16). Additionally, a diagnosis of HPV may be associated with adverse psychological, behavioral, and sexual effects; this translates into a significant number of individuals being exposed to negative psychological and behavioral consequences. This has led many researchers to investigate the impact of an HPV diagnosis on mental health and sexual behavior. To date, much work in this field has been carried out, yet, there have still been contradictory findings due to differences in the study designs, heterogeneity in the cervical screening programs, and lack of a well-accepted definition of emotions (17). For instance, the randomized controlled trial on the psychosexual impact of HPV testing in primary cervical screening conducted in 2008 by Kitchener et al. from England found that psychosexual functioning did not differ between those who received an HPV+ or HPV− result (18). In another study reported by Maggino et al. among 72 women attending a gynecological clinic, there were no significant differences in interest in sex, sexual arousal, sexual satisfaction, frequency of sex, sexual satisfaction, and sexual pleasure/orgasm between women who were HPV+ and HPV− over 6 to 12+ months (10). A third study by Reed et al. found no difference in sexual satisfaction, frequency of sex, or sexual arousal (19). These findings are in contrast to those reported by Ferenidou on 51 women whose HPV-positive results were recently disclosed, 41% reported a decrease in the frequency of sex, 22% reported feeling dissatisfied with their sex life, 22% reported difficulty in reaching orgasm, and decreased sexual desire following an HPV diagnosis (20). Our findings suggest that among women with HPV+ or HPV- results, testing positive for HPV may have no significant adverse sexual effects on both frequencies of sex, sexual desire, and orgasm; however, the difference reaches significance only with a sexual interest in HPV+. While there is a large amount of clinical, socioeconomic, and demographic data on HPV testing, this particular area of psychosexual outcomes is lacking and might have been overlooked to date. One possible explanation was that the study participants ranged from 18 to 72 years. Since sexual function in postmenopausal women over the age of 50 is different from women of childbearing age, this may affect the results obtained.

The majority of prior research found an increase in psychological morbidity, particularly short-term anxiety, following HPV-positive test results (10, 21, 22). However, we

did not observe a significant difference in anxiety levels after an HPV-positive test result. On the contrary, we found higher depression levels in women diagnosed with HPV compared to those without HPV. Our results support previous research in this area (18, 23). As we evaluated the patients shortly after their HPV-positive results, anxiety and depression may not have yet developed in such a short time. In addition, patients with severe lesions undergoing treatment for HPV may have experienced a deterioration in mental health. Unfortunately, we were unable to evaluate patients after a gynecological procedure. Moreover, it should be kept in mind that clinical and statistical heterogeneity makes it difficult to conclude the psychosexual impact of testing HPV-positive (23, 24).

Several limitations to this study are worth considering when interpreting the results. Firstly, the study's cross-sectional design did not allow us to infer causality between testing positive for HPV and emotional response. Due to the short duration of the study, we could not conclude the long-term effects of HPV infection on mental health status and sexual dysfunction. Unfortunately, we were unable to obtain relevant data on the patients' cytologic results, presence of genital warts, HPV vaccination status, and their partner's or husband's sexual function. Lastly, considering Türkiye's context, these findings may not apply to other settings due to cultural, religious, and ethnic diversity. Notwithstanding these limitations, this study has several strengths in that it has a large sample size comparing healthy and HPV-positive subjects that maximizes the interpretability of the study findings. A reliable, validated instrument was used to measure anxiety, depression, and sexual dysfunction since emotional responses are difficult to categorize, and healthcare providers and patients are reluctant to talk about sexual issues and sexually transmitted diseases.

The present study was designed to evaluate the psychosexual impact of testing positive for HPV. This study showed that being diagnosed with HPV made no significant difference in anxiety levels and sexual function, except for sexual interest, but increased depression scores. We believe that our findings add substantially to our understanding of the short-term psychosexual consequences of being diagnosed with HPV. Considering the large number of women participating in cervical cancer screening programs each year and are likely to be affected by psychosocial and sexual aspects, further studies are needed to investigate the psychological, social, and sexual effects of being diagnosed with HPV.

Ethical statement

The Research Ethics Committee of the Zeynep Kamil Training and Research Hospital approved the study (Approval number: 21.06.2021-21/211).

Conflict of interest

The authors have no conflicts of interest.

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Authors' contributions

Concept: E.K., M.A., Design: E.K., M.A., Data Collection or Processing: U.K.Ö., C.M.A., D.Y., Analysis or Interpretation: K.N.B., E.K., Literature Search: E.K., U.K.Ö., M.A., C.M.A., D.Y., K.N.B., Writing: E.K., U.K.Ö., M.A., C.M.A., D.Y., K.N.B.

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Correlation of blood glucose and prostatic-related parameters in patients with prostate enlargement

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Abstract

Enlarged prostate, either in benign prostatic hyperplasia or prostate cancer is the predominant urological disease affecting older men. Metabolic syndrome including high blood glucose was considered to take part in the development of prostate enlargement, although their association is still unclear. This study aims to investigate the correlation between blood glucose with prostatic-related parameters. A cross-sectional study was conducted involving 91 men with prostate enlargement from Urology Polyclinic, Tabanan Hospital, Bali Province, Indonesia. Prostatic-related parameters collected include patient's international prostate symptoms score using the questionnaire, prostate-specific antigen and random blood glucose analysis from serum blood sample, and prostate volume determined with transabdominal ultrasonography. Patient's mean age was 65.4 ± 10 years, mean prostate volume 52.7 ± 26 ml, mean prostate-specific antigen (PSA) 9.2 ± 17 ng/ml, mean international prostatic symptoms score (IPSS) 14.31 ± 7.1, mean random blood glucose 121.1 ± 40.5 mg/dl. This study found weak positive correlation between random blood glucose and prostate volume ($r=0.269$; $p=0.01$). PSA and prostate volume showed moderate positive correlation ($r=0.365$; $p<0.001$). No correlation between random blood glucose with PSA or IPSS was found in this study. This study found random blood glucose has positive correlation with prostate volume. We recommend enhancing blood glucose control to prevent the progress of prostate enlargement.

Keywords: blood glucose, prostate, prostate-specific antigen, urology

1. Introduction

Enlarged prostate is the most common cause of lower urinary tract symptoms in elderly men. As life expectancy increases, the elderly population is increasing and so does the incidence of prostate enlargement. Research in Asia showed the prevalence of benign prostatic hyperplasia (BPH) increases after the age of 40 years, with a prevalence of 8-60% at the age of 90 years (1). Prostate enlargement causes various morbidity and high financial burden, thus making prevention efforts mandatory. Risk factors thought to affect prostate enlargement are divided into non-modifiable factors and modifiable factors. Non-modifiable factors include age, race, and genetics, while modifiable factors include sex steroid hormones, metabolic syndrome, obesity, diabetes, physical activity, diet, and inflammation (2). Investigating the association of those risk factors with prostate enlargement, particularly the modifiable one, can be beneficial to prevent and treat prostate enlargement.

Besides the increase in the prevalence of prostate enlargement, diabetes mellitus prevalence was also increasing (3). By 2030, the global prevalence of type 2 diabetes mellitus is expected to increase up to 7079 cases per 100,000 people, demonstrating an ongoing rise in all regions (4). Type 2 diabetes mellitus was commonly seen in developed countries, but there are alarming tendencies in lower-income nations. The prevalence of diabetes in hospital-based studies may be underestimated because blood glucose measurements were never carried out by many people until there is a symptom. A lot of diabetic patients also do not comply with treatment, resulting in consistently high blood glucose (5).

Evidence showed that hyperglycemia is considered to take part in the development of prostate enlargement (6). Although, the association between the two is still unclear. Understanding the potential relationship of hyperglycemia with prostate enlargement could improve men's quality of life significantly. This study aims to investigate the correlation between random blood glucose with prostatic-related parameters including

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prostate volume, international prostate symptoms score (IPSS), and prostate-specific antigen (PSA).

2. Materials and Method

This study was a cross-sectional study conducted at the Urology Polyclinic of Tabanan Hospital, Bali Province, Indonesia from January-March 2022. This study methodology has been approved by the Institutional Review Board of Tabanan Hospital with the ethical clearance letter number 800/3102/KEPEG/RSUD. Informed consent has been obtained from all patients. We included all patients with prostate enlargement (prostate volume >20 ml) which was determined by transabdominal ultrasonography. Patients with repeated admission, incomplete data, and who refused to participate were excluded from this study. Research participant selection was done using total sampling methods. The independent variable in this study was random blood glucose (RBG), while the dependent variables were prostate volume, IPSS, and PSA. The patients were asked to fill out and submit the IPSS questionnaire, with the total score being the sum of the scores in each question. Serum blood samples were used to measure PSA and RBG on a clinical chemistry analyzer (Thermo Scientific Indiko Plus, Finland). Prostate volume was measured by transabdominal ultrasonography (GE® LOGIQ P9, South Korea) and calculated from transverse images by using the prostate ellipsoid formula (0.524 × height × width × length).

Data analysis was performed by using IBM Statistical Package Software for Social Science® version 23.0. The normality of numerical data distribution was measured using the Kolmogorov-Smirnov test. The correlation between random blood glucose and prostate-related parameters was analyzed using the Pearson correlation test for normally distributed data. Otherwise, the Spearman correlation test will be used. Further analysis was carried out using multiple linear regression analysis to investigate the association between prostate volume and other parameters. A two-tailed $p<0.05$ was considered a statistically significant association.

3. Results

There were a total of 114 men with prostate enlargement visiting the Urology Polyclinic at Tabanan Hospital from January-March 2022. 23 patients were excluded due to refusal to participate. There were a total of 91 patients who fulfilled the inclusion criteria. Baseline characteristics of the patients were shown in Table 1.

Table 1. Baseline characteristics

Variables (n=91)	Mean	SD	Range
Age (years)	65.41	10.074	48-91
Random Blood Glucose (mg/dL)	121.14	40.469	79-303
IPSS	14.31	7.136	1-28
PSA (ng/mL)	9.20	17.01	0-123
Prostate Volume (mL)	52.69	26.09	20.4-148

IPSS: International Prostate Symptoms Score, PSA: Prostate-Specific Antigen

Kolmogorov-Smirnov test revealed that the data was not normally distributed, therefore correlation analysis was carried out using Spearman Test. A significant correlation between RBG and prostate volume was found, but there was no correlation between RBG with IPSS and PSA (Table 2).

Table 2. Correlation test between variables

Variables	Correlation	p
RBG and IPSS	0.137	0.197
RBG and PSA	0.063	0.551
RBG and Prostate Volume	0.269	0.010

RBG: random blood glucose

Random blood glucose was found to be associated with prostate volume only, therefore only prostate volume was investigated in the multivariate analysis. Multiple linear regression revealed that there was a significant correlation between random blood glucose and prostate volume. There was also a significant correlation between PSA and prostate volume (Table 3).

Table 3. Multivariate analysis of possible risk factors affecting prostate volume

Variables	Correlation	95% CI	p
Age	0.056	-0.378 - 0.670	0.582
Random Blood Glucose	0.279	0.053 - 0.307	0.006
IPSS	0.062	-0.500 - 0.954	0.537
PSA	0.239	0.053 - 0.680	0.022

IPSS: International Prostate Symptoms Score, PSA: Prostate-Specific Antigen

4. Discussion

This study revealed that random blood glucose has a positive correlation with prostate volume. This finding was consistent with previous studies which found that there was an association between hyperglycemia and prostate size and that abnormal glucose was correlated with prostate enlargement. In patients younger than 70 years, compared with the normal glucose group, the adjusted odds ratio for prostate volume enlargement in the prediabetic group and the diabetic group was 2.27 (95% CI 1.29 - 4.00) and 4.74 (95% CI 2.18 - 10.30) respectively (6). Prostate volume was found to be higher in patients with HbA1c levels ≥6.5% (45.69±3.97 vs. 36.64±3.30) (7). Another study by Qu et al. showed that BPH patients with fasting glucose ≥7 mmol or oral glucose tolerance test >110 mg/dl had increased prostate volume (41.18 vs 51.52 $cm^{3,}$ p=0.005) (8). Opposite findings were reported in the study by Zhang et al. that found no correlation between fasting blood glucose and HbA1c with prostate volume, except fasting insulin (r=0.421). In the study by Yim et al., increased prostate volume is associated with fasting blood glucose (9).

While the precise mechanism was still unclear, there are several proposed mechanisms on how blood glucose could impact prostate enlargement. First, hyperglycemia was found to induce an inflammatory state in vitro and in vivo (10). A study showed an increase in c-reactive protein and increased reactive oxygen species in hyperglycemic patients compare to control (11). Inflammatory cells are frequently found in BPH and are also present in prostate cancer. Findings of T lymphocytes, B lymphocytes, and macrophages were reported, which are associated with prostate size in prostatectomy specimens. Second, hyperglycemia was found to be related to

raised sympathetic nerve activity, which increased the smooth muscle tone of the prostate and subsequent blockage of the bladder outlet and cause obstructive symptoms. Third, related to the increase of sex hormones in prostate cells. Hyperglycemia leads to hyperinsulinemia which is related to a lower level of sex hormone-binding globulin and leads to the increased quantity of sex hormone entering prostatic cells, thus influencing the growth of the prostate in size (10). Another proposed mechanism is that hyperinsulinemia (as a result of hyperglycemia) might induce prostate growth through the insulin-like growth factor (IGF) pathway that stimulates cell proliferation. Additionally, elevated insulin levels (as in diabetic patients) increase catecholamine levels in plasma and tissue, which may have a trophic effect causing an enlarged prostate by lowering the progression of apoptosis (12).

This study revealed that there was no correlation between blood glucose and IPSS. Chen et al. revealed that IPSS were higher in patients with HbA1c levels ≥6.5% (16.30±3.31 vs. 9.87±1.0) (7). Another study by Ferreira et al. stated that patient with type 2 diabetes mellitus has significantly higher IPSS (13). This discrepancy might be due to the difference in blood glucose parameters being used. This study also revealed that there was no correlation between blood glucose and PSA, although a previous study found PSA was higher in diabetic compared to the non-diabetic group (3.23 vs 1.94, p=0.013) (8). A study by Duarsa et al. in the Balinese population also found that increased PSA and age were correlated with prostate volume progression (14). Meanwhile, age was not correlated with prostate volume in this study.

The blood glucose parameter was chosen over the history of diabetes mellitus because of several conditions met in our patient. First, we were lacking the diagnostic standard to diagnose diabetes mellitus such as fasting blood glucose, because most of the patients were not fasting during the examination. Second, a lot of patients who admitted to not having a history of diabetes mellitus, never actually check their blood glucose levels which turned out to be high. Third, patients with a history of diabetes mellitus may have low blood glucose due to diabetic medication and still have high blood glucose due to non-adherence to medication.

This study has several limitations. First, this study design is cross-sectional and thus was unable to reveal causal associations between blood glucose and the prostatic parameters measured. Second, this study was a single institutional study and all patients have not undergone transurethral resection of the prostate (TURP) procedure, therefore in this study, we were unable to identify the cause of the prostate enlargement and differentiate between BPH and prostate cancer by biopsy. Although, prostate cancer was found to be associated with a higher prostate volume than BPH (15). Third, several variables that might be a significant factor for prostate volume such as previous medication was also not investigated and might be confounding factors. 5-a reductase inhibitor was known to be associated with prostate volume reduction (16), metformin was also found to inhibit prostate growth in an animal study (17), while insulin is associated with prostate cell growth (18). Oral hyperglycemic drugs were also not investigated and may have affected the levels of blood glucose measured.

This study showed that random blood glucose has a positive correlation with prostate volume. This study has demonstrated the necessity for blood glucose control to prevent the progress of prostate enlargement. We recommended further studies conducted with a better methodological approach, a larger sample size, and including more variables in a multivariate analysis, to better understand the association between blood glucose and prostate volume.

Ethical statement

This study methodology has been approved by the Institutional Review Board of Tabanan Hospital with the ethical clearance letter number 800/3102/KEPEG/RSUD.

Conflict of interest

The authors declare there is no conflict of interest.

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Authors' contributions

Concept: I.B.G.T.Y.Y., A.A.S.M.P., Design: I.B.G.T.Y.Y., A.A.S.M.P., P.M.W.T., Data Collection or Processing: I.B.G.T.Y.Y., I.B.T.Y., A.A.N.O.D., K.C.M., Analysis or Interpretation: I.B.G.T.Y.Y., A.A.S.M.P., P.M.W.T., Literature Search: I.B.G.T.Y.Y., A.A.S.M.P., P.M.W.T., Writing: I.B.G.T.Y.Y., A.A.S.M.P.

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Relationship between psychiatric symptoms, childhood traumas, and types of crime of convicts in Elazig closed prisons

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Abstract

Our study aimed to examine the childhood traumas and psychiatric symptoms of prisoners and their relationship with crime types. Persons convicted in Elazig Penitentiary Institutions were included in the study. The Demographic and Clinical Evaluation Form, Beck Depression Inventory (BDI), Beck Anxiety Inventory (BAI), Childhood Trauma Questionnaire (CTQ) and Symptom Checklist (SCL-90) were administered to all participants. A total of 370 people were included in the study. For prisoners who have been imprisoned before, CTQ-physical abuse, BAI, SCL-90-somatization, anxiety, and hostility subscale scores were higher than those who entered the prison for the first time (p values: 0.020, 0.003, 0.016, 0.017, 0.047, respectively). Prisoners with a family history of prison entry had higher SCL-90 test all subscale scores, total scores and CTQ physical abuse subscale scores than the group without a family history of prison entry. Those exposed to violence in childhood and those who had attempted suicide before had higher scores on all scales. Those with a history of alcohol and substance use had higher total scores on the CTQ-emotional abuse and sexual abuse scale. According to the type of crime committed, only the CTQ-sexual abuse subscale scores differed. These subscale scores of the prisoners involved in more than one crime were higher than those involved in a single crime (p=0.030). The CTQ-sexual abuse subscale scores of those who were involved in the crime of willful homicide were calculated to be high (p=0.030). It was thought that preventing abuse, violence, and traumas in childhood may be necessary in reducing the tendency to crimes.

Keywords: prison, prisoner, depression, anxiety, psychiatric symptoms

1. Introduction

Crime defines all of the actions for which criminal law sanctions are envisaged. The Criminal behavior concept is as old as human history, affects all societies, and is widespread (1). Individuals obliged to stay in prison after any crime and legally deprived of their freedom are defined as “detainees”. A “convict” is a person whose conviction has been decided by judicial organs (2). According to the Ministry of Justice’s data in Turkey for 2022, there were 314.502 detainees and 275.965 convicts (3). According to the 2018 data from the Turkish Statistical Institute (TSI), the number of people in prisons increased by 10.1% in 2019 (4).

The individual imprisoned for the crime committed is alienated from society. For the detainees and convicts, the crime committed, the punishment received, and being in a closed environment are stress factors. When all these come together, it is reported that the incidence of mental problems in detainees and convicts increases (5). Depressive disorder, schizophrenia, other psychotic disorders and anxiety disorders were found to be high in detainees and convicts in studies in the literature (5, 6, 7). It was also reported that the frequency of traumas and traumatic experiences was high in studies conducted with detainees and convicts (1). Also, detainees and convicts who have inadequate coping attitudes have difficulty overcoming their problems and have a risk of suicide (5, 8). Studies were conducted on coping attitudes, the effects of regular exercise programs on violent behavior, and the severity of depressive and anxiety symptoms in prisoners and convicts in prisons in our country (4, 9, 10, 11). However, to the best of our knowledge, a study in which general psychiatric symptoms and childhood traumas were evaluated together and their relationship with crime types was not detected in the literature. In light of this information, the present paper examined the childhood traumas and psychiatric symptoms of convicts in prisons and their relationship with crime types.

2. Patients and Method

2.1. Sampling and the intervention

The study was conducted in Elazig E Type, T Type, No 1 High-Security, and No 2 High-Security Penal Institutions. People convicted in Elazig E Type, T Type, No 1 High-Security, and No 2 High-Security Prisons were informed about the study.

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The study included people who agreed to participate, were literate and could fill in the forms. People who did not agree to participate in the study, those who had mental retardation or neurodegenerative diseases, and those who were illiterate were excluded.

The approval of the Fırat University Non-Interventional Local Ethics Committee was received for the study on 23.05.2019 with the number 09/02 and from the Republic of Tükiye Ministry of Justice with the date 12.08.2022 and the number 112444. The study was conducted in accordance with the Declaration of Helsinki principles. Each participant signed a written informed consent form. Then, the demographic and clinical evaluation form, Beck Depression Inventory (BDI), Beck Anxiety Inventory (BAI), Childhood Trauma Questionnaire (CTQ), and Symptom Checklist (SCL-90) were applied.

2.2. Data collection tools

The Demographic and Clinical Evaluation Form: This form was prepared by the researchers and includes demographic data on age, gender, marital status, and education levels. It also includes clinical evaluation questions such as how many years the participants had been in prison, whether they had received psychiatric treatment, whether they had taken substances before, and if so, what they used.

Beck Depression Inventory (BDI): Developed by Beck to measure the presence and severity of depressive symptoms as a self-report scale with twenty-one questions. As the calculated total score increases, the severity of depression increases. The Turkish validity and reliability study was conducted by Hisli et al. (12, 13).

Beck Anxiety Inventory (BAI): It was developed by Beck et al. to determine the frequency and severity of anxiety symptoms experienced by an individual on a self-report scale with 21 questions, scored between 0-3. Ulusoy et al. adapted the inventory into Turkish (14, 15).

Childhood Trauma Questionnaire (CTQ): The scale evaluates the experiences of abuse and neglect during childhood and adolescence. Developed by Bernstein et al., it is a self-report scale with twenty-eight questions. Turkish validity and reliability study was performed by Şar et al. It has five sub-dimensions; emotional neglect, emotional abuse, physical neglect, physical abuse, and sexual abuse. A total scale score is calculated based on the sub-dimension scores (16, 17).

Symptom Checklist (SCL-90): It is a ninety-question, 11-subscale, self-report scale. Symptom distribution is evaluated with subscale scores with nine sub-dimensions consisting of somatization, obsessive-compulsive symptoms, interpersonal sensitivity, depression, anxiety, anger/hostility, phobic anxiety, paranoid thoughts, and psychotic symptoms. In addition to these subscales, there is an additional scale that evaluates sleep and eating problems and a general subscale that makes a general assessment. The net score is obtained by dividing the calculated score of each subscale by the number of questions. The scores of one or more indicate psychopathological conditions. The scale was developed by Derogatis et al. and translated into Turkish by Dağ (18, 19).

2.3. Statistical analysis

All the analyzes were evaluated with the Statistical Package for Social Sciences version 22 program. The descriptive analyzes of the demographic data and applied scales were performed. Numerical data were presented as mean and standard deviation, and categorical data were presented as numbers and percentages. The Kolmogorov-Smirnov Test was used to analyze whether the data were suitable for normal distribution, and it was found that the data did not fit the given normal distribution. The Mann-Whitney U Test was used to compare the scales between two independent groups, and the Kruskal-Wallis Test was used to compare the scales between three or more independent groups. The linear relationship between the variables was analyzed using the Spearman Correlation Analysis. When a ***p***-value calculated for our study was less than 0.05, it was considered statistically significant.

3. Results

3.1. Demographic characteristics of the participants

The forms were given to 500 prisoners for the study, and 370 individuals who met the inclusion criteria were recruited. All participants were male, and their mean age was calculated as 34.51±10.26. The occupations before entering prison were as follows. The majority of the participants were self-employed (craftsmen) (151 people), and their economic situations were bad (225 people) with less than one thousand TL. The crimes due to which the prisoners were imprisoned were as follows: 131 people (35.40%) were selling drugs, 81 (21.89%) were injured, 57 (15.40%) were deliberately murdered, and 53 (14.32%) were extortion. One hundred people (27.02%) were involved in multiple types of crime.

Receiving psychiatric treatment status before entering prison was as follows: A total of 66 (17.83%) participants received psychiatric treatment, 304 (82.16%) did not have psychiatric treatment, and 47 (12.70%) of those who received psychiatric treatment received inpatient treatment. Although 60 (16.21%) had people who received psychiatric treatment in their family, 310 (83.78%) did not. While 272 (73.5%) never experienced self-harm/suicide attempt in any period of their life, 98 (26.5%) experienced self-harm/suicide attempt. The ways the participants tried suicide were as follows: 33 (8.9%) tried to commit suicide with drugs, 26 (7%) with sharp objects, 12 (3.2%) with firearms, 11 (3.0%) jumped off a height, and the remaining 16 participants tried multiple methods. The demographic characteristics of the participants are given in Table 1.

3.2. The scale scores of the participants

The Beck Depression Inventory scores were calculated as 19.21±13.19. The number of participants who scored 30 points

or more was 95 (25.67%). For BAI, the calculated value was 16.99±13.87. Two hundred twenty-seven participants (61.35%) received a score of 1 or higher in the SCL-90 supplementary items in which physiological symptoms such as appetite and sleep were questioned. SCL-90 general psychopathology level was calculated as one or more in 194 (52.43%) participants. The mean, standard deviation, and minimum-maximum values of the participants' scale scores are given in Table 2.

Table 1. The demographic characteristics of the participants

		N	(%)
Marital status	Single	188	50.8
	Married	134	36.2
	Divorced	40	10.8
	Spoused dead	8	2.2
Level of education	Literate	29	7.8
	Primary school graduate	76	20.5
	Secondary school graduate	130	35.1
	High school graduate	91	24.6
	Üniversity graduate	19	5.1
	Still in education	25	6.8
Occupation before going to prison	Employee	91	24.6
	Craftsman	151	40.8
	Civil servant	15	4.1
	Unemployed	94	25.4
	Retired	5	1.4
	Farmer	9	2.4
	Student	5	1.4
Income status (TL)	< 1000	225	60.8
	1000-2000	79	21.4
	2000-3000	41	11.1
	3000-4000	8	2.2
	> 4000	17	4.6
Type of prison	Open prison	144	38.9
	E type	114	30.3
	T type	112	30.8
Entering prison before	Yes	237	64.1
	No	133	35.9
A person who went to prison in the family	Yes	149	40.3
	No	221	59.7
Was s/he exposed to violence as a child?	Yes	160	43.2
	No	210	59.7
Type of violence to which they were exposed	Physical violence	130	35.1
	Sexual violence	3	0.8
	Other	27	7.3
Alcohol intake before entering prison	Yes/No/Quit	151/59/160	40.8/15.9/43.2
Smoking status	Yes/No/Quit	288/40/42	77.8/10.81/11.35
Drug intake before entering prison	Yes/No/Quit	170/47/153	45.9/12.7/41.4
Type of substance used	Marijuana	106	28.6
	Heroin	7	1.9
	Cocaine	4	1.1
	Methamphe amine	6	1.6
	Green prescription drug	5	1.4
	Multiple substances/drugs	69	18.6

Table 2. The scores received in the scales applied to the participants

	Mean	SD	Min-Max
BDI	19.21	13.19	0-60
BAI	16.99	13.87	0-57
SCL-90 Somatization	1.29	0.96	0-4
SCL-90-Anxiety	1.10	0.93	0-3.9
SCL-90-Obsession	1.34	0.90	0-4
SCL-90-Depression	1.26	0.94	0-4
SCL-90-Interpersonal sensitivity	1.21	0.94	0-3.69
SCL-90-Psychoticism	0.96	0.84	0-4
SCL-90-Paranoid thought	1.30	1.01	0-3.6
SCL-90-Hostility	1.11	1.04	0-3.63
SCL-90 Phobic anxiety	0.73	0.79	0-4
SCL-90-Addition	1.43	0.95	0-4
SCL-90-General psychopathology	1.19	0.82	0-4
CTQ-Emotional abuse	8.27	4.02	5-24
CTQ Physical abuse	8.21	4.49	5-25
CTQ Physical neglect	9.06	3.42	5-21
CTQ Emotional neglect	12.80	5.22	5-27
CTQ Sexual abuse	6.68	3.68	5.23
CTQ- Total	45.03	15.33	25-109
CTQ-Denial	0.79	0.83	0-3

3.3. The relationship between clinical variables and scale scores

The relationship between the participants' demographic data and clinical variables and the scale scores was examined. In the results, CTQ physical abuse score was higher in singles than in married people (p=0.046). Prisoners imprisoned before had CTQ-physical abuse, BAI, SCL-90-somatization, anxiety, and hostility subscale scores were higher than the inmates who entered prison for the first time (p values: 0.020, 0.003, 0.016, 0.017, 0.047, respectively). Prisoners whose families had a prison entrance had higher CTQ physical abuse scores than those with no family history of prison entry (p=0.009). They also had higher SCL-90 somatization, anxiety, obsession, depression, interpersonal sensitivity, psychoticism, paranoid thought, hostility, phobic anxiety, additional symptoms, and general symptom screening subscale scores than the group without prison entry in their families (p values=0.015; 0.045; 0.003; 0.010; 0.046; 0.001; 0.004; 0.013; 0.047; 0.000; 0.003, respectively). All scale and subscale scores of prisoners exposed to violence in childhood were high. Prisoners who had alcohol intake before entering prison also had higher CTQ physical abuse, emotional abuse, sexual abuse, total trauma scores, and SCL-90 hostility subscale scores than those without alcohol intake (p values = 0.000; 0.005; 0.001; 0.004; 0.005, respectively). Prisoners who used substances before entering the prison had higher CTQ-emotional abuse, physical abuse, sexual abuse, total scores of the scale and SCL-90 somatization, anxiety, psychoticism, hostility, and general

subscale scores than the group without substance use (p values = 0.005; 0.000; 0.013; 0.012; 0.034; 0.047; 0.021; 0.005; 0.042, respectively). All scale and subscale scores of the prisoners who had attempted suicide before were higher. According to the type of crime committed by the prisoners, only the CTQ-sexual abuse subscale scores differed. The subscale scores of prisoners involved in many different crimes were higher than those involved in one type of crime (Mean Rank=176.74; p=0.030). Those who were involved in the crime of willful homicide had high CTQ-sexual abuse subscale scores (Mean rank=163.26; p=0.030).

3.4. Spearman correlation analysis results

The depression and anxiety scale scores of the participants were positively and moderately correlated (r=.582; p=0.000). All sub-dimensions of the CTQ were positively correlated with BDI and BAI. BDI and BAI were moderately positively correlated with all subscales of the symptom screening list (SCL-90). The Spearman Correlation Analysis results of the participants are given in Table 3.

Table 3. Spearman correlation analysis results

	Prison duration	BDI	BAI	CTQ-P Neglect	CTQ-E Neglect	CTQ-P Abuse	CTQ-S Abuse	CTQ-E Abuse	CTQ-Total
Prison duration	1	.097	.221*	.136*	-.012	.083	.043	.031	.078
BDI	.097	1	.582*	. 364*	.228*	.351*	.188*	.374**	.409*
BAI	.220*	.582*	1	.393*	.183*	.417*	.201*	.451*	.428*
SCL-90-1	.195*	.502*	.746*	.312*	.116*	.363*	.189*	.367*	.347*
SCL-90-2	.161*	.529*	.746*	.389*	.185*	.419*	.266*	.455*	.441*
SCL-90-3	.157*	.491*	.635*	.338*	.084	.376*	.215*	.404*	.359*
SCL-90-4	.127*	.576*	.642*	.362*	.188*	.363*	.231 *	.423*	.399*
SCL-90-5	.164	.620*	.699*	.379*	.159*	.243*	.406*	.460*	.477*
SCL-90-6	.157*	.486*	.626*	.362*	.191*	.387*	.292*	.446*	.424*
SCL-90-7	.131*	.440*	.579*	.352*	.134*	.371*	.197*	.431*	.381*
SCL-90-8	.178*	.453*	.583*	.394*	.150*	.450*	.267*	.465*	.455*
SCL-90-9	.155*	.438*	.596*	.334*	.207*	.326*	.262*	.392	.399*
SCL-90-10	.147*	.524*	.596*	.290*	.085	.339*	.182*	.379*	.328*
SCL-90-11	.176*	.559*	.726*	.390*	.160*	.421*	.257*	.469*	.435*

Abbreviations in the table: BDI: Beck Depression Inventory, BAI: Beck Anxiety Inventory, SCL-90-1: Symptom Checklist-Somatization; SCL-90-2: Symptom Checklist-Anxiety; SCL-90-3: Symptom Checklist-Obsession; SCL-90-4: Symptom Checklist-Depression; SCL-90-5: Symptom Checklist-Interpersonal sensitivity; SCL-90-6: Symptom Checklist-Psychoticism; SCL-90-7: Symptom Checklist-Paranoid thought; SCL-90-8: Symptom Checklist- Hostility; SCL-90-9: Symptom Checklist-Phobic anxiety; SCL-90-10: Symptom Checklist-Appendix; SCL-90-11: Symptom Checklist-general symptomatology; CTQ-P. Neglect: Childhood trauma questionnaire-Physical neglect; CTQ-E. Neglect: Childhood Trauma questionnaire-Emotional neglect; CTQ-P. Abuse: Childhood trauma questionnaire-Physical abuse; CTQ-E. Abuse: Childhood trauma questionnaire-emotional abuse; CTQ-S. Abuse: Childhood trauma questionnaire-Sexual abuse; CTQ-Total: Childhood trauma questionnaire-total score. Spearman Correlation Analysis Test was used in the calculations. The ***r*** values are given in the table. *p<0.05

4. Discussion

The present study examined the psychiatric symptoms of the prisoners, the traumas they experienced during their childhood, and their relationship with the types of crimes. The majority of our participants were single and low-income people. The number of people who received outpatient and inpatient psychiatric treatment before entering prison was relatively low. Suicide attempt was experienced at a rate of 26.5% in the past. In the evaluations made with their current state, the depression scores of the participants were calculated as 25.67%, at a level that can be called severe depression. The psychiatric symptom scores of those who had been in prison before were higher. The SCL-90 somatization, anxiety, hostility scores, BAI scores, and CTQ-physical abuse scores were higher in those with prison admission before. Similarly, some subscale scores of those with a family history of prison entrance were high. Also, prisoners exposed to violence during childhood and those who had attempted suicide before had higher scores on all scales. When the results were examined according to the types of crimes committed by the prisoners, only the CTQ-sexual abuse subscale scores differed. These subscale scores of the prisoners involved in multiple crimes were higher than those involved in one. Finally, those who were involved in the crime of willful homicide had higher CTQ-sexual abuse subscale scores when compared to the prisoners who were involved in other crimes.

Both the crime and closed environment are a source of stress for the individual in prison because of a crime committed and whose freedom is restricted, making the prisoners mentally risky groups (5). The results obtained here confirm this data. The present study calculated the SCL-90 general psychopathology level as one or higher in 194 (52.43%) prisoners. An SCL-90 general symptom level score of 1.00 and above indicates that the symptoms are at a psychopathological level (18, 19). It was found that 227 prisoners (61.35%) received a score of 1 or higher from the SCL-90 supplementary sub-scale, in which physiological symptoms such as sleep quality and appetite were questioned. In a study very similar to the present study, SCL-90 additional symptoms, somatization,

and depression scores of a group of convicts were found to be in the first three ranks (5). Additional symptoms, obsession, and paranoid thought made up the first three lines in our results and were followed by somatization and depression. It was considered that psychiatric symptoms might increase with stress factors such as being in a closed environment and punishment.

It was shown that people become lonely and depressed after a while in prisons where freedoms are restricted, and inmates live within the framework of specific rules (5, 20). It was also determined that suicidal thoughts are high (5, 21). In a study conducted abroad with 236 newly imprisoned people, anxiety scores were high, with a rate of 66.5%, and depression scores with a rate of 85.2% (22). Similarly, it was shown in another study that prisoners have a risk of suicide rate of 13.2%. The same study also listed depressive disorder, dysthymic disorder, panic disorder, generalized anxiety disorder diagnoses, and childhood traumas as predictors for the probability of suicide (23). Another study conducted abroad reported the rates of depression in prisoners in developing and developed countries as 39.2% and 33.1%, respectively. Moreover, studies that used severity-determining scales for depressive disorder have shown a significant rate of 19.1% to 54% (24). In our results, the depression scores were high in the third rank for SCL-90. Also, the BDI scores of the prisoners participating in the study were calculated at a rate of 25.67%, at a level that can be called severe depression, and the participants tried suicide attempts with a rate of 26.5%. Although the scale scores for both depressive symptoms and suicide were high, people were less likely to seek treatment. In addition to all these, all scale scores of prisoners exposed to childhood violence were higher than those who did not experience violence. In other words, the depression scores of prisoners exposed to violence as children were much higher. These rates obtained here were generally compatible with the literature, and this was interpreted as an increased susceptibility to depression in deprived prisoners.

When the literature was reviewed, the number of prison entrances was examined in a limited number of studies, and the psychiatric symptoms of prisoners were questioned (22, 25). In a previous study, it was reported that the depression scores of those who had a prison entrance before were higher than those who did not have a prison entrance (22). Another study showed that the idea of suicide was less among prisoners entering prison for the first time (251). In our results, psychiatric symptom scores of prisoners admitted to prison before were higher than those who entered prison for the first time. SCL-90 somatization, anxiety, hostility scores and BAI scores, and CTQ-physical abuse scores were higher in those admitted to prison. This was interpreted as exposure to prison stressors more than once with different crimes increases mental symptoms.

Finally, when the prisoners were grouped according to the types of crimes they committed, it was found that there was only a difference in the CTQ-sexual abuse subscale scores. The subscale scores of prisoners involved in multiple crimes were higher than those involved in one. Those who were involved in the crime of willful homicide had higher CTQ-sexual abuse subscale scores than the prisoners who were involved in other crimes. In previous studies conducted in the literature on this subject, it was determined that the rates of childhood traumas were high in prisoners (26, 27, 28). Many studies show that traumas can increase the risk of mental illness. It was demonstrated that traumas might pose a risk of suicide (28). A previous study reported that all childhood traumas and abuse rates were high in both male and female prisoners. Again, a study conducted abroad reported that the rate of post-traumatic stress disorder was high in prison inmates and that people had anger control problems with the effect of traumas, and the risk of suicide increased (29). The present study found that those who were in prison more than once were exposed to more traumas, which supports these studies.

The most important limitation of the present study was the unequal distribution between the genders. Other limitations were the relatively insufficient number of participants, the self-report scale of the scales, and the inability to apply structured clinical interviews with the participants. These limited the interpretation and generalization of the results. It is necessary to conduct more extensive studies with larger sample groups for findings obtained to gain importance.

As a result, it was concluded that prison inmates might have mental symptoms and that their anxiety and depressive complaints might increase. It was also found that going to prison more than once causes more mental symptoms. Also, prisoners who were exposed to violence in childhood and had a previous suicide attempt had more psychiatric symptoms, and prisoners involved in more than a thousand different crimes and willful homicides were exposed to more childhood sexual abuse. In light of all these findings, it was considered that people who had a healthy childhood and were not exposed to abuse, violence, or trauma during childhood could be mentally healthier. It was thought that exposure to trauma in childhood might lead a person to commit a crime, which may affect both the person's life and society. For this reason, it was considered that the psychiatric follow-up of children exposed to childhood trauma and providing the necessary psychosocial support could reduce the delinquency rate. It was also considered very important to follow up on the prisoners' psychiatric symptoms, reveal the traumas they experienced, and intervene correctly and promptly. To generalize and interpret the findings obtained in the study, there is a need for studies in larger sample groups that also examine different psychiatric symptoms.

Ethical statement

The study was approved by the Fırat University Non-Interventional Local Ethics Committee on 23.05.2019 with the number 09/02 and by the R.T. Ministry of Justice with the date 12.08.2022 and the number 112444.

Conflict of interest
The authors declared no conflict of interest.

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Research Article

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The effects of the COVID-19 pandemic on pediatric patients with otitis media with effusion

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Abstract

Otitis media with effusion (OME) is a frequently self-limiting middle ear fluid accumulation. During the COVID-19 pandemic, there was a decrease in patient referrals to pediatric Otorhinolaryngology (pENT) outpatient clinics. The aim of this study was to compare the patients who presented at our pENT outpatient clinic with OME during the pandemic with patients who presented during the equivalent period before the pandemic, and to investigate the effects of pandemic measures on OME. The study included patients aged 1-15 years who presented at the pENT Outpatient Clinic due to OME. Four groups were created based on the date of March 2020, when the first COVID-19 case was recorded in Turkey. The groups were formed as one for each year from March 2018 to March 2022. The total number of patients admitted to the pENT outpatient clinic was recorded. Group 1 (March 2018-March 2019) included 1338 patients diagnosed with OME, which constituted 12% of the total number of patients. Group 2 (March 2019- March 2020) included 1238 patients, Group 3 (March 2020-March 2021) 241 (8%), and Group 4 (March 2021-March 2022) 432 (9.4%). From this study, it was observed that the greater attention paid to the mask, social distance and hygiene rules during the COVID-19 pandemic, and the implementation of distance education decreased the frequency of upper respiratory tract infections, and had a positive effect on the number of OME cases.

Keywords: COVID-19, children, otitis media, effusion

1. Introduction

Otitis media with effusion (OME) is fluid accumulation in the middle ear, which is frequently self-limiting and has an acute or chronic pattern. The presence of effusion for a period of longer than three months is defined as chronic OME. Before the age of 10 years, an average of 80% of children experience an attack of OME at least once, and it is seen most often in the first two years of life (1, 2). OME is seen at the highest rate in childhood because the Eustachian tube is anatomically and functionally immature, and there is a higher rate of upper respiratory tract infections associated with exposure to viruses and bacteria at school and creche and the presence of adenoid hypertrophy. The persistence of OME for longer than three months despite treatment is a cause of hearing loss and a primary indication for the application of a ventilation tube (3).

During the COVID-19 pandemic, there was a great decrease in patients presenting at or being referred to paediatric Ear, Nose, and Throat (pENT) polyclinics because of OME (4). This can be attributed to a decrease in infections that can cause OME due to the closure of schools, creches, curfews, and instructions to stay at home.

This study aimed to investigate the effects of the COVID-19 pandemic precautions on OME by comparing patients who presented at the pENT polyclinic because of OME during the pandemic with patients who presented in the equivalent period before the pandemic.

2. Material and Method

Approval for the study was granted by the Ethics Committee of Ondokuz Mayıs University (decision no: KAEK 2022/213). The study included patients aged 1-15 who presented at the pENT of Samsun University Medical Faculty Hospital, a tertiary-level regional reference centre. The patients were separated into four-year groups, based on March 2020, when the first COVID-19 case was recorded in Turkey.

Group 1 (pre-pandemic) included patients diagnosed with OME between March 2018 and March 2019.

Group 2 (pre-pandemic) included patients diagnosed with OME between March 2019 and March 2020.

Group 3 (pandemic period) included patients diagnosed with OME between March 2020 and March 2021.

Group 4 (pandemic period) included patients diagnosed with OME between March 2021 and March 2022.

The diagnosis of OME was made in our clinic as stated in the international guidelines, from the presence of fluid in the middle ear, retracted tympanic membrane, air bubble, or the air-fluid level in the otoscopic examination, together with the presence of Type B curve in the tympanometric examination.

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The data of the patients diagnosed with OME in our clinic were retrieved from the hospital database and patient files. The total number of patients in the same age group who presented at the pENT for any reason in the same time periods was also recorded.

The study exclusion criteria were incomplete records, the presence of acute mastoiditis, cholesteatoma, cleft palate or lip, or any diagnosed syndrome or chronic disease.

The acoustic impedance measurements of the patients were taken using Interacoustics AZ26 and AT235H clinical tympanometry devices.

2.1. Statistical Analysis

Descriptive statistics were stated as number (n) and percentage (%) for categorical variables. The age distribution between the groups was evaluated with the Kruskal Wallis test, and the gender distribution with the Chi-square test.

3. Results

Group 1 (pre-pandemic: March 2018 - March 2019) included 1338 patients, comprising 792 (60%) males and 546 (40%) females. The patients diagnosed with OME constituted 12% of the total 10.810 presentations at the pENT in that period.

Group 2 (pre-pandemic: March 2019 - March 2020) included 1238 patients, comprising 768 (62%) males and 470 (38%) females. The patients diagnosed with OME constituted 11% of the total 10.728 presentations at the pENT in that period.

Group 3 (pandemic period: March 2020 - March 2021) included 241 patients, comprising 137 (56%) males and 104 (44%) females. The patients diagnosed with OME constituted 8% of the total 2905 presentations at the pENT in that period.

Group 4 (pandemic period: March 2021 - March 2022) included 432 patients, comprising 251 (58%) males and 181 (42%) females. The patients diagnosed with OME constituted 9.4% of the total 4571 presentations at the pENT in that period.

No difference was determined between the groups regarding age and gender distribution (Table 1).

During the COVID-19 pandemic, there was observed to be a significant decrease in the total number of patients and patients diagnosed with OME.

4. Discussion

From March 2020, when the World Health Organisation declared COVID-19 a global pandemic, there was an increase in COVID-19 case numbers, and a series of precautions were taken to restrict social life in Turkey, as throughout the world. In that period, due to greater attention given to hygiene rules, mask-wearing, social distancing, curfews and lockdowns, and the implementation of distance education, there was a decrease in upper respiratory tract infections and, therefore, hospital presentations because of OME (5). This study aimed to investigate the effects of the COVID-19 pandemic on OME by evaluating the patients diagnosed with OME in the pENT before and during the pandemic.

Table 1. Distribution of the demographic data according to the groups

		Group 1 (n=1338)	Group 2 (n=1238)	Group 3 (n=241)	Group 4 (n=432)	P
Age (years)		6.08±3.1 (min:1, max:15)	6.21±3.1 (min:1, max:15)	6.09±3.2 (min:1, max:15)	5.98±3.2 (min:1, max:15)	0.748¥
Gender	M	792 (59.2 %)	768 (62 %)	137 (56.8 %)	251 (58.1 %)	0.246β
	F	546 (40.8 %)	470 (38 %)	104 (43.2 %)	181 (41.9 %)	

The prevalence of OME is greater in younger children as the Eustachian tube has not reached full anatomic and functional maturity. Moreover, an increase in upper respiratory tract infections also increases the frequency of OME (1, 2). In England, it has been reported that OME is the most common reason in childhood for referral to a surgeon (6). In another study in England, the frequency of patients with OME consulting a general practitioner was reported to be 15.2% (7). In two different studies in Turkey, the OME frequency was reported as 8.7% and 6.8% (8, 9). The OME frequency in the current study was determined as 11% and 12% in the two years before the COVID-19 pandemic.

Studies in literature have reported that OME is seen more in males (3). Although the reason for this is not fully known, several hypotheses have been developed. Genetic factors, different effects of the sex hormone on cytokines, and impaired mastoid ventilation in males have been suggested as possible reasons (10-12). According to the gender distribution data of the current study, OME was observed more in males, which was consistent with the data in the literature.

Although tympanograms are an important diagnostic tool in diagnosing OME, false positivity has been reported at 8% in previous studies (13, 14). Therefore, in our clinic, the evaluations of all the patients diagnosed with OME are made with physical examination, tympanogram, and audiogram findings together.

In a study by Nguyen et al. (4), the numbers of patients diagnosed with OME before and during the COVID-19 pandemic were compared, and there was reported to be a 50% decrease. Alde et al. (15) examined 2-month periods before and during the pandemic (May-June 2019, January -February 2020, May-June 2020) to investigate the effects of the COVID-19 restrictions on OME. The tympanogram data (Type B) were determined as 46% and 52% and fell to 2.3% during the pandemic. In a study by Toretta et al. (5), the period of February-April was compared in 2019 and 2020, and there was shown to be a significant decrease in the number of OME attacks and the number of antibiotics prescribed. In the current study, the two years before the pandemic were evaluated separately, and the numbers of patients diagnosed with OME were determined to constitute 11% and 12%, respectively, of

the total patient numbers. The number of patients who presented during the pandemic significantly decreased, with an 80% decrease in the number of patients compared to the previous year. The total number of presentations revealed that the rate of OME was 8% during the COVID-19 pandemic.

It was observed that wearing masks, social distancing, greater attention paid to hygiene rules, and the implementation of distance education with a decrease in upper respiratory tract infections (5) positively affected the number of OME cases during the pandemic.

This study had some limitations, primarily that it was retrospective and was conducted in a single centre. It should also be considered that even if a child was ill at that time, overly cautious parents might have been hesitant to visit a hospital because of the fear of infection during the pandemic.

This study differs from previous similar studies as two years were evaluated before and during the pandemic. The number of patients and percentage of the total presentations in the second year of the pandemic (Group 4) were significantly low, showing that preventative measures such as mask-wearing and social distancing could play a preventive role in developing OME. In light of these findings, taking these protective measures in addition to treatment could positively contribute to the follow-up period in managing chronic OME.

Ethical Approval

Approval for the study was granted by the Ethics Committee of Ondokuz Mayıs University (decision no: KAEK 2022/213, dated: 27.04.2022).

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

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Research Article

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The value of preoperative inflammatory markers in patients through total laryngectomy for advanced stage larynx carcinoma

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Abstract

The aim of this study is to evaluate the difference between neutrophil, lymphocyte, platelet and RDW (red cell distribution width) counts and NLR (neutrophil/lymphocyte ratio) and PLR (platelet/lymphocyte ratio) between patients who underwent total laryngectomy for advanced stage laryngeal squamous cell carcinoma and healthy control group. The study group consisted of 37 patients (36 male, 1 female) who underwent total laryngectomy and bilateral neck dissection for advanced stage laryngeal squamous cell carcinoma between January 2017 and December 2019. As a control group, complete blood count records of 35 healthy individuals who were routinely examined in 2019 within the scope of occupational health and safety were compiled. Blood was collected preoperatively from the patient in the study group. The data obtained from the whole blood counts of the two groups were analyzed statistically by comparing neutrophil, lymphocyte, platelet, RDW, NLR and PLR values. The age difference between the study and control groups was not significant ($p>0.05$). Neutrophil, platelet and RDW counts were higher in the study group compared to the control group and this difference was statistically significant ($p=0.001$, $p=0.035$, $p=0.001$, respectively). Although the lymphocyte count was high in the study group, the difference was not significant ($p=0.061$). The difference between NLR and PLR between the two groups was high in the study group and this difference was statistically significant ($p=0.001$, $p=0.007$, respectively). Neutrophil, platelet, RDW, NLR, PLR values were significantly higher in patients with advanced stage laryngeal cancer than in the control group. NLR, PLR and RDW values, which can be obtained cheap, simple and rapidly at the diagnosis stage of laryngeal cancers, may be biomarkers in determining prognosis. For this, multicenter, prospective studies are needed.

Keywords: laryngeal carcinoma, neutrophil lymphocyte ratio, platelet lymphocyte ratio, red cell distribution width

1. Introduction

Laryngeal cancer accounts for 30-40% of malignant tumors of the head and neck, and 1-2.5% of all malignant neoplasms in the human body. Histopathologically, 95-98% of laryngeal cancers are squamous cell carcinoma (SCC). Laryngeal cancer is more common between the 5th and 7th decades of life and in male gender. Many factors such as viral infections, smoking and alcohol exposure, exposure to carcinogens, gastroesophageal reflux and heredity are effective in the etiology (1).

Although the most important factor determining the prognosis of laryngeal carcinoma is the pathological TNM stage, vascular and perineural invasion, depth of invasion, patient's general condition, age, sex and treatment-related factors affect the process (2). In studies conducted, it has been reported that inflammation that occurs as a host response in tumor tissue affects tumor development, metastasis and response to treatment (3). Neutrophils, T and B lymphocytes and thrombocytes (platelets) play an important role in tumor inflammation and immunology (4).

Increased neutrophil count, lymphopenia and thrombocytosis have been associated with malignancies. While lymphocytes prevent tumor growth, cytokines secreted by tumor cells cause an increase in the number of neutrophils. Neutrophils play an important role in enlargement and metastasis. Platelets contribute to the development of the tumor by increasing angiogenesis by means of vascular endothelial growth factor (VEGF) (5). NLR and PLR are considered to be an important prognostic factor in many carcinomas and it is accepted that NLR is a better predictor than PLR (6). There are studies showing that RDW adversely affects the clinical outcomes of various cancers as an indicator of systemic inflammatory response (7).

The aim of this study was to evaluate the difference between neutrophil, lymphocyte, platelet, RDW counts, NLR and PLR between patients undergoing total laryngectomy for advanced stage laryngeal carcinoma.

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2. Materials and Methods

This retrospective study was approved by the Health Sciences University Samsun Training and Research Hospital Ethics Committee with the 2019/3/5 numbered decision. Between January 2017 and December 2109, 37 patients who underwent total laryngectomy and bilateral neck dissection for advanced stage laryngeal squamous cell carcinoma were included in the study. As a control group, complete blood count records of 35 healthy individuals who were routinely examined in 2019 within the scope of occupational health and safety were compiled.

Of the 37 patients in the study group, 36 were male and 1 was female, while the control group consisted of 35 men. Patients who had autoimmune disease, impaired thyroid function tests, diabetes mellitus, chronic renal failure, second malignancy, hematologic disease, active infection, chemotherapy-radiotherapy, steroid treatment in the last month and immunsupressive were not included. All patients in the study group had transglottic tumors and were in the T4a stage clinically and pathologically according to the American Cancer Committee Association (AJCC) 8th edition. Thyroid cartilage invasion by the tumor was reported pathologically in all patients.

Preoperative examination of the patients in the study group was performed with 2cc venous blood from the forearm. The control group included complete blood counts from venous blood. Blood counts were performed with Coulter LH 780 Analyzer (Beckman, USA). Neutrophil, lymphocyte, platelet, RDW counts were recorded separately for the study and control groups and then NLR and PLR were calculated.

SPSS 21.0 analysis program was used for statistical analysis. The normal distribution of the data of the study and control groups was analyzed by using the Shapiro-Wilk test. The values of normally distributed and abnormally distributed groups were shown as mean ± sd. The difference between the groups was evaluated by Independent Student-t test and Mann-Whitney U test. Logistic regression analysis was used to investigate the effects of the parameters on the groups. Descriptive statistics were expressed with Odds ratio and 95% confidence intervals. $p<0.05$ was considered statistically significant. The diagnostic decision-making characteristics of serum NLR and PLR values in advanced stage laryngeal cancers were examined by receiver operating characteristics (ROC) curve. Sensitivity, specificity, area under curve (AUC) of these limits were calculated in the presence of significant limit values and $p<0.05$ was considered statistically significant.

3. Results

The age of the patients in the study group of 37 patients with total laryngectomy ranged from 44 to 75, with an average of 59.89 years. In the control group of healthy individuals, mean age was 59.49 between 45 and 68 years. The mean age of all subjects was 59.69 years. Of the patients in the study group, 36 were male and 1 was female. All subjects in the control group were male. Age difference between the two groups was not statistically significant ($p>0.05$) (Table1).

Table 1. Age distribution of the study and control groups

Groups	n	Male/ Female	Mean age	Sd	Min.	Max	p
Patient	37	36/1	59.89	7.214	44	75	
Control	35	35/0	59.49	5.431	45	68	
Total	72	71/1	59.69	6.368	44	75	0.05

All patients in the study group had transglottic tumors and thyroid cartilage invasion in the pathology report. Seven patients had perineural invasion, 6 had perivascular invasion, 16 had subglottic extension, 13 had extralaryngeal involvement, and 7 had neck lymph node metastasis (bilateral metastasis in 2 patients).

Neutrophil, platelet and RDW counts were higher in the study group compared to the control group and this difference was statistically significant ($p=0.001$, $p=0.035$, $p=0.001$, respectively). Although lymphocyte counts were high in the study group, the difference was not statistically significant ($p=0.061$). The difference between NLR and PLR between the two groups was high in the study group and this difference was statistically significant ($p=0.001$, $p=0.007$, respectively) (Table 2).

Table 2. Distribution and analysis of hematological parameters and inflammatory markers

	Patient group (n=37)	Control group (n=35)	p	OR	95% CI	p
Neutrophil	6.43±2.45	4.54±1.55	0.001	2.55	0.751-8.665	0.133
Lymphocyte	1.88±0.71	2.15±0.62	0.061	0.55	0.058-5.295	0.607
Platelet	291.95±94.26	249.23±64.45	0.035	0.99	0.972-1.233	0.651
RDW	14.66±2.05	13.522±0.89	0.001	2.25	1.093-4.639	0.028
NLR	4.021±2.82	2.31±1.10	0.001	0.51	0.076-3.459	0.493
PLR	175.65±86.75	123.77±42.23	0.007	1.01	0.974-1.059	0.462

Neutrophil, lymphocyte, platelet and RDW values are calculated as $x10^3$
NLR: neutrophil/lymphocyte ratio PLR: platelet/lymphocyte ratio OR: Odds ratio

The sensitivity, specificity and AUC values for NLR were 75.7%, 62.9% and 0.772, respectively. The sensitivity, specificity and AUC for PLR were 70.3%, 51.4%, 0.685 respectively.In addition, the diagnostic cut off value for NLR and PLR calculated with data from both groups is summarized in Table 3.

Table 3. Cut-off value of parameters

	Cut-off Value	AUC	95 % CI Sensitivity	Specifity	p
NLR	>2.26	0.772 (0.664-0.880)	%62.9	%75.7	0.001
PLR	>121.08	0.685 (0.560-0.809)	%51.4	%70.3	0.007

4. Discussion

Laryngeal carcinomas accounts for 30% to 40% of malignant tumors of the head and neck; 1% to 2.5% of all malignant neoplasms in the human body. Histopathologically, 95-98% of laryngeal carcinomas are epithelial-originated squamous cell carcinoma (1). Advances in the diagnosis and treatment of laryngeal carcinomas provide protection of the larynx and prolonged survival. However, the 5-year survival rate remains low for patients with advanced laryngeal carcinoma (8). As a result of the intensive treatments applied to advanced staged patients, the patients' swallowing, respiratory and speech functions deteriorate, social problems are experienced and eventually their quality of life decreases (9). Total laryngectomy and bilateral neck dissection were performed in all 37 patients constituting the study group. Pathological evaluation revealed that all patients had T4a stage tumor and all of them had thyroid cartilage invasion.

There are many studies in which chronic inflammation contributes to the initiation and progression of cancer (4,10). Increasing neutrophil count contributes to tumor growth by reducing the cytolytic activity of lymphocytes and natural killer (NK) cells, producing proangiogenic factors such as VEGF, chemokines and proteases (11). Lymphocytes play an important role against the tumor progression by inhibiting proliferation-migration of tumor cells and inducing cytotoxic cell death.In animal studies, the deficiency in the development and / or function of CD4 + Th1 helper cells, CD8 + cytotoxic T cells or NK cells has been shown to increase susceptibility to cancer formation in the host (12). Reduced lymphocyte count negatively affects prognosis in some cancers (13). Tumor cells can increase the number of peripheral platelets through thrombopoetin, IL-6 or leukemia-inhibiting factor.The increase in the number of platelets contributes to the progression of the tumor by increasing angiogenesis through VEGF. Brown et al. reported that thrombocytosis is a negative prognostic factor in different malignancies (14). In our study, neutrophil and platelet counts were higher in the patient group compared to the control group, and this difference was statistically significant ($p = 0.001$, $p = 0.035$, respectively). Although the lymphocyte counts were lower in the patient group compared to the control group, the difference between the two groups was not statistically significant ($p = 0.061$).

Rise of RDW has been reported as a finding of inflammation and oxidative stress (15). In a meta-analysis consisting of eight cohort studies, increased RDW level has been reported as an indicator of poor prognosis in upper aerodigestive tract cancers (16). Bozkurt et al. (17) reported the results of 132 patients with laryngeal cancer that the risk of local and regional recurrences increased in patients with high RDW. Hsueh et al. reported that disease-free survival, cancer-specific survival and overall survival were low in patients have squamous cell laryngeal carcinoma with high RDW levels (18). In our study, RDW level was found to be significantly higher in the patient group compared to the control group ($p = 0.001$).

NLR and PLR values have been shown to be associated with poor prognosis in terms of mortality and recurrence (7). Increased NLR and PLR has been shown to be associated with poor prognosis in liver, parotis, oropharyngeal SCC and esophageal cancers. These markers were found to be independent prognostic factors in the head and neck SCC (19). Kara et al. (10) reported in their study on patients with laryngeal cancer that those who were at the T4 stage found PLR higher than those at the T1-2 stage, and those with higher NLR had higher local recurrence and survival was low. Tu et al. (20) showed that patients with advanced laryngeal cancer have high NLR and PLRvalues, NLR or PLR not only reflects tumor burden, but can also stimulate tumor progression by affecting tumor metabolism and microenvironment. They found that the combination of these markers, rather than using them separately, was more valuable in predicting prognosis. In our study, the specificity for NLR was 62.9% and the sensitivity was 75.7%. PLR specificity was calculated as 51.4% and sensitivity as 70.3%. NLR and PLR were found high in favor of the patient group, and this difference was found to be statistically significant ($p = 0.001$, $p = 0.007$, respectively). The limtations of our study wereto be retrospective, single-centered, and less number of cases.

In our study, neutrophil, platelet, NLR, PLR, RDW values were found to be statistically significantly higher in the patient group with advanced laryngeal cancer compared to the control group. NLR, PLR and RDW values, which can be examined easily, cheap and fast at the diagnosis step of laryngeal cancers, can be biological markers to determine the prognosis. To support these findings, multi-center, prospective studies need to be done.

Ethical Statement

This retrospective study was approved by the Health Sciences University Samsun Training and Research Hospital Ethics Committee with the 2019/3/5 numbered decision.

Conflict of interest

The authors declared no conflict of interest.

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No funding was used for the study.

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None to declare.

Authors' contributions

Concept: M.Ç., Design: D.M.M, Data Collection or Processing: S.V., Analysis or Interpretation: S.N.C., Literature Search: D.Ö., Writing: A.Ö.

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An evaluation of the laboratory and clinical data of the Crimean-Congo hemorrhagic fever patients during the COVID-19 pandemic

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Abstract

In this study, the purpose was to investigate the demographic, laboratory, and clinical characteristics of the Crimean-Congo Hemorrhagic Fever (CCHF) cases that were treated in our hospital during the Coronavirus disease-2019 (COVID-19) pandemic. It was also investigated whether the patients hospitalized with a provisional diagnosis of CCHF who tested negative for CCHF PCR were infected with COVID-19. In our study, data (epidemiological, clinical, laboratory, prognosis) from 38 patients diagnosed with CCHF through reverse-transcriptase polymerase chain reaction (PCR) and viral-RNA and/or Immunoglobulin M antibodies using ELISA between May 2020 and November 2022 were investigated retrospectively. Of all the patients, 23 were CCHF PCR (+) and 15 were CCHF PCR (-).15 (65.2%) of PCR (+) patients and 9 (60%) of PCR (-) patients were engaged in farming. 65.2% of PCR (+) and 26.7% of PCR (-) patients presented with a history of tick bites. 21 (91.3%) of PCR (+) and 12 (80%) of PCR (-) patients had a history of rural living. Among the PCR (+) patients, 65.2%, 17.4%, and 17.4% received treatment for CCHF in 2022, 2021, and 2020 respectively. 87% of PCR (+) and 60% of PCR (-) patients were discharged after full recovery. The most common symptoms were fever, diffuse body ache, weakness, and headache. Significant differences were found between PCR (+) and PCR (-) patients in terms of leukocytes, LDH, INR, NEU, PLT, fibrinogen, and NLR values. 3 patients hospitalized with a provisional diagnosis of CCHF tested negative for CCHF PCR and positive for COVID-19 PCR. Thoracic CT, clinical, and laboratory findings of these patients showed no differences from the other patients. The possibility of misdiagnosis should be considered in CCHF and COVID-19 infections due to their similar symptoms and indications. Extensive multicentric studies need to be conducted to investigate the causes of the increased number of CCHF cases during the pandemic.

Keywords: Crimean-Congo hemorrhagic fever, COVID-19, pandemic, tick

1. Introduction

The coronavirus disease-2019 (COVID-19) that has affected millions of people worldwide has been effective in our country since March 2020. The common symptoms observed in COVID-19 patients are highly similar to the symptoms in patients with Crimean-Congo Hemorrhagic Fever (CCHF) (1). There have also been cases of mixed infections of CCHF and COVID-19 during the COVID-19 pandemic (2). Thus, CCHF cases and epidemics are an additional threat to the pandemic coronavirus infection in endemic countries such as Türkiye, Iran, Oman, Russia, and Pakistan. This may lead to misdiagnosis and improper treatment in these regions (3).

The World Health Organization Regional Office for the East Mediterranean presented various risk factors for the emergence of zoonotic diseases and arboviruses in the background of the COVID-19 infection. Climatic and environmental changes, humanitarian emergencies, conflicts, insufficient healthcare systems, poor supervision and inadequate laboratory facilities, domestication, and animal slaughter during religious holidays are some of these risk factors (4).

CCHF is zoonotic disease caused by the *Nairovirus* that is transmitted to humans through *Ixodidae* tick bites (*Hyalomma marginatum* in our country) and unprotected contact with the blood or tissue of animals in the viremia stage or infected humans (5). There are also studies reporting nosocomial and sexual transmission of the disease (6). Moreover, it has been reported that the transportation of virus-infected ticks through migratory birds influences CCHF transmission (7).

The COVID-19 infection has many common clinical, laboratory, and radiographic characteristics with the CCHF (8). Fever, weakness, nausea, vomiting, stomachache, myalgia, diffuse bleeding, petechia, ecchymosis, hepatic dysfunction, and diffuse body ache are some of the common symptoms of CCHF. On the other hand, fever, dry cough, weakness and shortness of breath (dyspnea), and loss of taste and smell

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perception are common symptoms of COVID-19 (9). Both diseases are diagnosed with PCR tests. An important finding for both CCHF and COVID-19 is the ground-glass opacification in Chest Tomography (CT) scan findings. However, there is no evidence for the CCHF virus' direct invasion to the pulmonary interstitial tissue (10).

In this study, the goal was to investigate the laboratory and clinical characteristics of the CCHF cases treated in our hospital during the COVID-19 pandemic. Moreover, it was aimed to demonstrate that the patients hospitalized with a provisional diagnosis of CCHF who tested negative for CCHF PCR may have been subject to misdiagnosis and are infected with COVID-19.

2. Materials and Methods

Laboratory and clinical characteristics of the cases treated at our hospital for Crimean-Congo hemorrhagic fever between 2020-2022 were investigated retrospectively. Patients who were tested in the Microbiology Reference Laboratory of the Public Health Institution of Turkey and were diagnosed with positivity of CCHF virus IgM antibodies by ELISA and/or detection of CCHF virus RNA positivity by real-time reverse transcriptase (RT) polymerase chain reaction (PCR) were included in the study. Patients' demographic data including sex, occupation, and place of residence; their contact with ticks, onset year of the disease, symptoms, and findings; laboratory findings including hemogram, C-reactive protein (CRP), creatine kinase (CK), lactic dehydrogenase (LDH), activated partial thromboplastin time (aPTT), and international normalized ratio (INR); and the recovery and mortality rates were investigated.

Ethical permission for the study was obtained by Ethic Committee from Elazig Firat University, with decision numbers 12-23 and 18.11.2021.

2.1. Statistical Analysis

Statistical Package for Social Science for Windows (SPSS) 24.0 package was used to analyze the data. A chi-square test of independence was performed to investigate whether there were statistically significant differences between CCHF PCR (-) and PCR (+) patients regarding demographics, anamnesis, physical examination findings, laboratory findings, and epidemiological history findings. Furthermore, an independent samples t-test was performed to investigate the statistically significant differences in the laboratory findings of CCHF PCR (-) and PCR (+) patients. The results were considered significant with 99% ($p<0.01$) and 95% ($p<0.05$) confidence levels.

3. Results

A total of 38 patients were included in the study. 23 of these patients were PCR (+) and 15 were PCR (-). The demographic characteristics of the patients show that 65.2% of the PCR (+) patients and 80% of the PCR (-) patients were male. 8 (34.8%) of the PCR (+) patients and 3 (20%) of the PCR (-) patients were female. There were no significant differences between groups in terms of sex ($p=0.326$) (Table 1). 15 (65.2%) of the PCR (+) patients and 9 (60%) of the PCR (-) patients were engaged in farming. There were no significant differences between groups in terms of occupation (housewife, student, teacher, retiree, technician) ($p=0.147$) (Table 1). There were no significant differences in the cities that patients were residing in. However, 7 (30.4%) of the PCR (+) patients and 10 (66.6%) of the PCR (-) patients came to our hospital from the city of Elazığ (Table 1). Of the PCR (+) patients, 65.2%, 17.4%, and 17.4% received treatment for CCHF in 2022, 2021, and 2020 respectively. Of the PCR (-) patients, 46.7%, 33.3%, and 20% received treatment for CCHF in 2022, 2021, and 2020 respectively ($p=0.461$) (Table 1). 65.2% of the PCR (+) and 26.7% of the PCR (-) patients had a history of tick bites. 21 (91.3%) of the PCR (+) and 12 (80%) of the PCR (-) patients had a history of living in rural areas. 87% of PCR (+) and 60% of PCR (-) patients were discharged after full recovery. Inpatients in the clinic and the intensive care unit were given outpatient treatment and discharged. We did not have any patients who died (Table 1).

Table 1. Epidemiological and demographic characteristics of Crimean-Congo Hemorrhagic Fever cases

		PCR (+) n:23	PCR (-) n:15	*p*
Year	2020	4 (%17.4)	3 (%20)	0.461
	2021	4 (%17.4)	5 (%33.3)	
	2022	15 (%65.2)	7 (%46.7)	
Gender	female	8 (%34.8)	3 (%20)	0.326
	male	15 (%65.2)	12 (%80)	
Job	Farmer	15 (%65.2)	9 (%60)	0.147
	Housewife	6 (%26.1)	2 (%13.3)	
	Student	0 (%0)	1 (%6.7)	
	Teacher	2 (%8.7)	0 (%)	
	Retired	0 (%)	1 (%6.7)	
	Technician	0 (%)	2 (%13.3)	
Clinical status	service	1 (%4.3)	4 (%26.7)	0.134
	*ICU	0 (%)	2 (%13.3)	
	service+healing	2 (%8.7)	0 (%)	
City	Healing	20 (%87)	9 (%60)	0.449
	Elazig	7 (%30.4)	10 (%66.6)	
	Bingol	5 (%21.7)	1 (%6.7)	
	Tunceli	3 (%13)	2 (%13.3)	
	Bitlis	4 (%17.4)	1 (%6.7)	
	Diyarbakir	1 (%4.3)	0 (%)	
	Sivas	1 (%4.3)	1 (%6.7)	
	Kahramanmaras	1 (%4.3)	0 (%)	
	Agri	1 (%4.3)	0 (%)	
Tick Bite		15 (%65.2)	4 (%26.7)	0.063
Country side life		21 (%91.3)	12(%80)	0.235

***ICU:** Intensive care units, ***p<0.05**

Investigating the symptom and findings of the patients, 73.9% of the PCR (+) and 66.7% of the PCR (-) patients presented with fever; 78.3% of the PCR (+) and 73.3% of the PCR (-) patients presented with a headache; and 87% of the PCR (+) and 73.3% of the CCHF PCR (-) patients presented with diffuse body ache. There were no significant differences between PCR (+) and PCR (-) patients in terms of fever, headache, and diffuse body ache variables. PCR (+) and PCR

(-) patients showed significant differences in weakness (p=0.025) such that weakness was observed in all of PCR (+) patients and only 80% of PCR (-) patients. Other symptoms and findings did not differ significantly in PCR (+) and PCR (-) patients (Table 2).

Table 2. Symptoms and findings of Crimean-Congo Hemorrhagic Fever cases

	PCR (+) n:23	PCR (-) n:15	*P*
Fever	17 (%73.9)	10 (%66.7)	0.630
Headache	18 (%78.3)	11 (%73.3)	0.727
Diffuse body ache	20 (%87)	11 (%73.3)	0.290
Weakness	23 (%100)	12 (%80)	**0.025***
Nausea/vomiting	10 (%43.5)	8 (%53.3)	0.552
Diarrhea	7 (%30.4)	4 (%26.7)	0.802
Stomach ache	5 (%21.7)	6 (%40)	0.225
Ecchymosis	0	1 (%6.7)	0.210
Debris	2 (%8.7)	2 (%13.3)	0.649
Bleeding	2 (%8.7)	2 (%13.3)	0.649
Bloody diarrhea	1 (%4.3)	1 (%6.7)	0.754
Consciousness disorder	0	1 (%6.7)	0.210
Bleeding gums	1 (%4.3)	0	0.413

There were significant differences between PCR (+) and PCR (-) patients in terms of anemia (p=0.002), leukopenia (p=0.037), LDH level (p=0.004), and INR level (p=0.047). 4.3% of the PCR (+) and 46.7% of the PCR (-) patients presented with anemia. Leukopenia was observed in 73.9% of the PCR (+) and 40% of the PCR (-) patients. High LDH level were observed in all PCR (+) patients whereas only in 66.7% of the PCR (-) patients. The INR value was 1.2 and above in 73.8% of the PCR (+) and 40% of the PCR (-) patients (Table 3).

Table 3. Laboratory parameters of Crimean-Congo Hemorrhagic Fever cases at the time of application-1

		PCR (+) n:23	PCR (-) n:15	*P*
Anemia		**1 (%4.3)**	**7 (%46.7)**	**0.002****
AST/ALT increase		**21 (91.3)**	**11 (%73.3)**	0.138
Lung Infiltration (CT)		**1 (%4.3)**	**1 (%6.7)**	0.754
Leukopenia		**17 (%73.9)**	**6 (%40)**	**0.037***
CK increase		**17 (%73.9)**	**9 (%60)**	0.367
Creatinine	1.4 and below	**21 (%91.3)**	**14 (%93.3)**	0.242
	1.5 – 2.4	**2 (%8.7)**	**0**	
	2.5 and above	**0**	**1 (%6.7)**	
Thrombocytopenia		**22 (%95.7)**	**13 (%86.6)**	0.315
LDH increase		**23 (%100)**	**10 (%66.7)**	**0.004****
INR increase	0.9 and below	**2 (%8.7)**	**2 (%23.4)**	**0.047***
	0.9-1.2	**4 (17.5)**	**7 (%46.6)**	
	1.2 and above	**17 (%73.8)**	**6 (%40)**	
aPTT	11 sec and under	**20 (%87)**	**9 (%60)**	0.056
	15 sec and above	**3 (%13)**	**6 (%40)**	
aPTT rate	75% and below	**21 (%91.3)**	**14 (%93.3)**	0.821

Significant differences were found in terms of NEU (p=0.0001), CRP (p=0.002), fibrinogen (p=0.001), and NLR (p=0.002) between PCR (+) and PCR (-) patients. Accordingly, the mean value of NEU was determined as 1.22±0.82 in PCR (+) patients and 5.35±3.68 in PCR (-) patients. NEU value was found to be higher in PCR (-) patients compared to PCR (+) patients. The mean value of PLT was determined as 49.30±28.14 in PCR (+) patients and 95.06 ±58.76 in PCR (-) patients. PLT value was found to be higher in PCR (-) patients compared to PCR (+) patients. The mean value of CRP was determined as 99.34 ±51.25 in PCR (+) patients and 21.21±13.26 in PCR (-) patients. CRP value was found to be higher in PCR (+) patients compared to PCR (-) patients. The mean value of fibrinogen was determined as 2.31 ± 0.56 in PCR (+) patients and 3.93±2.06 in PCR (-) patients. Fibrinogen value was found to be higher in PCR (-) patients compared to PCR (+) patients. The mean value of NLR was determined as 2.32±1.81 in PCR (+) patients and 7.58 ± 7.53 in PCR (-) patients. NLR value was found to be higher in PCR (-) patients compared to PCR (+) patients. No significant differences were observed between the two groups in terms of MPV, LYM, D-Dimer, and PLR values (Table 4).

Table 4. Laboratory parameters of Crimean-Congo Hemorrhagic Fever cases at the time of application-2

	PCR (+) n:23	PCR (-) n:15	*P*
MPV	8.73 ± 0.74	8.59 ± 0.87	0.586
NEU	1.22 ± 0.82	5.35 ± 3.68	**0.0001***
LYM	0.75 ±0.58	1.01 ± 0.89	0.286
PLT	49.30 ± 28.14	95.06 ±58.76	**0.003***
CRP	99.34 ±51.25	21.21 ± 13.26	**0.002***
D-DIMER	6.39 ± 5.21	4.63 ± 0.22	0.402
FIBRINOGEN	2.31 ± 0.56	3.93 ± 2.06	**0.001***
PLR	109.28 ± 91.25	118.95 ± 78.85	0.753
NLR	2.32 ± 1.81	7.58 ± 7.53	**0.002***

3 patients hospitalized with a provisional diagnosis of CCHF tested negative for CCHF PCR and positive for COVID-19 PCR. However, there were no significant differences in the clinical, laboratory, and thoracic computerized tomography (CT) findings.

4. Discussion

The COVID-19 pandemic has led to various arbovirus epidemics in different regions and countries such as Brazil, Kenya, and Asia (10,11). There has been a CCHF epidemic in addition to the COVID-19 pandemic in our country as well. The majority of the cases were detected in the northern regions of Middle and Eastern Anatolia and middle regions of the

Black Sea region (10). Barkay and colleagues (12) reported an increase in the number of CCHF cases, especially in Eastern Türkiye, compared to previous years after the start of the COVID-19 pandemic in 2020. They argued that this increase in the number of CCHF cases is related to several factors including the increased residence in rural areas during the COVID-19 pandemic, the possibility of developing resistance to acaricides used against ticks, and ecological and climatic causes. In our study, we also detected an increase in the number of CCHF cases during the pandemic and especially in 2022.

CCHF is an important public health issue in Türkiye due to high mortality rates (1). CCHF can be seen in people of any age and sex. However, it has been reported to be more common in middle-aged working people as well as people who have a high risk of tick contact due to working in animal husbandry (13). The majority of patients included in our study are engaged in farming (PCR (+) = 65.2%, PCR (-) = 60%). Studies show that approximately 60% of CCHF patients have a history of tick bites (14). A study reported that 67.6% of patients had a history of tick bites and all of them lived in the rural areas (15). Kadanalı and colleagues (16) reported that 50.8% of patients had a history of tick bites and 93.6% of them lived in the rural areas. In our study, we found that 65.2% of CCHF PCR (+) and 26.7% of PCR (-) patients during the pandemic had a history of tick bites. Furthermore, 21 (91.3%) of CCHF PCR (+) and 12 (80%) of the PCR (-) patients had a history of living in the rural areas. It is easier to diagnose patients who have a history of tick bites with CCHF. However, it may be challenging to diagnose patients with CCHF when they do not have a history of tick bites and show symptoms similar to COVID-19 infection.

There is a possibility that COVID-19 conceals or imitates other inflammatory diseases in endemic regions (17). Thus, viral infections such as CCHF can lead to diagnostic confusion in patients infected with COVID-19 (10). There are also studies reporting co-infections of COVID-19 and CCHF (18,19,20). Our data includes 3 patients who were hospitalized with a provisional diagnosis of CCHF and tested positive for COVID-19. One of these patients had a history of tick bites and all of them had a history of visiting rural areas.

COVID-19 infection has atypical characteristics that complicate the diagnosis. The initial symptoms of COVID-19 are fever, cough, myalgia, and fatigue (21). A study showed that 83% of COVID-19 patients had fever, 82% had a cough, 31% had dyspnea, 11% had muscle pain, 8% had a headache, 2% had chest pain, 2% had diarrhea, and 1% had nausea and vomiting (22). CCHF also presents with non-disease-specific symptoms such as weakness, fever, headache, myalgia, nausea, and vomiting (10). Similarly, the most common symptom in our patients was weakness and it was 100% in CCHF PCR positive patients and 80% in CCHF PCR negative patients. Other most common symptoms were diffuse body ache (87%), headache (78.3%), and fever (73.9%) in CCHF PCR positive patients. Our COVID-19 PCR positive patients also displayed nonspecific symptoms such as fever, headache, diffuse body ache, diarrhea, and stomachache.

The most common abnormal laboratory findings observed in COVID-19 infection are thrombocytopenia, lymphopenia, prolonged prothrombin time, active partial thromboplastin time prolongation (hypercoagulability), high levels of D-dimer, and increase in ALT, AST, and LDH (23). Similarly, CCHF laboratory findings also show thrombocytopenia, lymphopenia, high levels of liver enzymes, and prolonged aPTT (17). A study by Baran and colleagues (24) reported as high LDH and CK (100%), thrombocytopenia and lymphopenia (90%), high AST levels (90%), and high ALT levels (70%). In a study by Alkan-Çeviker and colleagues (25), thrombocytopenia (95%), increased levels of AST and ALT (72%), leukopenia (69%), neutropenia (42%), increased levels of LDH (47.9%), and PTZ (25.3%) and aPTT prolongation (16.9%) have been reported in CCHF patients. The increase in neutrophils in CCHF patients leads to excessive cytokine release, decrease in the number of lymphocytes and monocytes, impaired immunity, and decrease in humoral antibody response. Inflammation emerges in tissues and organs. The increase in proinflammatory cytokines in CCHF patients causes hemophagocytic cell activation which leads to a rapid decrease in the number of leukocytes and thrombocytes. Thrombocytopenia is one of the primary laboratory parameters of CCHF (26). In a study, it was reported that the leukocyte and thrombocyte levels of CCHF patients are lower compared to the patients in the control group (27). Similarly, Doğan and colleagues (28) also reported lower levels of leukocyte, lymphocytes, and platelet in the CCHF patient group. Our study shows similar results to the literature. Our CCHF patients showed a decrease in leukocytes and thrombocyte levels. There were significant differences between CCHF PCR (+) and PCR (-) patients in terms of anemia, leukopenia, high LDH levels, and high INR levels. These indications were higher in the CCHF PCR (+) group. Thrombocytopenia was also observed in high levels in CCHF PCR (+) (95.7%) and PCR (-) (86.6%) patients but no significant difference was found between the two groups. NLR and PLR are biomarkers that indicate inflammation. Erturk and colleagues (29) reported similar levels of NLR and PLR in CCHF patients and the control group. Turkdogan and colleagues (30) reported significantly low levels of NLR in CCHF patients and that this relates to the severity of the disease. In our study, NLR levels were found to be significantly low in the CHHF PCR (+) group. No significant differences were found between the two groups in terms of PLR levels.

It has been shown that COVID-19 and CCHF do not only show similarities in clinical symptoms but also in CT findings (10). No direct invasion of the CCHF virus into the lung interstitial tissue was shown, but the ground-glass opacification is an important finding of the CCHF. It is co-observed with pleural effusion and consolidation due to

alveolar hemorrhage (9). In our study, there were nonspecific imaging findings in the CT results of CCHF patients. There were also no discriminatory findings in the CT results of COVID-19 positive patients.

It is possible to lose co-infected patients who have both COVID-19 and CCHF due to bad clinical course (19). In our study, all patients were discharged after full recovery. We did not have any patients who died. Patients who were COVID-19 PCR (+) and who were receiving inpatient treatment in the clinic or the intensive care unit were discharged with outpatient follow-up.

The constant attention to COVID-19 by public health officials and distraction from other infectious agents have led to different epidemics in various countries. It is important to keep in mind that there may diagnostic confusions between CCHF and COVID-19 due to the similarities in the clinical picture where CCHF is endemic, and that these infections may co-occur. Further research is needed for the development of diagnostic algorithms for the differential diagnosis of these diseases.

Ethical statement

Elazig Firat University Ethics Committee, dated:18.11.2021, approval number:12-23

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept and design: P.Ö.; data collection or processing: P.Ö., M.Ö.; analysis and interpretation: P.Ö., M.Ö.; writing: P.Ö., M.Ö.

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Epidemiological and clinical characteristics, management, and outcomes of antenatal hydronephrosis: A single-centre experience of 229 cases

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Abstract

The advances in the utilization of USG in routine antenatal follow-up resulted in an increased diagnosis of antenatal hydronephrosis (ANH). This study was conducted to elaborate on the ANH's epidemiological and clinical characteristics, management, outcomes, and possible risk factors. Two hundred twenty-nine cases diagnosed with ANH were included during the antenatal follow-up at the Obstetrics and Gynecology Department of Ondokuz Mayıs University between 2004 and 2022. The ANH was a USG finding suggesting a hydronephrosis ≥7 mm. The epidemiological and clinical characteristics, risk factors, treatment and outcomes in the postnatal period were assessed retrospectively. About 75% of the cases were male, 8% were premature births, and the mean gestational week of diagnosis was 22±3 weeks. About 43.7% of mothers had urinary tract infections, and a family history of any kidney disease was present in 24.5% of mothers' and 20.5% of fathers' family histories.38.7% of cases underwent surgery. At the end of the 6-month follow-up, 37.3% had regressed, 38.7% of them had stable hydronephrosis, and 18.9% of them had normal findings in USG. Male gender increased gestational urinary tract infections, and parents' family histories for any kidney disease were found as possible risk factors for the development of ANH. However, close follow-up and timely intervention, including surgery, provide favourable outcomes in these cases.

Keywords: antenatal hydronephrosis, antenatal ultrasonography, ANH epidemiology, risk factors, ANH surgery, ANH outcomes

1. Introduction

One of the most often observed anomalies on standard prenatal ultrasounds (USG) is antenatal hydronephrosis (ANH), which affects 1 to 4.5 per cent of all pregnancies (1). ANH is typically defined by an anterior-posterior diameter of 3 of 4 mm at less than 28 weeks of gestation and 37 mm after 28 weeks (2). Unfortunately, many patients cannot receive a conclusive diagnosis even though it is frequently regarded as an indication of congenital abnormalities of the kidney and urinary tract (CAKUT). Even though the knowledge of the causes and effects of CAKUT is growing, there has yet to be a consensus regarding the clinical importance, postnatal assessment, and treatment of newborns with AHN (3).

The main obstacle against a consensus on ANH management is partly associated with the difficulties in classifying a fetus's or a newborn's upper urinary tract dilatation, which is also a cause for the inconsistent reports on the degree and management of this condition (4). Thus, elaborating on the epidemiological features, patient characteristics, management strategies, and relevant outcomes in ANH is crucial for clinical decision-making. Furthermore, keeping the population characteristics may also play a role in the disease's epidemiology in mind, this study aimed to evaluate the general demographic and clinical characteristics, management and outcomes of our patients with ANH.

2. Material and Method

This study was conducted at the Gynecology and Obstetrics department of Ondokuz Mayis University Faculty of Medicine. Patients diagnosed with ANH between 2004 and 2022 were retrospectively evaluated. Demographic and clinical characteristics, parent characteristics, clinical management and follow-up records were obtained from electronic hospital databases and patient records. The ANH was an antenatal USG finding of hydronephrosis ≥ 7 mm.

Descriptive statistics were presented using frequency and per cent for categorical variables, and mean and standard deviation for continuous variables. All analyses were done using SPSS 25.

This study was conducted with the approval of Ondokuz Mayıs University ethics committee (Approval number: 2023/26, approval date: 04.02.2023).

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3. Results

A total of 229 patients with ANH were included in the study. The general parental characteristics of patients are presented in Table 1. Sixteen patients' parents were close relatives, and the mean ages of the mothers and fathers were 31±6 and 34±7 years, respectively. Urinary tract infections were present in 100 (43.7%) mothers but none of the fathers. However, kidney disease was present in 24.5% of mothers' and 20.5% of fathers' family histories.

Table 1. General characteristics of parents of the ANH cases

	n (%) / Mean ± SD
Parent consanguinity	16 (7)
Mother's characteristics	
Age (year)	31 ± 6
Weight gain during pregnancy	13.2 ± 4.44
UTI history	100 (43.7)
Gestational diabetes	13 (5.7)
Gestational hypertension	10 (4.4)
Medical treatment/drug use	61 (26.6)
Smoking	33 (14.4)
Kidney disease in mother's family	56 (24.5)
Father's characteristics	
Age (year)	34 ± 7
Medical treatment/drug use	21 (9.2)
Hypertension	3 (1.3)
Smoking	126 (55)
Kidney disease father's family	47 (20.5)

The demographic and clinical characteristics of the babies diagnosed with ANH in prenatal USG are presented in Table 2. Accordingly, most babies were male (73.4%), and 7.9% were premature births with a mean birth weight of 3218±497 grams. The mean gestational week of diagnosis was 22±3 weeks of pregnancy. Postnatal USG was unable for one patient, hydronephrosis diagnoses were confirmed in 224 patients (97.8%), and the remaining four patients (1.7%) had abnormal findings other than hydronephrosis.

Table 2. Demographic and clinical characteristics of patients

	n (%) / Mean ± SD
Sex	
Female	61 (26.6)
Male	168 (73.4)
Prematurity	18 (7.9)
Birth weight (gr)	3218 ± 497
Gestational week of diagnosis	22 ± 3
Postnatal USG	
None	1 (0.4)
Hydronephrosis	224 (97.8)
Abnormal finding	4 (1.7)

Follow-up USG findings in the 1st, 2nd, 3rd, and 6th-month USG assessments are presented in Table 3. Accordingly, hydronephrosis regressed from 3.1% to 37.3% and progressed in 0.9% to 5.1% of patients. At the end of the 6-month follow-up, 38.7% had stable hydronephrosis, and 18.9% had normal findings in USG.

The two approaches in clinical management included close follow-up and surgical management in indicated cases. As a result, about 61.3% of patients were followed-up without surgical intervention, and 38.7% underwent surgery. At the end of the follow-ups, ANH was resolved in 21.6%, regressed in 38.3%, and remained stable in 40.1% of the patients. Data presented table 4.

Table 3. Clinical progression of patients during 6-month follow-up USG assessments

	1st month	2nd month	3rd month	6th month
	n (%)	n (%)	n (%)	n (%)
Follow-up USG				
Normal	1 (0.4)	6 (2.6)	21 (9.3)	41 (18.9)
Hydronephrosis - regressed	7 (3.1)	47 (20.7)	86 (38.2)	81 (37.3)
Hydronephrosis - stable	217 (95.6)	138 (60.8)	101 (44.9)	84 (38.7)
Hydronephrosis - progressed	2 (0.9)	36 (15.9)	17 (7.6)	11 (5.1)

Table 4. Clinical management and outcomes of ANH

	n (%)
Surgical management	
None	138 (61.3)
Yes	87 (38.7)
Patient outcomes	
Resolved	49 (21.6)
Regressed	87 (38.3)
Stable	91 (40.1)

4. Discussion

To our knowledge, this is the most extensive case series of ANH patients in Turkey. In this study, we retrospectively evaluated our patients with ANH, and to summarize our findings, mothers and fathers of the patients with ANH were in their 30s of ages, about 15% of mothers and 55% of fathers were smoking, medical treatment for other comorbidities were present in about a quarter of mothers and 10% of fathers. Urinary tract infections were present in about half of the mothers. Regarding an inheritance pattern of kidney disease, almost 25% of mothers' and 21% of fathers' family histories had kidney disease. Most babies were male, and about 8% were premature births. About 40% of the patients underwent surgical intervention for ANH, and during the follow-up of 6 months, about one-fifth of them recovered completely, 40% regressed, and 40% remained stable.

Widespread utilization of prenatal USG during routine antenatal follow-ups resulted in increased diagnoses of ANH worldwide, and early diagnosis also provided timely interventions and decreased severe complications (5). The diagnosis is made using a 5-MHz transducer USG in the 3rd trimester of gestation (6). A USG finding suggesting visualization of the fetal urinary system and ureters, which should not be visible under normal development, indicates the presence of hydronephrosis. Various classification systems and management strategies were reported for ANH, and identifying

patients' epidemiological and clinical characteristics is essential for clinical decision-making in different populations. Based on our findings, male gender, presence of urinary tract infections during pregnancy, and a family history of kidney disease in the parents' families were found to be increased in the babies with ANH.

Several previous studies evaluated the risk factors associated with CAKUT. In one of those, Liu et al. (7) reported that male gender, preterm birth, abnormal antenatal USG, gestational hypothyroidism, and oligohydramnios were associated with increased CAKUT risk during pregnancy. Our results partly supported these risk factors, which the majority of our cases were male, and all had abnormal antenatal urinary system USG findings. However, we found no gestational hypothyroidism and oligohydramnios among our patients. The previous reports suggest that the most common causes of ANH are transient and physiological hydronephrosis, which may occur in more than half of the cases and have a benign prognosis after birth (8). Likewise, almost all of our cases in the postnatal USG had hydronephrosis remained, but more than half of them had regular or regressed USG findings in postnatal 6th-month assessments.

Besides the risk factors, there are discrepancies in ANH cases' management strategies. In general, the management strategies are associated with the persistence of hydronephrosis in the postnatal period, bilateral involvement and the severity of the disease. Antibiotic treatment is suggested to prevent urinary tract infections, but evidence for continuous antibiotic prophylaxis lacks (9). In an extensive series of 208 hydronephrosis patients with a mean follow-up of 11 years, Anderson et al.(10) recommended further evaluation and interventions for cases with symptomatic cases. Another study by Koff and Campbell (11) reported that only 5% of their cases undergone pyeloplasty surgery, however a review by Agras reported that about one-quarter of patients with an anteroposterior renal diameter of 3 cm were reported to undergo surgery in the first year (12). This was also consistent with our results that almost 40% of our patients underwent surgery in the postnatal first year.

The increased antenatal routine follow-up and widespread utilization of USG assessment during pregnancy resulted in a high diagnosis of ANH. This study evaluated our cases with ANH and reported the epidemiological and clinical characteristics, possible risk factors, treatment approaches, and outcomes in this patient group. Our results support the available evidence in the literature and suggest population characteristics in our region. To the best of our knowledge, this is the most extensive case series reported yet in Turkey. However, future studies with different areas and larger sample sizes are needed to make conclusions on the epidemiology of this prevalent disorder.

Ethical statement

This study was conducted with the approval of Ondokuz Mayıs University ethics committee (Approval number: 2023/26, approval date: 04.02.2023).

Conflict of interest

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Authors' contributions

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

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Review Article

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Hemiplegia and oral health

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Abstract

Oral health problems are often neglected due to other problems that are thought to be more vital in stroke patients. However, oral health is a significant quality of life determinant and needs to be protected in stroke patients as well as general population. Moreover, problems such as hemiparesis, hemiplegia, dysphagia, balance and coordination disorder and cognitive dysfunction due to strokes have a very negative impact on the self -care and/or care support of the caregivers. In this study, we aimed to search a large number of health databases and make a compilation on oral health problems, impact on quality of life and solutions in stroke patients. Keywords 'oral, dental, health, care, hygiene, dental, prosthesis, denture, tooth brushing, stroke, cerebrovascular and cardiovascular' and combinations of these words were searched in the literature. Studies involving at least one subject for oral health in stroke patients were included in the review. In the literature, it was seen that the oral health of the stroke patients was discussed more about mastication, swallowing, dysphagia, nutrition, hygiene, prosthesis and quality of life. It was concluded that there are no sufficient assessment and care guides that can be used by stroke patients or caregivers, and the information of dentists, patients and caregivers about special care for stroke patients was not sufficient.

Keywords: : dental health, oral hygiene, stroke, teeth

1. Introduction

Stroke is a disease characterized by the formation of focal neurological deficits as a result of brain lesions developed by embolism, ischemia or hemorrhage (1).Considering that approximately 15 million people have strokes each year and about one third of them die and one third of them are permanently disabled, it will be realized that the community affected by the stroke is too crowded (2).Stroke is the most common cause of neurological disability worldwide (3,4).It is the third cause of death after coronary artery disease and cancer (5).World Health Organization describes oral health as a person's being free of problems of biting, chewing, smile, speech and psychosocial, oral and facial pain, oral and throat cancer, oral infection and wounds, periodontal disease, dental decay, dental loss and other diseases (6).Factors such as hemiparesis, hemiplegia, spasticity, dysphagia, coordination disorder and cognitive dysfunction that occur with the influence of the central nervous system adversely affect the general life of the patients and seriously affect their oral health (2,7-9).In particular, techniques for oral hygiene, use of auxiliary devices and training of patients and caregivers are gaining importance. In addition, interventions for mastication, swallowing, nutritional problems and dental problems are required. Oral health problems are often neglected due to other problems that are thought to be more vital in stroke patients. However, oral health is a significant quality of life determinant and needs to be protected in stroke patients as well as general population. In this regard, health professionals, patients and caregivers need to be informed.

Due to the limited publications containing all the details related to oral health in patients with strokes in the literature and the lack of existing information that has been brought together, this study aims to present the details of oral health in patients with strokes in the light of the current literature, ie the effect of oral health problems in stroke patients, the quality of life and solution suggestions to problems. We also aimed to contribute to the education of all stakeholders of this disease.

1.1. Material and Methods

Keywords 'oral, dental, health, care, hygiene, dental, prosthesis, denture, tooth brushing, stroke, cerebrovascular and cardiovascular' and combinations of these words were searched in a large number of health databases (PubMed, Cochrane, Embase, MEDLINE and others). Studies involving at least one subject for oral health in stroke patients were included in the review. Current approaches and standards were investigated.

The fact that the populations examined in the studies contain any of the ischemic or hemorrhagic stroke were deemed sufficient to be involved in our study. The references of the articles included in order to determine the articles that may have been overlooked were searched, too.

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2. Results and Discussion

2.1. Oral health problems in stroke patients

Some studies have shown that oral health problems especially tooth loss, dental caries, and periodontal diseases are more frequent in stroke patients than the healthy individuals (10-15).

Factors such as hemiparesis, hemiplegia, spasticity, ataxia, apraxia, dysphagia, coordination disorder and cognitive dysfunction that occur with the influence of the central nervous system adversely affect the general lives of the patients and seriously affect their oral health (2,7-9,16).

Hemiplegic patients are insufficient to use their affected hands to brush teeth (2). It is often not possible to brush an effective brushing with non -dominant hand. As a result, dental caries and periodontal diseases can be seen (2). Stroke patients, who cannot perform their self -care because of their physical and/or cognitive status, are dependent on their nurses or caregivers (17). Some previous studies have shown that more than two -thirds of the patients had difficulty in brushing teeth in a way in relation to the severity of stroke. Although there is a gradual decrease in the post -stroke healing process, it is still stated that approximately half of the patients could not perform the ideal teeth brushing action (18,19). As a result of hygiene decreasing in the oral cavity, plaque formations, tooth decay, infection and halitosis may develop (20-22). Studies have shown increased bacterial colonization and fungal formation in stroke patients compared to non –stroke patients (23). In stroke patients who perform worse in daily life activities it has also been reported that the development of *Staphloccoci spp.* and *Candida spp.*is higher. This result shows the importance of self -care and nutrition in stroke patients in preventing bacterial colonization and thus protecting oral health (24).

It has been shown that colonization has increased due to dental prosthesis and as the device usage prolongs in intubated patients. There are also studies showing that gingival bleeding is higher in stroke patients (25). Another problem in stroke patients is dry mouth-xerostomia. It is usually seen as a side effect of drugs used and has been shown to develop more in stroke patients than non -stroke patients. Bacterial and fungal colonization and plaque formation may increase as a result of xerostomia (21,26-29). *Candida albicans* development is more common in hospitalized stroke patients, especially under antibiotic treatment (30).

In the studies, it was emphasized that the impact of the possible healing process after the stroke and the efforts of the caregivers decrease the yeast colonization and plaque formation. The contribution of rehabilitation to healing cannot be denied (18,25).

Patients with strokes may experience chewing, eating, drinking, swallowing and oral hygiene problems because of the affected lip, tongue, chewing muscles, soft palate and pharynx (20,26,31-34). Chewing and swallowing activities may be seriously impaired especially in moderate and advanced stroke patients. The biting force may be reduced on the affected side. In addition, it is possible to develop atrophy in chewing muscles in patients who cannot be fed for a long time. In both cases it disrupts nutrition. Previous studies have shown that up to 70% of stroke patients could not be fed sufficiently due to chewing and swallowing problems. The condition of the chewing muscles and temporomandibular joint in patients with stubbornness may cause malocclusion, impaired appearance and orofacial disorder (35).

Especially dysphagia is a serious risk factor for aspiration pneumonia, an important cause of death in stroke patients. In the presence of non -appropriate hygiene conditions in the mouth, advanced bacterial colonization occurs, especially aerobic gram-negative bacteria *Escherichia spp*., *Enterobacter spp*., *Klebsiella spps*, *Morganella spp*. and *Proteus spp*. (2,20).

In case of facial paralysis, the patient's buccal sulcus may not be aware of the accumulation of food residues. The tongue will naturally have the ability to clean the oral cavity and will not be able to reveal this feature in case of paralysis. For this reason, it is important to clean the oral cavity and sulcus with a toothbrush or with the help of a gauze wrapped in a finger. If the patient uses dental prosthesis, the cleaning of the prosthesis is also very important (21,27).

It should be remembered that problems such as aphasia will adversely affect the communication between the patient and the dentist and caregivers (10).

Stroke is more common in men (36). In accordance with this information, there are studies showing that the frequency of dental decay is more frequent in male stroke patients (37,38).

In the normal population of sixty and over, there are studies showing that some tasks related to personal care are approximately 10% and the rate of need for assistance in the field of daily living activities is approximately 40%, and as the age progresses, there are studies showing that dependence on others increases in daily living activities (39-41). In patients over sixty years old, there are studies that indicate that there is more gingival disease than younger patients. In addition, it has been reported that gingival health is worse in the elderly with acute strokes compared to the healthy elderly in the same age group. The frequency of applying to the dentist urgently due to the gingival problem among individuals with stroke history has been reported as high compared to the normal population (42-44).

Various studies for the perception of oral health status in the elderly have demonstrated different results. In some elderly populations, dissatisfaction with oral health came to the forefront, while in some studies, it was concluded that oral health is not cared for (45-47). This can be attributed to the socio-cultural state of the populations studied or the perception that other diseases are more vital. Since stroke is mostly seen in older individuals, the perception of oral health of stroke individuals may vary as in the general elderly population.

Due to the high frequency of stroke in the old age group, the possibility of using dental prosthesis increases, which has been reported in a study that approximately half of the patients with strokes were reported to use dental prosthesis (18,42). In another study, it was reported that the rate of patients who can use prosthesis instead of the lost teeth was lower than expected, and that the reason for this may be the inability to reach the health institution due to the bad socioeconomic condition that prevented intervention and the bad functional condition (37). It is obvious that stroke patients cannot perform the care of dental prostheses due to motor losses. In patients with hyperactive gag reflex, the problem of inability to tolerate dental prosthesis may also occur. For this purpose, even palatal anesthesia may be required to solve this problem of glossopharyngeal nerve (48). In some patients, revision of dental prosthesis may be required after stroke to adapt to the mouth better (27,49). The frequency of patients who needed this was as 42% in a study (50). It should be kept in mind that problems such as bad appearance, wound, infection, aspiration and nutritional failure due to inappropriate dental prostheses may develop (21,51).

It should be remembered that diabetes, which is a risk factor for stroke, are also a risk factor for the oral health problems of xerostomia, dental caries, fungal infections, dysphagia and glossodynia (52). In addition, another risk factor for stroke was reported to be associated with more teeth loss in stroke patients (37).

In a study with four hundred and ten strokes, low socioeconomic condition, unemployment and low education level were associated with impaired oral health. In addition, low functional independence and permanent function disorders were also associated with poor oral health (37).

The number of publications investigating the frequency and order of dentist control after stroke is limited. Among these studies, there are studies indicating that the proportion of stroke patients going to the dentist control was approximately three - quarters, the reason for this is that there are problems due to stroke such as dependence to the house, dependence to a wheelchair, inability to drive and inability to climb stairs (19).

2.2. Oral health related quality of life in stroke patients

These problems adversely affect the quality of life of the affected individual. Hemiparesis, which is frequently encountered in stroke patients, reportedly causes permanent insufficiency affecting the quality of life in approximately 70% of patients (53,54). Physical function and psychosocial deterioration of the affected individual should adversely affect the quality of life and cause an increase in the disability (55-57). Oral health is effective on the quality of life in physical and psychosocial context (58,59). Oral health was also found to be associated with the quality of life in patients with strokes (60,61). Decomposition in general appearance, eating, drinking and speaking abilities due to oral health will adversely affect the psychology, social participation and quality of life of individuals with strokes. A study has shown that almost all of the patients with strokes are not satisfied with the general appearance of their teeth. In the studies that evaluate the quality of life associated with oral health, the quality of life is bad in both acute and progressive rehabilitation stages in patients, and the quality of life associated with oral health is much worse in patients with strokes than non-stroke patients. In addition, it has been reported that the quality of life in edentulous patients is worse than the dentulous patients (19).

2.3. Assessment and management of oral health in stroke patients

Among the scales used frequently to evaluate oral health in patients with stroke, Oral Health Impact Profile 14 (OHIP-14) Oral Assessment Guide, Oral Health-Related Quality of Life (OHRQol), Geriatric Oral Health Assessment Index (GOHAI) and Medical Outcomes Study 12-item Short Form (SF-12) are included (62). There is no data showing the superiority of these scales. These scales are the scales that can be completed by the individual or by their caregivers. When it is evaluated whether there is a difference in the completion of the scale by the person himself or the caregiver, it is concluded that there is no difference in general except for subjective parameters such as pain and sensitivity and that the scale can be completed by the patient himself or the caregiver.

In the literature, methods to reduce bacterial colonization, plaque formation, gingival bleeding and halitosis are mentioned and there are no detailed results. These methods include manual or mechanical tooth brushing, sponge brushes, mouthwashes, brushes for dental prosthesis and ultrasonic irrigation (2).

The full and accurate training of health professionals, patients and caregivers about oral health of patients with strokes seems to be a must (2,63). It is known that separate special clinics are formed for dental and oral health of individuals with obstacles (2,64,65). For these patient groups that require special interest and care, it seems important to encourage and experience dentists (2,66). Information of the patient and the caregiver and training should be performed by the dentist. Dentist should be cautious in terms of bleeding, considering that patients with strokes are prone to gingival bleeding and using anticoagulants for prophylaxis (67). The appropriate time for elective interventions after stroke is controversial. In addition to the opinion that elective interventions should be avoided up to 3-6 months after the stroke, there are also opinions that argue that if the patient is stable, it can be intervened within a few weeks after stroke (21,50,68,69)

Oral care is usually performed by nurses in patients with stroke in acute periods and is generally known to be reluctant in this regard. For this reason, it is also left to personnel who do not have authority and knowledge. In intubated patients, oral care may be disrupted due to the fear of damaging intubation. The education of nurses who will do oral care is given by seniority from their own professional group not by

professional dentists, and continues to be applied by traditional methods and patient care cannot be performed according to current guidelines (2,70,71). The absence of equipment such as toothbrushes, toothpaste, rinses, artificial saliva and floss in the units where patient follow -up is performed is also a distinct problem.

In a randomized controlled study, the quality of life associated with oral health was found better in the group receiving an electric toothbrush, mouthwash and oral hygiene training than the group receiving manual tooth brushing and oral hygiene (62).

Oral health risk assessment should be performed in patients with strokes. Oral care of patients should be performed at least twice a day (72,73). Regular oral health assessments should not be neglected.

In patients with strokes, the protection of oral health and the solution of problems that may develop are as important as in the general population. However, considering the special needs of stroke patients, all the health professionals, patients and caregivers should be educated, encouraged and experienced. For interventions to stroke patients, it is necessary to develop some standardized guidelines, and in order to develop these guides, there is need for studies on the oral health of patients with stroke.

Conflict of interest

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Authors' contributions

Concept: M.I., I.I., P.C., Design: M.I., I.I., P.C., Data Collection or Processing: M.I., I.I., Analysis or Interpretation: M.I., I.I., Literature Search: M.I., I.I., Writing: M.I., I.I., P.C.

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Stem cell applications in female infertility – A rewiev

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Abstract

Infertility is a problem that affects approximately 15% of couples today. Although assisted reproduction techniques are widely used today, only 40-50% of couples who apply can have children with their own genetic structure. Especially in people with premature ovarian failure, the rate of conception does not exceed 5-10% with the treatments applied today. For this reason, many studies are carried out to obtain oocyte from stem cells with their proliferation and differentiation feature. In addition, regenerative cellular therapies that can replace assisted reproductive techniques and correct impaired fertility are also being investigated in both animal and human studies. In recent years, research has been carried out on stem cell-derived extracellular vesicles, which will eliminate immunological problems. In terms of safety and efficacy, clinical studies involving large populations are needed.

Keywords: infertility, premature ovarian failure, embryonic stem cells, adult stem cells

1. Introduction

Infertility is not being able to conceive despite one year of regular, unprotected intercourse (1). Infertility is encountered approximately in 12–15% of couples and 72 million people worldwide are diagnosed with infertility (2).

Despite all the developments in assisted reproduction today, most infertile patients cannot get pregnant with these methods. For this reason, stem cells are intensively investigated as an alternative method in the treatment of infertile patients (3,4,5,6,7,8,9,10,11).

It can be used as an alternative method in the treatment of infertility, as well as in the treatment of pathologies that indirectly cause infertility. These four diseases are as follows:

Premature Ovarian Failure (POF): According to the definition made by the European Society of Human Reproduction and Embryology (ESHRE), it is defined as a condition under 40 years of age, defined as oligo/amenorrhea lasting at least 4 months and Follicle Stimulating Hormone (FSH) value >25 IU/l measured at least 4 weeks apart. Its incidence has been reported to be 1% on average (13). The etiology of the disease has not been fully elucidated and genetic, environmental, enzymatic, infectious and iatrogenic factors are blamed (14). Current treatments are insufficient and different treatment modalities are needed.

Polycystic ovary syndrome (PCOS): It is a cause of infertility that causes increased ovarian function and anovulation. It is characterized by high androgen levels, irregular menstruation and the presence of numerous small cysts in the ovary. Its incidence is among 5-10% women of reproductive age and it is the most common endocrine disorder among women of reproductive age (15,16). Current treatments are inadequate in many patients. Regenerative medicine applications are being investigated in the treatment of inflammatory and immunological processes.

Endometriosis: It is a chronic inflammatory disease in which the basal layer of the endometrium is located outside the uterine cavity. Its frequency is between 6% and 10% and it is a multifactorial disease (17). Genetic, environmental and immunological mechanisms are involved in its pathogenesis. Today, treatments for infertility and pain cannot provide sufficient success for these patients.

Asherman's Syndrome: It is characterized by intrauterine adhesions, hypomenorrhea, or amenorrhea. Scar tissue formed in the uterus may disrupt embryo implantation and cause infertility or recurrent pregnancy loss (18). There is a history of pregnancy-related curettage in 90% of the patients. Although hysteroscopy and hormonal treatments are applied today, developments in regenerative methods may increase the success in the management of the disease.

Stem cells are undifferentiated cells found in embryo or adult tissues. These cells are cells that can renew themselves or differentiate when necessary.

Stem cells are divided into embryonic stem cells, induced pluripotent stem cells, adult mesenchymal stem cells, spermatogonial stem cells and ovarian stem cells according to

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their origin.

Embryonic stem cells are cells that can transform into all three germ sheets obtained from the inner cell mass of the embryo. It has been observed that both human and mouse embryonic stem cells are transformed into primordial germ cells in vitro, from which male and female gamete cells are formed by meiosis (19). It has also been shown that embryonic stem cells are effective in the restoration of endometrial tissue (20). However, due to ethical concepts, studies on the subject are limited.

Induced pluripotent stem cells were obtained from mouse fibroblast culture in 2006 and it has been shown that they can form all three germ sheets and maintain the same karyotype as embryonic stem cells (21). Since these cells are produced from adult cells, ethical problems caused by embryos are eliminated. In addition, since it is produced from people's own cells, immunological reactions are also less (22). Eguizabal et al. produced haploid gamete-like cells from keratinocytes and cord blood. They used a culture medium containing retinoic acid. Then forskolin, human recombinant leukemia inhibiting factor (LIF) and CYP26 inhibitor R115866 were used (23). Ramathal et al., on the other hand, transplanted skin cells from azoospermic and fertile men, together with Bone morphogenetic protein 4 (BMP4), Bone morphogenetic protein 8 (BMP8), retinoic acid (RA) and LIF, into mouse testis and studied germ cells. Ramathal et al. transplanted skin cells from azoospermic and fertile men together with BMP4, BMP8, RA and LIF into mouse testis and obtained germ cell-like cells (GCLCs) (24). Sasaki et al. human induced pluripotent stem cells in the presence of Activin A, CHIRON, BMP4, stem cell factor (SCF), epidermal growth factor (EGF) and LIF in human primordial stem cells. demonstrated that they can transform into germ cells. These cells are epithelial cell adhesion molecule (EpCAM) and Integrin α6 are distinguished from other cells by markers (25). Another study showed that human fibroblast-derived induced stem cells were transformed into spermatogenic cells both in a normal culture medium and by xenotransplantation (26). In their study, Yamashiaro et al. (27) and Gell and Clark (28) first transformed somatic cells into induced stem cells, then incipient mesoderm-like cells (iMeLCs) with Activin A and Chiron and then into human primordial germ cells and these cells were transformed into female mouse embryonic cells. Oogonia cells were obtained by culturing with ovarian-derived gonadal cells. Although induced pluripotent stem cells are promising, they carry teratogenic potential and the use of nucleic acid integration procedures, epigenic changes and genomic instability limits their use in treatment (29,30,31).

Mesenchymal stem cells are cells that can transform into osteoblasts, adipocytes and chondroblasts containing CD105, CD73 and CD90 surface antigens (32). These can be stem cells from bone marrow, stem cells from adipose tissue, stem cells from menstrual blood, stem cells from the umbilical cord and stem cells from amniotic fluid, depending on the tissue from which they are obtained. Mesenchymal stem cells contribute to the restoration of the ovary by going to the damaged ovarian tissue and secreting various cytokines. It increases new vessel formation with insulin-like growth factor (IGF-1), vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) and reduces apoptosis and fibrosis. The proliferation and differentiation abilities of mesenchymal stem cells decrease with age (33). Increased telomerase activity causes fetal stem cells to live longer than adult stem cells (34). Fetal mesenchymal stem cells can also be obtained from extraembryonic tissues such as amnion, umbilical cord, and placenta. Fetal stem cells contain CD105, CD29 and CD90, which are the surface markers of mesenchymal cells, as well as Octamer-binding transcription factor 4 (Oct-4), Nanog and Rex-1 markers of pluripotent cells (34). Fetal and adult mesenchymal stem cells use different pathways (35). For example, it was observed that fetal mesenchymal cells act through melatonin membrane receptor and antioxidant activity in mouse ovary damaged by cyclophosphamide (36).

Bone marrow-derived stem cells were obtained by Owen and Friedenstein in 1988 (37). Jing et al. showed that bone marrow-derived stem cells increased endometrial thickness after intravenous injection in mice while increasing anti-inflammatory cytokines and decreasing inflammatory cytokines (38). Wang et al. found that bone marrow-derived stem cells injected intravenously or directly into the uterus of mice with which they formed intrauterine adhesion increased the estrogen-progesterone receptor of the endometrium and decreased the fibrotic area. has shown to increase glands (39). Abd-Allah et al. provided the restoration of ovarian follicles by downregulating the FSH receptor of bone marrow-derived stem cells, downregulating the estrogen and upregulating the VEGF receptor in mice, in which they caused ovarian failure by giving cyclophosphamide in rabbits (3). Santamaria et al., in a prospective study of patients with Asherman syndrome, gave CD133+ bone marrow-derived stem cells to spiral arterioles and 10 of 16 patients became pregnant spontaneously or after the transfer (5).

Menstrual blood-derived stem cells are cells that can differentiate and proliferate like other mesenchymal stem cells and are obtained noninvasively. Liu et al. found that in the treatment with menstrual blood-derived stem cells in mice with premature ovarian failure with cyclophosphamide, Anti-Müllerian Hormone (AMH), Inhibin and estrogen levels improved and ovarian functions improved compared to the control group (40). Zheng et al., in their study, transformed MB-Mesenchymal stem cells (MB-MSC) cells into endometrial cells in vitro and then transplanted them into mice with adhesions in vivo to regenerate the endometrium (41). The ability to differentiate cells of menstrual origin comes from the presence of the OCT-4 transcription factor. Tan et al. used menstrual stem cells together with hormonal therapy in seven Asherman patients, while five patients became pregnant on

their own, while two patients became pregnant after the transfer (42).

Endometrial stem cells stromal consists of epithelial and endothelial cells. Stromal ones contain CD44, CD73 and CD90 but CD34 and CD45 are negative, epithelial ones are stage-specific embryonic antigen (SSEA-1), N- cadherin and NTDPase2 are positive, endothelial ones are in CD 31 and CD 34 phenotype (43). endometrial in the absence of injury, these cells remain silent. In the presence of endometrial damage, these cells are directed to the damaged area by means of chemokines and C-X-C chemokine receptor type 4 (CXCR4) (44).

Umbilical cord-derived stem cells are CD29, CD44, CD73, CD90 and CD105 positive, CD31, CD45 and HLADR-85 negative. It is very advantageous to obtain easily, low tumor risk and low immunological response. In animal POI models, the ovarian function has been shown to provide ovarian restoration by antiapoptotic activity in granulosa cells, decreasing FSH levels and increasing estrogen and progesterone (45,46). In addition, the dehydroepiandrosterone-induced mouse PCOS model has been shown to reduce inflammatory cytokines and improve infertility (47). Umbilical-derived stem cells prevent granulosa cell apoptosis by using various signaling pathways. Mitogen-activated protein kinase signaling pathway, G protein-associated receptors and insulin signaling pathways (48). In animal models, umbilical cord-derived stem cells have been shown to be beneficial by interfering with damaged endometrial cells by changing vascularity and inflammation (49). In addition, it was seen that it showed the same effect by activating matrix metalloproteinase 9 (50). In premature ovarian failure patients, it phosphorylates transcription factor forkhead box protein O3a (FOXO3a) and Forkhead Box protein O1 (FOXO1), providing primordial follicle activation and thus increasing the number of follicles (51)

Amniotic fluid-derived stem cells are frequently used in regenerative medicine due to their immunoregulatory properties and their ability to differentiate. VEGF, *Transforming growth factor* alpha and beta (TGFα and β) of these cells' growth It has been shown to improve ovarian function by activating factor (EGF) and bone morphogenic protein (BMP) signaling pathways (48). In mouse POF models, these cells are follicular especially if they have CD4C/CD105+ antigen. It has been shown to prevent atresia and restore ovarian function (52,53).

Amnion-derived mesenchymal stem cells ovarian in POF animal model's dysfunction prevents (54.55). Besides, inflammatory it decreases cytokines, increases neovascularization, and reduce apoptosis.

Placenta-derived mesenchymal stem cells increase folliculogenesis by activating the Phosphatidylinositol-4,5-bisphosphate 3-kinase/protein kinase B (PI3K/Akt) signaling pathway in animal POF models and restore ovarian function by changing the expression of hormones and receptors (56,57). Li et al. inositol-requiring ovarian cells using enzyme 1 (IRE 1) α pathway. showed that it corrects the dysfunction (58).

Adipose tissue-derived mesenchymal stem cells are frequently preferred because of their easy availability. In animal experiments, snowed that when the ovarian graft is applied together with the stem cell, it gains function faster by increasing VEGF expression (59). It has been shown to increase neovascularization and follicle proliferation in mouse ovarian defects induced by chemotherapy (60). It has been observed that mesenchymal stem cell and hormone therapy accelerate endometrial regeneration in Asherman syndrome (61).

Ovarian stem cells: In 2012, White et al. obtained stem-cell-specific marker VASA-positive cells from the human ovarian cortex and showed that follicle synthesis occurred after xenotransplantation of these cells into diabetic immunodeficient mice (62). However, the fact that these cells are very few in number and decrease with age has unfortunately hindered the progress of research. In addition, differentiation of these cells in vitro culture medium takes quite a long time.

Today, mesenchymal stem cell-derived extracellular vesicles are used to reduce the immunological reactions of stem cell therapy (63). However, although all these methods are promising, many studies are needed before they can be put into routine practice.

Conflict of interest

The authors have no conflicts of interest to declare.

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Authors' contributions

Concept:A.Z.Ö.,A.Ş.T.,Design:A.Z.Ö.,A.Ş.T.,Data Collection or Processing:A.Z.Ö.,A.Ş.T.,Analysisor Interpretation:A.Z.Ö., A.Ş.T.,Literature Search:A.Z.Ö., A.Ş.T.,Writing:A.Z.Ö., A.Ş.T.

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Review Article

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Overview of hematopoietic stem cell transplantation

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Abstract

Hematopoietic stem cell transplantation (HSCT) is the intravenous administration of hematopoietic stem cells (HSC) to restore blood cell production in individuals whose bone marrow or immune system is damaged or dysfunctional. This approach has been used to treat various malignant and nonmalignant disorders for the past half-century.

HSCT is performed in two ways, autologous and allogeneic. In the clinic, the type of stem cell transplant is decided according to the patient's diagnosis. Both kinds of transplantation have advantages as well as disadvantages. Knowing and managing the HSCT process well affects the success of the transplant. Knowing the complications that may occur after transplantation will facilitate the patient's treatment process. In this short review, the HSCT process has been tried to be explained with general titles.

Keywords: hematopoietic stem cell transplantation (HSCT), stem cell (SC), human leukocyte antigen (HLA), graft versus host disease (GVHD).

1. Introduction

1.1. What is Hematopoietic Stem Cell?

HSCs are cells found in bone marrow (BM), peripheral blood (PB), and umbilical cord blood (UCB) that can self-renew and differentiate into all adult blood cell types (Fig. 1). The spongy BM contains HSCs. In the bone marrow, HSCs create cells of the myeloid and lymphoid lineages, which are essential components of the immune system. The lymphopoiesis begins in the lymphoid series, and T, B, and natural killer (NK) cells, which are components of innate and adaptive immune cells, are created. The myeloid series gives rise to other blood cell components. Characterization of HSCs in clinical and research laboratories is done using CD markers. The existence and definition of HSCs were made 50 years ago, and studies on HSCs are still ongoing (1–3).

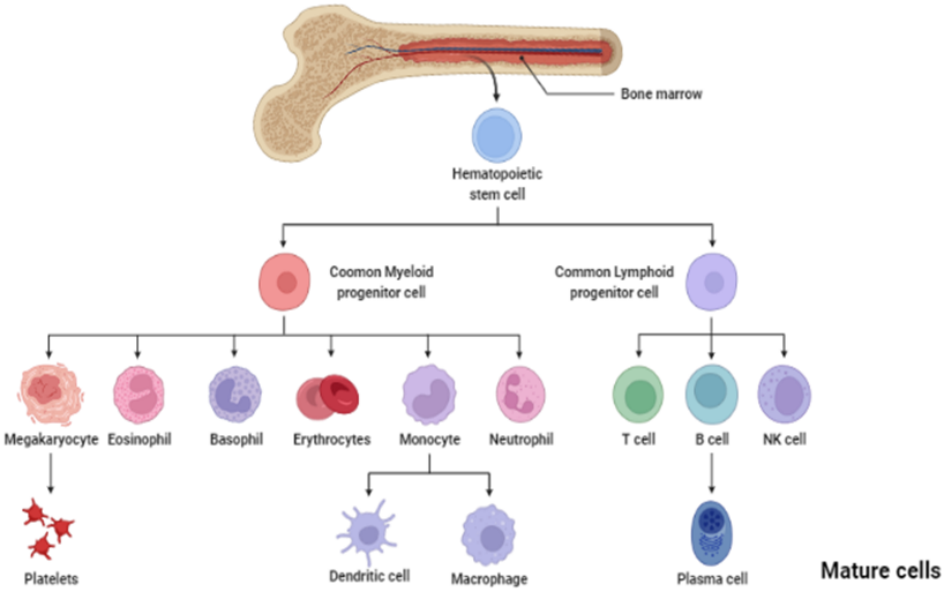

Fig. 1. Scheme of formation of mature blood cells from HSCs. Mature blood cells are formed from the blood-forming HSCs in the BM. Stem cells (SCs) are characterized by antigens on their surface (immunophenotyping)

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1.2. Hematopoietic Stem Cell Transplantation

Bone marrow transplantation, also known as HSCT, involves the transfer of healthy HSCs to patients with damaged bone marrow (4). In recent years, HSCT has entered the clinic as a life-saving treatment for many diseases, including autoimmune and genetic diseases, especially malignant and non-malignant hematological cancers. Since the 1940s, many developments have been recorded in the field of stem cell transplantation (SCT). Today, HSCT, which is accepted by many authorities to be used especially in treating hematological diseases, is performed in three different ways. They are allogeneic (from siblings, relatives, and HLA-matched unrelated donors), autologous (use of patient's own SCs in solid tumors), and syngeneic (from twin siblings). HSCs are derived from BM, PB, and UCB (5).

1.3. Autologous Stem Cell Transplantation

Using the patient's own SCs for transplantation is the main focus of autologous SCT. To increase the number of SCs in the peripheral blood, either cytokine (granulocyte colony-stimulating factor (G-CSF)) or cytotoxic agents in combination with cytokine are given to the patient as a mobilization agent to allow the SCs to leave the BM and enter the PB. Plerixafor, a CXCR4 antagonist, is a highly potent mobilizing agent. The primary function of G-CSF in this context is to trigger the release of proteolytic enzymes, particularly metalloproteinase-9, from BM stromal cells, allowing HSCs to be separated from the stroma. The presence of a sufficient number of SCs in the patient's PB is referred to as mobilization, and it is measured using a flow cytometer and the CD34+ cell surface antigen. The patients' apheresis process begins when they achieve a suitable amount of SCs in the calculation made using the CD34+ SC count and the patient's white blood cell count. The apheresis equipment collects SCs from the patient, freezes them under specific circumstances, and stores them in liquid nitrogen tanks until the transplantation procedure. A series of high-dose chemotherapy and radiotherapy are administered to the patient to kill the blasts identified as undesirable cells. Following that, similar to a blood transfusion, healthy SCs are delivered to the patient on the day of transplantation (Fig.2). For the treatment of Hodgkin Lymphoma (HL), Non-Hodgkin Lymphoma (NHL), and Multiple Myeloma (MM), autologous transplantation is often favored (MM). The patient's hematopoiesis flow is regulated by SCs reinfused into the patient. This process, in which healthy blood cell production begins again, is called engraftment. This procedure is monitored by the daily blood counts of transplant recipients. Although the incidence of problems following transplantation is lower than allogeneic transplantation, the risk of illness recurrence is considerable. Because autologous transplantation uses the patient's own SCs, graft failure (the inability of the transplanted cells to grow and divide in the BM) is uncommon, and graft versus host disease (GVHD), which occurs in allogeneic transplantation and is extremely difficult to treat, is not seen (6).

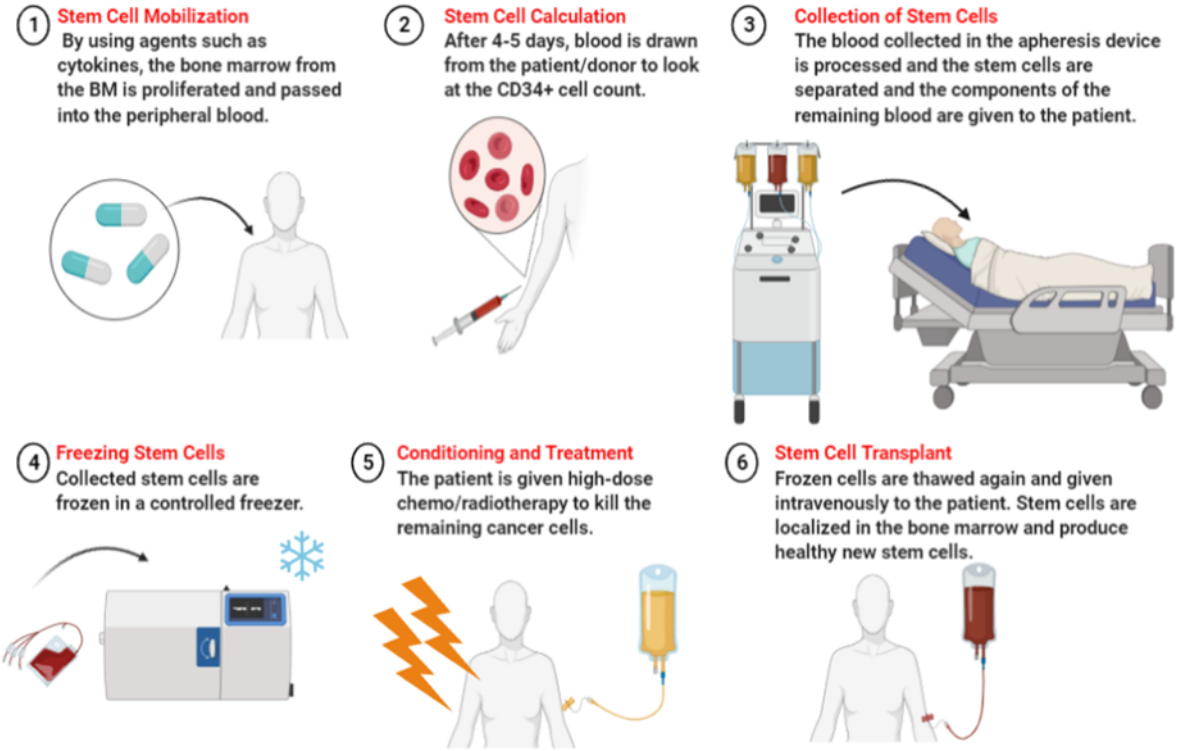

Fig. 2. Autologous SCT process

1.4. Allogeneic Stem Cell Transplantation

Patients with malignant and non-malignant hematological blood disorders are treated with allogeneic SCT when autologous SCT is not possible. In this type of transplant, human leukocyte antigen (HLA) compatibility between the patient and the donor is critical. Acute Myeloid Leukemia

(AML), Acute Lymphoblastic Leukemia (ALL), Chronic Myeloid Leukemia (CML), Chronic Lymphocytic Leukemia (CLL), Multiple Myeloma (MM), Non-Hodgkin Lymphoma (NHL), and anemia are all treated with allogeneic SCT (3).

1.4.1Types of Allogeneic Stem Cell Transplantation

Twins undergo *syngeneic SCT.* The community has a low rate of identical twins. The main benefits of syngeneic SCT are the minimal risk of developing GVHD and the lack of graft rejection risk. Because of these factors, the patient is not receiving long-term immunosuppressive medication (7).

Haploidentical SCT is performed on at least 50% HLA-matched relatives. The likelihood of graft rejection and GVHD is significant, depending on the degree of HLA incompatibility. Furthermore, after this transplant, the immune system takes a long time to rebuild (8).

Sibling SCT is a form of transplantation between siblings. Because of the genetic compatibility in HLA, it is frequently preferred. Transplantation from HLA-matched siblings is the clinic's first choice.

Unrelated SCT is an allogeneic SCT performed between individuals with unrelated HLA matching. When there are no HLA-matched family members, this is the transplant method of choice.

1.5. Sources of Hematopoietic Stem Cells

BM, PB, and UCR are the SC sources used for autologous or allogeneic HSCT. The BM source collects BM from the donor via aspiration while the donor is under general anesthesia. Using specific biopsy needles, BM is carefully aspirated from the posterior iliac crest region under operating room circumstances. The donor may undergo bleeding, infection, and localized pain throughout the 2.5-hour operation. Patients and donors who choose to collect SCs from bone marrow are subjected to a thorough physical assessment and any relevant tests. The patient does not need to introduce a specific catheter in BM transplants, and SCs are collected in one go under surgical settings. Another advantage is that no agents, such as G-CSF, are required, as with peripheral SC sources. On the other hand, the engraftment of neutrophils and platelets happens more slowly. Because of their quick engraftment timeframes and numerous other advantages, PB-derived SCs are the most often used source of HSCT in clinics. The quantity of SCs in the PB is normally modest. G-CSF and plerixafor are utilized to proliferate and transport SCs from the BM to the peripheral circulation. If sufficient CD34+SCs (5 to 15 g/kg/day) are calculated 4-5 days following the use of drugs, SCs are harvested from patients or donors in the apheresis unit. Because the veins of some patients or donors (children, patients undergoing continuous intravenous therapy, etc.) are insufficient for collecting enough SCs, the collection is done via a central venous catheter. Because not enough stem cells can be harvested in one go, SC collection (apheresis) may continue for a few days. In addition to being simple and inexpensive, acquiring PB-derived SCs has several drawbacks. The patient or donor may experience bone discomfort and flu-like symptoms using mobilization agents. Furthermore, citrate or anticoagulant chemicals used to prevent coagulation in apheresis unit sets may produce unwanted effects on the patient or donor (hypocalcemia). Another concern is the possibility of central venous catheter bleeding and infection. However, compared to BM, the recovery of all of these adverse effects occurs concisely.

The chord is constricted from a certain point after the infant is born to collect SCs from the UCB. CB is contained in a sterile blood bag with the anticoagulant. It is extracted from erythrocytes and plasma and frozen for future transplantation. Because HLA type is not an issue with UCB transplants, the risk of GVHD is lower than in other sources. The most extended engraftment timeframes are caused by the low quantity of stem cells (CD34+) derived from UCB. At least stem cells (CD34+) are isolated from cord blood using the above procedures. PBSCs produce the most significant number of stem cells. However, the danger of GVHD, the most common issue in allogeneic transplants, is substantial in all three donors. These sources of SCs contain GVHD-inducing T lymphocytes. The ratios of lymphocytes, monocytes, T cells, and dendritic cells are very high in PBSCs (9,10).

1.6. Steps of HSCT;

1.6.1. Preparation Regime: The patient is first readied for travel at this stage by employing particular preparation regimens. The preparation regimen is based on the use of chemotherapy, radiation, and other biological treatment procedures for antineoplastic or immunosuppressive reasons before autologous and allogeneic transplantation. These treatments, typically including high-dose chemotherapy, significantly contribute to the pulmonary problems reported following transplantation. Because there would be no genetic variations in autologous transplants, the goal of the preparation regimen is to remove the patient's undesirable cells and tumors. When SCs from the donor (relative-unrelated) are given to the patient in allogeneic SCs, a response may develop due to differences in HLA tissue compatibility. Priming regimens reduce the patient's immune system to prevent the transplant rejection reaction. Furthermore, the patient's defective hematopoiesis is removed using the preparation regimen used before allogeneic SCT (myeloablation). Preparation regimens are chosen in various centers by parameters such as patient diagnosis, condition, other illness factors, and transplant-related mortality risk (11–13)

1.6.2. Allogeneic Transplant Donor Selection: Allogeneic HSCT is the preferred treatment for a wide range of malignant and non-malignant disorders. Because the purpose of allogeneic HSCT is to replace the patient's ineffectively functioning hematopoiesis with that of a donor, so finding a compatible donor is a prerequisite. It is a time-consuming and complex process for both recipients and donors (10). Donor selection is crucial for allogeneic-HSCT success. When

making this decision, both relatives and non-relatives are considered. Tissue typing of fathers, mothers, siblings, and other relatives looks at the donor and recipient's HLA. When there is HLA compatibility, a stem cell source, and several donors available, donor age, gender, sex factor, weight, ABO blood group, and virus serological status should be evaluated in the donor selection process (8).

The phases of selecting a suitable donor in allogeneic SCT can be summarized as follows:

1. The tissue type of the patients is determined.

2. Family members' tissue type is determined (transplantation process starts at appropriate HLA match)

3. If compatible tissue typing cannot be established in family members, SCT centers apply to institutes that maintain national and international donor databases in search of suitable donors (National Marrow Donor Program (NMDP), German Bone Marrow Donor Center (DKMS), Turkish Stem Cell Coordination Center (TÜRKÖK), etc.)

4. Following the completion of the necessary procedures, the SCs are harvested from the compatible donor and sent to the transplant center under appropriate conditions (13).

1.6.2.1 Human Leukocyte Antigens (HLA)

Tissue typing (HLA screening) should be performed by experts in accredited laboratories as soon as possible when it is decided to perform allogeneic SCT for the patient. The HLA system is found on chromosome 6's short arm (6p21.3) and contains the most polymorphic gene cluster in the whole human genome. HLA system allows our immune system to recognize non-self. HLA is evaluated in the laboratory using molecular methods to determine whether there is tissue compatibility between the recipient and donor in allogeneic HSCT. Our HLA type is passed down from our parents. As a result, relative (family) screening is initiated first in the hunt for a donor. The HLA complex comprises Class I, Class II, and Class III sections and is found on the short arm of the sixth chromosome. HSCT screens for Class I genes HLA-A, B, and C and Class II genes HLA-DR, DQ, and DP. Donors should be entirely compatible in inbred screening and have an HLA match of 6/6 or 10/10 in unrelated screening. There is a 3/6 or 5/10 concordance in cases of one or two antigen mismatches or haploidentical cases (10,13–15).

1.6.3. Harvesting and Storage of Stem Cells from Peripheral / Bone Marrow

Under general anesthesia, BMSCs are extracted from the pelvis bone. Special bone needles used in BM extraction are inserted with injectors into multiple sections of the bone, and roughly 5 ml of BM is aspirated so that it does not mix with blood. Heparin (5,000 U/mL) must be used to clean injectors with used bone needles. To avoid coagulation, the aspirated BM contains ACD anticoagulant solution and is collected in unique bags. According to popular belief, 2 -3 x10^8 nucleated cells/kg are collected. The obtained BM is given to the patient the same day or the following day (16).

PBSCs are typically detected in tiny numbers. The number of PBSCs is enhanced approximately 100 times by utilizing different chemotherapeutic and growth agents (G-CSF, GM-CSF) (17). When sufficient SCs are obtained, they are harvested using special machines in the apheresis unit. If the harvested SCs are to be administered to the patient within 72 hours, they can be kept in the refrigerator at 2-8 °C. If the storage time is more than 72 hours, the SCs must be frozen to maintain viability. To prevent cellular dehydration and the production of ice crystals in the cells, 5-10% dimethyl sulfoxide (DMSO) is given to the collected SCs after centrifugation. Mechanical freezers (≤ -80°C) are used to freeze stem cells, which are then preserved in vapor-phase liquid nitrogen (≤−150°C) until the day of transplantation (6).

1.6.4. Transfusion of Stem Cells to the Patient

Frozen stem cell samples are thawed in a 37 °C water bath in the patient's room on the day of transplantation and infused immediately. Most adverse reactions during SCT are due to DMSO, fragmented granulocytes, or ABO incompatibility. Mild reactions such as nausea, vomiting, abdominal cramps, cough, hypertension, hypotension, and sometimes cardiac symptoms can be observed during SCT (Figure 2) (6).

1.7. Factors Affecting Transplant Success and Complications After Transplantation

Numerous factors influence transplant success. These factors may be related to the disease (disease resistance, disease phase, clonal abnormalities, and so on in malignancies, and the type of disease and associated rejection risk in non-malignant diseases), as well as the patient himself (age, comorbidities, infectious diseases/colonization, and so on). Furthermore, donor-related difficulties and SC sources can impact disease control and transplant-related mortality (18).

Another critical aspect is the occurrence of adverse responses and problems following transplantation. Despite advances in research and technology, severe and fatal reactions to drugs used in recipients' pre-transplantation preparation regimens, GVHD developing after allogeneic transplantation, infections resulting from recipient immune suppression, and respiratory failure in some patients are significant complications (12).

Post-transplant infections are one of the most serious of these problems. Cytomegalovirus (CMV) infection is one of the most prevalent after allogeneic transplantation. CMV infection can induce fever or cytopenia in patients, as well as pneumonia, hepatitis, gastroenteritis, retinitis, and encephalitis. CMV infection and the immune system both occur concurrently. For example, whereas GVHD disease increases the likelihood of CMV infection, CMV infection may have a role in the development of GVHD. The protein level in the patient's sample (plasma, urine, serum, tissue, etc.) or DNA using polymerase chain reaction is used to determine CMV (PCR). Although numerous factors influence CMV treatment,

antiviral medications, high-dose immune globulin therapy, and particular T cells are employed (19).

GVHD is one of the most prevalent problems following allogeneic SCT. GVHD, which is extremely difficult to treat, is studied in acute and chronic stages. It is referred to as acute GVHD if it occurs during the first 100 days of HPSCT and chronic GVHD if it occurs after 100 days. Age of the patient and donor, female donor option, unrelated donor option, and, as previously indicated, the presence of CMV in the patient or donor are all factors that contribute to the emergence of GVHD. The reactivity of the donor's T cells as a result of seeing the patient's tissues as antigenically foreign is critical in GVHD illness. GVHD causes serious harm to the patient's skin (scaling), liver (change in bilirubin level), and digestive system (severe diarrhea). The severity of GVHD and the extent of organ damage it produces are closely proportional to the level of HLA incompatibility (20).

1.2 Stem Cell Transplantation in Turkey

The journey of SCT in Turkey began in 1978. Prof.Dr.Korkut Zerkan performed the first allogeneic BMT in Turkey at Hacettepe University Medical Faculty Hospital in 1978, and Prof.Dr. Onder Berk and his colleagues performed the first autologous BMT at Gülhane Military Medical Academy in 1984. In 1992, the first autologous PBHSCT was conducted. Prof.Dr. Gündüz Gedikolu and Prof.Dr. Made by Sema Anak completed the first SCT in pediatric instances. Dr. Atila Tanyeli performed the first pediatric autologous SCT on a patient with recurrent HL in 1992 (21).

At the current level, Turkey competes with European countries on HSCT. Turkey, which has attained world standards due to qualified professionals trained in developing technical infrastructures, offers more appropriate and quality treatment options than other European countries and the USA. With the rise of health tourism, people worldwide are flocking to Turkey for transplants. The number of patients seeking transplantation services grows in lockstep with the expansion of stem cell transplantation centers. Of course, establishing the Turkish Stem Cell Coordination Center (TÜRKÖK) under the Ministry of Health to encourage unrelated SC donations and construct a donor database pool was critical to this accomplishment.

HSCT is a safe form of therapy for the treatment of hematological diseases. Having information about the transplant types will enable the selection of the right transplant type. It is very important in the search for alternative donors and HLA-matched allogeneic transplants. Technological developments to prevent complications that may occur after transplantation will further increase the use and effectiveness of HSCT.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

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

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Beyond cognition and sleep: Stop the domino effect

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Abstract

Sleep problems, particularly sleep deprivation and fragmentations, are common hazards in modern lifestyles and may be an unavoidable occupational drawback. Different metabolic or hereditary causes may induce sleep problems. Cognitive functions and closely related metacognition, which depend significantly on decision-making, are essential for learning and problem-solving. When impaired for any reason, the quality of life deteriorates, and the person may become dependent on others in severe cases of dysfunction. Sleep and cognition/metacognition are related, and an insult to one function may lead to the other domain's collapse. The present review highlights the significant causes and conditions of sleep problems, cognitive and metacognitive dysfunctions, the possible mechanisms, interactions, and the potential tools or agents that may improve them.

Keywords: sleep, cognition, metacognition, obesity, probiotics

1. Background

Poor sleep is a common health hazard in modern societies, and it impairs the rhythmic expression of clock genes responsible for circadian rhythm regulation for the central clock in the central suprachiasmatic nucleus (SCN) and the peripheral clocks throughout the body (1). Moreover, sleep deprivation is associated with impaired cognition, metacognition (2), stress coping, and affective disorder (3). These cases are summarized in Fig. 1.

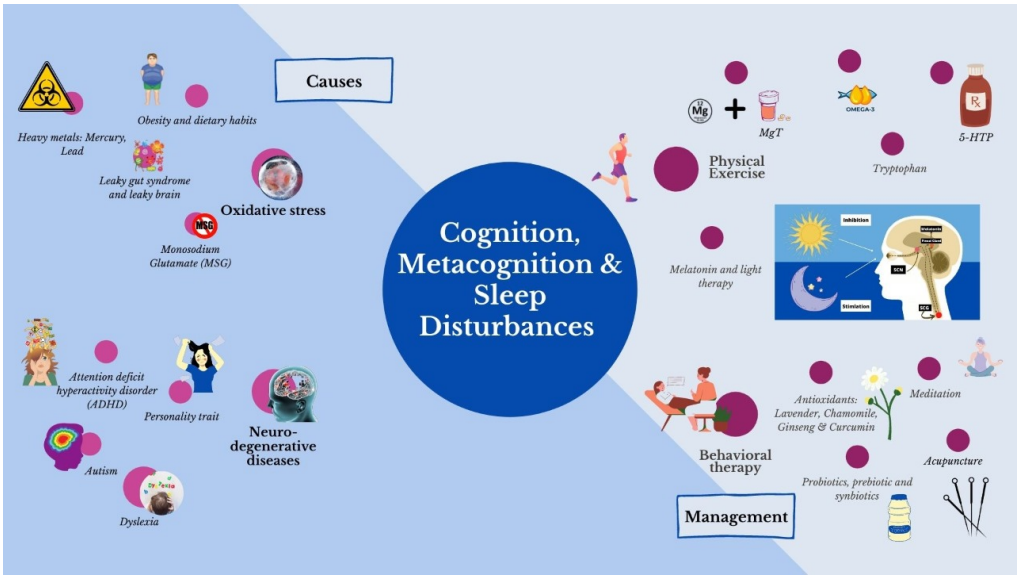

Fig. 1. Causes and management of sleep disturbances

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During sleep, there are ongoing processing and running activity for different cognitive domains like consolidation of motivationally relevant information, which depends on sleep spindles and slow-waves oscillatory activity (4), dopamine system activation (5), decision-making, and conditioned learning (6).

According to Caputo et al. (7), learning and cognitive memory mechanisms play a vital role in developing and maintaining anxiety since exposure to cues related to aversive situations induces high arousal and anticipatory anxiety. Memory becomes unstable following reactivation, and modification is then possible via reconsolidation and extinction, and inhibition of these processes results in attenuation of contextual cues on the anticipatory anxiety (8).

Circadian rhythm refers to all the physiological and behavioral activities repeated in a cyclic manner around 24 hours. The periodicity patterns depend on external factors or cues called zeitgebers, such as exposure to light or dark and temperature changes. The circadian phase determines multiple aspects of sleep physiology, including total sleep duration, the phasic alternation between rapid eye movement (REM) sleep and non-rapid eye movement sleep (NREM), sleep continuity, and spindle activity (9).

Accordingly, the circadian phase affects wakefulness and sleep duration, reflected in synaptic plasticity markers (10). Daily stress, hyper-arousal, and pre-sleep cognitive activities significantly affect sleep latency and insomnia (11).

Melatonin and cortisol levels are hormonal markers of circadian phases. Melatonin is the primary regulator of sleep-wake timing (12). In contrast, cortisol is a stress hormone strongly linked to anxiety and depression and plays a role in arousal (13). Sleep effects on cognition are not related to age. However, aging makes the person more susceptible to the hazards of sleep deprivation and interruption of circadian rhythm and cognition (14, 15). Accordingly, maintaining normal sleep could be a protective or therapeutic tool for certain cognitive disorders (16).

Metacognition is a higher level of managing and controlling cognition; it is firmly related to decision-making and problem-solving with neural systems located in the prefrontal cortex (17). Metacognition is a powerful academic tool that can lift academic achievement and learning if used properly. It represents our driving and control of thinking through two significant steps: metacognitive knowledge and regulation (18). Metacognitive knowledge is what we all know about our thinking, which approach is the best and differentiates between what we know and what we understand (19). The second step for metacognition is metacognitive regulation, which involves the actions and procedures we perform to learn (20).

The direct relationship between metacognition and sleep deprivation is not well studied relative to cognitive functions. Baranski (21) reported that sleep deprivation affects cognition and metacognition with more stress on cognitive functions. In another study by Aidman et al. (2), Using 40 hours of sleep deprivation in 13 Australian Army male volunteers aged 20 - 30 years, tested metacognition functions many times. It showed that fatigue-inducing states affected metacognition rather than cognitive mechanisms.

The present review highlights the significant causes and conditions of sleep problems, cognitive and metacognitive dysfunctions, the possible mechanisms, interactions, and the potential tools or agents that may improve them.

The methodology used for the present review involved electronic searches on PubMed and googled scholar between 1985 and 2021 using the keywords; sleep, cognition, metacognition, obesity, dietary habits, leaky gut syndrome, leaky brain, heavy metals, MSG, exercise, Omega-3, magnesium, probiotics, oxidative stress, antioxidants, herbs, acupuncture.'. We excluded articles in languages other than English and unrelated ones, assessed two hundred eighteen articles for eligibility, and included them in the review.

2. Common causes of impaired sleep and metacognition

2.1. Obesity and dietary habits

Cultural dietary habits are essential determinants for weight gain, sleep quality, and duration consequences. Moreover, in a cross-sectional self–reported questionnaire on African American 1837 adults (75% females), with a mean age of 48.2 + 13.7 years and a mean BMI of 32 + 7.5 Kg/m2, Wu et al. (22) demonstrated that decreasing sleep was related to increasing weight and Body Mass Index (BMI). This creates a vicious pathological circuit of weight gain and sleep abnormality.

Ding et al. (23) revealed an association between high consumption of sugars and sleep deprivation with modified leptin hormone profiles. They explained that shift work affected the hypothalamic-pituitary-adrenal axis and triggered circadian disturbances causing hyperglycemia and excessive fat deposition. Leptin and ghrelin are essential for controlling hunger and sleep, and both hormones are reciprocally regulated in sleep deprivation with higher production of ghrelin and lower production of leptin, resulting in disruption of energy balance (24, 25).

Metacognitive dysfunction is well described with eating disorders like anorexia nervosa (26). By evaluating 44 adults, Quattropani et al. (2016) illustrated that emotional and affective disorders were commonly associated with obesity.

On the molecular level of the neurons, repeated sleep disruption produces oxidative stress, neuronal injury, and loss, especially among the neurons involved in arousals like hypothalamic orexinergic neurons and the locus ceruleus noradrenergic neurons (27).

Obstructive Sleep Apnea (OSA) interrupts or pauses breathing cycles during sleep with airway obstruction. It is a common disorder among obese, related to numerous health

issues (28). Sleep duration and quality are essential to maintain cognitive performance homeostasis (29). It is common in obese individuals with decreased sleep duration or sleep quality, usually with dysfunction of cognitive processes (14, 30), especially consolidation of memories (31) through synaptic remodeling (32). Holloway et al. (32) studied 16 male subjects randomly assigned in a crossover design. They used a high-fat (75%) diet compared to a standard diet (23% fat) for five days. After a 2-week washout period, subjects consumed the opposite diet. They showed that a seven-day high-fat diet reduced attention and reaction time with depression and impaired retrieval speed associated with neuroinflammation. Besides, after studying 4- year cognitive change in 6183 older women, Okereke et al. (33) demonstrated that high intake of saturated fats in young adults impaired memory and cognitive function, causing neurological disorders like dementia and Alzheimer's disease in mid and later life.

Fats increase oxidative stress and change apoptosis. Free radicals cause the progression and development of cognitive dysfunction via interfering with synaptic transmission, mitochondrial function, neuroinflammation, and axonal transport, with neuronal loss in dementia disease (34).

2.2. Monosodium glutamate (MSG)

Monosodium l-glutamate (MSG), a sodium salt of l-glutamate, is commonly used as a taste-enhancing additive. Glutamate, an excitatory neurotransmitter, is a part of its molecule (35). MSG produces excitatory and inhibitory responses according to dose, route, and intake duration (36). Onaolapo et al. (36) compared the effect of food–added MSG on neurobehavior, serum biochemical measures, cerebral cortex, liver, and renal morphology in mice fed a standard diet and high-fat diet for eight weeks.

Glutamate transport across the BBB is well-regulated to protect against glutamate-induced excitotoxicity (37). Prolonged dietary consumption of glutamate in the form of MSG in 64 adult male rats increased glutamate levels in the brain in the long run and impaired hippocampal function (38). This produced forebrain activation in various areas, including the insular cortex, basal ganglia, limbic system, and hypothalamus (39). In studying ten adult rats, MSG prolonged Rapid Eye Movement (REM) sleep duration and its episode frequency (40) and was associated with snoring and sleep breathing disorders in non-obese subjects (41).

MSG causes hyperactive and inattentive behavior (35). Akataobi. (35) demonstrated that MSG affected neonate and adult rats after six weeks of study similarly. Memory impairment with MSG is either a result of interference with glutamate synthesis in the hippocampus or inhibition of the cholinergic system (42).

The effect of MSG on cognition has also been linked to its attenuating effect on the cyclic - AMPK level in the hippocampus (43). Hippocampal AMPK protects neurons and attenuates the damage by b-amyloid and glutamate excitotoxicity (44).

2.3. Leaky gut syndrome and leaky brain

There is a complex bidirectional interaction between the gut microbiome and the brain. This microbiome involves trillions of human microorganisms, including bacteria, fungi, viruses, and protozoa. It has the most significant and vulnerable surface to prevent a leak (penetration) of some food components, environmental factors, and others. Disruption of this barrier causes leaky gut and brain, resulting in neurological diseases such as Alzheimer's, autism spectrum disease, stress, and Parkinsonism (45).

Leaky gut (intestinal hyperpermeability) triggers include physical or psychological stress, nutritional deficiencies, food allergy, food irritants such as casein or gluten, food additives, intestinal dysbiosis, infections, autoimmune diseases, toxins, and NSAIDs.

When significant bacterial or food particles penetrate the intestine, they release inflammatory and immune mediators, initiating more inflammatory and allergic responses, with more intestinal permeability and changes in CNS functions, including mood and behavior (46). In a review by Julio-Pieper et al. (46), they reported that about half of clinical and animal model studies showed intestinal barrier damage in schizophrenia, autism spectrum disorders, and neurodegenerative diseases.

2.4. Oxidative stress

After intracerebroventricular injection of radiolabeled arachidonate into mouse models of Alzheimer's disease, Furman et al. (47) revealed increased biomarkers of oxidative stress in many neurodegenerative diseases. Redox imbalance can cause a leaky gut and leaky brain via immune cells modulating oxidative stress. Ischemia may initiate disruption of the blood GIT and blood-brain barrier and occurs via reperfusion and production of reactive oxygen or free radical formation. This is antagonized by antioxidants (48).

In humans, excessive energy, especially high-fat food, causes oxidative stress and impairs cognitive function. Oxidative stress is more likely to develop in the brain due to the diversity of reactive species to modulate heterogeneous signaling pathways (49). Cognitive dysfunction is linked to low-grade inflammatory stress, inducing cell-mediated immunity and oxidative stress.

2.5. Heavy metals

Mercury

Mercury (Hg) exposure induces aversive memory injury and recognition memory deficits (50). A review by Bjørklund et al. (50) revealed neurological symptoms in dental workers who are occupationally exposed to chronic low levels of metallic Hg. In dentists and dental personnel, memory, neurobehavioral, cognitive, and attention disturbances are more common. Long-standing exposure to HgCl2 impairs memory and induces anxiety (51). Mercury accumulation has

been shown in the pineal gland, which participates in circadian function through melatonin and serotonin (52).

Hg can cause Alzheimer's disease (53), and the high penetrability of Hg into the brain alters sleep patterns by dysregulating the extracellular concentrations of glutamate, acetylcholine, and dopamine. These neurotransmission changes are reflected in non-REM sleep, REM sleep, awakening, and decreased night-time melatonin levels (54).

After intoxicating 20 rats for 45 days, Teixeira et al. (51) showed that $HgCl_2$ accumulates in the hippocampus and cortex regions with a higher affinity for the cortex. Furthermore, Mercury inhibits serotonin binding to brain receptors (53). The 5-HTergic system in the prefrontal cortex and basal ganglia plays a significant role in neuroprotection and cognitive regulation (55).

Mercury exposure in the prenatal period impairs the dopaminergic and glutamatergic system, impairing learning and memory (56) with decreased IQ scores and other attention and spatial tests memory (57).

Methyl mercury, which has sufficient permeation to the brain, causes Minamata disease, producing damage and neurological manifestations of the disease (58).

Additionally, there is an association between mercury toxicity and autistic spectrum disorder (ASD) diagnosis. Of 91 studies from 1999 to 2016, 74% suggested a direct and indirect relationship between Hg and autistic spectrum disorder (59).

Lead

Lead (Pb) is another heavy metal that can penetrate the CNS and negatively affect metacognition and sleep. Lead precipitation in front-hippocampal circuits impairs the acquisition, consolidation, and recall of memories, especially emotional memories, which causes emotional and behavioral dysregulation frequently seen in Pb-exposed children (60). Moreover, lowered intellectual scores, learning and memory scores, visual memory scores, verbal memory scores, and inadequate sleep with inadequate performance during the day have been recorded with lead exposure (61). In a cross-sectional study, Mohammadyan et al. (61) measured the occupational exposure of 40 soldering workers to lead fumes through their blood lead levels and sleep quality and recording of digestive disorders. Impaired sleep quality and gastrointestinal disorders were prevalent.

According to Kalinchuk et al. (62), nitric oxide (NO) donors induce sleep through adenosine production. This means that reduced NO production causes sleep deprivation. Pb changes the activity and expression of neuronal nitric oxide synthase (nNOS) and brains endothelial nitric oxide synthase (eNOS) as it simulates Ca^{+2} and blocks its binding sites for NOS, reducing cerebral NO levels.

In addition to its effect on sleep, the reduced NO production impairs long-term potentiation (LTP), forming the molecular base for learning and memory (63). Lead exposure causes hippocampal damage by denaturating myelin and neuronal nuclear irregularities (64). Allen et al. (65) described memory and metacognition dysfunction using a high–resolution multi-parameter mapping technique in 48 healthy individuals. Furthermore, Lead is a non-competitive N-Methyl-D-aspartate receptor (NMDAR) antagonist. NMDA-Rs are ligand-gated receptors stimulated by glutamate and are vital players during neural development, neuronal plasticity, learning and memory, and LTP (66). Rocha et al. (66) summarized clinical and preclinical studies with various research techniques. They showed that low lead levels decreased cognitive functions and produced maladaptive behavior in human and animal models.

It is worth noting that lead exposure is a risk factor and a common association with Attention Deficit Hyperactive Disorder (ADHD) (67).

2.6. Personality trait

Lack of impulsivity affects sleep behavior, and McGowan and Coogan's (68) study showed that individuals with impulsivity suffered from shorter sleep duration, less efficient sleep, delayed sleep timing, and greater diurnal arousal. Social Anxiety Disorder (SAD) is a common finding in modern societies. It is believed that the way of thinking may affect the severity of this disorder. In clinical and experimental samples included in cross-sectional investigations, Gkika et al. (69) showed that negative beliefs and the dangerousness of thoughts were positively and significantly correlated with SAD.

Using metacognition models, McEvoy (70) stated that Meta-Cognitive Therapy (MCT) was associated with a better outcome in the anxiety and depression symptoms than the usual treatment.

Ronfeldt et al. (71) investigated the effects of third-wave cognitive constructs (mindfulness, psychological inflexibility, and meta-cognitions) on a person's psychological status, stating that mindfulness was inversely proportional to anxiety.

Additionally, after completing an online questionnaire at baseline and one year on 76% of 2291 participants from universities, Sun et al. (72) showed that negative meta-cognitions contributed to developing anxiety and paranoia.

Anxiety and depression symptoms are common in people with physical health conditions, with increased anxiety symptoms in cardiac and cancer patients (73) and diabetes (74). Sleep deprivation is considered a factor that affects mental health; Pires et al. (75) observed that significant anxiogenesis resulted from lack of sleep.

Interestingly, most sleepwalking or talking children also had an anxiety disorder (76). Sleep problems have several adverse health outcomes. Gould et al. (77) study on 109 adults aged 66-92 years revealed that the Geriatric Anxiety Scale (GAS) affective and somatic sub-scales were significantly associated with global sleep quality, suggesting that personalized treatment improved specific anxiety –symptom

domains or vice versa. A study by Nadorff et al. (78) showed that Generalized Anxiety Disorder (GAD) was significantly correlated with alarming dream frequency. Moreover, sleep loss affects brain regions, which are essential for decision-making, such as the prefrontal cortex.

Short and Weber (79) described an association between time of sleep and risk-taking, while Benard et al. (80) revealed that the proportion of suicides among patients with bipolar disorders (BD) was higher than in the general population, with more circadian rhythms and sleep disturbances compared to healthy control.

2.7. Autism

Autism spectrum disorder (ASD) has severe social communication impairments and restricted and repetitive behavior and interests (81). Grainger et al. (82) suggested that the metacognitive ability to express our mindset depended on the same processes that we could anticipate others' mindset ("mindreading"), which was impaired in ASD. They found that people with ASD had mindreading problems and could not precisely monitor their memory details and components. However, they showed that some people with ASD had superior metacognitive abilities relative to neurotypical people.

Furthermore, Griffin et al. (83) reported that adults with ASD had high levels of alexithymia, "a difficulty identifying and labeling one's emotional states". Autism is usually associated with a learning disability and lower levels of IQ, impacting all learning and severely impairing adaptive behavior compared to deficits in overall or general intelligence (84).

Adults with ASD hardly develop independent life with moderate mental capabilities (85). The metacognitive impairment in an ASD can be explained by understanding the representational nature of belief, even their own beliefs. They also have more difficulty understanding desire than neurotypical individuals (86). Additionally, individuals with ASD struggle with 'strategic' aspects of social learning, selecting the relevant and be-imitated information during imitation tasks (87).

Sleep problems are common lifelong comorbidity in ASD and can increase latency, sleep fragmentation, and circadian phase disorders (88, 89). This results from neurotransmitter abnormalities or other commonly associated comorbidities like epilepsy (90).

Sleep difficulties lead to negative consequences during the daytime, like physical aggression, irritability, inattention, hyperactivity (91), impaired cognitive performance, and quality of life (88).

2.8. Dyslexia

Dyslexic readers' abilities are lower than typically developing readers in all forms of self-reported metacognitive knowledge and reading motivation, with lower performance than typically developing readers. However, dyslexic children have normal vocabulary consolidation abilities and can recall novel words like normal children (92). Children with dyslexia have slow-wave sleep with a longer duration and higher frequency of sleep spindles, explaining the affected vocabulary consolidation (93). Carotenuto et al. (94) found that children with dyslexia showed higher rates of Sleep Disturbances Scale for Children (SDSC) pathological scores in the total SDSC score, higher rates in the number of times they woke up, nocturnal hyperkinesis, and snoring.

A recent study by Huang et al. (2020) (95) revealed that salivary melatonin in dyslexic children was less than the average children, and the rhythm of day low/night high disappeared.

2.9. Attention Deficit Hyperactivity Disorder (ADHD)

Attention deficit hyperactivity disorder (ADHD) is associated with academic retardation, the dysfunctional social interaction that results in a poor quality of life. ADHD in adulthood suffers from inattention, hyperactivity, impulsivity, and low academic achievement (96). ADHD management includes cognitive-behavioral therapy (CBT) and stimulant drug treatment with methylphenidate (97).

ADHD negatively impacts different aspects of cognition, including attention, executive functioning, memory, and learning. Objective psychometric tests may provide information about the individual's cognitive efficiency, whereas subjective self-reports might indicate success in individual goal achievement (98)

Gregory et al. (99) study on 2232 twin children associated ADHD with low sleep quality. Caregivers of children with ADHD develop sleeping problems due to their effort to care for them (100). Their children also develop sleep abnormalities resulting in dysfunction (101). Fortunately, poor sleep remits over time in ADHD, which may reassure parents and children with ADHD (99).

2.10. Neurodegenerative diseases

Alzheimer's disease (AD), the commonest dementia worldwide, is associated with sleep deprivation. In 18 longitudinal studies that included 246.786 subjects at baseline and 25.847 dementia cases after an average of 9.49 years of follow-up, patients with insomnia, sleep-disordered breathing, or other sleep disturbances were found to have higher risks of developing AD (102).

A post-mortem study by Lim et al. (103) on 45 older adults with a mean age at death of 89.2 years demonstrated that repeated sleep interruption caused a decreased number of hippocampal neurons in AD than normal controls.

Sleep abnormalities in Parkinson's Disease (PD) are common non-motor symptoms significantly impacting patients' quality of life (104) and precede any motor or cognitive manifestations (105). Abbott and Videnovic (106) hypnotized that circadian and sleep changes may strike the neurodegenerative process in PD that increases the sleep and

circadian abnormalities in a vicious circle pattern with more neurodegeneration and sleep disorders (107)

Sleep problem management may be a potential means to slow disease progression from the early stages of PD (108). Moreover, in PD, there is a dysfunction of circadian rhythm markers such as cortisol, melatonin, C-type natriuretic peptide amino-terminal (NT-proCNP), and Tumor necrosis factor-alpha (TNF-α), and assessment of these markers can be considered as markers for onset and stage of PD (109).

3. Management of dysfunctional metacognition and sleep disorders

3.1. Physical Exercise

A healthy diet and lifestyle play a crucial role in delaying aging health issues and maintaining an acceptable cognitive function level, especially in vulnerable seniors (110). A higher level of total daily physical activity is associated with a reduced risk for neurodegenerative diseases (111).

Regular exercise may play an essential role in the upregulation of brain-derived neurotrophic factor (BDNF) and upregulates the production of several neurotransmitters, like serotonin, associated with mood enhancement and reduced depressive symptoms (112).

Physical exercise positively impacts sleep in both normal conditions and the presence of sleep disorders; it enhances sleep quality and decreases the time to fall asleep (113). Moreover, physical exercise has a restorative property on the brain's prefrontal lobe, positively impacting sleep quality (114).

These exercise-induced actions may indirectly affect cognition as sleep improves neurotransmitters and neurotrophic systems such as; norepinephrine, serotonin, endorphins, BNDF (113), and melatonin (115). The systematic review and meta-analysis reported by Banno et al. (115) included nine studies with a total of 557 participants.

There is a positive correlation between physical exercise and academic performance with higher cognitive and metacognitive skills (116), and this is linked to the remapping of the brain as a part of brain plasticity that is improved and upgraded by physical exercise(117). Physical exercise performance may affect the brain through factors released into circulation with physical exercise.

The bone hormone Osteocalcin level is upregulated by physical exercise, and it is essential in the construction of bones and the regulation of blood sugar (118). Moreover, Osteocalcin affects sleep length, diurnal rhythm, brain signaling, and metacognition. It stimulates the secretion of serotonin, which influences mood and cognition.

The Osteocalcin is composed of a protein gel matrix made from Vitamin D repositories in the body. Therefore, individuals with Vitamin D deficiency are obese and have sleep problems because it is correlated to Osteocalcin's function and its impacts on sleep and body mass(119).

3.2. Omega-3

Omega-3 polyunsaturated fatty acids (PUFA n3) are neuroprotective agents because of their anti-inflammatory and anti-apoptotic actions. Furthermore, Omega-3 regulates the function of growth factors that influence synaptic plasticity and function (120). Omega-3 regulates membrane fluidity and gene expression (121). Low dietary Omega-3 causes impaired glutamate and monoamine synaptic function (122), contributing to depression, cognitive decline, or dementia, especially for AD. These effects of Omega-3 deficiency are related to manipulating the endocannabinoid and inflammatory molecules that result in microglia engulfment of hippocampal synapses. That explains the neuroprotective effect of Omega-3 and its attenuating action for cognitive decline in the elderly (119).

Oily fish is necessary for normal serotonin production and sleep control. Additionally, in 395 healthy children aged 7-9 years, 16 weeks of Omega-3 supplementation elevated the Omega-3 concentration of red blood cells and decreased the severity of obstructive sleep apnea with better sleep patterns (123). Oily fish also provide vitamin D that regulates the sleep-wake cycle. In a trial involving 677 people consuming oily fish, Del Brutto et al. (124) showed a link between dietary fish consumption and sleeping in a population where caught fish formed the primary dietary protein source.

Another study by Komori (125) treating depressed individuals with phosphatidylserine and O3PUFAs showed normalized salivary cortisol (circadian and basal secretion) in responders compared to non-responders. Chronic insomnia is associated with spikes in the release of cortisol (126). In support of the previous studies; Alzoubi et al.'s (127) eight weeks study on rats demonstrated the protective and antioxidant effects of Omega-3 on long and short memory in sleep deprivation.

3.3. Melatonin and light therapy

Dysfunction of the timekeeping system causes Circadian rhythm sleep-wake disorder (CRSWD), leading to the loss of synchrony and harmony between endogenous components of the circadian system and the external ques (128). It may be a primary disorder like circadian phase disorder or secondary to abnormal melatonin secretion in psychiatric or neurological diseases (129).

Various drugs are introduced for sleep disorders, like chloral hydrate, barbiturates, benzodiazepines, modafinil, antidepressants, and anxiolytics. However, these medications have unavoidable side effects, like daytime sleepiness, cognitive dysfunction, and dependency.

It is crucial to consider synchronizing the circadian components with the given therapy, for example, considering the time of melatonin or light therapy for circadian rhythm sleep disorders (128).

The wavelength determines the light therapy efficiency, the strength of the applied light, the time of application, and whether the person was previously exposed to light or not (130). The same light intensity may delay the sleep phase of the circadian cycle if administered before the core body temperature minimum or advance it if administered after it. For the same reasons, exogenous melatonin administration should also be timed by circadian phase for non-24 hours sleep-wake phase disorder. In blind individuals, Tasimelteon (a selective dual melatonin receptor agonist) showed promising results (128).

The pineal gland secretes melatonin into the blood exclusively in the dark following biological time. It is tolerated and has a lower risk of dependence than other sleep medications (131). Melatonin therapy for chronic insomnia is a safe and effective method to improve sleep onset, latency, duration, and quality. Exogenous melatonin stimulates the naturally secreted melatonin, binds to melatonin receptors, and triggers the target signaling pathways; hence, it treats insomnia (132). The pineal gland secretes melatonin into the blood exclusively in the dark following biological time. It is tolerated and has a lower risk of dependence than other sleep medications (131). Melatonin therapy in chronic insomnia is a safe and effective method to improve sleep onset, latency, duration, and quality (133); (134). Exogenous melatonin stimulates the naturally secreted melatonin, binds to melatonin receptors, and triggers the target signaling pathways; hence it treats insomnia (132).

Furthermore, melatonin ameliorates the complications caused by sleep-breathing disorders. The hypoxia-induced hyperglycemia in experimental studies for sleep apnea included 36 mice injected with IP melatonin for 21 days (135). It inhibits the expression of inflammatory cytokines (Tumor necrosis factor-alpha, Interleukin-6, and Cyclooxygenase-2) and fibrotic markers (PC1 and TGF-beta) (136).

On the other hand, melatonin may improve hypersomnolence's central disorder as it alters sleep architecture in narcolepsy, a disorder of circadian rhythm and REM sleep deficit. Changes in REM patterns in narcolepsy patients are like those seen in patients and animal models with the pineal gland removed. Additionally, melatonin's exogenous doses significantly increase REM sleep time in normal cohorts and patients with a central hypersomnolence disorder (132). Melatonin also relieves shift workers' sleepiness (137).

3.4. Tryptophan

A diet rich in Tryptophan and antioxidants improves affective and cognitive domains (138). Tryptophan plays a crucial role in protein synthesis. It is a precursor of biologically active compounds like serotonin, melatonin, quinolinic acid, kynurenic acid, tryptamine, and also coenzymes essential for electron transfer reaction (redox balance of metabolism), such as nicotinamide adenine dinucleotide (NAD^+) (139).

A deficiency in Tryptophan, caused by malnutrition, may affect the central and peripheral serotoninergic pathways, although different nutrition-derived hormonal molecules may rescue some of this deficiency (140). Serotonergic dysfunction has been related to panic, depression, aggression, and suicidality symptoms. Because the serotonin system is involved in various psychiatric disorders and is also involved in the regulation of satiety, it can be important in the pathophysiology of eating disorders such as anorexia nervosa.

Tryptophan is used to treat various disorders but has been withdrawn in most countries. During the treatment of tryptophan preparations, undesirable symptoms include various pulmonary, cutaneous, and neurologic symptoms, eosinophilia-myalgia syndrome, and disease-related muscle pain. Various diseases and disorders are linked with Tryptophan and its metabolites. Increased metabolism of Tryptophan, or undesirable effects of low Tryptophan, such as decreased absorption or intake and signs of depression and neurovegetative complaints, has been observed in different pathology types (141,142).

Murr et al. (143) measured serum concentrations of free Tryptophan and CRP in 1196 patients with coronary artery disease. Lower serum tryptophan levels in patients undergoing coronary angiography were predictive for higher total, cardiovascular, and non-cardiovascular mortalities. Thus, the increased risk in patients with major depression for developing cardiovascular disease, the inadequate response to treatment, and increased morbidity and mortality could relate to more significant disturbances of tryptophan metabolism (144).

The biosynthesis of the most important neurotransmitters for mood stability is serotonin, dopamine, epinephrine, norepinephrine, and nitric oxide (NO), which is achieved by enzymes tryptophan 5-hydroxylase, phenylalanine 4-hydroxylase, tyrosine 3- hydroxylase, nitric oxide synthase, and all these enzymes require BH4 as a cofactor (145).

BH4 is a strong reductant and therefore undergoes oxidation readily (146). Consequently, antioxidants prolong the life span of BH4 and contribute to increasing the activity of the BH4-dependent enzymes (147), and the biosynthesis of the mentioned neurotransmitters increases; this may explain why foods rich in antioxidants are considered as mood enhancers and improve cognitive abilities.

Tryptophan can be used for the management of sleep problems. It is interesting to note that dietary Tryptophan produces therapeutic effects through melatonin. Tryptophan treatment's crucial feature is that it does not directly reduce cognitive ability (148).

Mood and cognition are closely related in older persons (149) and linked to serotonin's biochemistry (150). Classical antidepressants like selective serotonin reuptake inhibitors (SSRIs) increase serotonin levels in the brain leading to enhanced postsynaptic neuronal activity (138).

A meta-analysis of several clinical trials observed a

precognitive effect of antidepressants in patients with major depressive disorder. Accordingly, antidepressants significantly affect psychomotor speed and delayed recall (150). A potential role of tryptophan depletion in cognitive ability has already been suggested and is further strengthened in patients with Alzheimer's dementia and Huntington's disease (151).

3.5. 5-hydroxytryptophan (5-HTP)

The density of serotonergic fibers increases in the hippocampus and decreases in the thalamic paraventricular nucleus (PVN) due to brain serotonin depletion. In contrast, the serotonin precursor 5-hydroxytryptophan (5-HTP) administration could rescue these defects following the reestablishment of brain 5-HT signaling (152).

A study that included 4-week-old mice considered that serotonergic systems affected sleep, and the combined GABA and 5-HTP had synergistic effects on sleep duration and quality (153).

Meloni et al. (154) revealed that scores of the Beck Depression Inventory (BDI-II) and the Hamilton Depression Rating Scale (HDRS21) showed substantially more significant improvement with 5- HTP. Supplementation for night terrors examined the influence of L-5-hydroxytryptophan on sleep terrors. More than half-night terror episodes have been observed in over 93% of children within a month. These results confirm that arousal levels might be positively influenced by treatment with L-5-hydroxytryptophan, resulting in reduced sleep terror behaviors in children (155). The trial of these authors included 45 children aged 3.2-10.6 years.

3.6. Magnesium and magnesium l-threonate (MgT)

Magnesium (Mg^{+2}) is a cofactor for many enzymes, and many organs need it to maintain proper function (156). Furthermore, Mg^{+2} is critical for numerous cellular processes, including enzymatic reactions, ion channel functions, metabolic cycles, cellular signaling, and biomolecules' stability, such as RNA, DNA, and proteins (157).

Mg^{+2} is essential for regulating the structural and functional synapses and synaptic plasticity (158). The intracellular concentration of Mg^{+2} positively correlates to synaptic branches' arborization (156).

Besides, Mg^{+2} regulates NMDA receptors (NMDAR) block, which is essential in controlling long-term potentiation and synaptic plasticity (156).

Several studies indicated that synaptic connections in the hippocampus decline during aging, with the degree of loss of synapses correlating with memory function impairment. The reduction seems to be specific to certain hippocampal subregions. Furthermore, in aging rodents, the dentate gyrus (DG) is the most affected area in the loss of synapses (159). Altering the hippocampus's synaptic efficacy is an initial event in cognitive disorders such as AD (160).

Magnesium deficiency and depression are linked in experimental and clinical studies (161, 162). However, prospective cohort studies failed to find an association between magnesium status and later risk of depression (163). Some intervention studies suggested a beneficial role of magnesium supplementation in treating depression (164), while others have not (165).

Moreover, Lai et al. (166) investigated children's cognitive functions and correlated the results to maternal blood zinc and magnesium levels during pregnancy. They revealed that deficiency of minerals during pregnancy was linked to the cognitive dysfunction of their children. A low Mg^{+2} intake has been associated with poor-quality sleep and inflammatory stress (161).

Magnesium l-threonate (MgT) consists of a magnesium ion and threonate, which exists physiologically within the brain. MgT significantly increases magnesium ion levels within the CSF (156) and improves memory recall and spatial memory (156).

Besides, MgT prevented reducing glutamatergic synaptic transmission under AD-like pathological conditions characterized by excitotoxicity (167). A study of the hypoxic zebrafish model assessed the effects of magnesium on cognitive functions. It showed that pre-treatment with MgT upregulated glutamate transporter EAAT4, improved neuronal survival, and maintained learning following hypoxia induction (168).

3.7. Probiotics, prebiotics, and synbiotics

The gut microbiome helps digestion, the immune system, and mental health (169). Enteroendocrine cells transmit signals from GIT to the brain via bacterial metabolites, hormones such as serotonin, and vagal afferent fibers (170). Roberts et al. (171) explained the role of the gut microbiome as a dynamic factor in the etiology of Alzheimer's disease by demonstrating metabolites from microbiota in the cerebrospinal fluid of Alzheimer's patients.

Chong et al. (172) did a randomized, double-blind, and placebo-controlled multicenter trial on 63 healthy elders over 65 years to study the effects of 12 weeks of probiotics on cognitive functions and mood. They demonstrated that probiotics promote mental flexibility and relieve stress in healthy older adults. This can encourage probiotic use in a healthy diet in adults.

Probiotics supply billions of beneficial bacteria directly and are derived from fermented food. Prebiotics are mainly fibers derived from vegetables, fruits, and cereals. They help the growth and multiplication of beneficial bacteria. Synbiotics are combinations of probiotics and prebiotics. These act by producing neurotransmitters such as gamma-aminobutyric acid (GABA), dopamine, acetylcholine, serotonin, and neurochemicals such as BDNF. All these modulate cognition and mood (173).

In addition, intestinal dysbiosis causes inflammation via the

microbiota-gut-brain axis. Five studies with an aggregated sample of 297 individuals (174) showed that probiotics benefit cognitive function in Alzheimer's disease and mild cognitive impairment subjects by decreasing inflammatory and oxidative markers.

Li et al. (175) clarified that probiotics, prebiotics, and synbiotics are very beneficial therapeutically in improving cognitive function and behavioral and psychological symptoms in patients with dementia.

3.8. Antioxidants

Although oxidative stress is critical in the pathogenesis of many cognitive diseases, some studies revealed that antioxidant therapy is clinically ineffective in these disorders.

Certain precautions are required to be effective clinically. Firstly, dose adjustment is required. Higher doses of antioxidants may be harmful (176). Secondly, some antioxidants have low solubility and absorption with rapid metabolism, so they require new delivery systems. Thirdly, advanced delayed damage cannot be reversed by antioxidants (177); thus, they should be given at earlier stages of the disease.

3.9. Herbs

Lavender

Aromatherapy is a method based on plant-derived essential oils. In 39 elders, a week's daily smell of lavender oil improved cognition and sleep disorders (178). Rafii et al. (179) showed the anxiolytic and sleep-promoting effects in burn patients with lavender and chamomile oil during massage.

Lavender (Lavandula angustifolia) essential oil (EO) is used in emotional disorders and consists primarily of linalool and linalyl acetate (180) and belongs to Lamiaceae's family (181). Linalool is a monoterpene with antioxidant, anti-inflammatory, and anti-convulsant activities, protects neurons from toxicity, and attenuates cognitive and affective disorders in the transgenic model of AD (182)

The anxiolytic action of lavender essential oil depends on its serotonin and glutamate-like characteristics (180) involved in the mechanisms for anxiety, learning, and reconsolidation of memory (183). However, lavender, coriander, and linalool's neuroprotective effects against Aβ- induced neurotoxicity in vitro cellular models are still unknown (7).

Coelho et al.'s (184) study demonstrated the inhibitory effect of vaporized lavender on the conditioned contextual fear memory without affecting the consolidation of tone fear memory, indicating lavender's potential use to manipulate the cognitive aspect of anxiety. Although memory updating depends on reconsolidation, the former process is a consequence of the latter, and the inhibition of memory updating does not necessarily imply impairment of the primary memory (185).

On the other hand, lavender oil reduces daytime sleepiness by improving sleep quality, thus improving mental functions (186), as shown in EEG changes in response to its antianxiety action (181). The Power Spectral Density of alpha and theta waves in EEG showed an increase in response to lavender inhalation, thus is associated with mood enhancement and calming effect (181, 187).

Chamomile

Chamomile is a common anxiolytic, sleep-inducing herb (188). Chang and Chen (189) studied the chamomile effect in postnatal women and showed that consumption of chamomile tea attenuated depression and sleep problems. However, these effects were noticed only for two weeks. On the other hand, Moss et al. (190) demonstrated that chamomile aroma impaired alertness and cognitive performance.

Ginseng

Ginseng has a therapeutic effect on improving cognitive function as learning and memory in neuroinflammatory diseases such as cerebral ischemia, stroke, traumatic brain injury, Parkinsonism, and Alzheimer's disease. It has anti-inflammatory and regenerative effects and improves cell-cell communication via the induction of neurogenesis and angiogenesis (191).

Curcumin

Preparations of curcumin with high bioavailability improve working cognitive function, memory, learning, and mood in healthy older individuals (192). Clinical results of Katherine et al. (193) showed improvement in hippocampal function, suggesting relief of cognitive decline in some populations.

Multiple systemic and central mechanisms are involved via various nutrients, including flavonoids (194).

3.10. Behavioral therapy

Cognitive Behavioral Therapy for insomnia (CBT-i) improved multiple aspects of sleep, like sleep latency, efficiency, and insomnia severity (195, 196).

Sweetman et al.'s (197) trial included 145 participants treated for six months and compared the clinical data (symptoms, signs, investigations, outcome) using Continuous Positive Airway Pressure (CPAP)-which is considered a treatment for sleep apnea- with or without Cognitive and Behavioral Therapy for insomnia (CBT-i) before initiating CPAP treatment. They showed better compliance with CPAP use in the CBT-i group and developed attenuated global insomnia, cognitive enhancement, and a positive effect on sleep impairment six months later.

Despite the supportive data on the benefits of CBT on sleep disorders, the effect on metacognition and cognition is still unclear; meta-cognition and cognition decrease in Meta-Cognitive Therapy (MCT) and Cognitive-Behavioral Therapy (CBT). Specifically, in a study on 74 patients, meta-cognition was more affected in MCT than CBT, but cognition did not show marked alternation (198).

3.11. Meditation

Yoga promotes the mind and self-regulation's meta-cognitive capacity, such as potentiating attention and memory, improving cognitive functioning scores, and sensory awareness. In mild cognitive impairment, patients who received the mindfulness-based stress reduction program showed better neuronal circuits of the cingulate cortex, medial prefrontal cortex, and left hippocampus (199).

Aging is linked with neural structure, function, and cognitive performance; they naturally decline over time. The study by Gard et al. (200) found that Yoga and meditation decreased the fall-off rate for fluid intelligence.

Afonso et al. (201) revealed that 21 elderly women yoga practitioners showed a significantly greater Cortical Thickness (CT) in a left prefrontal lobe cluster. The brain's Default Mode Network (DMN) has three operational modules: two occipital-parietal-temporal subnets and one frontal subnet. Moreover, Fingelkurts et al. (202) showed an enhancement of the frontal DMN module via body scan with repeated yoga meditation, focus on breath, and mantra repetition.

Gard et al. (200) stated that Yoga improved memory, concentration, attention, speed, and accuracy in math computations with less emotional lability, excitability, and aggressiveness. Furthermore, Yoga increased students' self-compassion, emotion regulation skills, and non-judgmental self-reflection. Additionally, Pozuelos et al. (203) showed that a relatively short mindfulness practice period significantly changed brain dynamics related to the internal monitoring of response conflicts and errors.

Furthermore, Xiao et al. (204) showed that 121 perimenopausal women involved in meditation training showed significant improvement in their scores on the Self-Rating Anxiety Scale (SAS) and Pittsburgh Sleep Questionnaire (PSQ). Varghese et al. (205) studied meditation's effect on patients with Type 2 Diabetes Mellitus (T2DM) and revealed a significant improvement in the PSQ score, sleep quality, duration, and function during daytime activities.

Another study on the impacts of Integrated Yoga (IY) Intervention on Sleep Quality among professional caregivers of older adults with AD showed significant sleep quality improvement after one month than baseline (206).

3.12. Acupuncture

Acupuncture is considered complementary medicine, usually used as an additional treatment. It has been reported to improve performance in several tests (Insomnia Severity Index, Sleep Efficiency, Total Sleep Time) (207) and the Hamilton Depression Scale (HAMD) (208).

Acupuncture also improved persistent sleep disturbance in mild Traumatic Brain Injury (mTBI) and Posttraumatic Stress Disorder (PTSD) (209) and reduced insomnia after stroke (210). Wang et al. (211) studied the effect of acupuncture with nimodipine versus nimodipine alone and presented the best scores in the Montreal Cognitive Assessment (MoCA) score in the acupuncture and the drug combination compared to acupuncture or drug alone.

Moreover, electro-acupuncture and body acupuncture combined with cognitive function training increased patients' Mini-Mental State Examination (MMSE) scores compared to the cognitive function training alone (212). Patients had better scores in the Mini-Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA) compared to western medications (213).

The present review presented the common cause and comorbidities associated with sleep, cognition, and metacognition dysfunction. Some of these conditions are initiated by affecting either sleep or cognitive functions; others simultaneously hit both domains (sleep and cognition). Primary therapeutic tools to restore sleep and cognitive function have been presented as investigated by previous studies. We recommend further clinical and experimental studies to clarify more data on the missing parts of the molecular mechanism of action for some of the presented drugs and procedures, to evaluate the combination of these factors to assess if there is any synergistic effect, and to check these factors concerning broader and diverse populations (age or sex-based).

Conflict of interest

The authors declare that they have no conflicts of interest.

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Authors' contributions

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Experimental intervertebral disc degeneration models

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Abstract

Intervertebral disc degeneration is a major health problem of close concern to both young and old. The problem is also growing as the global population ages. Intervertebral disc degeneration is defined as progressive changes affecting the spine as a component of natural aging under the effect of multiple factors (such as smoking, obesity, and incorrect exercise). For a solution to be found, experimental disc degeneration must first be induced, the causes of the disease must be identified, and early diagnostic and therapeutic methods must then be developed. Methods of inducing intervertebral disc degeneration with high applicability in rats were identified from the previous literature. This review discusses four methods of disc degeneration induction. It also discusses how to detect degeneration formation and development times. As a result of the literature review, information about four different and reliable intervertebral disc degeneration methods is presented.

Keywords: intervertebral disc, degeneration, animal models, rat

1. Introduction

Intervertebral disc degeneration (IDD) is a disease that may be seen at any age and may not be fully treatable. Cheung et al. (1) examined the distribution of IDD by age using magnetic resonance imaging and detected the condition in 40% of individuals aged under 30, and in more than 90% of those aged 50-55. This shows that IDD can be seen at any age, but that the incidence increases with age. However, some clinicians have reported no association between IDD and age, and describe the condition as having other pathological causes. There is, therefore, no current consensus on whether IDD develops due to age or pathological causes (1).

The elderly population is increasing worldwide. This is also leading to an increase in age-related health problems. In addition to old age (2), IDD can also emerge as a result of environmental factors (such as smoking, obesity, and incorrect exercise) (3). IDD is one of the principal causes of low back pain (4). More than 80% of adults approached health institutions due to low back pain at some time in their lives. Back pain is also one of the most common causes of restricted activity among individuals aged under 45 in particular (3). Disc degenerations are extremely painful, especially those seen in the early period (5). Back pain also affects the adult population, in addition to low back pain (6).

IDD caused low back pain has become an important problem due to increased costs and loss of working time for diagnosis and treatment (4). One study from the USA reported total low back pain-associated health costs of $91 billion. The health costs of individuals with low back pain exceed those without low back pain by approximately 60% (7). In addition, the incidence of IDD-related spinal surgery in the USA increased by 500% between 1990 and 2011 (8).

It is extremely difficult to develop and apply models for examining the pathogenesis of disc degeneration and assessing potential treatments (9). Disc degeneration is difficult to detect in humans and is usually identified after pain has occurred. Detection is particularly impeded by the slow progression of degeneration, the lack of disc degeneration tissue, and multi-factor underlying causes. Animal experiments are, therefore, fundamental in this context. The anatomical and biomechanical properties of the discs of both large and small animals are relatively similar to those of humans (10, 11). The purpose of this review is to summarize viable animal models of IDD.

2. Models of experimental intervertebral disc degeneration in animals

2.1. Animals Used

Both Sprague Dawley and Wistar rats have been used in these experiments (12-15). All animals were anesthetized during the surgical procedures.

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2.2. Methods Used

2.2.1. Intervertebral disc degeneration model induced with ovariectomy

Wang et al. (16) discovered during an experiment that rats subjected to ovariectomy also developed IDD. This method was subsequently developed based on that finding. Female Sprague Dawley rats were used for the model, and IDD has been reported to occur 12 weeks after surgical intervention (13).

Surgical procedure

Experiments begin when rats are three months old. The ovaries are removed (dual ovariectomy), and the incision area is sutured. Rats are sacrificed after 12 weeks of survival (13).

2.2.2. Intervertebral disc degeneration model created in the rat tail

This method is performed using the tail region of Sprague Dawley rats. IDD has been reported two weeks after the procedure Fernández-Susavila et al. (15).

Surgical procedure

During the procedure, a tourniquet is attached to the tail to reduce blood circulation in that region and the blood supply of the area to be studied.

Once the tail skin has been sterilized, a dorsal 4-cm midline incision is made between the C6 and C10 caudal vertebrates identified by digital palpation in the tail. A 1-mm diameter hole is created under the vertebral endplates using a precision drill. To control the depth of drill penetration, attention is paid to the resistance of the contralateral wall of the vertebral bone. The bone cavity is filled with cyanoacrylate to barrier any revascularization to the endplate, and the incision site is closed. The animals are sacrificed after two weeks (15).

2.2.3. Intervertebral disc degeneration model created by needle puncture

This method is applied to the coccygeal intervertebral region of Wistar rats weighing 300-350 g. IDD takes place 1-4 weeks after the procedure.

Surgical procedure

The coccygeal intervertebral spaces Co6-7, Co7-8, and Co8-9 are identified by digital palpation and confirmed by fluoroscopy. The intervertebral Co7-8 level is left untouched for control purposes, and no procedure is performed. The tail skin is first cleaned with antiseptic. A fluoroscopy-guided 20-gauge needle is then inserted at the Co8-9 (distal) and Co6-7 (proximal) annulus fibrosus level. After penetration, the needle is rotated 3600 and held in place for 30 seconds. The depth of penetration of the needle is controlled. For this, the resistance of the contralateral annulus fibrosus is checked during the procedure. After the procedure, the area is closed, and the animals are sacrificed after four weeks (12).

2.2.4. Intervertebral disc regeneration model created by Dedifferentiated Fat (DFAT) cell transplantation

Sprague-Dawley male rats (12 weeks old, bodyweight 300 g) were used in this model. IDD occurs in the eighth week after the procedure. Intervertebral disc degeneration is created as a result of a two-stage application.

Dedifferentiated Fat cell preparation

A new preadipocyte cell line, named dedifferentiated fat (DFAT), is created with mature adipocytes from adult rats (17). DFAT cells are easily isolated from about 1 g of subcutaneous adipose tissue and can be increased easily (14). 1g adipose tissue is taken under the skin of the rats. The adipose tissue is gently shaken at 37 ° C for 1 hour and kept in 0.1% (W / V) collagenase solution (Collagenase type 1, Koken, Tokyo, Japan). After filtration and centrifugation at 135g for three minutes, the floating top layer containing unilocular adipocytes is collected. Afterwards, the collected cells are washed with phosphate-buffered saline. Then, the cells (5x104) are placed in 25 cm2 culture flasks filled with Dulbecco's modified Eagle medium (DMEM; Invitrogen, Carlsbad, CA) supplemented with 20% fetal bovine serum (FBS; JRH Bioscience, Lenexa, KS, Lot 6G2146). It is then incubated at 37 °C in 5% CO2. Cells float on medium and are waited until they stick to the upper inner ceiling surface of the bottle. After 7 days, the flasks are inverted, with the media removed and the cells at the bottom. The media are changed every 4 days until the cells merge. When cells divide occurs, the cells become available (14, 18). Using these methods, DFAT cells are obtained. These cells are then transferred to the intervertebral discs by surgical operation.

Dedifferentiated Fat cell transplantation

In rats, disc damage is created by the needle model (12). One week after intervertebral disc damage is done, DFAT cells (5 x104/50 ml PBS, DFAT group, n ¼ 13) are transferred to the damaged area. After the incision area is closed, animals are sacrificed eight weeks later (14).

2.3. Detection of intervertebral disc degeneration

Methods such as histology evaluations (Van Gieson, collagen VI, collagen fiber orientation using picrosirius red staining, hematoxylin-eosin, and immunohistochemistry), magnetic resonance imaging, micro-computed tomography, polarized light microscopy, light microscopy can be used to determine whether IDD is occurring (12-15, 19)

3. Result

IDD is still an unsolved problem for society. Scientists resort to all possible methods to solve this problem. Since there are restrictions on applications to the human body, the most appropriate approach is experiments on animals. In this review, we tried to bring together animal models of IDD.

Conflict of interest

There is no conflict of interest including any financial, personal

or other relationships with other people or organizations that could inappropriately influence, or be perceived to influence this work.

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Authors' contributions

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

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Emergencies in movement disorders

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Abstract

Movement disorders can be defined as the abnormality of the speed and form of body movements. Although movement disorders often occur chronically, they can sometimes develop acutely or sub-acutely, and some can be fatal if not diagnosed and treated early. Here, emergencies in movement disorders are discussed under two main headings, as emergencies related to hyperkinetic movement disorders and emergencies related to hypokinetic movement disorders. This review draws attention to the importance of that accurate diagnosis and early treatment can be life-saving in emergencies in movement disorders. It also provides recommendations for diagnosis and therapy.

Keywords: movement disorders, emergencies, hyperkinetic movement disorders, hypokinetic movement disorders

1. Introduction

Movement disorders can be defined as the abnormality of the speed and form of body movements. Most of this group of disorders are due to deviations from the typical basal ganglia-cortex cycle (1). However, some movement disorders, such as myoclonus, may arise from pathologies in the spinal cord and other structures (1). Movement disorders are divided into two groups (1): 1) Hyperkinetic movement disorders are excessively unwanted movements and include tremor, chorea, ballism, dystonia, stereotypy, tics, and myoclonus. 2) Hypokinetic movement disorders include akinesia (loss of movement), hypokinesia (decreased amplitude of movement), bradykinesia (slowing of movement), and rigidity. Sometimes akinesia can be used broadly to include hypokinesia and bradykinesia. Therefore, hypokinetic movement disorders can also be expressed as akinetic-rigid disorders (1). Parkinsonism is an example of a hypokinetic movement disorder. Although movement disorders often occur chronically, they can sometimes develop acutely or sub-acutely, and some can be fatal if not diagnosed and treated early. Studies on the frequency of emergent movement disorders are few. In a prospective study (2), 6690 of 131,537 patients admitted to the emergency department underwent neurologic evaluation, of which 1.4% were diagnosed with acute movement disorders. It was revealed that 73.9% of these patients had hyperkinetic, 26.1% hypokinetic and 19.8% mixed movement disorders (2). There are few publications about emergencies in movement disorders in English literature. Here, emergencies in movement disorders have been discussed under two main headings, emergencies related to hyperkinetic movement disorders and emergencies related to hypokinetic movement disorders. Here, we have discussed the clinical features (ie, definition, brief pathogenesis, causes, and diagnosis) and emergency treatment of each emergency movement disorder.

2. Emergencies related to hyperkinetic movement disorders

This group includes acute chorea and ballismus, immediate myoclonus, emergencies in tic disorder, and acute dystonia and dystonic storm.

2.1. Acute chorea and ballismus

Clinical features

Chorea is irregular, unpredictable, short, and non-stereotypical aimless movements that flow rapidly from one body part to another (3). Ballismus is a proximally dominant, irregular, abrupt, rough and bouncing rocking or throwing movement and is closely related to chorea (4). Indeed, ballismus and chorea are two hyperkinetic movements that differ in speed and amplitude, one of which may represent the continuity of the other (5). Hemiballism is ballistic movements on one side of the body. Movements can be self-destructive, tiring, or troublesome. Movements often improve after days to weeks, and ballistic movements often become choreiform (3). The most common cause is stroke [affecting the contralateral subthalamic nucleus (STN)], followed by non-ketotic hyperglycemia (3). Acute severe chorea and ballismus, if left untreated, can cause hyperthermia, dehydration, and rhabdomyolysis (5). Table 1 shows the causes of acute chorea and ballismus (5).

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Table 1. Causes of acute chorea and ballismus

Vascular	Autoimmune	Metabolic	Infectious	Structural	Drug/toxin
Stroke (Ischemic / hemorrhagic)	Sydenham / chorea gravidarum	Uremic encephalopathy	Cryptococcal granuloma	Basal ganglia lesion/mass	Alcohol
Malformation	Paraneoplastic*	Hypoglycemia	Toxoplasma	Cerebellar lesion	Antiepileptics
Cerebral anoxia	Multiple sclerosis	Hypoparathyroidism	Tuberculoma	Subthalomotomy	Methadone
Postpump chorea	SLE	NKHG	HIVE	Thalamotomy	Amphetamine
	Scleroderma	Hyperthyroidism			Morphine
	Behcet's disease	Polycythemia vera			Oral contraceptive
	APS				Levodopa
	Sarcoidosis				Cocaine
	PAN				

*May be anti CV2/CRMP-5 antibody-positive; SLE, Systemic lupus erythematosus; NKHG, Non-ketotic hyperglycemia; HIVE, Human immunodeficit virus encephalitis; APS, Anti-phospholipid antibody syndrome; PAN, *Polyarteritis nodosa*

Diagnosis is supported by laboratory investigations appropriate to clinical findings after clinically evaluating patients (5). Magnetic resonance imaging (MRI) is often the first step, and a change in intensity in the basal ganglia may be seen. Auto-antibodies [e.g., α-streptolysin-O (ASO) and α-DNAase-B antibodies for a patient with Sydenham chorea] and other biochemical studies may be required for diagnosis and differential diagnosis (6). Sometimes functional imaging [e.g., *fluorodeoxyglucose*-positron emission tomography (FDG-PET)] may be needed. It has been reported that striatal hypermetabolism in FDG-PET is present in the acute phase of the disease in patients with Sydenham's chorea (6, 7), whereas striatal hypometabolism has been reported in FDG-PET in degenerative chorea (e.g., Huntington's disease) (7).

Treatment

Here, we will first define the general principles of the approach and then add specific treatments in some special clinical situations. In the general approach, the dose of the causative drug, if any, should be reduced first, or the drug should be temporarily stopped. Rehydration should be provided to meet the fluid deficit and prevent complications such as acute renal failure due to rhabdomyolysis. Dopamine receptor blockers (e.g., antipsychotics) or dopamine depleters (e.g., inhibitors of the presynaptic vesicular monoamine transporter type 2 - VMAT2 such as tetrabenazine) can be used to reduce symptomatic chorea and ballism (4, 6). If there is hyperthermia, antipyretic approaches (e.g., cooling the body) and drugs (e.g., paracetamol) should be given. If necessary, sedation (e.g., diazepam) should be provided. Since movements may disappear over time in hemiballism, treatment should be discontinued after three months, and the patient should be re-evaluated (3). On the other hand, it is crucial to investigate the underlying pathological condition or disease (Table 1.) and perform specific treatment accordingly (3).

Chorea due to non-ketotic hyperglycemia

Clinical features

Chorea due to non-ketotic hyperglycemia is more common in the elderly, women and people of Middle Asian origin (5, 8). It has been reported that GABA insufficiency may play a role in the mechanism of emergence of chorea in this group of patients since it causes deterioration of the basal ganglia functions (8). Because it has been reported in these patients that GABA is rapidly depleted due to both the use of GABA as energy and the lack of acetoacetate, which can be used to synthesize GABA (8). The clinic often presents as hemichorea (often ipsilateral extremity, sometimes predominant in the facial muscles, jaw and tongue) or hemiballism, with an acute/subacute onset (3, 5). Biochemical examination reveals high blood sugar and negative ketones in the urine, while T1-weighted MRI shows a change in intensity in the basal ganglia (Fig. 1) (3, 5).

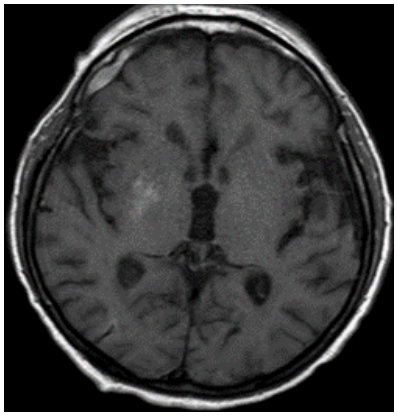

Fig. 1. T1-weighted MRI of a 67-year-old male patient with left hemichorea due to non-ketotic hyperglycemia shows hyperintensity in the right globus pallidus.

Treatment

Blood glucose regulation, supportive care, and symptomatic treatment (e.g., dopamine receptor blockers and, if necessary, sedation) should be given (mentioned in the general approach to chorea) (4,6,8).

Sydenham's chorea

Clinical features

Sydenham's chorea may begin abruptly 1 to 6 months after streptococcal pharyngitis (3, 9). It has been reported that autoimmune pathologies in persons with genetic predispositions are involved in the pathogenesis of Sydenham's chorea (6). It has been reported that anti-basal ganglia antibodies (e.g., antibodies developed against epitopes of M-

proteins) against A β-hemolytic streptococcus cause pathological changes that will lead to loss of function by acting against neuronal tubulin and D1 and D2 receptors in basal ganglia (6). Although chorea is often generalized clinically, it can also occur as hemichorea at a rate of 20% (6). Behavioral changes such as obsessive-compulsive disorder, hyperactivity, anxiety and emotional lability may accompany chorea (6). Weakness and hypotonia may be prominent, and even paralytic chorea may develop in 1.5% of cases (6, 10). Oculogyric crisis, hypometric saccades, and rarely vocal tics and dysarthria have also been reported (6, 10). The diagnosis is made clinically, but high ASO and anti-DNAse B antibody titers support the diagnosis (5, 6, 10). It has been reported that FDG-PET and Single Photon Emission Computerized Tomography (SPECT) performed in the acute phase of the disease revealed the striatal abnormality (striatal hypermetabolism and hyperperfusion, respectively) (6).

Treatment

In addition to supportive treatment, GABAergic drugs (e.g., benzodiazepines, valproic acid at a dose of >1500mg/day or carbamazepine), dopamine receptor blockers or dopamine depleting agents can be used in the symptomatic treatment of chorea (6, 10). In Sydenham's chorea, immunomodulatory therapy is recommended to shorten the disease duration and prevent relapses and complications (e.g., 25 mg/kg/day intravenous methylprednisolone for five days followed by oral deflazacort 0.9 mg/kg/day for three months, especially in severe or persistent cases of chorea, 1-2 gr/kg/day for two days or 400 mg/kg/day for five days intravenous immunoglobulin or plasma exchange) (10-12). It has been reported that penicillin can prevent cardiac complications of rheumatic fever (sometimes it may take several years) (10-12).

Chorea gravidarum

Clinical features

Chorea gravidarum (CG) often begins in the first trimester of pregnancy and may rarely cause an emergency (3). It is estimated that chorea may be related to increased sensitivity to dopamine in the striatum due to hormonal changes in patients (12). On the other hand, it has been reported that antiphospholipid antibody syndrome (APAS) is a common cause of CG in some people (3). It has been reported that it may also be associated with previous Sydenham's chorea (5, 12). Chorea can be unilateral or bilateral, often, the face and extremities are affected, and dysarthria is common (3). Psychiatric complaints may accompany chorea (13).

Treatment

In CG, chorea usually resolves in the last trimester or disappears shortly after birth (14). In severe cases, general acute chorea therapy (e.g., haloperidol symptomatically) is administered (5). Abortion or premature delivery may be indicated rarely (15).

Antiphospholipid antibody syndrome (APS)

Clinical features

Antiphospholipid antibody syndrome may cause acute generalized chorea (3). Antiphospholipid antibody syndrome may be primary or secondary to systemic lupus erythematosus (3). It has been reported that antiphospholipid antibodies, which cause inflammation and increase the permeability of the blood-brain barrier by binding to the intracranial endothelium, cause chorea due to direct binding to the basal ganglia neurons (12). Antiphospholipid antibodies (i.e., lupus anticoagulant, anticardiolipin, and anti-β2-glycoprotein-I) are used for diagnosis (16).

Treatment

Treatment consists of general acute chorea treatment, immunosuppressive/immunomodulatory treatment of APS, and follow-up and treatment of its complications (deep vein thrombosis, pulmonary embolism, stroke, thrombotic microangiopathy, thrombocytopenia, and hemolytic anemia) (12).

2.2. Immediate myoclonus

Clinical features

Myoclonus can be defined as a sudden-shock-like involuntary contraction (positive myoclonus) or inhibition (negative myoclonus) of muscle (agonist or antagonist) or muscle groups (3, 17). The pathogenesis of myoclonus is related to the source of the stimulus (e.g., it can be cortical, subcortical, brain stem, spinal cord or peripheral) (1, 18). Table 2 shows the causes of immediate myoclonus (3, 18).

Table 2. Causes of immediate myoclonus and clinical pictures

Etiology	Clinical picture(s)
Cerebral anoxia	Postanoxic myoclonus, Myoclonus SE
Metabolic disorders; Hepatic and uremic encephalopathy	Asterix
Toxic disorders; MAO inhibitors, SSRI, SNRI and tricyclic antidepressants, opiates, levodopa, gabapentin, triptans, LSD, amphetamines, cocaine, MDMA or ecstasy	Serotonin syndrome and NMS
Structural disorders; Thalamic lesion (contralateral)	Acute focal Asterix, positive myoclonus

SE, Status epilepticus; SSRI, Selective serotonin reuptake inhibitor; SNRI, Serotonin noradrenaline reuptake inhibitor; NMS, Neuroleptic malignant syndrome; LSD, Lysergic acid diethylamide; MDMA, 3,4-Methylenedioxymethamphetamine

Postanoxic myoclonus (Lance-Adams syndrome)

Clinical features

Lance-Adams syndrome is seen after recovery from anoxia (delayed onset) (3). It is action myoclonus (often intentional), absent at rest, and stimulus sensitive (3, 19). Myoclonus is of cortical origin, and it may be accompanied by mild cognitive impairment, dysarthria, ataxia, pyramidal signs, rigidity, and epilepsy (3, 19). It may resolve spontaneously over the years (3, 19).

Treatment

Clonazepam, primidone, valproic acid, or levetiracetam can be used alone or in combination for the symptomatic treatment of myoclonus (3).

Myoclonus status epilepticus

Clinical features

Myoclonus status epilepticus is a persistent generalized or multifocal myoclonus involving the face, extremities and axial muscles in coma patients and is spontaneous or sensitive to sound (3, 20). It is reported that it is seen in 30% of comatose adults who develop after cardiac arrest (21). It may begin in the hours immediately after cerebral anoxia (3). It may be of cortical (giant SEP; abnormal EEG) or subcortical (SEP and EEG normal) origin (20). Myoclonus status epilepticus is often considered to be a poor prognostic sign of cerebral anoxia (22). Additional poor prognostic features are defined as 1) death, 2) persistent unconsciousness after one month, or 3) severe disability requiring complete care after six months (3).

Treatment

After venous access is established, respiratory-circulatory support and therapeutic hypothermia should be applied to the patient as supportive treatment. While levetiracetam or Pracetam is the first choice for the cortical origin for the symptomatic treatment of myoclonus, valproic acid, phenytoin, phenobarbitone, and benzodiazepines can be used in combination with levetiracetam or Pracetam if there is no adequate response (23). Subcortical origin is often treated with clonazepam (23). In cases that cannot be controlled with these treatments, anesthesia should be provided with propofol (supports GABA) infusion. However, propofol can cause myoclonus (case reports) (20).

2.3. Emergencies in tic disorder

Clinical features

Tic disorder is defined as semi-voluntary, sudden, rapid, non-rhythmic, intermittent, repetitive movements (motor tics) or sounds (voice tics) (3, 24, 25). Pathogenetically, it results from the disinhibition of the loop between the cortex-basal ganglia-cortex (24). Conditions that require urgent evaluation in tic disorder include tic exacerbation (i.e., increase in amplitude, efficacy, or frequency) and neurological effects associated with severe exacerbation (e.g., spacing-subdural hematoma, compressive neuropathy, and myelopathy) (3). Factors that worsen tics include fatigue, physical or mental stress, anger, infection, stimulants and tricyclic and SSRI group antidepressants (for the treatment of comorbid conditions such as attention-deficit/hyperactivity disorder or OCD that may accompany tics) (3).

Treatment

First of all, removing the precipitating factor is very important to reduce the severity of deterioration (2, 3). Neuroleptics such as pimozide, haloperidol and risperidone, anti-hypertensives such as clonidine and guanfacine and tetrabenazine (dopamine-depleting agent) can be used as symptomatic treatment of tic attack (2,3). Botulinum toxin injections may be appropriate for focal tics (25). For tics resistant to pharmacological treatment, surgical treatment (e.g., deep brain stimulation) may be an option (26). In addition, the treatment of complications is essential.

2.4. Dystonic emergencies

Dystonic storm or status dystonicus

Clinical features

Dystonia is defined as a movement disorder characterized by sustained or intermittent muscle contractions that cause abnormal, frequently repetitive, patterned movements, postures, or both (25). A dystonic storm is defined as continuous severe dystonic contractions in patients with dystonia (2). Dystonic storms can cause acute worsening (2). Aggravating factors include infection, drug changes, and trauma (2).

Treatment

Pharmacologically, anticholinergics, dopamine receptor blockers, dopamine depleters, sedative and paralytic agents can be given (2). In cases where there is no response to drug treatment, surgical methods such as deep brain stimulation can be applied (2).

Dystonic reaction

Clinical features

Acute dystonic reactions may occur 24 hours after exposure to dopamine receptor blockers (2). It has been reported that the risk increases in men and when the dose is titrated rapidly (2). Blepharospasm, cervical, laryngeal or limb dystonia, and oculogyric crisis may occur singly or in combination (2). When the larynx or pharynx is affected, there may be a danger of respiratory failure (2).

Treatment

First of all, the etiological drug should be discontinued, and then parenteral anticholinergics or antihistamines (e.g., diphenhydramine 25-50 mg) should be administered (3). It is recommended to take oral anticholinergics for a few more days to prevent the risk of relapse (3).

3. Emergencies related to hypokinetic movement disorders

When emergencies related to hypokinetic movement disorders are mentioned, emergencies in Parkinsonism and Parkinson's disease come to mind first. Parkinsonism is defined as the condition where bradykinesia is accompanied by one or more rest tremors, rigidity, and impaired postural reflexes (27). Parkinson's disease (PD) is the most common cause of parkinsonism and presents with non-motor symptoms and motor symptoms like parkinsonism (28). The emergencies in this group of diseases can be grouped under two headings: Specific and nonspecific (2, 3, 29). Specific ones will be discussed under hyperpyrexia syndromes, acute parkinsonism, acute worsening of parkinsonism, severe motor complications such as severe Off and severe dyskinesia, falling and psychosis (29). Nonspecific include infection (21-32% of PD patients presenting to the emergency department), cardiovascular or cerebrovascular events (12-26% of PD patients presenting to the emergency department), gastrointestinal disorders (8-11% of PD patients presenting to the emergency department), and metabolic disorders (2-6% of PD patients admitted to the emergency department) (29).

3.1. Hyperpyrexia syndromes

Parkinsonism hyperpyrexia syndrome

Clinical features

Parkinsonism hyperpyrexia syndrome (PHS) is defined as a sudden clinical worsening in which UPDRS scores increase by ≥20 points and temporary unresponsiveness to therapeutic doses of dopaminergic drugs or rescue drugs for three days (30). PHS was first described in 1981 (31). PHS is clinically characterized by severe akinesia, severe rigidity, hyperthermia, dysautonomia (tachycardia, fluctuation in blood pressure, incontinence and sweating) and altered consciousness, which mimics neuroleptic malignant syndrome (NMS) and does not respond temporarily to dopaminergic therapy (2, 32). Studies have reported that dopamine active transporter (DAT) activity is markedly decreased in PD patients with akinetic crises (30). It has been reported that the decrease in DAT expression may occur in response to the sudden decrease in striatal dopamine levels and mitochondrial function due to the abrupt discontinuation of exogenous DA'ergic drugs (30). It is clear that DA transmission to the striatum, hypothalamus, and cortex decreases due to the decrease in DAT expression. It has also been reported that a decrease in binding to post-synaptic striatal D2 receptors (due to decreased striatal dopamine receptor expression and affinity) and an increase in calcium and pyrogen release from the sarcoplasmic reticulum in skeletal muscle are also involved in the pathogenesis (30, 33). From clinical history, determining the presence of one or more of the triggers such as reduction or stopping of dopamine agonists, levodopa and amantadine (18 hours to seven days later: malignant withdrawal syndrome), closure of STN-deep-brain stimulation (DBS) (malignant STN-DBS withdrawal syndrome), trauma, gastrointestinal system diseases, infections, excessive heat and dehydration may be a clue for diagnosis (2, 3, 30, 33). Although the diagnosis of PHS is considered when the neurological examination reveals severe akinesia, severe rigidity, hyperthermia, dysautonomia, and altered consciousness, the diagnosis should also be supported by determining the increase in serum white blood cell (WBC), creatine kinase (CK) and liver enzyme levels (2, 3, 32, 33). Complications of PHS include venous thrombosis, pulmonary embolism, aspiration pneumonia, DIC (disseminated intravascular coagulation) and kidney failure (30, 34).

Treatment

As in NMS, supportive measures such as intravenous (IV) hydration, antipyretic, and, if necessary, mechanical ventilation and hemodialysis are crucial. Dopaminergic therapy needs to be restarted. For this, liquid levodopa, bromocriptine (7.5-15mg 3 times a day), ropinirole (1-2mg 3 times a day), pramipexole (0.18-0.36mg 3 times a day), subcutaneous apomorphine (1- 2mg/hour) and transdermal rotigotine (2-4mg/day) are recommended (3, 34). Solving the underlying etiological problems will accelerate the healing process. As additional treatments, IV administration of dantrolene (relaxes muscles at muscle level), which prevents calcium release from the sarcoplasmic reticulum and thus muscle contraction, is recommended in severe or resistant rigidity (starting with 1 mg/kg, maximum 10 mg/kg/day in 3-4 doses) (34). High-dose IV methylprednisolone (e.g., 3-day pulse therapy) has been found to be effective (e.g., reduction in disease duration and significant clinical improvement) in small randomized trials (35, 36). Electroconvulsive therapy was found to be effective in case samples (36). Follow-up and treatment of complications are crucial. Despite treatment, 10-30% of permanent worsening and death have been reported (37).

Dyskinesia hyperpyrexia syndrome (DHS) in Parkinson's disease

Clinical features

DHS can be defined as the presence of severe and persistent dyskinesia, which causes mental status changes, hyperthermia, and elevated serum CK and BK, without rigidity, in patients with advanced Parkinson's disease (2, 38). Non-physiological (intermittent) dopaminergic stimulation predisposes to DHS, especially in long-term disease (38). In preclinical studies, an increase in ambient temperature has increased dopamine receptor sensitivity and dopaminergic transmission (38). Chauhan et al. (39) showed an increase in dopamine and glutamate in the blood and hypothalamus and the presence of hypothalamic inflammation at high ambient temperature (45 ± 0.5 °C) in rats. The abnormality of thermoregulation, one of the autonomic impairments in PD, may cause a more noticeable increase in dopaminergic receptor sensitivity at high ambient temperatures (38). On the other hand, dehydration may exacerbate the abnormality of thermoregulation (38).

Increasing the daily dose of DA'ergic therapy and multiple concomitant therapies, presence of infection, high ambient temperature (especially during long illness), trauma and dehydration are reported as risk factors (38). DHS is defined in a case where severe diffuse chorea and dystonia developed and body temperature increased to 42°C, serum CK to 16040 U/L and BK to 14200 while taking 1500 mg/day of L-dopa carbidopa intestinal gel (LCIG) in the summer heat (38).

Treatment

General approach methods such as antipyretic measures, IV copious fluids, and reducing or treating the number of risk factors (e.g., reducing or stopping the number and dose of antiparkinsonian drugs, administering antibiotics if there is an infection) can be life-saving (3, 38). Treatment (e.g., respiratory-circulatory support, dialysis) of developing complications (e.g., rhabdomyolysis, renal failure, and heart failure) is crucial (3, 38). Despite treatment, there is a risk of increased mortality (38). Table 3 shows the comparison of clinical and laboratory features of hyperpyrexia syndromes (2, 3, 30, 34, 38, 40, 41).

Table 3. Comparison of clinical and laboratory features of hyperpyrexia syndromes

Feature	PHS	DHS	NMS*	SS	MH
Age	Old	Old	Young	All ages	Child
Underlying disease	Parkinsonism	Parkinsonism	Psychosis	Psychiatric	Gene mutation**
Onset	Acute	Acute	Acute	Subacute	Acute
Triggering factor (s)	DBS and DA'ic drug withdrawal, surgery, trauma, infection	DA'ic drug, trauma, infection, high ambient temperature, dehydration	Antipsychotic use (Idiosyncratic)	SSRI, SNRI, MAOi, TCA Amphetamine, Cocaine	Volatile anesthetic, Succinylcholine
Fever	-/+	++/+++	+++	++	+++
Rigidity	+++	-	+++	++	+++
Other motor sign(s)	Other parkinsonian sign(s)	Dyskinesia	Tremor	Myoclonus, stereotypy, hyperreflexia	-
Autonomic instability	-/+	-/+	++/+++	+++	+++
Confusion	++/+++	-/+/++	+++	+++	+
Serum CK elevation	++	+++	+++	++	+++
Metabolic acidosis	+	-/+	+	+	++
Medication	LD, BC, PP, AM, DR	Dose reduction or interruption	BC, ATN, DR	CHDN, MTGT	DR

PHS, Parkinsonism hyperpyrexia syndrome; NMS, Neuroleptic malignant syndrome; SS, Serotonergic syndrome; MH, Malignant hyperthermia; DBS, Deep brain stimulation; DA, Dopamine; SSRI, Selective serotonin reuptake inhibitor; SNRI, Serotonin-norepinephrine reuptake inhibitor; MAOi, Monoamine oxidase inhibitor; TCA, Tricyclic antidepressant; - None; + Mild; ++ Moderate; +++ Severe; CK, Creatine kinase; LD, Levodopa; BC, Bromocriptine; PP, Pramipexole; AM, Apomorphine; ATN, Amantadine; DR, Dandrolen; CHDN, Cyproheptadine; MTGT, Methysergite; *Three major (fever, rigidity, elevated CK) or two major and four minor (tachycardia, variable blood pressure, tachypnea, diaphoresis, mental status change, leukocytosis) criteria are required for the diagnosis of NMS **Type 1 ryanodine receptor (RYR1) gene encoding the ryanodine receptor found in skeletal muscle

3.2. Acute parkinsonism

Clinical features

The terms acute worsening of parkinsonism or abruptly worsening OFF periods are also used synonymously with acute parkinsonism. Potential causes of abrupt change include parkinsonian drugs, particularly dopamine receptor blockers (e.g., antipsychotics and antiemetics), concomitant infection (e.g., urinary tract infection and pneumonia) or metabolic disruption (e.g., central pontine myelinosis), subdural hematoma (a history of falling can be obtained in patients with suddenly worsening PH), hypoxic-ischemic encephalopathy, toxicity (e.g., carbon monoxide, methanol, and manganese), acute hydrocephalus, spinal cord lesion, and brain tumor (2, 3, 42).

Treatment

Correction or elimination of the underlying cause is very important (2). When necessary, symptoms should be treated with antiparkinsonian drugs (2).

3.3. Severe motor complications

It has been reported that 8% of Parkinson's patients admitted to emergency departments have motor complications (29).

Severe OFF periods

Clinical features

OFF periods can be defined as the reappearance of signs and symptoms of the disease seen in the later stages of PD. In severe OFF periods, significant akinesia may be accompanied by nonmotor symptoms such as abdominal discomfort, pain, and dysautonomia (e.g., diaphoresis, variations in blood pressure, and tachycardia) and panic attacks (2, 43). Potential triggers or aggravating factors include dopaminergic drug changes, the addition of antidopaminergic drugs to treatment, and concomitant infections (2). Severe prolonged OFF periods may lead to complications such as aspiration and deep venous thrombosis (43).

Treatment

Crushing L-dopa tablets in acidic liquid (e.g., by adding

vitamin C) or administering dispersible or controlled-release forms of L-dopa, dividing the daily dose of L-dopa, adding an MAO B inhibitor to L-dopa, and administering L-dopa with COMT inhibitor can shorten the off-time (2, 44). Subcutaneous injection of apomorphine or inhaled L-dopa powder without carbidopa (antiparkinsonian effect begins within 10-30 minutes) accelerates recovery (44). Prophylactic anticoagulants may be required. Stereotactic surgery can be considered as an additional alternative treatment (2).

Severe Dyskinesia

Clinical features

Severe generalized dyskinesia can cause rhabdomyolysis, hyperthermia, and dehydration (3). Dyspnea, tachypnea, dyskinesia of respiratory muscles (chest wall discomfort and involuntary grunting sounds) and anxiety may accompany (3).

Treatment

Reducing (or stopping) each levodopa dose is the first strategy in Peak-dose dyskinesias (44). Benzodiazepines can be given temporarily for accompanying anxiety (Neuroleptics should not be used) (3). Hydration and antipyretic approaches can be life-saving (3). An intermittent 'rescue' subcutaneous injection of apomorphine immediately before an on/off state transition may be a viable alternative in treating biphasic dyskinesias (43). Amantadine, DBS and LCIG are recommended for dyskinesia prophylaxis (3, 44).

3.4. Acute psychosis in Parkinson's disease

Clinical features

It has been reported that 8% of Parkinson's patients admitted to emergency departments have psychosis (29). Psychosis is the name of the clinical picture accompanied by hallucinations (visual>auditory), delusions (commonly paranoid) and agitation in addition to confusion, and it is seen in more than half of the cases (45-64%) during the course of the disease (3, 43). The most common triggers are the conditions associated with antiparkinsonian drugs (drug change, addition or dose increase), followed by acute clinical conditions (infection, metabolic disorders) or concomitant dementia (3, 43).

Treatment

After treating comorbidities, starting from anticholinergics, MAO-B inhibitors, DA agonists, amantadine, and COMT inhibitors should be gradually reduced or discontinued (3). If psychosis persists, antipsychotics (pimavanserin, a selective 5-HT2A receptor inverse agonist/antagonist, clozapine or quetiapine) may be required (3, 43, 45). Cholinesterase inhibitors such as rivastigmine and donepezil reduce hallucinations (43).

3.5. Fall in Parkinson's disease

Clinical features

It has been reported that 13-27% of PD patients admitted to the emergency department have a history of falling (29). As PD progresses, falls increase, and the frequency of falls is reported to be around 70% annually (46). Postural instability or freezing causes 80% of falls (29). Injuries (often hip fractures) occur in approximately 25% of falls (29). Severe dyskinesias and orthostatic hypotension can also cause falls (47).

Treatment

Although adjusting the levodopa dose in the treatment of falls (e.g., reducing the dose in the on-stage freezing and increasing the dose in the off-stage freezing) is the first strategy, freezing and postural instability are often not sensitive to levodopa (29, 48, 49). In levodopa-resistant freezing, the benefit of DBS [STN, globus pallidus internus (GPi) singly or combined with pedunculopontine nucleus] is controversial. However, high-frequency stimulation such as 130Hz in STN-off period freezes and low-frequency stimulation such as 60Hz in STN-on period freezes can be recommended (49, 50). Table 4 shows non-dopaminergic pharmacological treatment in freezing (49, 50). Exercise (reduces falls) and assistive devices (Walker, laser cane) can be preventative (49). Treatment of comorbidities that negatively affect mobility and may cause freezing can reduce falls (48). For example, SSRI or SNRI for depression and anxiety, rivastigmine for cognitive dysfunction, compression stockings and domperidone for orthostatic hypotension, and appropriate treatment for poor vision and musculoskeletal problems can be recommended. It has been reported that new non-drug treatment methods such as transcranial magnetic stimulation (TMS), noninvasive vagus nerve stimulation (VNS), and transcranial direct current stimulation (tDCS) are also effective in freezing (49). In addition, the treatment of possible complications is also critical.

Table 4. Non-dopaminergic pharmacological treatment in freezing

Drug	Group	Effect/Result
Rivastigmine	Ach-esterase inhibitor	Ineffective / Not recommended
Amantadine	Glutamate antagonist	Additional therapy in L-dopa-responsive freezing*
Methyl phenidate**	CNS stimulant	Freezing despite optimal L-dopa and STN DBS therapy*
Atomoxetine**	Strattera	Ineffective/ Not recommended
Droxidopa	Prodrug of norepinephrine	Combined with entecapone in DA resistant freezing*
Caffeine / Istradefylline	Adenosine antagonist	Recommended for research*
Botulinum toxin	Chemical denervation	Off period freezing*

* Not clear: There are positive and negative results; **It is used in the treatment of hyperactivity attention deficit syndrome; CNS, *Central nervous system;* STN-DBS, Subthalamic nucleus-*deep brain stimulation;* DA, dopamine

4. Conclusion

In summary, accurate diagnosis and early treatment can be life-saving in emergencies in movement disorders. Finally, complications related to device-aided therapies, including DBS, LCIG, and apomorphine subcutaneous infusion, may present extraordinary emergencies such as severe motor complications, psychosis and hyperpyrexia syndromes refractory to standard therapy (30, 38, 43). We discussed approaches to these complications in detail above.

Conflict of interest

None to declare.

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Authors' contributions

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

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Review Article

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CRISPR/Cas9 system in hematopoietic stem cells: Basic research and clinical applications

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Abstract

Clustered regularly interspaced short palindromic repeats (CRISPR) approach adapted from the prokaryotic adaptive immunity system against pathogen attack is so valuable and promising tool for treatment of human malignant and non-malignant hematological disease and disorders through gene editing in hematopoietic stem cells (HSCs). Moreover, the CRISPR/Cas9 approach is not only useful for therapeutic purposes; it is considerably preferred for the generation of in vitro and in vivo animal disease models. CRISPR/Cas9 approach has been developed for highly efficient on-target cleavage, and low off-target effect via delivery systems and manipulation of CRISPR components including single guide RNA (sgRNA) and Cas enzymes. In this review, we discussed the CRISPR/Cas9 system applications on hematopoietic stem cells in basic research and clinical area with basic research and clinical perspectives.

Keywords: hematopoietic stem cells, CRISPR/Cas9, gene editing, genome engineering, hematologic diseases, hematologic malignancies

1. Introduction

1.1. CRISPR System as Prokaryotic Adaptive Immunity System

The CRISPR system is found in prokaryotic organisms as a natural defense system against viral infection and exogenous plasmids. CRISPR system was discovered in Escherichia coli in 1987 (1) and it was found that this system cooperates with CRISPR-associated (Cas) proteins. We know that the prokaryotic organisms have a high ability for survival against to many difficult environmental conditions and viral attacks in spite of their simple cellular structure. This ability is associated with the management of genome homeostasis and protection from viruses using many defense systems (2). The CRISPR system, which is one of the defense systems, is mainly used against viruses, called as bacteriophages, in a prokaryotic cell. This system works in three stages including specific sequence recognition, targeting and degradation of exogenous foreign nucleic acid, respectively. CRISPR system is based on the recognition of the specific features of pathogens and thus, reminiscence of this pathogen features such as recognition of pathogen-derived structures by human immune memory B cells and rapidly reaction against to pathogens by adaptive immune system cells (3). The recognition occurs through the addition of the nucleic acids of viruses and exogenous plasmids into the CRISPR locus. This locus consists of short palindromic repeated sequences (25–35 bp). These sequences are separated by spacers (typically 30–40 bp each), also called as CRISPR array, and also the cluster of Cas genes (4). The spacer sequences of the CRISPR array that belong to different viruses provide targeting of the viral nucleic acids and destroy them when the viral attack is repeated. The insertion of the viral nucleic acid parts into the CRISPR array is also called as adaptation stage (2). In the adaptation stage, Cas protein complex first interacts with the target nucleic acid by recognition of the specific short (2–4 bp) sequence (known as protospacer-adjacent motif (PAM)) and then, double stranded breaks (DSBs) are generated in the target DNA. The ejected region of the target DNA is inserted into the CRISPR array (4). The adaptation stage is followed by the generation of a long precursor CRISPR RNA (pre-crRNA), also called as expression stage, through the transcription of the CRISPR array and expression of Cas genes. After the maturation of pre-crRNA by Cas proteins, the recognition of target viral nucleic acid results in the destruction of the target by crRNA and Cas endonucleases functions. Mature crRNA has a guide function to recognize and then cleave the foreign target nucleic acid which has similarity to the previously memorized sequences in CRISPR array (2, 3). Therefore, the crRNA is referred as guide RNA (gRNA) (4). This process is also called as RNA-mediated interference (2, 3).

Cas proteins encoded by Cas genes located in the CRISPR array are another essential components of CRISPR–Cas system. Cas proteins, which display effector role, are responsible for CRISPR/Cas system diversity. CRISPR/Cas systems are mainly grouped into two classes (Class 1 and Class 2) based on the structure of the effector complex and these

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classes consist of six major types (Type I-VI) based on the Cas protein variety and the CRISPR locus structure (5). The Class 1 consists of multi-protein effector complexes (Cascade, Cmr, Csm), and includes types I, III and IV. However, Class 2 contains effector proteins with a single subunit that promote effector complex functions and also includes types II, V, and VI (6). Type I in Class 1 is the most common system rather than other types. In Type I system, the targeting of DNA is promoted by Cascade and PAM-dependent manner and the target DNA is destroyed by using Cas3 protein. Besides, Type III system in Class 1 which is commonly found in archea, includes the multi-protein Csm or Cmr complexes. Thus, it promotes the recognition of foreign DNA or RNA regardless of the PAM sequence and the cleavage of targets by using Cas10 protein along with effector nucleases such as RNases Cmr4 and Csm3. The last system Type IV is rare system rather than the others (6, 7). In Class 2, Type II system is characterized by Cas9 endonuclease which is a multi-domain protein (7) and dual crRNA– transactivating crRNA (tracrRNA) guides which promote the guidance of the RuvC and HNH nickase domains to form the blunt-ended DNA DSBs in target DNA with 3′ PAM. Type V is rare system and includes Cpf1 (Cas12a) nuclease which promotes guidance of a single crRNA to RuvC-like endonuclease for generation of sticky-ends in target DNA with a 5′ PAM (6). The type VI CRISPR-Cas system, which encodes the HEPN domain (higher eukaryotes and prokaryotes nucleotide)-containing effector protein Cas13 (8), targets single-stranded RNA (ssRNA) with an RNase activity through the requirement for a protospacer flanking sequence (PFS) instead of the PAM sequence. Among Class 2, Types II and V are used for DNA editing, whereas type VI is used for RNA editing (9).

The diversity on CRISPR/Cas system leads to the diversity on its functions. CRISPR/Cas systems along with these properties have become so valuable for the development of new genome engineering tool. In particular, the CRISPR–Cas9 system among overall systems has become prominent with its simplicity (5) and has become the apple of the biotechnology.

1.2. CRISPR System as Genome Editing Tool

Genome editing has paved the way in the field of biotechnology by allowing for genetic manipulation. The development of genome editing tools provides the improvement of the treatment of monogenic diseases and disorders. Genome editing technologies are not only studied in animal and human cells but also, they are valuable tools for plant genome editing enabling the improvement of crop and nutritional value, resistance to crop disease and management of the biotic and abiotic stress in many crop species (10).

Until today, several genome editing tools have been developed. The overall tools are based on the sequence-specific programmable endonucleases including meganucleases, zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and CRISPR-Cas (11). These engineered nucleases provide genome editing through DNA repair mechanisms including non-homologous end-joining (NHEJ) or homology-directed repair (HDR) following the generation of the DSBs at the specific DNA site (12). These engineered nucleases are divided into two classes depending on the strategy of target DNA recognition. Meganucleases, ZFNs and TALENs enable the target DNA recognition with protein-DNA interactions and thus, are included in one class. The second class consists of CRISPR/Cas which provides the targeting to specific DNA site by a short RNA guide molecule (13). Therefore, meganucleases, ZFNs and TALENs are referred to the protein-based platforms while CRISPR/Cas system is referred to the RNA-guided targeting (14).

Let's briefly mention other engineered nucleases before deeply getting into the CRISPR/Cas9 system: Meganucleases are restriction endonucleases and have large recognition sites (13). ZFNs and TALENs are chimeric endonucleases and have fused two domains including the DNA binding domain and the FokI nuclease domain (11). ZFNs have a DNA-binding domain consisting of a tandem array of Cys2His2 zinc fingers while TALENs have a DNA-binding domain derived from the proteins found in Xanthomonas plant bacteria (15). Among them, ZFNs are more expensive technology rather than the others and also, they are difficult to design. Besides, they are known to cause cytotoxicity. In contrast to ZFNs, TALENs are not cytotoxic but, they have low off-target activity (16).

Genome editing is accelerating with the development of CRISPR/Cas system modifying the mechanism of the prokaryotic adaptive immune system. CRISPR/Cas system in biotechnology uses type II and Cas9 displays a vital role as an effector protein. The RNA-guided CRISPR-Cas9 system is originally derived from Streptococcus pyogenes bacteria and it does not require target-specific protein interaction. Its specificity depends on the guidance of 20 nucleotide length guide RNA sequence and Cas9 effector protein. Guide RNA hybridizes with target DNA sequences and also, Cas9 protein is responsible for the recognition of 5′-NGG-3′ sequences known as PAM (16). Cas 9 protein has six different domains including HNH, RuvC, REC I, REC II, Bridge Helix, PAM interacting domains. The nuclease domains that are responsible for cleavage of target DNA are HNH and RuvC domains. Besides, the Bridge Helix domain is associated with the cleavage activity. The Rec I domain is essential for binding to guide RNA while the function of the REC II domain remains unclear. The PAM-interacting domain plays a crucial role in binding to target DNA through contributing to PAM specificity (17). The CRISPR/Cas9 system, like other tools, benefits from natural DNA repair mechanisms such as NHEJ and HDR. NHEJ repairs the DSBs through relegation of the two DSB ends without any requirement for template and also, is used for gene suppression through the formation of insertion or deletion (Indel) mutations. HDR requires a homologous template for repair and thus, it is used for replacement of a mutated gene

and recovery of gene (13). Besides, the adaptation (spacer acquisition), expression (crRNA biogenesis) and interference stages of the CRISPR system in a prokaryotic cell are adapted in the engineered technology with the specific design of single guide RNA (sgRNA). Moreover, trans-activating crRNA is discovered as an anti-sense RNA and a cofactor for Cas9 nuclease and, it also contributes to processing crRNAs (15). Briefly, CRISPR/Cas9 system initiates with the binding of gRNA to Cas9 nuclease and thus, Cas9 protein is activated with the conformational changes. Cas9 protein screens the DNA sequences to find the target DNA sequences that can match PAM specific sequence and then, gRNA binds to the target region. Finally, Cas9 nuclease domains form the cleavage on the target DNA (17).

The CRISPR-Cas9 system is more applicable than protein-based nucleases with many advantages. Cas9 protein is a stable component for CRISPR/Cas applications however; other nucleases are newly synthesized depending on the target. CRISPR-Cas9 is a cheaper technology than the others. Importantly, multiple gene editing can be performed with CRISPR/Cas9 system through the use of different sgRNA specifically designed for the different DNA sites (18).

CRISPR/Cas9 system is mainly used with three strategies including the plasmid-based CRISPR-Cas9 system, transfer of Cas9 mRNA and sgRNA and also, transfer of Cas9 protein and sgRNA to the target cell. Overall strategies have been developed to increase the CRISPR-Cas9 efficiency and thus, focus on different points and have different advantages and disadvantages. Plasmid-based CRISPR/Cas9 system is developed to prevent multiple transfections and thus, single plasmid encoding Cas9 nuclease and sgRNA, which is specific to the target DNA site, is used in this strategy. However, plasmid transfer processes result in different challenges. Another strategy involves the transfer of Cas9 mRNA and sgRNA into the target cell. However, low stability of transferred mRNA can cause the failure of gene editing and thus, it is a major problem for this strategy. The direct delivery of Cas9 protein and sgRNA into the target cell has many advantages, including high stability, showing faster function and low immune response (18).

The efficiency of CRISPR/Cas9 technology not only depends on Cas9 and sgRNA forms but, it also significantly depends on the delivery methods including viral and non-viral delivery systems. Each system has different advantages and disadvantages. In viral system, viral vectors are used for the delivery of CRISPR/Cas9 components. The utilized viral vectors are mainly divided into two groups such as integrating (retroviruses and lentiviruses) and non-integrating vectors (adeno-associated viruses (AAVs), adenoviruses, and herpes viruses) (18). Among them, AAVs and Lentiviruses are more useful and popular depending on their non-pathogenicity, low immunogenicity, and high infection efficiency (19). However, the integration of viral vector into the host cell genome is the main handicap for this delivery system. Therefore, non-viral delivery systems including the physical delivery systems, microinjection, electroporation, hydrodynamic delivery, lipid transfection, and utilization of gold nanoparticles become more preferable (18).

The utilization of CRISPR/Cas9 system in biotechnology is limited by off-target and on-target issues. It is expected that CRISPR/Cas9 system having sgRNA and PAM sequences matching to target DNA site only perform the genetic manipulations on the interested site. However, CRISPR/Cas9 system can recognize the undesired DNA regions and cleave these regions. This circumstance is called off-target effect of CRISPR/Cas9 system. Off-target effects cause genomic instability and toxicity, functional destruction in genes, epigenetic alterations, cell death, transformation and accordingly carcinogenesis (18). For reduction of the off-target and increase of the on-target efficiency, many various strategies based on the main components of the CRISPR/Cas9 system such as sgRNA and Cas9 have been developed. These strategies include truncation of the 3′ end of sgRNA and the addition of two guanine nucleotides to the 5′ end of the sgRNA, optimization of Cas9-sgRNA concentration, replacement of wild type Cas9 nuclease with D10 nickase, which is a mutant version of Cas9 allowing cleavage of only one strand (also known as paired nicking strategy), generation of fused catalytically dysfunctional Cas9 with FokI nuclease domain (fCas9) leading to enhanced DNA cleavage specificity and also, co-transfer of chemically modified sgRNAs with Cas9 mRNA or protein. Among them, modification of sgRNA and Cas9-sgRNA concentration strategies may reduce on-target specificity along with minimizing off-target effect and thus, it requires a balance (20). CRISPR/Cas9 applications are also limited by the induction of DNA damage toxicity because of the trigger of CRISPR-induced DSBs to apoptosis (21). To overcome this, different Cas9 variants such as catalytically inactive endonuclease dead Cas9 (dCas9), which perform gene editing without the generation of DSBs and Cas9 nickase, which induces single-strand breaks rather than DSBs, have been developed. These variants may prevent this limitation through the elimination of the risks of DSBs (22, 23). In addition to the DNA-damage toxicity, immunogenic toxicity caused by anti-Cas9 antibodies and the antibodies against the AAV vector used for the delivery of CRISPR components can be seen as a problem for CRISPR/Cas9 applications. For that, researchers have investigated the different Cas9 orthologs and AAV serotypes that may be safer for gene editing through prediction of the binding strength to major histocompatibility complex (MHC) class I and class II (21).

Studies are still being conducted to improve the efficiency of CRISPR/Cas9 and to avoid its limitations. The CRISPR/Cas system is a promising and valuable tool for therapeutically treatment of viral infection, cardiovascular, hematologic and eye diseases, muscular dystrophy, neurological and immunological disorders and also, cancer therapy. The

CRISPR/Cas system is not only used for gene therapy but also, it is important for the generation of transgenic cell lines and animal models through the mimicking of various modifications of DNA. Besides, the CRISPR/Cas9 system is preferred for the manipulation of plant genomes for crop improvement (17).

In this review, we will specifically discuss the applications of CRISPR/Cas system, which has a wide application area, in HSCs and thus, improvements in the treatment of malignant or non-malignant hematological diseases and disorders with research and clinical perspectives.

1.3. Basic Research on CRISPR System in Hematopoietic Stem Cells

In 2013, the CRISPR/Cas9-mediated genome editing was firstly applied in the mammalian cells (24, 25). This milestone has accelerated the research on the treatment of various diseases with gene therapy and the generation of in vitro and in vivo disease models. Especially, gene editing applications in HSCs are highly curative approach for many hematological diseases and disorders.

Hematopoietic stem cell transplantation (HSCT) is a widely used approach for the treatment of many hematological diseases and malignancies with the ability to reconstitute the hematopoietic system. HSCT is divided into two categories, such as autologous transplantation, in which the patient's healthy blood stem cells are used, and allogeneic transplantation, which uses the stem cells from foreign healthy donors. The utilization of allogeneic HSCT has many limitations, such as the lack of a human leukocyte antigen (HLA)-matched donor, the risk of progressing graft versus host disease (GVHD) and infection depending on immune suppression. As a result, autologous transplantation is a viable alternative to allogeneic HSCT and has demonstrated efficacy in clinical trials for hematological diseases and malignancies, metabolic storage diseases, and immunodeficiency disorders with the development of gene therapy. The autologous transplantation of genetically edited HSCs provides more safety and efficiency for various genetic and oncological diseases (26).

The monogenic β-hemoglobinopathy diseases caused by mutations in the β-globin (HBB) gene, commonly consist of sickle cell disease (SCD) and β-thalassemia and are conventionally treated by HSC transplantation (27). However, these hemoglobinopathies could be ameliorated by CRISPR/Cas9 system-mediated gene correction in patient-derived HSCs. Gene editing on β-hemoglobinopathies with CRISPR/Cas9 system is based on gene correction, HBB gene insertion, and the disruption of genes suppressing the fetal hemoglobin (HbF). Among them, gene correction and gene insertion are more difficult strategies because of the quiescent properties of HSCs that are not eligible for HDR that occurs in the G2 phase of the cell cycle. Therefore, an NHEJ-based strategy is more applicable (28). However, Dever et al. showed that CD34+ HSPCs obtained from mobilized peripheral blood (mPB) can be genetically modified at the HBB locus through the utilization of Cas9 ribonucleoproteins (RNPs) along with recombinant AAV6 donor delivery in spite of their resistance and the enrichment of the cells might enhance the editing efficiency if it is combined with HSC expansion technologies such as small molecule drug utilization (29). Another study also used the enrichment strategy of targeted-HSPCs (hematopoietic stem and progenitor cells) to minimize inefficient HSC targeting and optimized a protocol by using CRISPR/Cas9 and recombinant AAV6 homologous donor delivery for improvement of the gene therapy for blood and immune system diseases and disorders (30). Moreover, researchers described the new method based on electroporation of RNPs instead of using lentiviral transduction to edit the genomes of murine and human HSPCs using the CRISPR/Cas9 system and thus, showed that this method improves the CRISPR/Cas9 applications on hematopoiesis and hematologic diseases with no requirement for the stages of lentiviral transduction, minimizing the risks of lentiviral integration in unwanted regions and also, no requirement of a mouse strain expressing Cas9 (31). Many studies have focused on the disruption of genes suppressing HbF such as BCL11A, KLF1, and ZBTB7A genes for HbF reactivation (32). In relation to that, Lattanzi et al. tried to optimize a new efficient protocol for CRISPR/Cas9 mediated gene editing on β-globin locus in mobilized CD34+ HSPCs by using plasmid or lentiviral mediated and DNA-free delivery methods and demonstrated that the plasmid-mediated delivery method had high cell toxicity and also, lentiviral-mediated delivery had a high level of off-target cleavage. However, RNA or RNP delivery had low off-target effect and toxicity on primary HSPCs, thus it could be used for gene editing (33). Another study developed an optimized protocol based on the editing of the BCL11A enhancer gene which is required for HbF repression by using modified synthetic sgRNA, SpCas9 protein (34). Besides, Samuelson et al. also investigated the new platform including multiplex CRISPR/Cas9-mediated gene editing on the BCL11A enhancer and the HBG promoter, which are required for HbF repression, in human HSCs. They showed that targeting and dual-editing on these genes provided the generation of HbF but, resulted in chromosomal rearrangement and thus, reported that this protocol had some safety concern resulting from the generation of chromosomal translocations in clinical usage despite HbF reinduction (35). Yen et al. developed a device (called TRIAMF) which is based on a filter membrane and provides cell permeabilization for the delivery of RNPs to HSPCs as an alternative to electroporation system. They reported that the usage of TRIAMF provided the in vitro HbF induction and also, protected the normal multi-lineage and engraftment potential in NSG mice [Non-obese diabetic (NOD) severe combined immune deficient (SCID) gamma mice]. Besides, the data showing that the erythrocytes derived from the engrafted edited HSPCs provided the maintenance of high level HbF induction until 20 weeks as a result of TRIAMF device support that this low cost and non-electroporated device

is so promising for delivery of RNPs into HSPCs (36). Moreover, the information about that the precipitation of α globin induces the apoptosis and death of erythroid lineages indicates the importance of the balance between of α and β globin subunits in treatment of β-thalassemia caused by the abnormalities on hemoglobin synthesis which consists of 2 pairs of α and β globin subunits. The study trying to ameliorate the β-thalassemia with genetically editing of the α-globin locus in HSPCs was based on the two strategies including downregulation of α-globin gene HBA2 and also, upregulation of β-globin gene by using Cas9 nickase allowing precise gene editing without InDel mutations. They performed the CISPR/Cas9 mediated gene editing on healthy HSPCs and then, on HSPCs of the patients with β-thalassemia. They showed that the edited healthy HSPCs had the long-term repopulation and multipotency capacities, as well as that CRISPR/Cas9 mediated gene editing provided the correction of α/β globin imbalance in HSPC-derived erythroblasts. This study revealed the novel approach on CRISPR/Cas9 application in HSPCs for β-thalassemia treatment (37). In relation to the balance between α and β globin chains, Mettananda et al. also used an alternative strategy based on knockdown α-globin expression through CRISPR/Cas9-mediated mutation generation on MCS-R2 gene, which is an enhancer of α-globin in human CD34+ LT-HSCs and they provided the knockdown of α-globin in erythroid cells generated by edited HSCs without any distruption on erythroid differentiation or off-target activity (38). SCD from β-hemoglobinopathies is also being investigated for CRISPR/Cas9 mediated gene editing. The study about the correction of SCD mutation used the RNP/ single-stranded oligodeoxynucleotides (ssODN)-based CRISPR system and revealed the efficient HDR-mediated correction of the mutation in SCD HSPCs and also, enhanced production of γ-globin and fetal hemoglobin (39). In another study, it was shown that the usage of high-fidelity (HiFi) Cas9 variant with sgRNA and ssODN provided the correction of HBB gene in CD34+ HSPCs derived from SCD-patient and also, the edited cells differentiated into normal erythroid cells having a normal level of hemoglobin. Besides, these edited HSPCs had the ability to engraft and maintain post 16 weeks of transplantation (40). CRISPR-mediated gene editing is also a curative approach against immunodeficiencies, hematologic disorders and malignancies in addition to hemoglobinopathies. There are many researches regarding this.

Wiskott-Aldrich Syndrome (WAS) caused by mutations in the WAS gene, which is associated with cytoskeleton of hematopoietic cells, is an X-linked severe primary immunodeficiency. CRISPR/Cas9 or ZFN systems provides the gene addition to K-562 cancer cells with WAS donor template (41). Rai et al. also developed a CRISPR/Cas9 platform based on the knock-in WAS complementary DNA (cDNA) in patient-derived CD34+ HSPCs and thus, provided the amelioration of WAS expression by using gRNA targeting WAS 5′UTR and an AAV6 vector (42).

The X-linked chronic granulomatous disease (X-CGD) from immunodeficiency syndromes was also genetically corrected to repair a mutation in CYBB gene by CRISPR/Cas9 system with HDR mechanism in CD34+ HSPC (43). Sweeney et al. also focused on gene therapy for X-CGD treatment and presented a CRISPR/Cas9-mediated HDR repair approach based on the targeted insertion of CYBB cDNA in X-CGD patient-derived HSPCs in combination with inhibitor of 53BP1, which is responsible for choosing NHEJ over HDR (44).

Severe combined immunodeficiency (SCID-X1) from X-linked disorders is caused by mutations in the IL2RG gene located on the X chromosome and is a target for gene editing. Pavel-Dinu et al. described a modified CRISPR-Cas9-AAV6 approach based on the integration of cDNA which results in the gene correction >97% of IL2RG mutations on CD34+ HSPCs derived from SCID-X1 patient compared to healthy donor-, PB- and umbilical cord blood (UCB)-derived CD34+ HSPCs and also, provided the clinical advantages with safety (45).

In a study about the immunodeficiency focused on the treatment of X-linked agammaglobulinemia (XLA) caused by a mutation in the Bruton's tyrosine kinase (BTK) gene through the integration of BTK cDNA into the 5′ end of BTK locus by using the HR mediated-CRISPR-Cas9 approach in mPB CD34+ cells and they showed the safety and efficiency of this approach for XLA treatment (46).

Friedreich ataxia (FRDA) caused by genetic abnormalities in the FXN gene is an autosomal recessive disorder (47). Researchers who reported that the HSPC transplantation can be used for the treatment of FRDA carried out a study based on the gene correction on CD34+ cells from a patient's peripheral blood with FRDA by using CRISPR/Cas9 system. They corrected the FXN gene in FRDA patients' CD34+ cells through the removal of GAA expansion with high efficiency, no cytotoxic effect in vitro or in vivo while the transplanted cells had engraftment and clonogenicity abilities (48).

Gomez-Ospina et al. firstly showed the application of CRISPR/Cas9 mediated gene editing for the treatment of Mucopolysaccharidosis type I (MPSI), which is a common lysosomal storage disease (LSD) from genetic disorders caused by lysosomal protein deficiencies and also, caused by iduronidase (IDUA) deficiency. They overexpressed IDUA protein using the CCR5 locus to deliver RNP and AAV6-mediated templates into human CD34+ HSPCs, revealing an efficient platform that allowed the edited HSPCs to provide the lysosomal protein while maintaining long-term repopulation and multi-lineage differentiation potential in the MPSI mouse model (49).

From metabolic disorders for which hematopoietic stem cell transplantation is a treatment option, Pyruvate kinase

deficiency (PKD) is caused by mutations in the liver and erythroid pyruvate kinase gene (PKLR). Fañanas-Baquero et al. used an RNP delivery-based CRISPR/Cas9 approach through rAAV6 transduction in human UCB HSPCs and showed the correction phenotype in erythroid cells derived from edited-PKD-HSPCs (49).

The CRISPR/Cas9 system is also used for gene knock-in, which is required for supplementation of a protein (also called protein replacement therapy) that is deficient because of a genetic defect for the treatment of many diseases. Pavani et al. developed an ex vivo editing platform to integrate the therapeutic transgenes into the genome under the transcriptional control of the α-globin promoter, which is a suitable locus for transgene knock-in, for enhancement of their expressions on human HSPCs. After knock-in of therapeutic transgenes, they demonstrated that erythroblasts derived from targeted HSPCs secreted the therapeutic proteins with maintained multi-lineage differentiation and long-term repopulation potential and thus presented the safety of the novel CRISPR-Cas9-based HSPC platform (50).

Moreover, CIRSPR/Cas9 approach is more beneficial for the contribution to immunotherapy. The study about the generation of resistance to immunotherapy targeting CD33 in normal hematopoietic cells demonstrated the efficient CRISPR/Cas9-mediated deletion of CD33 exon 2, which is responsible for expression of the V-set domain recognized by therapeutics targeting CD33, but not full-length CD33 to reduce the potential adverse effects caused by in vitro and in vivo disruption of the entire CD33 locus in hematopoietic cells and in immunodeficient mice (51). Another group also investigated the potential of CRISPR/Cas9 approach based on the NHEJ-mediated disruption of the CD33 gene to generate the resistance to CD33 CAR T therapy in normal HSPCs for leukemia treatment. They generated the CD33 knockout mPB-HSPC and thus, revealed that CD33 knockout HSPCs provided immune reconstitution post acute myeloid leukemia (AML) targeting with CD33 CART therapy (52).

The applications of CRISPR/Cas9 system are so common for human immunodeficiency virus (HIV-1) treatment. The study about the generation of HIV-1 resistance cells revealed that CRISPR/Cas9 with two sgRNA guiding SaCas9 (Staphylococcus aureus Cas9) that is known with the effective gene editing ability and ease of delivery provided the distruption on chemokine receptor 5 (CCR5) gene by using lentiviral delivery method in primary CD4+ T cells and human CD34+ HSPCs and thus, present an alternative approach for HIV-1 treatment (53). In addition, many studies have been carried out for the generation of CRISPR/Cas9 mediated-CCR5 ablation human CD34+ HSPCs to obtain the HIV-1 resistance cells (54-56).

Gene editing tools are important for the treatment of the hereditary disease Fanconi anemia (FA) which is caused by different mutations in 22 FA genes. Autologous edited-HSC transplantation is mostly curative therapy (57). CRISPR/Cas9 approach has been mostly investigated in fibroblasts or induced-pluripotent stem cells derived from patients (58-63). Moreover, gene correction in HSCs is another strategy for FA treatment. Roman-Rodriquez et al. showed the correction of FA phenotype through the generation of compensatory mutations on the coding frame of FA proteins, which is required for the FA pathway, in FA patient-derived HSPC by using NHEJ-mediated repair in their preclinical study (64).

The CRISPR/Cas9 system is also used for the generation of disease models. Regarding this, Jeong et al. successfully generated a leukemia model by using the CRISPR/Cas9 system through induction of the chromosomal translocation between the MLL and AF9 genes in human UCB-derived CD34+ cells via the delivery of CRISPR/Cas9 as RNPs using electroporation (65). Another group also generated the murine leukemia model through the induction of reciprocal translocation between MLL and AF9 genes in both mouse cell line and primary isolated HSPCs by using CRISPR/Cas9 approach with a dual-single guide RNA (66). To generate a leukemia model characterized by translocation between MLL and ENL genes (t[11;19]/MLL-ENL), researchers generated chromosomal rearrangements on these gene locus by using the CRISPR/Cas9 system. This lentiviral-mediated CRISPR/Cas9 delivery system provided the generation of leukemia model through transformation of human UCB-derived CD34+ HSPCs (67). Schiroli et al. presented SCID-X1 mouse model with the efficacy and safety of hematopoietic reconstitution arising from edited HSPCs through CRISPR/Cas9-mediated gene correction on IL2RG in human HSPCs (68). Another gene editing study on HSPCs was carried out to generate the GATA1 transcription factor expressed HSPC model. They provided the expression of GATA1 isoforms including long and short isoforms in neonatal cord blood-derived long-term (LT-HSCs) and short-term HSCs (ST-HSCs), and myeloerythroid progenitors (MEPs). Therefore, down syndrome associated with AMKL was modeled with editing of GATA1 short isoform in CRISPR/Cas9 edited-HSPCs (69).

The CRISPR/Cas9 approach has been improved for more efficient editing without adverse effects including a low level of on-target cleavage and a high level of off-target activity. Researchers have optimized and developed many protocols for this aim. In this context, Hendel et al. used chemically modified sgRNAs, which is specific to IL2RG, HBB and CCR5, to induce gene editing efficiency and reduce off-target effect in cell lines and primary isolated human T and CD34+ HSPCs. They revealed that chemically modified sgRNA enhanced genome editing efficiencies rather than unmodified sgRNA through co-delivery of chemically modified sgRNAs with Cas9 mRNA or protein (70). Mandal et al. investigated the on-target efficiency and off-target cleavage risks depending on the utilization of sgRNA and dual gRNA in the CRISPR/Cas9 system in primary isolated human CD4+ T and CD34+ HSPCs through generation of beta-2 microglobulin

(B2M) and CCR5 gene ablations. B2M, which is a component of MHC class I, ablation could be useful for generation of hypoimmunogenic cells for immunotherapy molecules and also, CCR5 ablation is important for the protection from HIV infection. They showed that the use of CRISPR/Cas9 with sgRNAs provided the efficient CCR5 ablation in CD34+ HSPCs, but not for B2M ablation in CD4+ T cells. However, the use of CRISPR/Cas9 with a dual gRNA enhanced gene deletion efficiency in CD4+ T and CD34+ HSPCs for B2M gene and also, for CCR5 gene in CD34+ HSPCs. This approach is promising for efficient on-target and low off-target mutagenesis (71). In this regard, a study on CCR5 ablation using CRISPR/Cas9 editing revealed that CCR5 was edited by a non-viral CRISPR/Cas9 system with minimal off-target effect, and thus CCR5 ablated LT-HSCs provided long-term reconstitution and improved resistance to HIV-1 infection in transplanted immunodeficient mice. These findings revealed an alternative strategy for gene therapy on HSCs (54).

1.4. Clinical Applications of CRISPR System for Hematopoietic Stem Cells

Hematopoietic stem cells are generally used for curative therapy as allogeneic or autologous HSCT for hematological malignant /non-malignant and monogenic diseases. As explained before, allogeneic HSCT has many limitations including lack of HLA-matched donor, risk of GVHD and infection arising from immune suppression occurring for transplantation. Herein, autologous HSCT presents an alternative approach for eliminating these adverse effects of allogeneic HSCT. Especially, autologous transplantation of CRISPR/Cas9 mediated-edited HSCs is so promising for many hematological and monogenic diseases and disorders. Gene therapy approach with viral vectors and gene editing tools such as ZFNs, TALENs and Meganucleases have recently been mostly preferable for treatment of many diseases. Especially, there are several ongoing and completed clinical trials about gene editing in HSCs (http://clinicaltrials.gov/) (summarized in Table 1). Among these, ZFNs-based gene editing in HSCs has been extensively clinically tested for inducing resistance to HIV infection via CCR5 disruption (NCT02500849) and reactivation of HbF for hemoglobinopathy treatment via BCL11A enhancer blocking (NCT03432364). The advantages and efficiency of CRISPR/Cas9 system have become a promising approach for gene correction in HSCs (72). Among them, CTX001, which is autologous CD34+ hHSPCs genetically edited by CRISPR-Cas9 system on BCL11A gene to produce HbF in HSCs, is particularly tested in phase I/II clinical trials in pediatric and general subject with β-hemoglobinopathies (β-thalassemia (NCT03655678; NCT05356195) and SCD (NCT03745287; NCT05329649). Another clinical trial (NCT04925206) has also been carried out for β-thalassemia treatment through targeting of BCL11A repressor gene. In addition to reactivation of HbF, the replacement of mutated β-globin through gene correction is another approach for treatment of β-hemoglobinopathies (β-thalassemia (NCT03728322; NCT05444894) and SCD (NCT04774536; NCT04819841). Other clinical trial has been carried out to investigate resistance to CD33-targeted-immunotherapy through the CD33 ablation with CRISPR system in HSCs (NCT04849910). Moreover, a clinical study (NCT03164135) has been performed for the evaluation of the safety of CRISPR-mediated CCR5 modified CD34+ cell transplantation for HIV-1 treatment.

Table 1. Clinical trials associated with CRISPR/Cas9-mediated gene editing in HSCs

NCT number	Phase	Disease	Target	Responsible party	Status
NCT03655678	II/III	β-Thalassemia (Pediatric Participants)	BCL11A	Vertex Pharmaceuticals Incorporated	Active
NCT04925206	I	β-Thalassemia	BCL11A	EdiGene (GuangZhou) Inc.	Active
NCT05356195	III	β-Thalassemia	BCL11A	Vertex Pharmaceuticals Incorporated	Recruiting
NCT04208529	LT-Follow-up Study	β-Thalassemia	BCL11A	Vertex Pharmaceuticals Incorporated	Enrolling by invitation
NCT03728322	Early Phase 1	β-Thalassemia	β-globin	Allife Medical Science and Technology Co., Ltd.	Unknown
NCT05444894	I/II	β-Thalassemia	β-globin	Editas Medicine, Inc.	Recruiting
NCT04774536	I/II	SCD	β-globin	Mark Walters, MD, Professor in Residence, University of California, San Francisco	Not yet recruiting
NCT04819841	I/II	SCD	β-globin	Graphite Bio, Inc.	Recruiting
NCT03745287	II/III	SCD	BCL11A	Vertex Pharmaceuticals Incorporated	Active
NCT05329649	III	SCD (Pediatric Participants)	BCL11A	Vertex Pharmaceuticals Incorporated	Recruiting
NCT04849910	I/II	AML	CD33	Vor Biopharma	Recruiting
NCT05309733	LT- Follow-up Study	AML	CD33	Vor Biopharma	Recruiting
NCT03164135	-	HIV-1	CCR5	Affiliated Hospital to Academy of Military Medical Sciences	Unknown

Note: Information from clinicaltrials.gov.tr; LT: Long-term, SCD: Sickle cell disease; AML: Acute myeloid leukemia, HIV-1: human immunodeficiency virus

In the light of all preclinical and clinical studies, it can be highlighted the power of CRISPR/Cas9 approach in therapeutic area and the importance of the improvement of new platforms to enhance the gene editing efficiency and clinically feasibility.

2. Conclusion

HSCs which are valuable for the treatment of malignant and non-malignant hematological disease and disorders via transplantation are offered as a target for gene editing. Therefore, the CRISPR/Cas9 system of genome editing tools has been developed as a curative approach for hematological malignancies and diseases. In spite of the many advantages of the CIRSPR/Cas9 system including its fast and cost-effective, this technology has several technical limitations. Many studies have developed new platforms and protocols to overcome these limitations. Until today, many preclinical and clinical studies have shown the potential of CRISPR/Cas9 approach in correction of genetic abnormalities in HSCs and the curative action of the CRISPR/Cas9-mediated edited HSCs. Along with all these properties, CRISPR/Cas9 gene editing tool is considered a valuable and promising therapeutic method for future gene therapy.

Conflict of interest

None to declare.

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Authors' contributions

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

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Review Article

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From laboratory studies to clinical applications mesenchymal stem cells in cancer treatment: Translational oncology

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Abstract

Stem cell-based studies have accelerated to treat various pathologies, particularly neurological, cardiovascular, orthopedic diseases and cancer with the understanding of their therapeutic potential after their discovery. Stem cells have recently aroused great interest as a promising treatment option in fighting to cancer with extensive study in the fields of cancer biology. Nevertheless, much uncertainty regarding the act of stem cells in cancer development and treatment remains obscure before the clinical use of stem cell investigation. Despite many obstacles, the migration ability of stem cells, the bioactive factors they secrete, and their immune regulatory properties make them advantageous for gene therapy strategies. However, unknowns and a lack of scientific data remain concerning the use of stem cell-based therapies. Therefore, more experimental data are needed to confirm the different study results presented by different scientific communities. In this review, we focused on summarizing the available experimental and clinical data on the potential uses of mesenchymal stem cells in cancer therapy.

Keywords: anti-tumoral activity, cellular therapy, cancer, mesenchymal stem cells

1. Introduction

Mesenchymal stem cells (MSCs) were defined 40 years ago by Friedenstein et al. as non-hematopoietic cells isolated from the bone marrow (1). MSCs, depending on their stem cell properties, which have the potential to renew themselves and differentiate into cells from the same or different embryonic layer, are easily accessible cells because they can be obtained from many adult tissues (2-7)

Although MSCs show different characteristics according to the tissue sources from which they are obtained, they are basically described by the International Cellular Therapy Association (ISCT) depending on several properties; including 1. adhesion to plastic surface when grown in vitro, 2. having no expression of CD45, CD34, CD14 or CD11b, CD79α or CD19 and HLA-DR but having high expression of CD73, CD90, and CD105, 3. differentiation to adipocytes, chondrocytes, and osteoblasts when cultured under certain conditions (8-10).

MSCs constitute a group of stem cells that are widely studied in clinical practice due to their ability to be cultured easily *in vitro*, their potential to differentiate into cells from different embryonic layers, their immunomodulatory properties, tissue repair, and their secretion of many paracrine factors (11).

MSCs can be induced into many different cell types of mesodermal and non-mesodermal origin (8). MSCs have significant therapeutic potential for stem cell transplantation, repair of damaged organs, and gene therapy because of their potential for self-renewal and multi-lineage differentiation, and they emerge as the most promising candidates in regenerative medicine and clinical therapy (12). Their low immunoreactivity and high immunosuppressive properties make MSCs an important source of cells for both autologous and allogeneic applications (13).

Until today, clinical applications have been encountered in the treatment of orthopedic fractures and osteoarthritis (14, 15), and in the treatment of cardiovascular diseases (16, 17). In addition, MSC-based studies as cellular therapeutics have been carried out for many diseases including neurological diseases (18, 19), liver regeneration (20), acute kidney injury (21), cardiac ischemia (22) and diabetes (23) in preclinical animal models. At the same time, ongoing preclinical studies propose that MSCs may be appropriate targets for cell therapy in many cancers. However, the antitumor potentials of MSCs are still unknown. In some of the studies targeting many types of cancer, MSCs were found to show protumorigenic effects (24-28), while in some others it was observed that they showed antitumorogenic effects (29-40).

It has been considered that the various findings showing stimulating or inhibiting results of MSCs on tumor growth may be related to change in tumor patterns, differences in MSC

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source, donor-dependent variables, dose or timing of MSC administration, cell isolation and culture method, number of stem cell passages used, and the other experimental conditions (25, 41). Therefore, more scientific data is needed to make these studies suitable for future clinical research. At the same time, it is important to standardize the studies and explain the underlying reasons for this variability in the data before the results obtained from laboratory studies are transferred to clinical applications.

2. The Sources of MSC and Their Role in Cancer Treatment

Despite significant successes in treatment, cancer is still one of the major causes of death worldwide. The nature of cancer, such as its aggressive nature and metastatic potential, as well as weakness in the host defense mechanism and failures in therapeutic management, may be important causes of treatment failure. In this respect, discovering the cellular mechanisms and signaling pathways that are effective in tumor formation and development will enable the development of new targeted anti-cancer therapies (35).

Understanding that MSCs migrate to damaged tissues and areas of inflammation has an important place in the emergence of their therapeutical aspect in the therapy of diseases. Various research has reported that MSCs can cause tumor growth inhibition or induction, depending on the types of tumor which MSCs contact and interact with (8, 42).

Studies have reported that MSCs originating from different tissues show different properties. According to the results of various phenotype and function studies, it has been reported that MSCs obtained from different tissues are not completely similar, but represent an extremely heterogeneous cell population that varies depending on various extrinsic and intrinsic factors (43). In a study comparing the therapeutical potentials of the MSC population obtained from different sources, they showed that MSCs gained similar phenotypic and functional properties after the first passes; however, bone marrow derived MSCs (BMMSCs) performed better than other sources for protection of tissue viability and has a paracrine effect. Although MSCs originating from different sources exhibit similar behavior in cell culture, they secrete different paracrine factors and exhibit different therapeutic potential (41). Accordingly, MSCs obtained depending on the tissue source are thought to show different anti-cancer properties. It has been shown that co-culture of human umbilical cord blood and adipose tissue-derived MSCs with primary glioblastoma multiforme cells revealed the inhibitory and apoptotic effect of umbilical cord-derived-MSCs (hUCMSC) on glioblastoma multiforme cell growth and caused apoptosis and the proliferative effect of adipose tissue-derived-MSCs (hATMSC) (44). In the experimental model investigating the therapeutic effect of ATMSCs on hepatocellular carcinoma, it was found that cancer cell proliferation decreased and also, the transcriptional expression of TIMP-1, -2 and -3 and TIMP-1 and -3 were significantly increased in HepG2 and PLC-PRF-5 cells, respectively. It has been reported that increased TIMP secretion may be a mechanism used by stem cells to restrict tumor invasion (45).

It was highlighted that hUCMSCs exhibited growth inhibition in xenograft models of human breast cancer cells and the same results were obtained after repeated experiments. In the study, it was observed that hUCMSC significantly inhibited DNA synthesis in breast cancer cells depending on the applied dose and the cells were arrested in the G2 phase. Accordingly, it has been suggested that hUCMSCs cause cell cycle arrest and cause growth inhibition of cancer cells (29).

There are studies showing that hUCMSCs induce apoptosis in cancer cells and inhibit the proliferation of tumor cells (46). Another research also reported that after 48 hours co-culture of hUCMSCs and Esophageal cancer cells (Eca109) resulted in significant upregulation of B-cell lymphoma 2 (Bcl-2), survivin, matrix metalloproteinase 2 (MMP-2) and matrix metalloproteinase 9 (MMP-9) expression levels according to controls (47). Besides, the study performed in rats suggested that hUCMSCs could be used as cellular therapy for breast cancer (34). The effect of MSCs on cancer proliferation varies according to the source obtained, cancer cell, application dose and duration, and application method. The studies from the literature were summarized in Table 1 according to MSC source, cancer model, and experimental protocol.

Table 1. Study summaries showing the effects of MSCs on cancer proliferation

MSCs source	Cancer model	Experimental protocol	Result	References
Human umbilical cord	Human breast cancer cell line	In vitro co-culture [1:6 (SC:CC)] and in vivo (xenograft model; 0.5×10^6 cells per injection)	Inhibition of the proliferation	(29)
Human umbilical cord	Human malignant glioma cell line	hUCMSCs and activated hUCMSCs (by interleukin-2, interleukin-15, granulocyte macrophage colony-stimulating factor); in vitro co-culture [0:1, 1:1, 5:1 and 10:1 (SC:CC)]	Inhibition of the proliferation	(48)
Human bone marrow, adipose tissue, and umbilical cord	Ovarian cancer cell lines	In vitro co-culture [1:1 (SC:CC)]	Inhibition of the proliferation	(36)
Human umbilical cord	Ovarian cancer cells line	In vitro co-culture [1:1 (SC:CC)] 24-96 hours	Inhibition of the proliferation	(46)
Human umbilical cord	Hepatocellular carcinoma cell line	In vitro co-culture [1:1 (SC:CC)] and CM (1:1 and 1:20); in vivo (xenograft model; 1:1 SC-CC injection)	Inhibition of the proliferation	(49)

Table 1. Study summaries showing the effects of MSCs on cancer proliferation (continue)

Human umbilical Cord	Esophageal cancer cell line	In vitro co-culture [0:1, 1:1, 2:1, 5:1 (SC:CC]	Inhibition of the proliferation	(50) (Abstract)
Human umbilical Cord	Esophageal cancer cell line	In vitro co-culture [1:1 (SC:CC)] and CM (1:1); in vivo (xenograft model; 5×10^6 cells per injection)	Promotion of the proliferation	(47)
Human umbilical Cord blood	Human chronic myeloid leukemia cell line (K562)	Co-culture of 5.000 K562 cells /well with human UC-MSCs at 1:1, 1:5, 1:10 and 1:100	Inhibition of the proliferation in a dose-dependent	(33)
Human umbilical Cord	Human breast cancer cell lines	Conditioned medium (0, 10 or 20% CM)	Promotion the proliferation and migration (via activation of the ERK pathway.)	(26)
Human bone marrow	Kaposi's sarcoma	In vitro [1:6 (hUCMSC: MDA231)] and in vivo (xenograft model; 0.5×106 cells per injection)	Inhibition of the proliferation	(51)
Human bone marrow	Head and neck cancer cell lines (SCC-25)	Co-culture of SCC-25 cells with human BM-MSCs at 1:1 ratio	Promotion of the proliferation	(27)
Human bone marrow	Primary patient-derived human acute myeloid leukemia cells	Culture of MSCs (2 x 104 cells/well) 1 x 106 with AML cells in Transwell system for 3 days	Promotion of the proliferation	(24)
Human bone marrow	Human brain tumor cell lines	Co-culture with Transwell system and conditioned media usage	Decrease in tumor proliferation	(40)
Human bone marrow	Colon cancer cell lines (HCT116 and LOVO CRC)	Condition media obtained from 1 x 106 MSCs	Increase in proliferation	(28)
Rat bone marrow	Human chronic myeloid leukemia cell line (K562)	Co-culture of K562 and MSCs at 10:1 ratio for 7 days	Inhibition of cell proliferation	(32)
Human adipose and bone marrow	Pancreas, liver, colon, prostate cell lines	Co-culture of MSC-CC at different ratio (1:1, 1:5, 1:100) and conditioned media; *in vitro* and *in vivo* model	Inhibition of the proliferation in a dose-dependent	(30)
Human palatine tonsil tissue	Head and neck squamous cell carcinoma cell lines (PNUH-12 and SNU-899)	48 hours culture of MSC-CC at different ratio (1:20, 1:10, 1:5, 1:2)	Inhibition of the proliferation in a dose-dependent	(38)
Amniotic membrane	Lung carcinoma cells (A549)	Conditioned media and heat-treated conditioned media (at 43°C for 45 minutes and then at 37°C for 24 hours)	The conditioned medium led to significant proliferation of tumor spheroids. Heat treated conditioned medium resulted in a reduction in both spheroid diameter and cell proliferation	(31)
Amniotic fluid and adipose tissue	Human kidney (786-0) and bladder (T24) carcinoma cell lines	24, 48, 72 hours incubation with conditioned media	It reduces the viability of bladder and kidney cancer cells, has been demonstrated to induce cell cycle disruptions in T24 cells; however, it may play a role in developing resistance to anticancer agents.	(39)

SC: stem cell; CC: cancer cell

The anti-tumor function of MSCs on hematological cancers has been less studied than on solid cancers and less data are available. However, there are data showing that MSCs inhibit (32, 52-54), induce proliferation on hematological cancers (52).

Results obtained by administering mouse bone marrow-derived MSCs to hematological cancer cells indicate that it may be safe and efficient in treating hematological malignancies (54). Additionally, proliferative effects of adipose tissue-derived MSCs on hematological cancer cell lines by secreting DKK-1 (dickkopf-1), a negative regulator of the WNT signaling pathway, which is known to be effective in tumor formation, are presented (55). Data have been provided that human bone marrow mesenchymal stem cell application has an anti-tumoral effect in the xenograft model of non-Hodgkin lymphoma (56), whereas it supports tumor growth of adipose tissue-derived MSCs in animal models of acute lymphoblastic leukemia cells (57). In a recent study, it was determined that bone marrow-derived MSCs caused a strong increase in the number of cells in the G0/G1 phase of stem cells after long-term co-culture with the K562 hematological cancer cell line, and significantly late apoptosis was induced in K562 cells. Analysis of 34 different cytokines suggested that the increase in metalloproteinase-1 tissue inhibitor (TIMP-1) and cytokine-stimulated neutrophil chemoattractant-1 (CINC-1) may be effective in the inhibition of K562 cell proliferation via BAX and caspase-3 cascade signaling pathway (32). It is known that secreted growth factors and cytokines vary according to the type of cell and culture conditions; however, the kind of cytokine, which is significant in cancer cell death, depends on both the stem cell and the target cancer cell type. It is known that the density of MSCs significantly affects morphology, proliferation rate and secreted factors. It has been suggested that the antitumor actions observed in solid cancers are associated with a low MSC dose. A similar association has not yet been proposed for hematological malignancies, possibly due to the paucity of studies (58).

In line with the data from different studies, the cytokines responsible for cancer cell death vary according to the stem cell and target cancer cells. Although a reduction in the growth curve of cancer cells due to UCMSC was observed, BMMSCs have been shown to stimulate the growth and metastatic ability of melanoma cells in a genetic tumor model. It has also been shown that MSCs participate in tumor stroma formation, thereby promoting tumor growth. The reasons underlying these conflicting results include the fact that MSC cultures contain differentiated cell populations, different pathophysiological conditions originating from the MSC obtained donors, or potential feeder cell contamination that may be present in the MSC culture supports different cellular processes in cancer cells (29).

MSCs show their therapeutic functions with immunosuppressive, anti-apoptotic, anti-fibrotic, angiogenic and anti-inflammatory effects through paracrine factors such as secreted growth factors, cytokines and extracellular vesicles. Tumors apply chemoattractive effects on MSCs, facilitating their movement to the tumor niche. One of the most frequently researched signaling pathways in the mobilization of MSCs into the tumor microenvironment has been identified as C-X-C Motif Chemokine Ligand 12/C-X-C chemokine receptor type 4 (XCL12/CXCR4). However, migration of MSCs towards cancerous tissue is also controlled by many molecules, including cytokines such as IL8, growth factors such as TGFβ1 or platelet-derived growth factor (PDGF), and extracellular matrix molecules such as MMP-2. Upon reaching the tumor site, MSCs interact with cancer cells through direct and indirect mechanisms that influence tumor development and growth. The paracrine effects of MSCs are one of the major mechanisms involved in cancer regulation and are regulated by many factors, such as growth factors and cytokines. It ultimately affects cellular processes including proliferation, survival, angiogenesis and immunosuppression/immunomodulation of cancer cells. Paracrine agents can be secreted directly into the extracellular space or are packaged into extracellular vesicles for release into the tumor environment. At the same time, MSCs can induce cell cycle arrest by inhibiting various cellular mechanisms such as the phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) pathway and negatively affect cancer proliferation (8).

3. Mesenchymal Stem Cells Conditioned Media

Co-culture studies can sometimes be contradictory due to the likely growth superiorities of one cell kind over another, caused by the culture medium, rather than a true anti-cancer effect. As a result of recent studies, it is known that MSCs generally exert their therapeutic effects with various paracrine factors such as cytokines, growth factors, extracellular matrix proteins and different extracellular vesicles secreted by these cells. MSC conditioned medium (MSC-CM), also called MSC secretome, constitutes an intensive source of paracrine factors. So far, it has been extensively studied for regenerative treatments in various diseases such as cardiovascular diseases, wound healing and bone regeneration (59, 60).

Using stem cells in translational oncology and regenerative medicine has many advantages. Besides, it has some disadvantages that come with the use of cells, such as immunological responses in the recipient after administration, movement of the cell to non-target tissue and a decrease in its regenerative potential. In vitro culture of target stem cells is mandatory to obtain the required amount of cells for clinical applications. However, factors such as minimal media variability in culture conditions and changing pH conditions encountered by enlarged stem cells following the transplantation process adversely affect their regenerative capacity. It has also been reported that in vitro cultured stem cells acquire properties similar to cancer cells when applied to normal tissues (61).

The important advantage of using CM as a cell-free cellular therapy is no requirement of donor-recipient compatibility, which is important in cellular therapy. At the same time, MSC-CMs will have an advantage that is more accessible and applicable as it will require less controlled conditions as an

application procedure when compared to the use of stem cells, which require sterile and special production conditions in the application process to the patient. In addition, it will be easily packaged, commercialized after being freeze-dried or lyophilized, and will be more accessible and portable as it will eliminate the need for cryopreservation compared to cell-based therapy (59). Based on all these properties, CMs are promising candidates as biological pharmaceuticals, but with many unknowns (62).

Since stem cells obtained from different sources have the potential to secrete different paracrine factors, the choice of tissue in which stem cells will be used in the treatment of diseases is important (59). In addition, different paracrine factors can be secreted at different rates according to the age of the cells and the culture status (hypoxic / normoxic, time taken for MSC-CM collection, 2D/3D cultures) (61) . Severe stress levels to which cells may be exposed can induce the secretion of resistance-related factors to toxic conditions. It has also been reported that the secretome obtained in serum-free conditions will accelerate the secretion of angiogenic factors. In a study, it was shown that 3D culture of cells and hypoxic culture conditions changed secretion levels in conditioned media. In particular, CMs from 3D spheroid cultures showed significant differences compared to 2D cultures in terms of 11 cytokines and IL-2Rα (59, 62, 63).

Different studies have been conducted to determine the effects of conditioned media of MSCs on cancer cell growth and different results have been observed (35, 64). It has been shown that CMs obtained from human lung-derived mesenchymal stem cells inhibit tumor cell growth (65), CMs obtained from human Wharton's jelly-derived MSC (hWJMSCs) had no effect on proliferation and apoptotic potential of A549 lung cancer cells (66), and CMs obtained from BMMSC can inhibit lung cancer cell proliferation and induce apoptosis of tumor cells in vitro (67).

In another research evaluating the anti-cancer effect of the secretome of hUCMSCs on breast cancer cells, it was shown to cause depending on the dose administered cytotoxic effect on the cancer cell line (68). Besides, the findings of in vitro studies investigating the therapeutic potential of MSCs from different tissues on ovarian cancer cell lines showed variability in the effects of MSC secretomes from different tissues, and conditioned media obtained from hUCMSCs was more effective in necrosis, while the supernatant derived from hATMSC was found to play a more important role in apoptosis. As a result, it has been reported that MSC-CMs cause a significant decrease in tumor markers (CA-125, LDH, beta-HCG) (36).

4. Mesenchymal Stem Cells Exosomes

Exosomes, which are secreted by cells and are approximately 30-100 nm in diameter, are defined as lipid bilayer extracellular membrane vesicles found in numerous body fluids, including blood, amniotic fluid, cerebrospinal fluid, saliva, and lymph and secreted by a variety of cells in culture, including epithelial cells, stem cells, immune cells, and tumor cells (69, 70). These exosomes are known to function in the regulation of cellular interaction through various molecules, including cytokines, growth factors, nucleic acids such as protein, lipid, mRNA, miRNA, lncRNA (71) . At the same time, most of the exosomes have tetraspanins such as CD81, CD63 and CD9, heat shock proteins such as HSP60, HSP70 and HSP90, ALG2-interacting protein X (ALIX), tumor susceptibility gene 101 (TSG101), and additionally tissue-specific proteins from which they are derived (72).

The molecular composition of exosomes varies considerably according to the donor cell, epigenetic changes, and physiological and pathological conditions to which the cell is exposed. Exosomes derived from MSCs also have anti-inflammatory, immunomodulatory and regenerative properties, which are similar to MSCs (71) and have been reported to have both pro-tumorigenic and anti-tumoral effects (73).

There are studies reporting that MSCs and MSC-CMs induce or inhibit tumor cell proliferation, migration and growth and are discussed in detail in the sections above. Studies investigating the effect of MSC-derived exosomes on tumor proliferation and migration have accelerated in recent years, and it has not been resolved whether or not they contribute. In a study in which MCF7 breast cancer cells were treated with MSC exosomes, it was determined that MSC exosomes applied at different concentrations induced an increase in cell migration in a dose-dependent manner (74). However, MSC exosomes derived from mouse bone marrow have been shown to inhibit angiogenesis as a result of downregulation of vascular endothelial growth factor (VEGF) expression in mouse breast cancer cell lines in in vitro and in vivo experimental models (75).

The results of the study evaluating the effect of exosomes obtained from hBMSCs on human gastric and colon cancer cell lines showed that the expression levels of VEGF and CXCR4, which are known to be important in tumor angiogenesis, growth and metastasis, was increased by the activation of ERK1/2 and p38 MAPK pathways. Besides, they showed the induction of tumor growth and promoting angiogenesis in *in vivo* models (76). Similarly, it has been shown to induce proliferation in gastric cancer cells and increase the migration and metastatic potential of cancer cells by stimulating epithelial-mesenchymal transition (77).

In addition to studies supporting that MSC-derived exosomes stimulate tumor growth, there are also a significant number of studies that inhibit tumor growth. In vitro and in vivo experimental models in bladder cancer cells demonstrated that the increase in caspase-3 expression and decrease in Akt phosphorylation of microvesicles derived from hWJMSCs induce apoptosis and cause cell cycle arrest in cancer cells (78). Another data demonstrating the antitumoral effect of exosomes

was obtained from lung cancer xenograft models, and it was demonstrated that MSC-EVs increase apoptosis in lung cancer cells and have the potential to be a therapeutic agent (79).

Despite inconsistent results regarding the effect of exosomes on cancer proliferation, the use of these molecules as carriers for the specific and safe delivery of drugs or nucleic acids (miRNAs, siRNAs, and LncRNAs) is increasing interest in exosomes. Particularly, their nanoscale dimensions and their use in the intercellular transfer of various cellular components provide important advantages in the efficient transfer of therapeutic agents to cancer cells. It has been reported that siRNA-loaded exosomes targeting oncogenic Kras have a strong anti-tumoral effect in models of pancreatic ductal adenocarcinoma (PDAC), and importantly, it has been shown that large-scale production under good manufacturing practice (GMP) conditions in cancer treatment may be possible (80).

5. Clinical Applications of MSCs in Cancer Therapy

A limited number of clinical studies have been conducted, inspired by successful preclinical results in the cancer treatment of MSCs. In the study conducted by MD Anderson Cancer Center as a Phase 1 clinical trial, it was aimed to find the highest tolerable dose of human mesenchymal stem cells containing interferon beta (MSC-INFβ) that can be given to patients with ovarian cancer and to test the safety of MSC-INFβ (NCT02530047). Another Phase 1 clinical study was conducted to determine the maximum tolerated dose of allogeneic bone marrow-derived MSCs loaded with oncolytic adenovirus DNX-2401 in the treatment of glioma patients (NCT03896568). In addition to its potential for direct use in cancer treatment, there are clinical studies covering MSC applications to eliminate various side effects such as acute renal failure and cardiomyopathy that develop after treatment protocols such as chemotherapy and radiotherapy used in cancer treatment (NCT02509156, NCT03874572, NCT01275612, NCT02814864). The clinical studies carried out both for the treatment of MSCs and for the elimination of their side effects are summarized in Table 2. Despite the clinical trials, the lack of published studies reveals the need for more progress and more data to be obtained before MSCs can be used in cancer treatment.

Table 2. MSC clinical trials in the treatment of cancer and treatment-related adverse events

NTC Number	Treatment	Cancer type	Clinical stage
NCT01045382	Mesenchymal stem cells and HLA-mismatched allogeneic hematopoietic cells	Hematological malignancy	Phase II
NCT01092026	Cord blood transplantation with mesenchymal stem cell coinfusion	Hematological malignancy	Phase I/II
NCT01129739	Safety and efficacy study of umbilical cord/placenta-derived mesenchymal stem cells	Myelodysplastic syndromes	Phase II
NCT01844661	Safety and efficacy of bone marrow-derived autologous mesenchymal stem cells infected with ICOVIR5, an oncolytic adenovirus (CELYVIR)	Solid Tumors Metastases	Phase I/II
NCT01983709	Allogeneic human bone marrow derived mesenchymal stem cells	Prostate cancer	Phase I
NCT02270307	Mesenchymal stromal cells and cyclophosphamide as a GVHD prophylaxis	Leukemia Multiple Myeloma	Phase II/III
NCT02079324	Genetically modified mesenchymal stem cell (GX-051)	Head and Neck Cancer	Phase I
NCT02068794	Genetically modified mesenchymal stem cell (Oncolytic Measles Virus Encoding Thyroidal Sodium Iodide Symporter; MV-NIS)	Recurrent Ovarian Primary Peritoneal or Fallopian Tube Cancer	Phase I/II
NCT02181478	Cord blood and mesenchymal stromal cells	Hematological malignancy	Early Phase I
NCT02530047	Mesenchymal stem cells containing interferon beta (MSC-INFβ)	Ovarian cancer	Phase I
NCT03184935	Umbilical cord mesenchymal stem cells	Myelodysplastic syndromes	Phase I/II
NCT03298763	Mesenchymal stem cells expressing TRAIL	Adenocarcinoma of Lung	Phase I/II
NCT03608631	Mesenchymal stromal cells-derived exosomes with KrasG12D siRNA	Pancreatic cancer	Phase I
NCT03896568	Allogeneic bone marrow human mesenchymal stem cells loaded with a tumor selective oncolytic adenovirus, DNX-2401,	Glioma	Phase I
NCT02509156	Safety and efficacy of allogeneic mesenchymal stem cells	Anthracyclines-induced Cardiomyopathy	Phase I
NCT03874572	Evaluating the safety and feasibility of allogeneic mesenchymal stem cells	Radiation-induced hyposalivation and xerostomia	Phase I
NCT01275612	Ex-Vivo expanded mesenchymal stem cells	Chemotherapy-Induced Acute Kidney Injury	Phase I
NCT02814864	Mesenchymal stromal cell	Radiation-induced Hemorrhagic Cystitis	Phase II

6. Conclusion

This review focuses on the potential roles of mesenchymal stem cells, paracrine factors released from MSCs, and exosomes in cancer therapy, whose use in the treatment of many diseases in regenerative medicine is supported by clinical and preclinical studies. In the light of the data obtained so far, the potential effect of MSCs, paracrine factors released from MSCs and exosomes on cancer cells remains unclear due to the influence of various factors on tumor development and the variability between experimental applications. The fact that the results of the limited number of clinical studies have not been published appears to be an important shortcoming. Inconsistent results for the potential use of MSCs for cancer treatment reveals that their potential to be used in cancer treatment should be supported by more experimental processes and clinical studies.

Conflict of interest

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Case Report

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Brain imaging findings of methanol poisoning and detection of chronic stage nervous system damage: A case report

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Abstract

In this article, we presented the chronic neurological findings of an acute case of methanol intoxication due to the use of homemade alcohol. In the neurological examination performed 6 months after intoxication, bilateral vision loss, tremor, dysarthria, dysphagia, loss of taste sensation, and decrease in the Cognitive State Test score were detected. Peripheral polyneuropathy consistent with axonopathy was detected in the peripheral nerve conduction study, and optical neuropathy was detected in the examination of the pattern visual evoked potential. Brain magnetic resonance imaging revealed findings compatible with bilateral putaminal necrosis.

Keywords: methanol intoxication, optic neuropathy, putaminal hemorrhagic necrosis, polyneuropathy, cognition

1. Introduction

Methanol is called wood wine and obtained from the distillation of wood (1). It is contained in antifreeze, cleaning solutions, paint, and paint removers. Illegally produced alcohol or homemade alcoholic beverages may contain high methanol levels (2). Methanol itself is not very toxic, but its metabolites are. Methanol is converted to formaldehyde by the alcohol dehydrogenase enzyme, and formaldehyde is metabolized to formic acid by aldehyde dehydrogenase (2). Adverse effects due to methanol intoxication are caused by the effect of lactic acid, which increases due to formic acid and cellular hypoxia (3). There may be residual sequelae in the patients who have survived after acute methanol poisoning. We aimed to investigate the residual sequelae of methanol intoxication related to central nervous system (CNS), visual pathways, peripheral nervous system, and cognition.

2. Case

35-year-old male patient applied to the neurology outpatient clinic of our hospital with the complaints of speech disorder, difficulty in swallowing, tremor in the hands, inability to taste, and forgetfulness. In his anamnesis, it was learned that he consumed approximately 200 ml of alcohol produced at home six months ago and then applied to the hospital with the complaints of headache, nausea, vomiting, blurred vision, and tendency to sleep. The patient declared that he received treatment in the intensive care unit with the diagnosis of methanol poisoning. A detailed neurological examination was performed 6 months after the event. The right eye could count fingers at a distance of one meter and the left eye at a distance of 0.5 meters. In the fundus examination, the bilateral temporal region was pale, and the visible retina was normal. Direct and indirect light reflex was bilaterally minimal. Bilateral gag reflex could not be observed; there was no uvula movement. Sweet (1/1 diluted sugar water), salty (1/1 diluted salt water) and sour (lemon juice) taste test was performed with the patient who stated that he could not taste. The test was applied to the right and left sides of the tongue. He could not get the bilateral salty and sweet flavors, and he got the sour taste partially. He had complete muscle strength in four extremities. There was no rigidity or bradykinesia. Babinski's reflex was negative on the left and positive on the right. There was a 3-4 beat clonus on both feet. Cognitive State Test was applied upon the description of forgetfulness complaint. He scored 21/27 on the cognition test. A decline was observed in memory, recall, abstraction-judgement, attention, and language skills in the test. No hearing loss was detected in the audiometry test. Brain magnetic resonance imaging (MRI) revealed bilateral putaminal and frontal subcortical lesions. There was no evidence of myelopathy in cervical and thoracic MRI. Bilateral visual evoked potential (VEP) study revealed bilateral visual transmission disorder. Distal symmetrical polyneuropathy (PNP) findings were detected in the peripheral nerve conduction study (NCS). He was investigated for the etiology of PNP. The laboratory tests results as fallows: fasting blood glucose was 83 mg/dL, HbA1c was 5.2%, thyroid stimulating hormone (TSH) was 2.1 mIU/L, and vitamin B12 was 374 ng/L. Anti-nuclear antibody, PR3 ANCA, MPO ANCA, Anti-DsDNA, anti-Sm-D1 were negative. Monoclonal gammopathy was not detected in serum immune electrophoresis. No

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abnormal band was observed in urine immune fixation electrophoresis. Anti-human immunodeficiency virus (HIV), Anti HCV, HBsAg were negative.

Medical history: No known abnormality.

Family history: The mother was a type-2 diabetes mellitus patient.

3. Discussion:

Most cases of acute methanol toxicity result from oral intake. Ingestion of up to 30 mL of pure methanol results in permanent blindness. 30-240 mL is potentially fatal, but individual sensitivity varies greatly (4). Nausea, vomiting, dizziness, headache, and visual disorders are the most common complaints in the acute phase (5). Impaired consciousness, memory loss, Parkinsonism, convulsions, coma, and death may occur in severe poisonings (6). Extrapyramidal symptoms are typical neurological sequelae in the patients recovering from poisoning. These include dystonia and Parkinsonism (7). Our patient had mild-moderate essential tremor. He had no Parkinson's tremor and no dystonia. There was Babinski positivity and clonus. No epileptic seizures were diagnosed.

Characteristic brain imaging findings of methanol toxicity include bilateral putaminal hemorrhagic necrosis, cerebral and intraventricular hemorrhage, cerebellar necrosis, and diffuse cerebral edema. Cystic necrosis may occur in the chronic stage of the affected areas (8). Bilateral putaminal necrosis can also be observed in Wilson's disease, Kearns-Sayre syndrome, and Leigh's disease. Carbon monoxide, cyanide, trichloroethane poisoning, or diffuse hypoxia may also cause similar radiological findings and should be considered in the differential diagnosis (8). Our patient did not have any previously known metabolic or hereditary diseases. In addition, the history of vision loss and homemade alcohol use was characteristic for methanol intoxication.

The case had typical bilateral putaminal hemorrhagic necrosis images consistent with methanol intoxication in acute brain tomography (CT) (Fig. 1) and acute-subacute flair MRI (Fig. 2). In the chronic period, putaminal volume loss was observed (Fig. 3).

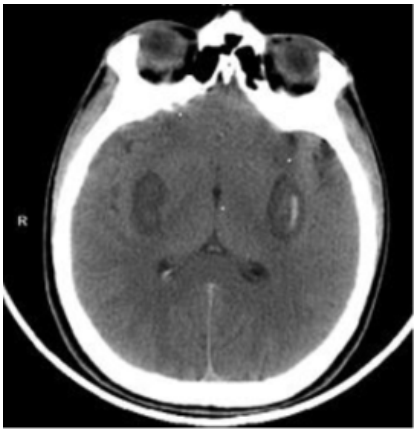

Fig. 1. Bilateral putaminal hemorrhagic necrosis accompanied by edema is observed in acute period brain CT

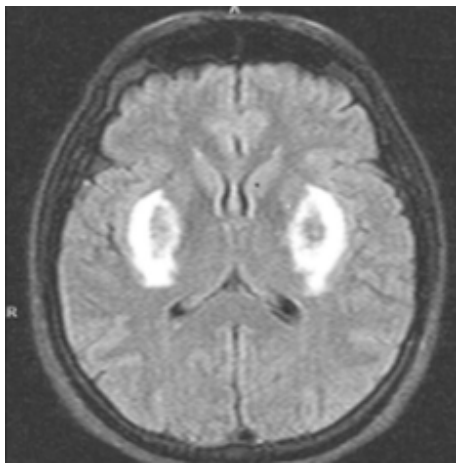

Fig. 2. Bilateral putaminal hemorrhagic necrosis areas in acute-subacute period flair MRI

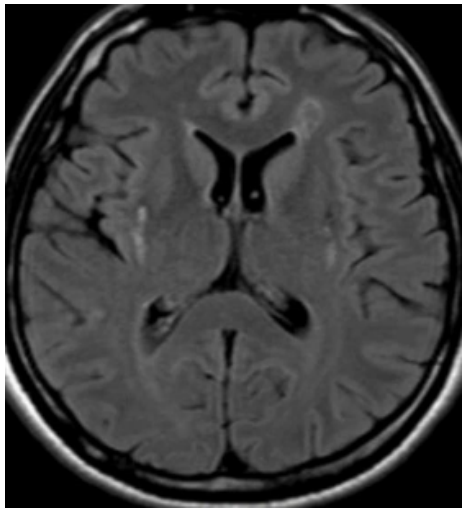

Fig. 3. Bilateral putaminal volume loss in chronic period flair MRI, lesions accompanied by hemosiderin pigments and gliosis, and hyperintense lesion in the left frontal subcortical white matter

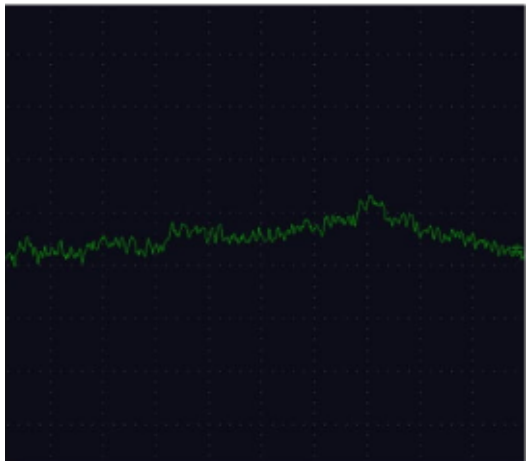

Fig. 4. Pattern VEP study showed there was no P100 wave on the left

In the literature, 5th, 7th and 9th cranial nerve involvement findings after methanol poisoning have been diagnosed (9). In our case, dysarthria, dysphagia, and loss of taste sensation (cranial 7th and 9th nerve involvement) were detected in accordance with cranial nerve involvement. Cognitive State Test was applied for the complaint of forgetfulness. He scored 21/27 on the cognition test. Although it is not common in the

literature, there are papers stating that there is a decline in cognition (6).

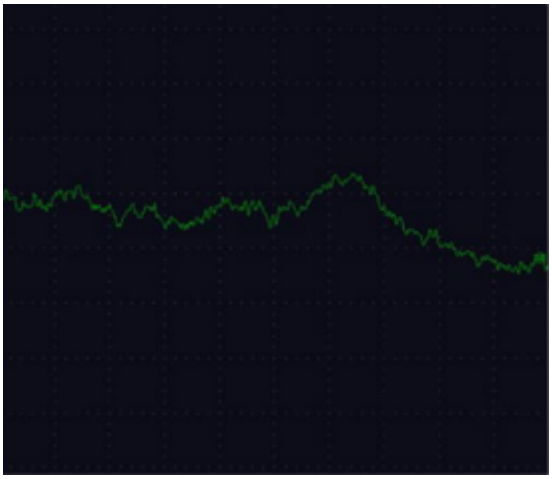

Fig. 5. Pattern VEP study showed the P100 wave on the right was torsioned, and its wave latency was prolonged

Methanol intoxication can typically cause damage to pigmented retinal epithelial cells, chiasm, and optic tract. Visual symptoms usually start within 12-48 hours and occur in approximately 50% of cases (10). In our case, visual symptoms emerged in the acute period and progressed to blindness. Pattern VEP study showed severe visual conduction disorder in both eyes (Fig. 4 and Fig. 5).

Distal symmetrical PNP findings were detected in the NCS performed on the case. Laboratory values for PNP etiology were within normal limits. He had no alcohol addiction and no diabetes. For this reason, it was thought that PNP may be due to methanol intoxication. Cases of polyneuropathy due to methyl alcohol intoxication have been reported in the literature (5).

This case showed classical, clinical and imaging findings consistent with methanol intoxication. Contrary to the literature, findings consistent with loss of sense of taste and decreased cognition were observed.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: F.Y.C., M.F.Ö., Design: F.Y.C., M.F.Ö., Data Collection or Processing: F.Y.C., Analysis or Interpretation: F.Y.C., M.F.Ö., Literature Search: F.Y.C., Writing: F.Y.C.

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Case Report

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A rare case: Coexistence of noncompaction cardiomyopathy and Ebstein anomaly

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Abstract

Noncompaction cardiomyopathy is a rare type of cardiomyopathy that can result in left ventricular failure, thromboembolic events, tachyarrhythmias, and sudden cardiac death. It is a congenital cardiomyopathy in which deep trabeculations and cavities in the left ventricle are formed due to the cessation of the development of myocardial tissue in the intrauterine period. Ebstein's anomaly is a congenital anomaly characterized by apical displacement of the tricuspid valve septal leaflet. Although the association of non-compaction cardiomyopathy and Ebstein is a rare disease, there are cases described in the literature. A 23-year-old male patient presented with palpitations and fatigue and was diagnosed with non-compaction cardiomyopathy and Ebstein anomaly. After the diagnosis, the patient is followed closely without complications with appropriate medical follow-up. Congenital heart diseases can be seen alone or in association with other cardiac malformations. When there are suspicious findings in a patient with Ebstein's anomaly, it may be associated with non-compaction cardiomyopathy, and screening with echocardiographic and cardiac MRI should be considered.

Keywords: noncompaction cardiomyopathy, Ebstein anomaly, congenital heart diseases

1. Introduction

Noncompaction cardiomyopathy is a rare type of cardiomyopathy that can result in left ventricular failure, thromboembolic events, tachyarrhythmias, and sudden cardiac death. It is a congenital cardiomyopathy in which deep trabeculations are formed, and cavities in the left ventricle are formed due to the cessation of the development of myocardial tissue in the intrauterine period. (1) Since normal compaction develops at the apex of the last ventricle, the apex is involved in the vast majority of cases. (2) End-systolic non-compact/compact myocardium ratio >2 in echocardiography is important in the diagnosis. (3) On the other hand, in cardiac MRI, according to Peterson's criteria, end-diastolic non-compact myocardium/compact myocardium rate ≥ 2.3 also makes the diagnosis. (4) Again, with cardiac MRI, Jacquier et al., in their study in which they measured trabecular and global left ventricular mass, showed that a trabecular myocardial mass greater than 20% of the global myocardial mass is important in diagnosis. (5)

Ebstein's anomaly is a congenital anomaly characterized by apical displacement of the tricuspid valve septal leaflet and may be associated with other congenital cardiac malformations. (6)

Although the association of non-compaction cardiomyopathy and Ebstein is a rare disease, there are cases described in the literature. We also planned to present this rare association in order to contribute to the literature.

2. Case Report

Our patient is a 23-year-old male patient with no known disease. He applied with the complaints of palpitation and fatigue, which lasted for 5-10 minutes and about 2-3 years, especially after exertion. In the cardiac examination of the patient, blood pressure was 120/70, and heart rate was 80 beats/min. A 2-3/6 systolic murmur was heard in the tricuspid valve focus. No significant additional pathology was detected in the physical examination. In the ECG of the patient, the rhythm was sinus, and QRS fragmentation was observed in lead D3. The ejection fraction was 55%, and mild global hypokinesia was observed in echocardiography. Increased trabecular structure in the LV and flow between these trabeculae with color Doppler were observed. In addition, it was noticed that the septal leaflet of the tricuspid valve was displaced apically, and the distance between the mitral and tricuspid annulus was measured as 18 mm. (Fig. 1). Cardiac MRI was requested from the patient with the preliminary diagnosis of non-compaction cardiomyopathy and Ebstein anomaly. Cardiac MRI showed a distinct trabecular appearance at the level of the apical and lateral walls of the left ventricle. The non-compact/compact ratio is approximately 2.3. The appearance was reported as compatible with light non-compaction. (Fig. 2) 100 mg of acetylsalicylic acid, 50 mg of metoprolol, and 1 mg of cilazapril were added to the patient's treatment. Our patient has been asymptomatic for about six months and can climb 3-4 flights of stairs. The patient is followed closely for possible complications.

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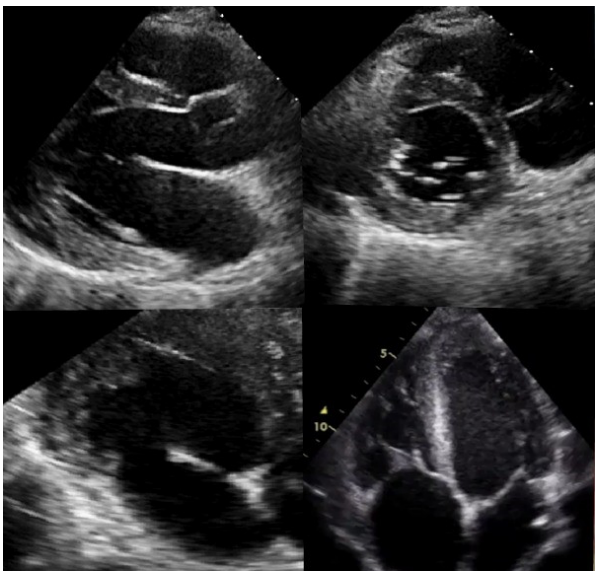

Fig. 1. Echocardiography images of the patient

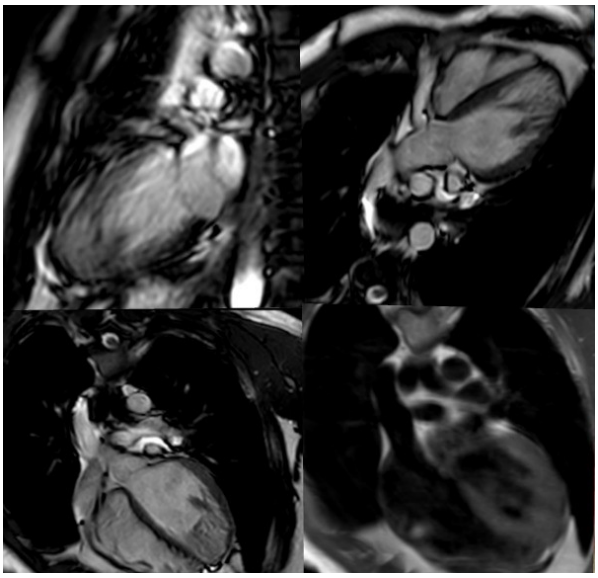

Fig. 2. Cardiac MR images of the patient, increased trabeculation structure in the LV apical

3. Discussion

This case is important because there are cases reported in the literature, even though there is a rare association with congenital heart disease. Both echocardiography and cardiac MRI are important in the association of Ebstein anomaly and left ventricular non-compaction cardiomyopathy. Cardiac MR imaging provides superior myocardial characterization and endocardial identification compared to echocardiography and is a more sensitive imaging modality in detecting left ventricular non-compaction. (7) Although current guidelines do not recommend routine cardiac In cases where there is increased trabecular structure in the left ventricle, cardiac MRI scanning can be considered in this sense to exclude left ventricular noncompaction. RI scanning may be considered in this context to rule out left ventricular non-compaction. In addition, this association is a condition that requires genetic screening. Both diseases may be alone or associated with other congenital heart diseases. Cases in which a mutation in the sarcomere gene MYH7 was associated with the coexistence of non-compaction cardiomyopathy and Ebstein anomaly have been reported. (8-9-10) In another literature study, this association was also shown in the sarcomere gene TPM1 genetic mutation. (11) In this study, it is recommended to perform both MYH7 and TPM1 gene analysis. In the same study, family screening was also recommended, and these gene mutations were detected and diagnosed in other family members. This shows us that genetic transmission may occur when the diagnosis is made, and it is important to detect other family members for early diagnosis. However, genetic screening was not performed in our case.

Congenital heart diseases can be seen alone or together with other cardiac malformations, as seen in our case. Noncompaction cardiomyopathy is a vital disease ranging from severe left ventricular dysfunction and malignant arrhythmias to life-threatening thromboembolic events and even sudden death. When there are suspicious findings in a patient with Ebstein's anomaly, it may be associated with non-compaction cardiomyopathy, and scanning with echocardiographic and cardiac MRI should be considered. It should also be kept in mind that it is hereditary and screening of other family members.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

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Case Report

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Post arrest bispectral index monitoring

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Abstract

The bispectral index is an electroencephalography-based technology used to monitor sedation. BIS is a processed EEG parameter designed specifically to assess patient responses to sedation and anesthesia. This case report aimed to discuss the bispectral index changes during resuscitation of a patient who applied at the emergency unit with cardiopulmonary arrest in light of the literature.

Keywords: bispectral Index, electroencephalography, cardiopulmonary arrest, cardiopulmonary resuscitation

1. Introduction

Cardiopulmonary resuscitation (CPR) includes medical support in circulatory and respiratory arrest. The quality of CPR, especially the quality of chest compressions and minimization of interruptions, are the most critical determinants of the cause of cardiac arrest and its treatment success. Cellular damage from ischemia/reperfusion injury is a dynamic process, and in cardiac arrest, there is a therapeutic window in which effects can be reduced after resuscitation. Neurological prognosis is very difficult to determine early after resuscitation from cardiac arrest. In most cases, attempts to determine prognosis are not possible from the return of spontaneous circulation (ROSC) to at least the 72-hour mark (1).

Bispectral index (BIS) is an electroencephalography (EEG) based technology used to monitor sedation. BIS is a processed EEG parameter designed specifically to assess patient responses to sedation and anesthesia. It is used primarily in an operating room setting and is increasingly used in the ICU. It has a sensor placed across the patient's forehead to detect the electrical activity in the brain (2). We performed the multivariate statistical analysis and combined relevant EEG features to produce the BIS, a number strongly correlated to the clinical endpoint of the hypnotic state and displayed on a linear scale (0-100). This scale includes arousal states such as awake, sedation, deep sedation, and coma. A BIS value as 100 means an awake state, while a value below 60 and 40 means deep sedation and deep anesthesia or hypnotic state, respectively (3).

This report aimed to present our case who applied at the emergency unit with cardiopulmonary arrest and was monitored by BIS during CPR.

2. Case

A 64-year-old man applied at the emergency unit with cardiopulmonary arrest. His family mentioned that he was admitted to the hospital several times and discharged from the coronary intensive care unit a week ago. The patient had a history of hypertension and congestive heart failure. We performed cardiopulmonary resuscitation successfully for half an hour. After resuscitation, his vital signs were the following: Body temperature 36.1°C; blood pressure 85/64 mmHg; and heart rate 74 beats per minute. We monitored the patient with BIS. BIS values remained between 0 and 10 for half an hour. Then his BIS started to rise, and it reached 61 in the fiftieth minute after resuscitation (figure 1). Widespread ST depression was present on post-resuscitation electrocardiography. Echocardiography evinced ejection fraction as 20%. We admitted the patient to the intensive care unit. He was extubated after three days of ventilatory support. We discharged the patient without any sequelae after 5 days of

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intensive care follow-up and 3 days of clinical follow-up. An informed consent form about publication of this case was obtained from the patient and his son.

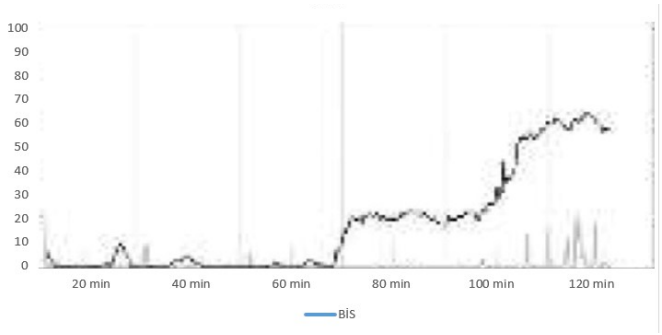

Fig. 1 Bispectral index

3. Discussion

BIS is a parameter originating from a mathematical analysis of data taken from the electroencephalogram. It is a simple, easy to interpret and noninvasive method. A BIS of 100 represents an awake individual, while a BIS of 0 represents complete electrical silence (2). Using BIS may greatly benefit clinical management by assessing the central nervous system function. A high BIS value reflects cerebral activity and should encourage the team to continue CPR. On the other hand, it is not clear to stop CPR and make death decisions according to a low BIS. Many care reports showed the potential benefit of BIS monitoring during resuscitation from cardiopulmonary arrest. Our case showed that BIS could be low, even zero in post-resuscitation, but it could rise after an hour.

EEG changes due to impaired cerebral perfusion and cerebral ischemia are defined as isoelectric EEG monitoring following voltage loss. In the literature, BIS changes due to cerebral hypoperfusion and stroke have been reported as case reports in patients who underwent perioperative BIS monitoring (5,6). Welsby et al. reported a patient with an unexpected BIS decrease during coronary bypass graft operation and a post-operative stroke. They claimed to have encountered a stroke and that this decrease might have been due to it (5). Morimato et al. suggested that this decrease might have been due to cerebral hypoperfusion in a patient who had a BIS decrease during an arteriovenous shunt operation (6). A study evaluating the relationship between ischemic stroke and BIS reported a low BIS in patients with acute ischemic stroke. The authors associated this lowness with low EEG waveforms caused by cerebral hypoperfusion (7). Similarly, Küsken et al. showed that it associated with pathological findings in patients with head injury (8).

Researchers in the literature have shown that ROSC was associated with sharp increases in cytokines and other inflammatory response markers (9). On the other hand, arterial carbon dioxide levels and partial pressure after ROSC have been shown to correlate with clinical outcomes. Additionally, specifically, hypocapnia is associated with worse neurological outcomes (10).

In conclusion, based on our clinical experience in this case, we think that BIS may be a predictor like other effective predictors of ROSC. We believe that researchers should be encouraged to do more research on the relationship between BIS and ROSC.

Conflict of interest

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Authors' contributions

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Curative breast cancer surgery with local anesthesia

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Abstract

Breast cancer has become an important health problem affecting mostly women, and its incidence is increasing. It is treated with a multidisciplinary approach involving medical oncology, radiation oncology and surgical oncology. Surgical removal of the tumor is the most important step in its treatment. General anesthesia used during surgery carries significant risks in patient groups with advanced age and comorbidities. In addition, treatment options such as chemotherapy and radiotherapy other than surgery may have significant side effects in these patient groups. Therefore, surgery plays an important role in the treatment of patients. Surgery with local anesthesia is a suitable alternative to general anesthesia in patients with early stage breast cancer because it carries less risk. Invasive Ductal Carcinoma was diagnosed in a 64-year-old female who presented with a mass in the right breast. In the anesthesia examination, she was evaluated as an ASA 3 because of her comorbidities and age. We present our patient with high comorbidity and advanced age who underwent curative cancer surgery using only local anesthesia.

Keywords: breast cancer, local anesthesia, breast-conserving surgery, radiotherapy

1. Introduction

In women, apart from skin cancers, cancer of the breast is the most common malignant disease and also the second most common cause of cancer-related death (1–3). While the surgical treatment of breast cancer was aggressively applied as radical mastectomy at the beginning of the 20th century, today it has evolved into a more conservative and minimally invasive surgery in the form of breast-conserving surgery (BCS). With the addition of radiotherapy, BCS has achieved similar survival rates to mastectomy and has replaced mastectomy, especially in early-stage breast cancer (ESBC) (4–6). BCS has an increased risk of mortality and morbidity due to anesthesia, especially for elderly patients and patients with comorbidities, as it is mostly performed under general anesthesia. In this study, we aimed to present a breast cancer patient who was considered to be at high risk in terms of anesthesia and therefore underwent curative surgery using only local anesthesia.

2. Case Presentation

A 64-year-old female patient was admitted to our clinic due to a right breast mass. Written informed consent was obtained from the patient for the publication of the case report. The patient's medical and surgical history had mitral valve replacement and total thyroidectomy surgeries, atrial fibrillation, diabetes mellitus, hyperlipidemia, and primary hypertension. On physical examination, an irregular mass was palpated in the outer middle quadrant of the right breast. No pathological lesion was palpated in the left breast and both axillae. On mammography and breast ultrasonography, the mass was interpreted as a 14x9 mm lesion with irregular borders and a thick echogenic capsule in breast imaging reporting and data system classification (BIRADS) 4. A tru-cut biopsy of the mass resulted in an invasive breast carcinoma. Surgery was planned for the patient whose whole body positron emission tomography did not reveal any findings suggestive of metastasis. Due to comorbidities, the patient was considered high risk by the American Society of Anesthesiologists (ASA) 3 in the anesthesia examination. Therefore, the patient's surgery was performed using 2% Prilocaine, a local anesthetic. No inhaler or intravenous anesthetic was used. Prilocaine was injected into the area where the incision was made for the sentinel lymph node biopsy (SLNB) after methylene blue injection to the subareolar region and 8 minutes of massage. A Sentinel lymph node was found and sent to the frozen section. Following prilocaine injection on and around the mass, the tumor was removed by obtaining a macroscopic clean margin and sent to frozen. As a result of the frozen section, the pathology reported that the closest surgical margin was 1,5 cm and no metastasis was observed in the sentinel lymph node. On the first postoperative

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day, we discharged the patient, and she had no postoperative complications. In the pathological examination of the specimen, a tumor with the morphology of an Invasive Ductal Carcinoma (grade III/ III, Modified Bloom Richardson) with the largest diameter of 1,5 cm was detected. In the immunohistochemical examination, it was revealed that estrogen receptor (ER): 100% positive, progesterone receptor (PR): 60% positive, Cerb B2: negative, and Ki-67: 40-50%. No recurrence or metastasis was detected in the 3-year follow-up of the patient who received adjuvant radiotherapy and hormonal therapy in the postoperative period.

3. Discussion

Breast cancer is an important health problem with an increasing incidence. A multidisciplinary approach is needed for surgery, which is the most important step in the treatment. Surgery is mostly performed under general anesthesia. In addition, regional or local anesthesia can be applied to patients who are at high risk of general anesthesia due to advanced age or additional diseases. Among these techniques, various epidural anesthesia and nerve blocks are used.

Surya et al. (7) presented five patients who underwent mastectomy and axillary dissection with a combination of more than one anesthesia techniques which were brachial plexus block, supraclavicular nerve block, and finally thoracic epidural anesthesia. All of these patients had diabetes mellitus and hypertension and two patients had coronary artery disease.

Colak and Alici (8) performed a modified radical mastectomy under sedation and local anesthesia on a patient with advanced-stage who was evaluated as ASA IV as a result of anesthesia examination.

Chongshan et al. (9) found that there was no relationship between the type of anesthesia and the prognosis of the disease in breast cancer patients operated under local anesthesia and general anesthesia. And local anesthesia was found to be more advantageous in terms of cost.

Hirokawa et al. (10) operated ESBC patients under general and local anesthesia and showed that there was no difference in terms of oncological outcomes due to the type of anesthesia. Axillary dissection was performed in a second session under general anesthesia after a positive sentinel lymph node was detected in a patient who was operated on under local anesthesia, and they emphasized this as one of the disadvantages of local anesthesia. In addition, they found local anesthesia advantageous in terms of hospital stays and cost.

It is known that in the process from the past to the present, minimally invasive methods have taken the place of radical methods in breast cancer surgery. BCS with SLNB have become routine procedures, especially in the treatment of ESBC. Even if the SLNB is positive, there are randomized controlled studies showing that the disease is well controlled with adjuvant treatment without axillary dissection (11). It has not been shown that there is an oncological disadvantage in patients who were operated on without using general anesthesia (10). In our opinion, this shows that local and regional anesthesia techniques can be used instead of general anesthesia.

In conclusion, it should be kept in mind that curative breast cancer surgery can be performed under local anesthesia to avoid the risks of general anesthesia in patients with advanced age and comorbidities.

Conflict of interest

The authors declare that they have no conflict of interest.

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Authors' contributions

Concept: Y.D., F.G., Design: Ö.D., D.Y.B., Data Collection or Processing: Y.D., Ö.D., D.Y.B., Analysis or Interpretation: Ö.D., F.G., Literature Review: Y.D., F.G., Writing: Y.D., Ö.D., D.Y.B.

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Lung cancer and choroidal metastase

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Abstract

Sixty-nine-year-old male patient, having treatment after one month due to lung adenocancer, was admitted to our clinic with the complaint of blurred vision in the left eye. Fundoscopy examination revealed irregular macula in both eyes and metastasis in both eyes choroid in Optical Coherence Tomography (OCT). In our case, it was observed that although both eye choroids had metastasized, the complaints of the eyes were lost with radiotherapy. In this case, a patient who developed eye metastases during the follow-up of lung cancer after completion of chemotherapy is presented. The patient with eye metastases improved after radiotherapy.

Keywords: lung neoplasms, choroid, radiotherapy, metastasis

1. Case

Our case was a 69-year-ol male patient who had no complaints other than cough. In the computerized tomography (CT) taken incidentally, an enlarged lymph node in the right peribronchial, subcarinal, right anterolateral subpleural 10 mm spicular extension, irregular nodule was observed (Fig. 1). A diagnosis of adenocarcinoma was made as a result of biopsy taken from the lymph nodes by mediastinoscopy. The patient with T1b N2 M0 (Stage 3A) did not have EGFR, ALK, ROS-1 mutations. The patient was initially treated with paclitaxel and carboplatin chemotherapy with curative radiotherapy (RT) a total dose of 61.2 Gy to the primaries and lymph nodes in our clinic. He was admitted to our clinic with the complaints of increased dyspnea and blurred vision in his left eye 24 months after the end of his treatment. In the fundoscopy examination in the Ophthalmology clinic, macular irregularity was observed. Multiple masses in both eyes were evaluated as metastasis to the choroid in Optical Coherence Tomography (OCT) (Fig. 2). At the same time, Positron Emission tomography (PET-CT) showed high FDG (SUV max: 8,31) uptake in the mass in the right hilar region, pleural effusion and metastases to the adrenal and bone (Fig. 3).10 x 3 Gy radiotherapy to both eyes with volumetric intensity modulated arc radiotherapy (VMAT) technique for 10 days treatment. It was observed that the eye complaints disappeared after the treatment. An improvement in the appearance of metastases was observed with control OCT (Fig. 4). The patient's visual impairment improved after radiotherapy applied to the eye and carboplatin paclitaxel chemotherapy was continued. Informed consent form was obtained from the patient and patient relatives in that case reports.

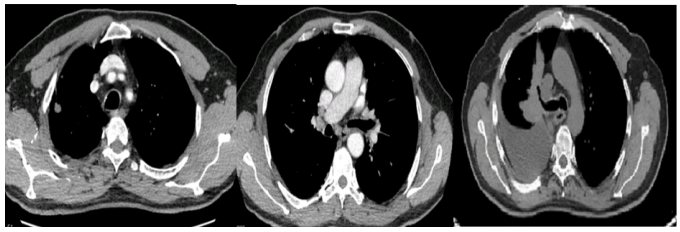

Fig. 1. Right peribronchial, subcarinal enlarged lymph node, right anterolateral subpleural 10 mm spicular extension, irregular nodule

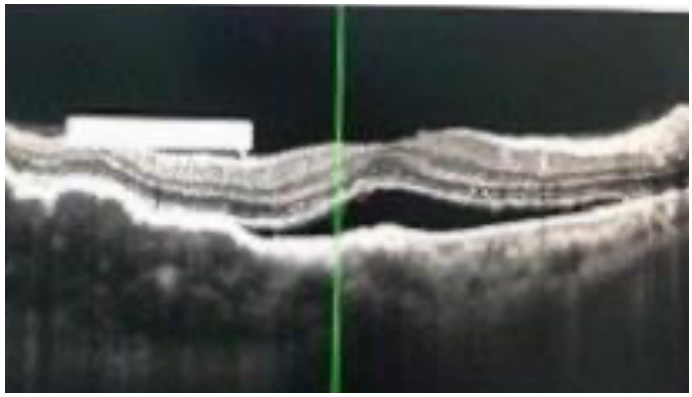

Fig. 2. Optical Coherence Tomography (OCT) image of choroidal metastasis

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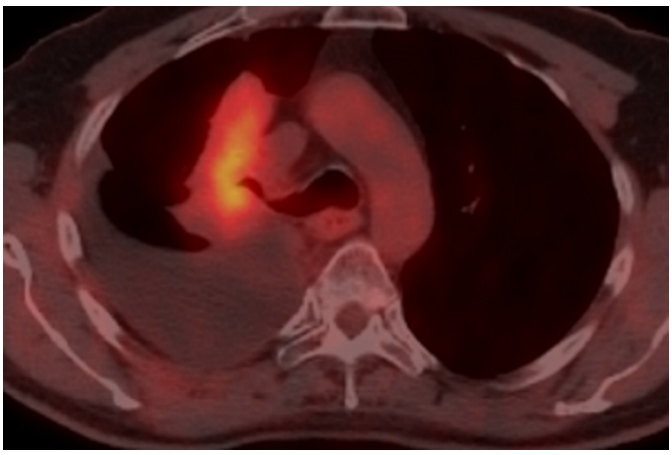

Fig. 3. High FDG uptake (Suv max :8.31) in the mass in the right hilar region and pleural effusion in positron emission tomography (PET-CT)

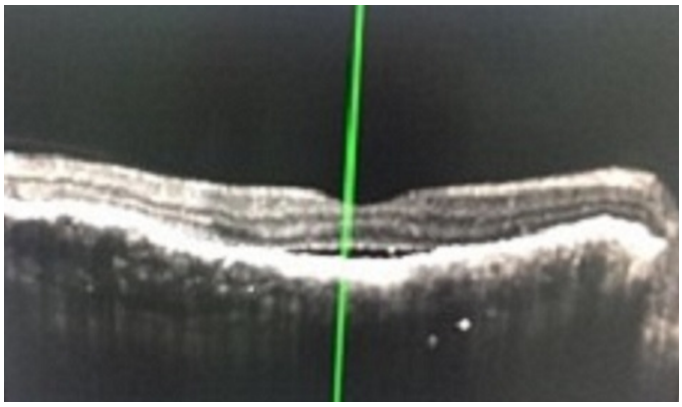

Fig. 4. Improvement in metastasis appearance with control OCT

2. Discussion

The majority of eye cancers develop from metastases of other organs to the eye.

It is quite rare, and its frequency has been reported to have increased in recent years. Choroidal metastasis; is more common than iris and ciliary body metastases (1). The number of studies evaluating the incidence of choroidal metastasis due to lung cancer is few, and the incidence of choroidal metastasis varies between 0.1-7%(2). Lung cancer, which metastasizes most frequently into the eye, is reported to be the histopathological type of adeno cancer in 41% (3).

The uveal system is the part of the eye where metastases are most common. In the uvea, the choroid (88%) is the most affected area, followed by the iris (9%) and the ciliary body (2%). Although the exact cause is not known, the high vascularity in the region may explain this situation (4-5). Lung cancer is responsible for 30% of metastases to the choroid (6). It is more common for choroidal metastases. It is more common for choroidal metastases to involve the eye as multiple foci and 20-40% of them are bilateral (7).

The majority of patients have symptoms of lung cancer. Eye metastases are usually seen in advanced stages of cancer. Presence of metastases in at least one or more organs other than the eye is common (8). The most common metastases are seen in the brain, kidney, and liver adrenal. At the time of presentation with ocular symptoms, the patient also had adrenal and bone metastases (9). In our case, the presence of metastasis in both eyes and the absence of symptoms in the other eye is due to the fact that the metastasis remained silent in that eye. It has been reported in fewer cases that patients without a diagnosis of lung cancer initially presented with ocular symptoms. In general, symptoms of blurred vision, pain, floaters, eye redness, epiphora, diplopia are seen. Our case, on the other hand, applied to our clinic with the complaint of blurred vision in the left eye after the end of the lung cancer treatment, but on examination, metastasis was found in both eyes.

Radiological methods such as OCT, fluorescence angiography of the fundus, CT, orbital MR ultrasonography, PET imaging are useful in the diagnosis of these metastases. OCT is more sensitive than ultrasonography in the evaluation of small metastases. Features of metastasis may include anterior compression, irregularity and posterior shadowing. In retinal involvement, retinal pigment epithelial abnormalities and subretinal fluid are found (10).

Purpose of treatment; to maximize the patient's quality of life and to restore or preserve vision. A multisystemic approach to the treatment of the disease is very important. Treatment can be systemic or local. Chemotherapy can be used in systemic treatment. However, there are no definite conclusions about its usefulness.

There are some studies in the form of more case reports, especially on adenocarcinoma, and it is reported that it responds well to various chemotherapies. In particular, tyrosine kinase inhibitors such as bevacizumab, gefitinib, erlotinib, crizotinib and pemekrexet are more effective chemotherapy drugs in adenocancer (10, 11).

However, in our patient EGFR, ALK, ROS-1 were negative, drugs mentioned in the literature could not be administered at the beginning of the disease and after metastasis developed. Systemic treatment is more preferred than local treatment because complications such as local infections, xerophthalmia and cataract formation are rare. Radiotherapy, enucleation, systemic steroids, less frequently transpapillary thermotherapy and vitreous bevacizumab are used in local treatment. It has been reported that good results will be obtained especially when bevacizumab is given intravitreal together with systemic treatment (12).

Surgery does not play an important role in treatment, as surgery has a high morbidity potential.

Radiotherapy is usually the most important form of local treatment applied when lesions do not respond to systemic therapy. In many studies, radiotherapy to the eye between 20 and 50 Gy is widely used and provides symptomatic relief by controlling tumor growth. Although External Beam

Radiotherapy (EBRT) is the most widely used technique in the treatment of choroidal metastases; newer modalities such as plaque brachytherapy, proton beam therapy, and stereotactic radiosurgery can be used.

External Beam Radiotherapy (EBRT), first applied in 1979, is a widely used treatment modality for uveal metastases that do not regress despite systemic therapy (13). There are many studies indicating that EBRT is very beneficial in the treatment of patient symptoms and life expectancy. The improvement in visual functions is more permanent due to the higher dose of EBRT compared to other radiotherapies. If the applied RT dose is more than 32 grays, a faster response to the treatment is observed and the symptoms improve significantly. Our case responded well to 3 Gy radiotherapy for 10 days visual acuity increased. The patient showed response to both palliative radiotherapy and subsequent chemotherapy. In choroidal metastases, survival time may vary depending on the primary tumor type and local spread of the tumor. The prognosis of patients who develop choroidal metastases due to lung cancer is generally poor and the average life expectancy is reported to be 7-12 months (14). The survival of the patient in choroid metastasis with lung primary is no more than six month (15).

In patients with a diagnosis of lung cancer, if there are ocular symptoms, metastasis to the choroid should be considered. Choroidal metastases have a poor prognosis. Primary choroidal melanoma, haemangioma, inflammatory granuloma can be the differential diagnosis. Treatment options should be reviewed in order to improve visual function and improve quality of life in these patients. A multidisciplinary approach seems important for the treatment and follow-up of patients. In conclusion, radiotherapy should be considered as another treatment option in patients with lung adenocarcinoma who do not respond to chemotherapy.

Conflict of interest

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Authors' contributions

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Letter to Editor

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Medical management during the pandemic period

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Abstract

We read with great interest the article titled "Diagnostic Accuracy of Clinical Gestalt of Doctors with Different Experiences in COVID-19 Suspected Patients" prepared by Özkan and published in the third issue of your journal in 2022. Thanks to the author and editorial board for this interesting and informative article. In addition, we would like to briefly touch on the parameters used in the perception of triage and patient management during the pandemic process.

Keywords: pandemic, SARS-CoV-2, COVID-19, medical management

Dear Editor,

We read with great interest the article titled "Diagnostic Accuracy of Clinical Gestalt of Doctors with Different Experiences in COVID-19 Suspected Patients" prepared by Özkan and published in the third issue of your journal in 2022 (1). Thanks to the author and editorial board for this interesting and informative article. In addition, we would like to briefly touch on the parameters used in the perception of triage and patient management during the pandemic process.

The increase in emergency service applications, especially during the pandemic, is a major problem worldwide and in Turkey. The fact that emergency health services are free of charge causes people to apply to emergency health services in non-emergency situations, and the growing patient volume causes many problems, such as the inability to intervene in time for patients who need urgent health care services and physician and patient dissatisfaction. In this case, the method of separating the emergency patients from the non-emergency patients and determining the priority of treatment of the patients gain importance (2).

In the Book of Travels of Evliya Celebi, Evliya Celebi talks about the soldiers waiting at the castle gate. Triage officers are not like soldiers waiting at the castle gate. Their key task is to ensure that the patient in need of emergency medical support reaches the physician immediately. They contribute to the management of health resources. Healthcare providers use triage scales to distinguish between emergency and non-emergency patients (2).

Concerns about the use of health resources have increased after the first SARS-CoV-2 case was reported in Turkey and the World Health Organization declared a pandemic on March 31, 2020 (3). Rapid Emergency Medicine Score (REMS), Modified Early Warning Score (MEWS), and Rapid Acute Physiology Score (RAPS) are parameters that can be used in prehospital and emergency departments based on vital parameters. In the pandemic, early warning systems such as REMS, MEWS and RAPS were studied for their ability to predict bad outcomes (4).

Scoring systems formed by adding laboratory parameters to the early warning systems based on vital parameters in the emergency room management of SARS-CoV-2 patients were studied. It has been shown that available scoring systems such as CURB-65 and pneumonia severity index, which are used in the management of pneumonia patients, can also be used in SARS-CoV-2 patients (5). It has been reported that C-reactive protein, lymphocyte count, interleukin 6, and D-dimer can be used in the follow-up of disease and in clinical management, such as the decision for intensive care admission (6,7).

In addition to all these available scoring systems and laboratory parameters, new markers have been developed using machine learning (8-10). Weng et al. developed the ADNC score for SARS-CoV-2 infected patients using age, neutrophil-to-lymphocyte ratio, D-dimer, and C-reactive protein recorded at admission (8). Xie et al. reported a prognostic model using lactate dehydrogenase, lymphocyte count, age, and SpO2 as predictors of SARS-CoV-2-related death (9). Yan et al. proposed a marker that could predict 10-day mortality using lactic dehydrogenase, lymphocytes, and high-sensitivity C-reactive protein (10).

As a result, the pandemic period is a period in which scarce

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resources should be used effectively. Researchers have developed new parameters to manage resources using vital and laboratory parameters.

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Authors' contributions

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Letter to Editor

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Prognosticating poor outcome in COVID-19

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Abstract

We have read the article titled "Comparison of liver biomarkers with N/L ratio, CRP, d-dimer in Covid 19 pneumonia and its effect on mortality" prepared by Haydar et al. with great interest. We thank the editorial board and the authors for publishing this successful and informative manuscript. We also would like to mention a few important points about prognosticating mortality in COVID-19 to contribute to the discussion of the study.

Keywords: COVID-19, SARS-CoV-2, pandemics,prognosticate

Dear Editor,

We have read the article titled "Comparison of liver biomarkers with N/L ratio, CRP, d-dimer in Covid 19 pneumonia and its effect on mortality" prepared by Haydar et al. with great interest (1). We thank the editorial board and the authors for publishing this successful and informative manuscript. We also would like to mention a few important points about prognosticating mortality in COVID-19 to contribute to the discussion of the study.

The COVID-19 pandemic has had negative social, economic, and psychological effects on health workers and society. The important reasons of these effects were not recognizing the disease in the early stages of the pandemic, not being able to predict how deadly it will be, and not being able to predict mortal cases (2). Authors studied early warning systems laboratory parameters and scores such as PSI and CURB-65 to prognosticate mortality in COVID-19 at early period of pandemic (3,4). Wang et al., one of these authors, worked to find the ideal prognostic score (5). As Haydar et al., they reported age, neutrophil-lymphocyte ratio, D-dimer and C-reactive protein as independent predictors. Based on this result, Weng et al. developed the ANDC early warning score. The score was calculated using the formula 1.14 × age − 20 (years) + 1.63 × neutrophil-lymphocyte ratio + 5.00 × D-dimer (mg/L) + 0.14 × C-reactive protein (mg/L). They reported the area under the curve of the model with a good value of 0.975 (95% Confidence Interval: 0.947–1,000). The authors suggested that 59 and 101 for ANDC could be used as cut-off values to classify COVID-19 disease.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

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Research Article

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SCUBE-1 as a novel predictor of thromboembolic event

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Dear Editor,

SCUBE-1 is a newly identified, produced cell surface protein determined through embryogenesis's first part. The protein contains cascading EGF-like repeats following the N-terminal signal peptide sequence, a spacer area, cysteine-rich recurrence motifs, and a CUB domain at the C-terminus. These molecules are collected in alpha granules in inactivated platelets, translocated to the platelet surface after stimulation by thrombin, secreted as small fathomable fragments, and incorporated into the thrombus. After platelet activation, SCUBE-1 expression increases, and SCUBE-1 migrates to the cell surface, cleaves, and is issued into the flow as minor soluble units. These circulating particles increase platelet-platelet bonds and agglutination in thrombotic situations. In addition, it has been shown that it is associated with acute ischemia and an increase in hypertension due to the increase in SCUBE-1 values in endothelial damage. SCUBE-1 accumulation was detected immune-histochemically in the subendothelial matrix of improved atherosclerotic lesions in humans. Platelet stimulation and accumulation are responsible for acute ischemic complications (1-3). It is thought that SCUBE-1 may be a new platelet endothelial adhesion molecule.

There are many findings in the literature investigating SCUBE-1 and thromboembolic events (1-4). For example, Yıldırım et al. described that SCUBE-1 is a marker of platelet activation in cases with ST-elevation myocardial infarction (STEMI) and a novel platelet-endothelial adhesion molecule who experienced primary percutaneous coronary intervention and they recommended that SCUBE-1 can use as a predictor of great thrombus burden in cases with STEMI (1). In one more study, Sönmez et al. showed high levels of SCUBE1 both in the STEMI and non-STEMI patients (2). On the other hand, Özkan et al. found that SCUBE-1 was not a useful marker for unstable angina pectoris and acute coronary syndrome. The results of Özkan et al. suggested that further studies are necessary to show the association between SCUBE-1 and acute coronary syndrome (3). Yılmaz et al. investigated the relationship between return of spontaneous circulation (ROSC) in cases with cardio-pulmonary resuscitation and serum SCUBE-1 levels and showed SCUBE-1 was higher in the ROSC group compared with the mortality cases (4). One of the important causes of mortality in COVID-19 is thromboembolic pathogenesis (5). Toprak et al. presented SCUBE1 as one of the main contributing factors of thrombotic complications, which is an increased cause of morbidity and mortality in COVID-19, according to the results of their study (6). Dirican et al. found high SCUBE-1 in cases with pulmonary embolism, and they recommended SCUBE-1 as a new diagnostic biomarker with high sensitivity (7). In an empirical study, Aköz et al. showed that SCUBE-1 is an early predictor of acute mesenteric ischemia and might be useful in detecting irreversible intestinal damage (8). In different experimental research, Türkmen et al. found that SCUBE-1 could use as an early predictor of acute ischemic stroke according to SCUBE-1 from the first hour of the persuaded stroke and continued elevated until the sixth hour verified (9).

In conclusion, in light of the mentioned literature, SCUBE-1 can be used as a predictor of thromboembolic events.

Keywords: SCUBE-1, thromboembolic events, myocardial infarction

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The authors declared no conflict of interest.

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GUIDE FOR AUTHORS

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Please keep text, tables and graphics, as separate files in other word do not import the figures or tables into the text file. Text files should be supplied in one of the following formats: Microsoft Word or WordPerfect, Windows or Macintosh formatted.

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Manuscripts should be prepared electronically using an appropriate MS Word compatible word-processing package, formatted for A4 or letter page size, double-spaced throughout with 2.5 cm margins on all sides, and using Times New Roman, 12-point font. Text should not be justified, but flush left. Words should not be hyphenated to fit on a line. Pages should be numbered sequentially.

1-TITLE PAGE: The title page should contain the following items:

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(4) the ORCID of all authors;

(5) the name and complete address of the corresponding Author that includes telephone number, facsimile number and E-mail address to whom correspondence and proofs should be sent.

2-MAIN TEXT:

a) Original articles: Introduction; Materials and methods (with explicit reference to compliance with ethical standards, including the name of the Ethics Committee that approved the study and the informed consent declaration); results; discussion; acknowledgments; funding; conflict of interest; references.

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The main text should contain the following items:

Abstract: This should provide a concise description of the purpose of the report or summary of the review and should not exceed 300 words. Avoid abbreviations, unless they are used universally.

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Introduction: The objectives of the research should be clearly stated in this section. Relevant background information and recent published studies should be described concisely, and be cited appropriately.

Materials and methods: This section should contain all the details necessary to reproduce the experiments. Avoid re-describing methods already published; only relevant modifications should be included in the text.

Ethical statement: Research carried out on human participants must be in compliance with the Helsinki Declaration as revised in 2013. (https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/). The written consent of each subject should be

taken. Therefore, for any research studies or evaluations involving human participants (including students, residents, fellows and faculty members), authors need to provide information regarding ethical approval for the research presented in the manuscript. A statement to this effect must be incorporated into the ‘‘Materials and Methods’’ section and should include the name of the approving committee (e.g., Institutional Review Board, Ethics Committee on Human Research, etc.), the name of the institution at which approval was granted and a reference number where appropriate. When experimental animals are used, the methods section must clearly indicate that adequate measures were taken to minimize pain or discomfort. Experiments should be carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC), or with the animals for experimental procedures.

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Conflict of Interest:

All potential conflicts of interest must be stated within this section. This pertains to relationships with industry and other corporations whose products or services are related to the subject matter of the submitted manuscript.

Funding:

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Acknowledgments:

Author acknowledgments should be written in the third person ("The authors wish to thank...") and written permission should be obtained from all individuals who are listed in the Acknowledgments section of the manuscript.

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