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Editor in Chief

Assoc. Prof. Dr. Fazile Nur EKİNCİ AKDEMİR

ntms.editor@gmail.com

Ağrı İbrahim Çeçen University, Turkey

Assoc. Prof. Dr. Mustafa Can GÜLER

mcanguler@yahoo.com

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mcanguler@yahoo.com

Atatürk University, Turkey

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ntms.editor@gmail.com

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aliahiskalioglu@hotmail.com

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Graphic Design

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Hilal KIZILTUNÇ ÖZMEN, hkiziltuncozmen@hotmail.com, Atatürk University

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In Vitro Effects of Boric Acid and Bevacizumab in Non-Small Cell Lung Cancer

Fatma Fırat[1], Tuğçe Aladağ[1*]

[1]Department of Histology and Embryology, Faculty of Medicine, Afyonkarahisar Health Science University Afyon, Turkey.

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[*]***Corresponding Author***
Tuğçe Aladağ
Department of Histology and Embryology
Faculty of Medicine,
Afyonkarahisar Health Science University,
Afyon, Turkey
Phone: +90 272 246 3301/5143
E-mail: tugcealadagg@hotmail.com

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Authors' ORCIDs
Fatma Fırat
http://orcid.org/0000-0003-0027-5138
Tuğçe Aladağ
http://orcid.org/0000-0003-3250-6113

Abstract: Lung cancer is one of the most common types of cancer worldwide and is responsible for the loss of more than 1 million people each year. It has been reported that the 5-year survival rate of lung cancer is approximately 15 % or less due to cell metastasis. Therefore, there is a need to develop adjuvant therapies to prevent death from lung cancer cell metastasis. The aim of this study is to evaluate the effects of boric acid and bevacizumab on the vascularization, apoptotic, and metastasis steps of A549 lung cancer cells, such as invasion, migration, and epithelial mesenchymal transition (EMT) abilities, either alone or in combination. The study was divided into 4 groups as control(CONT) and boric acid (BA), Boric acid+altuzan (BA+ALT) and altuzan (ALT). The IC50 dose of boric acid was determined by the MTT method. 30µM boric acid and 7 µM Altuzan were applied to BA, BA+ALT and ALT groups for 24 hours. Anti-VEGF for vascularization, Anti-Vimentin for EMT, Anti-MMP-9 for invasion, and Anti-Bax, Anti-Bcl-2 and Anti-Caspase-3 antibodies for apoptosis were stained immunocytochemically and H-Score analysis was performed. Cell migration was evaluated by the wound healing assay. It was observed that MMP-9 immunoreactivity and apoptotic markers increased in the direction of Cas-3 in the BA group, while the immunoreactivity of Vim and VEGF did not change significantly ($p<0,05$). When the migration was evaluated, it was observed that the cells did not migrate in the BA and BA+ALT groups at the end of the 24th hour, and the wound areas were closed in the other groups. It was observed that while BA affected the migration, invasion and apoptotic characters of A549 cells independently of bevacizumab, it had no effect on their vascularization properties © 2022 NTMS.
Keywords: A549; Apoptosis; Bevacizumab; Boric Acid; EMT.

1. Introduction

Because lung cancer is an aggressive type of carcinoma, it is in the first place of cancer-related deaths (1, 2). An estimated 1.1 million deaths due to lung cancer occur worldwide each year (3). Although different treatment options such as antiangiogenic, immunotherapy and radiation therapy are applied for lung cancer, survival rates are quite low. Because of metastases, the survival rate of lung cancer remains

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below 15 percent even for a period of 5 years (4, 5). If a metastasis-preventing treatment can be developed, it can be predicted that survival rates will increase. A recent study showed that transition epithelial to mesenchymal (EMT) plays a key role in cancer metastasis process (6). EMT is expressed by abnormalities in the regulation of EMT markers. Loss of the epithelial marker E-cadherin and increases in the mesenchymal markers vimentin and N-cadherin have been associated with metastatic and invasive behavior. The metastatic behavior of cancer cells was evaluated using these markers in studies. For example, propofol has been shown to significantly increase the E-cadherin expression, while decreasing the expression of vimentin, N-cadherin, and SNAIL, which is considered as evidence that propofol can inhibit the process of EMT (7).

Bevacizumab (altuzan), one of the molecularly targeted, new generation antineoplastic drugs; Its mechanism of action is to inhibit the biological activity of vascular endothelial growth factor (VEGF), and it's shown that it can be used in the treatment of cancers by suppressing the formation of tumor vessels (8). It is frequently used in clinic for the treatment of primary, intermediate and advanced tumors. In addition, recent findings in studies have concluded that bevacizumab may regulate its anti-tumor effect by activating signal cascades of ER's at the cellular level, apart from its ability to inhibit vascular formation (9). It was shown that bevacizumab treatment produced an antitumor effect at the cellular level in A549 cell xenografts (10, 11). It is reported that tumor progression, that is, growth, infiltration and metastasis steps, is supported by tumor neovascularization. VEGF expression has been found to be excessive in other types of cancer, including non-small cell lung cancer (NSLC) and has also been correctly associated with recurrence and metastasis rates (12-14). Bevacizumab is a monoclonal antibody (MAb) which binds specifically to VEGF and can block neovascularization (15). However, Kim et al. suggested that bevacizumab inhibits the apoptosis pathway through activation of Bcl-2 gene expression (16) .

Boron is a trace element for organisms and its derivative in the form of water-soluble inorganic acid, boric acid, is abundant in natüre (17, 18). In a study, boric acid shown to inhibit proliferation while the cell is still in the cell cycle stage, without causing cell death (19). The cytotoxic abilities of boric acid for cancer have been presented by some studies, but its cancer-related cellular effects have not been fully elucidated (20, 21). Knowing the cellular changes caused by boric acid will reveal its potential therapeutic effects and will guide other anticancer studies in this field (22).

We aimed to evaluate the possible effects of single and combined applications of boric acid and bevacizumab on A549 lung cancer cells in a two-dimensional cell culture environment using apoptotic and EMT transition markers.

2. Material and Methods

2.1. Preparation of Chemicals

Boric acid was used by dissolving 30 µM in a medium heated to 37 °C and passing through a 0.22 µm filter. Altuzan (100 mg/4ml, Roche) was used by dissolving 7µM in a medium heated to 37 °C.

2.2. Cell Viability Determination by MTT

MTT test was performed for determinening the cytotoxic effect of boric acid on cells A549 and IC50 dose. A549 non-small lung cancer cells used $2x10^4$ cells per each well, and 10, 30, 50 and 100 µM boric acid was applied 24 hours. After 24 hours, MTT (Sigma-Aldrich) performed as desciribed bey manifacturer before. Values were measured in a microplate reader (Shimadzu UV-1601). And the MTT assay performed for 8 times. For Altuzan, on the other hand, since there are sufficient number of dose determination studies in the literature, no dose determination was made and 7 µM was used in accordance with the literature (23).

2.3. Culture of Cells

A549 non-small lung cancer cell line (n=4) was incubated with DMEM F12 (SLM-243-B, Sigma) medium containing 10 % Fetal bovine serum (FBS)(10270106, Gibco) 1 % L-Glutamine (59202C, Sigma) and 1 % antibiotic- (penicillin-streptomycin) (P4333, Sigma) in a 37 ˚C humid incubator with 5% carbon dioxide. When 80 % confluency was reached, cells were divided into four treatment groups. The groups were grouped as Boric acid (BA), Altuzan (ALT), boric acid+Altuzan (BA+ALT), and control (CONT) groups, IC50 doses of the chemicals determined by MTT were applied to the cells for 24 hours.

2.4. Immunocytochemical Staining

The determined groups were were fixed with paraformaldehyde 4 % (PFA) 30 min. After fixation, hydrogen peroxide 3 % (H_2O_2) 5 min was applied. For permeabilization, was performed with Triton-X 0.1 % 100 and proteins were blocked for an hour. Anti-Vimentin (1/200, ab8978, abcam), anti-VEGF (1/200, ab76055, abcam), anti-MMP-9 (1/200, ab76002, abcam), anti- Bcl-2(1/200, sc-7382-santa cruz), anti-Bax (1/200, MA5-14003-thermo), and anti-Caspase 3 (1/200, sc-56053) incubated 1 night at +4 ºC. Secondary antibodies applied and visualized with DAB, photographed with a (NIKON Eclipse E600) light microscope. H-Score evaluation and statistical analysis were performed. H-score: 500 cells from each field were counted per groups. The staining intensities; The number of stained cells (%) evaluated using this formula X (Staining intensity 0, 1, 2, 3, 4, 5), with a H-score between 0 and 500 (24).

2.5. Assay of Cell Migration

Wound healing assay was applied to evaluate the migration tendencies of cells. A549 non-small lung cancer cells from ATCC (CCL-185) were incubated until 100 % confluent. Afterwards, the groups were applied at the determined drug doses, and a wound area was created between the cells by drawing a line from the middle area with the a sterile 10 µl pipette tip. In order to compare the tendency of the cells to migrate towards the to area and close the wound area and their speed during this migration, photographs were taken from the groups at 12 hour intervals with a phase contrast microscope (ZEISS Axio Vert iLED) and comparatively evaluated (25).

2.6. Statistical analysis

The results of the H-score evaluation and the measurements taken in the cell migration experiment were analyzed statistically using the SPSS 20.0 (demo version) statistical analysis program, using Kruskal Wallis test for comparison of groups and Dunn test for pairwise comparisons.

3. Results

3.1. Evaluation of the Effect of Boric Acid on Cell Viability by MTT Method

Cell viability was determined by MTT method to determine the IC50 dose of boric acid in A549 cells. A549 cells were treated with 10, 30, 50 and 100 µM boric acid for 24 hours. After 24 hours, it was seen that 100 µM dose killed all cells. In the cell groups treated with 10, 30 and 50 µM, the average viability rates were observed as 81.54 % and 27 %, respectively. The IC50 dose of BA for A549 cells was determined as 30 µM and applied to the study groups in other steps (Figure 1).

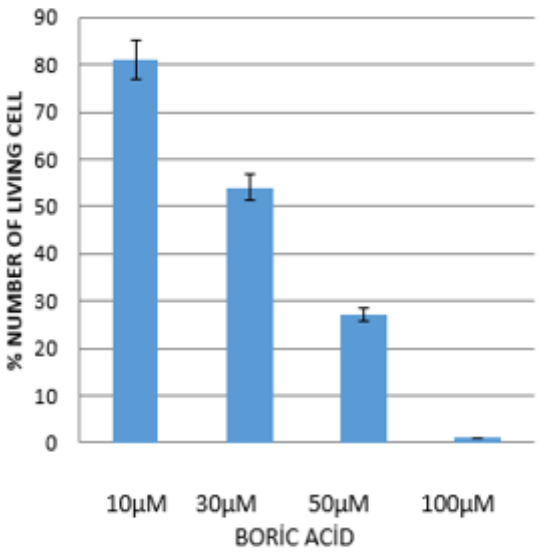

Figure 1. MTT analyzes of the effects of boric acid tretment on cell viability in A549 cells.

3.2. Effects of Boric Acid and Altuzan on Epithelial Mesenchymal Transition

In order to immunocytochemically evaluate the effect of BA and Boric acid+Altuzan on epithelial mesenchymal transition, boric acid, boric acid+Altuzan and altuzan were applied to A549 cells for 24 hours. The cell group that did not receive any treatment was accepted as the control. After the treatments, Vimentin protein expression was evaluated by immunocytochemical method and by H-Score analysis (Table 1). According to the H-Score analysis results, the immunoreactivity of Vimentin did not differ significantly in the other groups compared to the control group ($p>0.05$).

3.3. Effects of Boric Acid and Altuzan on Invasion

The effect of boric acid and Altuzan on cell invasion was evaluated immunohistochemically by the expression of MMP-9 protein. H-Score analyzes were performed on the differences in expression intensity of MMP-9 protein between the groups (Figure 2). There were a significant decrease in MMP-9 immunoreactivity in the BA and ALT groups (respectively $p=0.042$, $p=0.002$) and in the BA+ALT group compared to the control group ($p=0.004$) (Table 1).

3.4. Effects of Boric Acid and Altuzan on Cell Migration

A549 cells were incubated in 24-well culture dishes to allow proliferation of cells to cover 80% surface area. After providing full coverage of the cells on the surface, the wound healing test, which is often used to evaluate the migration ability, was performed. For BA, BA+ALT and ALT groups, doses determined by MTT were applied. The cell group that did not receive drug treatment was accepted as the control. The migration of cells to the wound area was monitored for 24 hours, and the wound areas were measured and photographed for each group at 0, 12, and 24 hours. At the end of the 24th hour, complete closure was observed in the groups and the experiment was terminated and the areas in the groups with gaps were measured in µm² and compared. The experiment was repeated 4 times for each group and statistical analyzes were made with pairwise comparison tests. Complete closure was observed in the control group and ALT group at the 12th hour. When BA and other groups were compared at the 24th hour, no significant difference was observed between the BA+ALT group and BA, but there was a significant difference between CONT and BA ($p= 0.002$), and ALT and BA ($P=0.017$). When statistical analyzes are evaluated, it is seen that this significant difference is due to BA (Figure 3, Table 2).

3.5. The Effects of Boric Acid and Altuzan on Apoptosis

In order to evaluate the effect of Boric acid and Altuzan on cell apoptosis in A549 cells, the expression of Bax, Bcl-2 and Cas-3 proteins was determined by immunocytochemical method and analyzed by scoring

with H-Score method after 24 hours of treatment. According to the results of the H-Score analysis, when Bax immunoreactivity was compared with the CONT group, an increase was observed in the BA and BA+ ALT groups (p=0.002, p= 0.042, respectively). The difference between the CONT group and the ALT group was not significant (p=0.308). When ALT and BA+ALT groups were evaluated, it was observed that there was a decrease in Bcl-2 immunoreactivity compared to the CONT group (p= 0.054). There was no significant difference in the BA group compared to the CONT group (p=0.258).

While Cas-3 immunoreactivity increased significantly in the BA group compared to the CONT group (p=0.024), the increase in the BA+ALT group was not significant (p>0.05).

3.6. The Effects of Boric Acid and Altuzan on Vascularization

In order to evaluate the effect of boric acid and Altuzan on vascularization in A549 cells, after 24 hours of treatment, the change in VEGF protein expression was evaluated by immunocytochemical method and H-Score analysis was performed (Figure 2). According to the results of H-Score analysis, there was a significant decrease in VEGF immunoreactivity in the ALT group (p=0.003) and BA+ALT (p= 0.029) groups when compared to the CONT group, and these differences were significant.

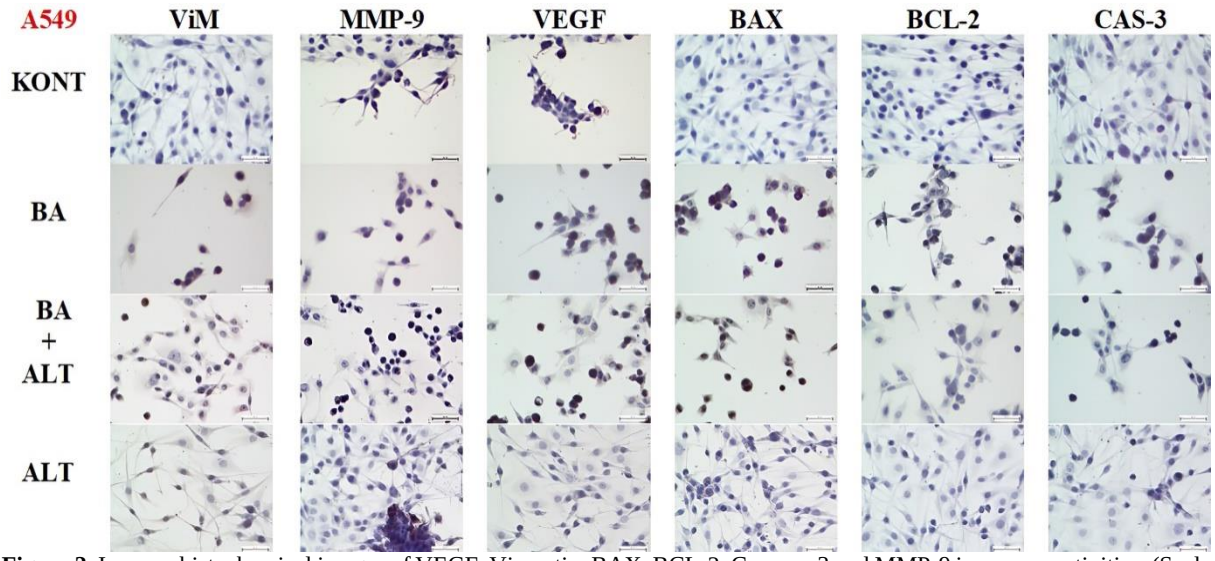

Figure 2. Immunohistochemical images of VEGF, Vimentin, BAX, BCL-2, Caspase-3 and MMP-9 immunoreactivities. (Scale Bar 100 µm).

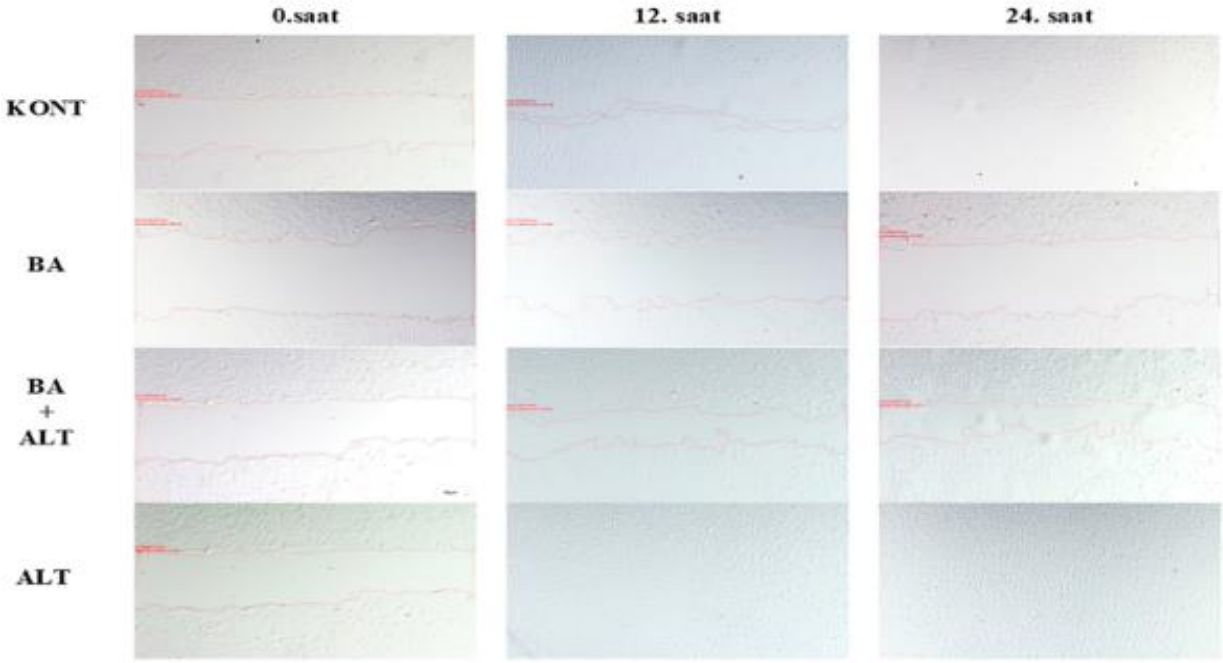

Figure 3. Evaluation of cell migration by wound healing assay (Scale bar 200 µm).

Table 1: H-Score pairwise comparison statistical differences between groups.

Groups	Vimentin	MMP-9	VEGF	Bax	Bcl-2	Cas-3
BA	255±3.60^{ab} (255)	186±1.90^{ab} (186)	234±5.13^{a} (236)	263±2.45^{bc} (264)	185±4.84 (185)	242±2.63^{ab} (243)
ALT	223±3.78^{a} (224)	202±4.16^{c} (200)	152±2.55^{ab} (152)	207±2.91^{c} (208)	180±2.33^{b} (180)	185±2.11^{a} (185)
BA+ALT	226±1.50^{b} (227)	234±3.61^{ab} (225)	183±2.55^{c} (184)	183±4.27^{a} (226)	177±1.80^{a} (177)	212±3.00 (212)
CONT	250±2.15 (250)	272±3.00^{abc} (272)	242±4.10^{bc} (242)	150±4.16^{ab} (150)	232±3.00^{ab} (232)	190±6.00^{b} (190)

Different letters in the line indicate significant difference between groups.

Table 2: Pairwise comparison statistical differences between cell migration experimental groups.

Groups	0. HOUR	12. HOUR	24. HOUR	P Value
CONT	51798.35±7559.54$^{\mathbf{aA}}$	1356.33±498.26$^{\mathbf{aA}}$		0.018
BA	70066.61±14729.28$^{\mathbf{aA}}$	77961.70±5319.95$^{\mathbf{aB}}$	70717.83±8413.03$^{\mathbf{aA}}$	0.174
ALT	55913.25±8994.52$^{\mathbf{bA}}$	1425.62±299.9^{abA}	1166.75±119.33$^{\mathbf{aB}}$	0.039
BA+ALT	55746.54±8981.36$^{\mathbf{bA}}$	35138.58±4387.13$^{\mathbf{abAB}}$	30247.35±8353.02$^{\mathbf{aAB}}$	0.039
P Value	0.126	0.005	0002	

Different lowercase letters in the row and different uppercase letters in the column indicate significant differences between groups.

4. Discussion

Lung cancer is the leading cause of cancer-related deaths worldwide, with approximately 2 million new cases and 1.76 million deaths annually. The treatment of cancer may include multiple options, such as radiation therapy, surgery, chemotherapy, hormone therapy, alternative medicine, or targeted therapy, depending on the patient's overall health. Many anti-cancer substances used in alternative medicine have been identified, produced and tested to date, and 70% of the anti-cancer drugs used are obtained from natural sources (26). Boron, after silisyum, is one of the most common elements in natüre (27). It is usually found in nature as a chemical compound such as boric acid (BA), and BA is the most common form of boron found in the human body (28). Many researchers have pointed out that boric acid has antioxidant, anticancer, antigenotoxic and hepatoprotective properties (29, 30). In the present study, the IC50 dose of boric acid application, which is considered a toxic dose, in A549 lung cancer cells was investigated by the MTT method and determined as 30 μM. The determined dose was used to establish the treatment groups. The possible effects of boric acid on the migration, invasion, apoptosis and EMT properties of non-small cell A549 lung cancer cells, alone and together with Altuzan, which is used in cancer treatment, were investigated by in-vitro methods.

Vimentin is an important component of the intermediate filament family of proteins and is expressed in normal mesenchymal cells. It is known to maintain cellular integrity and provide resistance to stress. Overexpression of vimentin in cancer is well associated with increased tumor growth, invasion, and poor prognosis. In recent years, vimentin has gained importance as a marker for epithelial-mesenchymal transition (EMT) (31). In our study, when compared with the control group, it was observed that there was no significant change in Vimentin immunoreactivity in all groups. It was thought that the use of boric acid alone or in combination with altuzan did not alter vimentin protein expression during 24-hour treatment, so it had no effect on mesenchymal transition through vimentin protein. The limited studies in the literature regarding the effects of boric acid on vimentin protein expression in non-small cell lung cancer cells make it difficult to evaluate epithelial mesenchymal transition in terms of vimentin protein. In addition, the study we presented seems to be a peereview study for other researchers. When the effects of BA on the migration of cells were evaluated with the cell migration-migration experiment, there were significant differences in the BA and BA+ALT groups at the end of 24 hours compared to the other groups and the cells migrated towards the wound area in the ALT and CONT groups and completely covered the area, the migration characteristics of the cells were determined by BA shows that it is affected. The absence of changes in vimentin proteins, which are used as markers for EMT transition, suggests that the invasion abilities of cells are suppressed and the EMT transition may have never been initiated.

Cancer cells secrete serine proteinases and matrix metalla proteinases (MMPs) that break down extracellular matrix proteins in order to migrate into the tissue. Highly expressed MMP-2 and MMP-9 are in malignant tumor cells, facilitating cell migration by playing a role in the degradation of the extracellular matrix, which is an important component of the basement membrane that leads to cancer metastasis (32). MMPs are genes involved in cancer disease functional promotion of angiogenesis, progression, metastasis, invasion, and escape from control of immune cells, these genes have been noted to be frequently upregulated in cancer (33). In our study,

MMP-9 immunoreactivities were also found to be significantly decreased in the BA, ALT and BA+ALT groups compared to the CONT group. Jiang et al. In this study, the effect of local treatment of bevacizumab, the active ingredient of altuzan, against triple negative breast cancer (TNBC) xenograft tumors was examined and they said that MMP-9 expression level decreased compared to the control groups (34). In our study, a significant decrease in MMP 9 immunoreactivity in the BA group compared to the CONT group suggests that it has a similar effect independent of the altuzan effect. Contrary to our results, Wang D. et al. in 2015 showed that bevacizumab strongly inhibited MMP 2 and MMP 9 in A549 cells in a dose-dependent manner at the mRNA level (35). The difference in our results is that the effective dose of bevacizumab used in this study showed a similar effect at twice the values we used. When we look at the literature studies, it seems that the number of studies showing and discussing the direct effects of boric acid on MMPs is quite limited. In a study conducted with boron, which is also a derivative of boric acid, the effects of boron on keratinocytes MMP 2 and MMP 9 were examined for the evaluation of wound healing in vitro, but contrary to our study, it was observed that it accelerated the wound healing process by increasing the amount of MMP (36). This difference was thought to be due to the experiment with non-cancerous keratinocytes.

Bevacizumab is the active ingredient of Altuzan, an anti-vascular endothelial growth factor (VEGF) monoclonal antibody (mAb) and a drug used in various types of cancer (37). Vascular endothelial growth factor (VEGF) is an endothelial cell-specific mitogen and an angiogenic inducer in various in vivo models. VEGF is believed to be one of the key molecules as it stimulates endothelial cell activation, survival and proliferation of tumor cells and facilitates invasion and migration (38). VEGF overexpression has been observed as a poor prognostic parameter in lung carcinomas (39). In our study, when VEGF immunoreactivities were compared with the control group, the decrease in the ALT group was found to be statistically significant ($p=0.003$). This result was similar to the results of previous studies and showed that altuzan also suppressed angiogenesis in A549 cancer cells. Compared to the control group, the decrease determined when the BA group was evaluated was not statistically significant ($p>0.05$), while there was a significant decrease in the BA+ALT group ($p=0.045$). The decrease in ALT and BA+ALT groups was thought to be due to altuzan. The studies of Özyarim and Karabağ Çoban support our results and show that the application of boric acid at different doses and hours in colon cancer cells did not cause a significant change in VEGF amounts compared to the control group. Karaca et al. in their in vivo study in 2022, they said that boric acid reduces VEGF expression, which is inconsistent with the results of our study. This difference is probably due to the fact that the study was performed in vivo and in non-cancerous tissues or due to the difference in the amounts of boric acid used.

There are studies supporting the use of boric acid in cancer treatment. In some epidemiological studies, BA exposure has been shown to reduce prostate cancer cell proliferation in men and cervical cancer cell proliferation in women, leading to a reduction in the incidence of lung cancer, as well as induce apoptosis in prostate, melanoma, and breast cancer cell lines (40–44). Apoptosis is one of the most accepted cellular death mechanisms for anticancer activity. Although it is believed that only a limited part of the mechanism of apoptosis has been elucidated, activation of caspase family proteins is shown as initiator and maintainer of apoptosis (45,46).It is known that caspase-3 plays the key role in the apoptotic process and that caspase-3, which causes DNAse activation, has a direct effect on DNA fragmentation (47,48). In a study, Boron Nanoparticle spheres were shown to induce apoptosis and inhibit cell proliferation for both androgen-sensitive LNCap and androgen-independent DU145 prostate cancer cells, and also increased caspase-3/7 activity (49). In the present study, changes in the immunoreactivity of the caspase regulatory proteins Bax, Bcl-2 and caspase-3 were evaluated for the evaluation of apoptosis. It was observed that Bax increased significantly in the BA and BA+ALT groups compared to the control, and simultaneously, Bcl-2 immunoreactivity was significantly decreased in the same groups. The increase in Cas-3 immunoreactivity also suggests that the apoptotic process of cells in these groups may be initiated by boric acid and may be regulated by disturbing the balance between Bax/Bcl-2 proteins. It is seen that there is a significant increase in Bcl-2 protein amounts in the ALT group compared to the control. This increase is thought to be due to the effect of bevacizumab, and in support of this idea, there are studies in the literature showing that bevacizumab can have a bidirectional effect at certain dose ranges (23).

In our study, we investigated the possible effects of Boric Acid and bevacizumab on the migration, invasion, apoptosis and EMT properties of non-small cell lung cancer cells A549 in-vitro. According to the findings and results of the methods we used, BA affects the migration, invasion and apoptotic characters of A549 cells independently of bevacizumab. In our study, it was not observed that there was any effect on VEGF-mediated vascularization. Although our study is among the pioneering studies in which the possibility of using BA for therapeutic purposes is questioned, it is a study that was conducted in an in vitro 2D environment and has limitations. On the other hand, we think that it will shed light on the literature as a pre-study.

5. Conclusions

Our study presents the effect of boric acid on the alteration of protein expressions, which are effective in the EMT process of lung cancer cells. Further 3D studies, animal and human experiments are needed to elucidate the therapeutic effects of boric acid on EMT.

Limitations of the Study

The limitations of this study are that it is a cell culture study arranged in a two-dimensional environment.

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Conflict of Interests

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Author Contributions

ECT and ZO designed the research. ECT participated in data collection and data analysis. ECT and ZO wrote the manuscript, read and approved the final script

Ethical Approval

No Ethics Committee Application İs Required.

Informed Consent

The authors accept their responsibilities in the study. There is no conflict of interest between the authors.

Availability of Data and Materials

The material used in the study and without the permission of the authors.

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The Effectiveness of the School-Age Hearing Screening Program in Turkey and the Effects of the COVID-19 Pandemic

Uğur Yıldırım[1]*

[1]Department of Otolaryngology and Head and Neck Surgery, Samsun Education and Research Hospital, Samsun, Turkey.

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****Corresponding Author***
Uğur Yıldırım
Otolaryngology and Head and Neck Surgery
Samsun Education and Research
Hospital, 55090, Samsun, Turkey
Phone: +90 (362) 3111500
+90 5056313911
E-mail: uguryildirimkbb@gmail.com

DOI: 10.56766/ntms.1055331

Authors' ORCIDs
Uğur Yıldırım
https:// 0000-0002-2373-6235

Abstract: The aim of the study is to evaluate the effectiveness of the hearing screening program in school-age children in Turkey, and to discuss alternative methods for children who cannot be screened due to the COVID-19 pandemic. Within the scope of the school-age hearing screening program, children who fail the screening are referred to reference centers. The effectiveness of the screening program was investigated by recording the ear examinations and definitive audiometric examination results of the children who were referred to a tertiery-level center. Evaluations were made of a total of 87 children, comprising 49 boys (56.3%) and 38 girls (43.7%) with a mean age of 6.97 years, and no risk factors for hearing loss. The physical examination results showed that 53 children were normal, 15 had cerumen impaction, 3 otitis externa, 13 otitis media with effusion and 3 acute otitis media. The audiometric examination results were recorded as 72 normal, 13 conductive hearing loss, and 2 sensorineural hearing loss. Thus, the false positive rate of screening audiometry performed at school was 82.7%. School-age hearing screening is an important test to ensure the cognitive and academic development of children. Pure tone audiometry is indispensable in the evaluation of children who fail the test. For the evaluation of children who could not be tested for 1.5 years due to the COVID-19 pandemic, alternative methods such as tele-audiometry may be utilized. © 2022 NTMS.

Keywords: Audiometry; Child; COVID-19; Hearing; Screening.

1. Introduction

Hearing loss is an important preventable and treatable cause of developmental disorders in childhood (1). Hearing loss can cause retardation in speech, cognitive and social development in children, regardless of its degree (2). In order to detect hearing loss, hearing screening programs (HSP) are performed for newborns and school-age children in many countries of the world. A European Consensus Statement was published in 2012 on what needs to be done in this regard (3).

In Turkey, the "Hearing Screening Program for School-Age Children" has been implemented since 2015 by the Turkish Public Health Institution, integrated with the Ministry of National Education. According to this program, screening audiometry is performed for first-grade primary school children, and those with suspected hearing loss or who are not tested are referred to reference centers. The main purpose of this study was to investigate the effectiveness of the testing method used in the HSP

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for school-age children and to develop a standardization for the evaluation of suspicious cases. The secondary aim was to discuss alternative screening methods for the detection of children with hearing loss at this time when face-to-face education and screening programs have been disrupted due to the COVID-19 pandemic.

2. Material and Methods

Screening audiometry is performed with a "Resonance R17A" device for all students who attend the 1st grade of primary school in Turkey with an informed consent form signed by their parents. In this test, hearing loss of 20 dB and above at frequencies of 500 Hz, 1, 2, 4 kHz is investigated. A child who fails at any frequency is tested again within 48 hours-1 week. Children who fail the repeated screening are referred to reference centers for a complete ENT examination and audiological evaluation.

According to the screening program, there are also direct referral criteria independent of the audiometric examination. These are a family history of late-onset hearing loss, craniofacial and/or ear anomalies, head trauma-loss of consciousness, ototoxic drug use, pre-existing sensorineural hearing loss, developmental disorder, speech and language delay, learning disability, cleft palate and lip, Down's syndrome, recurrent or chronic otitis media (COM), exposure to noise, receiving special education, or grade repetition.

In this study, evaluations were made of all children who failed the school-age hearing screening who were referred to the ENT outpatient department of a tertiary-level hospital between February 1st, 2019 and March 1st, 2020. As the risk factors for hearing loss were questioned before the screening test performed at school, only children who did not have risk factors but failed the test were included in the study. Otoscopic examination, tympanometry and pure tone audiometry (PTA) were performed and the results were recorded. Earwax aspiration was performed after the use of emollient drops in children with impacted plugs. Audiological tests were performed after earwax removal. Those with type B results in the tympanometry were diagnosed with otitis media with effusion (OME) and followed up for 6 months. In the audiometric examination, pure tone averages were determined at frequencies of 250, 500, 1000, 2000, 4000 and 8000 Hz in all children. According to the level of hearing loss, patients were classified as mild (20-39 dB), moderate (40-59 dB), moderately severe (60-84 dB), or severe (85 dB and above). The necessary treatments were applied to children who had hearing loss or who had abnormal examination results. The false-positive rate was determined by calculating the percentage of children with normal hearing despite having failed the hearing screening. Those who were found to have hearing impairment in PTA were categorized according to the conductive type and sensorineural type loss. The treatments applied and the results of the follow-up were recorded. There were no exclusion criteria in this study, as all patients who were refered to the outpatient clinic were included.

Data obtained in the study were analyzed statistically using SPSS version 22 Software (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY, USA). Descriptive tables containing the ratios and percentages of the data were formed.

Approval for the study was obtained from the Clinical Research Local Ethics Committee of a tertiary-level healthcare center (Decision No: GOKA/2021/6/12).

3. Results

Evaluations were made of a total of 87 children, comprising 49 boys (56.3 %) and 38 girls (43.7 %) with an average age of 6.97 years, and no risk factors for hearing loss. The results of the physical examination were that 53 children were normal, there were 15 cases of cerumen impaction, 3 otitis externa (OE), 13 OME and 3 acute otitis media (AOM) (Table 1). The audiometric examination results were recorded as 72 normal, 13 conductive hearing loss (CHL), and 2 sensorineural hearing loss (SNHL) (Table 2). Accordingly, the false positive rate of screening audiometry performed at school was 82.7 %.

Table 1: Otoscopic ear examination results.

Examination	Total (n, %)	Male (n, %)	Female (n, %)
Normal	53 (60.91)	28 (32.18)	25 (28.73)
Earwax	15 (17.24)	9 (10.34)	6 (6.89)
Otitis Externa	3 (3.44)	2 (2.29)	1 (1.14)
Otitis Media with Efusion	13 (14.94)	7 (8.04)	6 (6.89)
Acute Otitis Media	3 (3.44)	3 (3.44)	0

Removal of earwax was performed using aspiration after softening ear drops. Syringing was not performed on any patient. In all cases with earwax, the examination after aspiration showed normal tympanic membranes. The final audiometric examination was performed in 3 patients with AOM at 3 months after the treatment, and all 3 had normal hearing after treatment. In the 6-month follow-up of 13 patients with OME, bilateral ventilation tube application was performed in 6 patients, while spontaneous recovery was observed in 7 patients. Pure tone audiometry of these cases was also normal after surgery. Bilateral mild sensorineural hearing loss (SNHL) was detected

in 2 patients and hearing aids were provided. In the free field audiometry performed after hearing aid treatment, both cases had normal hearing with the aids. Abnormalities were detected in the examination or audiometric examination in 41.3 % of the children who failed the screening audiometry (39 % examination, 17.2 % audiometry). Treatment was required in 12.6 % of all subjects. All patients with abnormality achieved normal hearing after the necessary treatment

Table 2: Pure tone audiometry results.

		Right Ear	**Left Ear**
Normal Hearing		72 (82.7 %)	72 (82.7 %)
Conductive Hearing Loss	Mild	13 (14.9 %)	13 (14,9 %)
	Moderate	0	0
	Severe	0	0
	Profound	0	0
Sensorineural Hearing Loss	Mild	1 (1.1 %)	1 (1.1 %)
	Moderate	1 (1.1 %)	1 (1.1 %)
	Severe	0	0
	Profound	0	0

4. Discussion

Hearing impairment is a global public health problem that affects 350 million people around the world, according to data from the World Health Organization (WHO) (4). Approximately 10 % of these individuals are children and 60 % of hearing loss in children is due to preventable causes (4). Hearing loss is a rehabilitable and preventable disease, but when not treated, it can cause varying degrees of language and speech retardation, and cognitive and intellectual development disorders (5). All this information highlights the importance and necessity of childhood HSP.

In order to detect childhood hearing loss, newborn HSP has been implemented in Turkey since 2004 (6). Newborn hearing screening is performed using OAE and/or automated ABR but in these tests, hearing losses below 40 dB cannot be detected (7, 8). In addition, late-onset or acquired hearing losses are overlooked by only newborn screening. Therefore, a second screening should be applied in the pre-school period (4-7 years) in order to prevent social problems caused by hearing loss in children. The "European Consensus Statement on Hearing Screening of Preschool and School-age Children" was published in 2012 (3). Accordingly, even if the hearing loss is mild (20-40 dB), when it is not rehabilitated, it reduces social and academic achievement in adulthood. Therefore, hearing screening audiometry in preschool children should detect hearing loss of 20 dB and above. For this purpose, an HSP has been implemented for children in the 1st grade of primary school since 2015 in Turkey (9).

In a study conducted in Kyrgyzstan, hearing loss was found in 123 children (27.2 %) in the audiometric screening of 452 children aged 7-13 years (10). According to another study in India, 284 children aged 6-10 years were screened with audiometry, and hearing loss was detected in 34 (11.9 %) (11). A further study on the subject was conducted in Malatya, Turkey, in which a total of 812 5th grade students were screened and 24 students (3 %) failed this screening. Hearing loss was detected in 11 of the 24 children in the definitive audiometric examination (12). These studies show that hearing loss, which is directly related to the cognitive and behavioral development of children, can be diagnosed early with childhood screening tests, thereby preventing a major public health problem.

In previous studies in the literature, the screening test results of the children were directly reported. Unlike those studies, the current study evaluated the exact audiometric examination results of the children who were screened, not the screening test results. In this way, a very large population was investigated indirectly with a small number of subjects.

In the HSP for school-age children, how the screening tests will be performed and who will be referred to the reference center are prescribed in detail. However, there is no standardization for the evaluation of these children in the reference center. Sometimes only scans such as ear examination, acoustic immitance or otoacoustic emission are performed and hearing is evaluated accordingly. In this case, patients with hearing loss of 40 db and below cannot be detected (13). In this study, ear examination, tympanometric examination and pure tone audiometry were routinely applied to all children who were refered to the reference center. Applying pure tone audiometry clearly reveals even 20 db hearing loss. In this way, two patients started to use hearing aids in the previous year in the center. It is known that even with mild hearing loss, instrumentation is one of the most important factors that directly affect the future academic success of children (14, 15). Therefore, these children should be evaluated in a center equipped to perform pure tone audiometry.

Another handicap of the school age HSP is the requirement for one-on-one interviews. Due to the COVID-19 pandemic, face-to-face education was not conducted in Turkey for 1.5 years. Therefore, some of the children currently in the 2nd and 3rd grade of primary school has not passed the HSP. This situation

constitutes an important public health problem. Scanning with tele-audiometry, which does not require face-to-face interviews, can also be considered as an alternative method for these children. This is a scanning method that is performing by sending some standard sounds through a special internet-based computer program (16). It has been demonstrated in various studies that tele-audiometry is no different from face-to-face scanning audiometry devices (16-18).

5. Conclusions

School-age hearing screening is an important test to ensure the cognitive and academic development of children. As pure tone audiometry is indispensable in the evaluation of children who fail the test, patients should be referred to fully equipped hospitals. For the evaluation of children who could not be tested for 1.5 years due to the COVID-19 pandemic, alternative methods such as tele-audiometry may be utilized. This study will contribute to the literature in terms of raising awareness among both pediatrics and otolaryngologists about school-age hearing screening.

Limitations of the Study

A few participants and the lack of a multicenter study.

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Conflict of Interests

There are no financial interests or personal conflicts that may affect the study in this article.

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Author Contributions

The study has a single author and U.Y. contributed.

Ethical Approval

Ethics committee approval was obtained from the local ethics committee of the tertiary health center (Degree no: GOKA/2021/6/12).

Informed Consent

Informed consent forms were obtained from the patient's parents.

Availability of Data and Materials

Study data is appropriate and open to share for the development of other research.

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High Mean Platelet Volume and Mean Platelet Volume/Platelet Ratio Predict Mortality for COVID-19 Patients in Intensive Care Unit

Emel Saglam[1], Saime Ozbek Sebin[2]*, Songul Kocman[3], Cem Cemal Balaban[1], Can Yucelsen[1], Ahmet Engin Atay[1]

[1]Department of Internal Medicine, Bağcılar Training and Research Hospital, Health Sciences University, İstanbul, Turkey
[2]Department of Physiology, Faculty of Medicine, Ataturk University, Erzurum, Turkey
[3]Department of Family Medicine, Bağcılar Training and Research Hospital, Health Sciences University, İstanbul, Turkey

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****Corresponding Author***
Saime Ozbek Sebin
Department of Physiology
Faculty of Medicine
Atatürk University
Erzurum, Turkey
Phone: +90 5362335667
E-mail: saime.ozbek@atauni.edu.tr

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Authors' ORCIDs
Emel Sağlam
http://orcid.org/0000-0003-0444-586X
Saime Ozbek Sebin
http://orcid.org/0000-0002-1738-4800
Songül Kocaman
http://orcid.org/0000-0001-6239-3960
Cem Cemal Balaban
http://orcid.org/0000-0002-9736-0322
Can Yucelsan
http://orcid.org/0000-0001-7754-184X
Ahmet Engin Atay
http://orcid.org/0000-0002-3711-5157

Abstract: Mean platelet volume to platelet count ratio can be a new marker of mortality in critical COVID-19 cases in intensive care unit to retrospectively examine the factors predicting death rate in COVID-19 cases. 106 patients infected with COVID-19 in intensive care units were enrolled in this research. The patients' hospital records and the patient management tools were thoroughly examined. The Interleukin-6, C-reactive protein, procalcitonin, leukocyte count, neutrophil count, neutrophil %, neutrophil/lymphocyte ratio, and neutrophil/albumin ratio were significantly higher among nonsurvivors (p=0.0001, p=0.004, p=0.003, p=0.049, p=0.007, p=0.009, p=0.007, and p=0.0001, respectively). While the survivors had lower widths of platelet distribution and red blood cell distribution, as well as lower mean platelet volume and mean platelet volume to platelet count ratio (p=0.016, p=0.03, p=0.005, and p=0.049, respectively), they had higher hemoglobin, platelet, mean corpuscular hemoglobin concentration, lymphocyte % and monocyte % (p=0.022, p=0.033, p=0.042, p=0.008, and p=0.04, respectively). In the logistic regression, five features -including high levels of C-reactive protein, procalcitonin, pro-brain natriuretic peptide, mean platelet volume to platelet count ratio, and low level of platelet- were found to be mortality predictors for COVID-19 patients in intensive care unit (p=0.045, p=0.025, p=0.017, p=0.027, and p=0.041, respectively). Mean platelet volume to platelet count ratio, neutrophil count to lymphocyte count, and neutrophil count to albumin ratio predict mortality in critical cases of COVID-19 which will contribute to the early detection of the disease and to the effective treatment of the patients. © 2022 NTMS.

Keywords: COVID-19; Mortality; Mean Platelet Volume.

1. Introduction

The coronavirus family affects camels, cows, and bats causing outbreaks every decade in the last 30 years. A new strain of the family was defined in the early days of 2020 in Wuhan, China (1). World Health

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Organization (WHO) named this new strain SARS-CoV-2 on February 11, 2020 (severe acute respiratory syndrome coronavirus 2) (World Committee on Virus Classification) (2-4). Globally, as of April 3, 2022, just over 489 million COVID-19 patients with over 6 million registered deaths (5).

There is still no consensus on the modes of transmission, the treatment methods, and the follow-up protocols for COVID-19 infection which has become a burden for global public health. Severe pneumonia, acute cardiac injury, acute respiratory distress syndrome (ARDS), and septic shock are among the probable complications that may increase the mortality rates. Clinical findings and bilateral, multiple, and atypic ground glass appearance on thorax tomographic examination increase the probability of COVID-19 infection which can be confirmed by PCR-based tests. Poor prognostic factors are multilobular infiltration, lymphopenia, co-infection, smoking, hypertension, and advanced age (> 60 years).

Cases of COVID-19 fall into four different groups: mild, moderate, severe, or critical (6). Currently, COVID-19 researches have mainly concentrated on the risk factors and the prediction of mortality for the mild and moderate (81 %) COVID-19 cases, consisting of the vast percentage of the total cases (7-11). The average mortality rate of severe (14 %) and critical (20 %) cases is 50 % (1, 12, 13). Almost none of the researchers has announced risk factor prediction and mortality analysis for severe and critical cases. Therefore, identifying the risk factors related to the prognosis and severity of the infection might prove useful to determine the high-risk patients at the initial evaluation phase (14). Several predictive markers have been introduced to academic literature such as identifying high-risk groups in the general population (15), diagnosing COVID-19 (16), and predicting mortality and serious disease progression.

Mean platelet volume (MPV) to platelet count (MPV/Plt) ratio (MPR) was proposed as a prognostic marker in SARS-CoV-2. Thrombocyte count enhances inflammatory states due to cytokine-mediated rise of thromboepoietin levels. On the other hand, MPV reflects the increased production of megakaryocytes in the bone marrow and the expression of young thrombocytes in the circulation. Decreased thrombocyte count enforces megakaryocytes to augment more thrombocytes through immune system stimulation resulting in higher MPV. Negative consequences of activated thrombocytes tend to increase oxidative stress, thrombosis, and apoptosis (17, 18).

Understanding pathophysiologic mechanisms of progressive disease contributes to more aggressively approaching patients that are at risk of developing a critical infection. Our aim is to investigate the correlation between the hematologic and inflammatory parameters and the mortality rates of critical COVID-19 patients in the ICU.

2. Material and Methods

2.1. Study Design and Participants

106 COVID-19 patients in the ICU were included in the study. The patients' hospital records and the patient management tools were thoroughly examined.

Any verified case was assumed as a positive result on the reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assay of nasopharyngeal swab specimens. Those with COVID-19 evidence in thorax CT such as ground-glass opacities -either alone or in combination with pulmonary consolidations- were included.

Demographic features of the non-pregnant patients aged between 25 and 96 years old were recorded, and they were divided into 2 subgroups according to the mortality status of survivors and non-survivors. Written informed consent papers were received from all patients. The study was approved by the Ethics Committee of the Bagcilar Training and Research Hospital (Date: May 29, 2020; IRB Number: 2020.05.2.05.061) and was performed in accordance with ethical standards laid down in the 1964 Declaration of Helsinki.

Data was gathered through the patient management system and the COVID-19 records of the health care institutions. We compiled 59 specifications -containing the demographic data and the laboratory test results as well. The patients were mainly either discharged or deceased. Mortality count throughout the hospitalization period was acquired from the discharge registers.

2.2. Definitions

All covariates were obtained from the electronic records: gender, age, and laboratory results including all hemogram parameters (leukocyte/white blood cell-WBC-, hemoglobin (Hgb), hematocrit (Hct), red blood cell (RBC), Platelet (Plt), plateletcrit (PCT), red blood cell distribution width (RDW), platelet distribution width (PDW), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), basophil count and %, eosinophil count and %, lymphocyte count and %, monocyte count and %, MPV, neutrophil count, neutrophil %, interleukin-6 (IL-6), C-reactive protein (CRP), procalcitonin, d-dimer, fibrinogen (FBG), ferritin, glucose, lactate dehydrogenase (LDH), creatinine, urea, uric acid, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkalen phosphatase (ALP), gama glutamil transpherase (GGT), total bilirubin, direct bilirubin, total protein, albumin, anti-HBs, Anti-HCV, Anti-HIV, HBsAg, sodium (Na), Calcium (Ca), Clor (Cl), Potassium (K), pro-BNP, prothrombin time % (PT), INR, activated partial thromboplastin time (APTT), thyroid

stimulating hormone (TSH), 25-OH vitamin D, free T4, and free T3).

2.3. Statistical Analysis

The Number Cruncher Statistical System (NCSS) 2007 Statistical Software (Utah, USA) was used to analyze the data. All data were expressed as the mean ± SD. The Student's t-test was used to analyze the difference between the groups. The Chi-square test was used to analyze the quantitative data. For the evaluation of the correlation of variables, Pearson correlation and Spearman's rho correlation analysis were administered. The variables that proved to be significant in univariate analysis were also included in multivariate logistic regression. $p<0.05$ was accepted as significant.

3. Results

3.1. Demographic and Clinical Specifications

Of the 106 verified COVID-19 patients -including those in the ICU-, the prevalences of female and male patients were 37.7 % and 62.2 %, respectively. The demographics and laboratory findings were shown in Tables 1 and 2.

3.2. The Comparison of Survivors and Non-Survivors

Compared with survivors (median age=62.7), non-survivors (median age=69.6) were older (p=0.016), whereas the gender distribution of both groups was similar (p=0.122).

In the non-survivor group, while the mean IL-6, CRP, Procalcitonin, WBC, PDW, RDW, MPV, neutrophil count, neutrophil %, neutrophil count/lymphocyte count ratio (NLR), MPV/Plt ratio (MPR), neutrophil count/albumin ratio (NAR), urea, direct bilirubin, Pro-BNP levels were significantly higher compared to survivors (p=0.0001, p=0.004, p=0.003, p=0.049, p=0.016, p=0.03, p=0.005, p=0.007, p=0.009, p=0.007, p=0.049, p=0.0001, p=0.001, p=0.016, p=0.002) (table 1-2), they had lower hemoglobin, platelet, MCHC, lymphocyte %, monocyte %, albumin and calcium levels (p=0.022, p=0.033, p=0.042, p=0.008, p=0.04, p=0.0001, p=0.008) (Table 1-2).

Logistic regression analysis showed that CRP (p=0.045), Procalcitonin (p=0.025), MPR (p=0.027), Pro-BNP (p=0.017) and thrombocytopenia (p=0.041) are associated with higher mortality rates (Table 3).

Table 1: Age, Gender and Hemogram Characteristics of Critical Patients with COVID-19.

		Survivor (n:39)		Non-Survivors (n:67)		p
Age (Year)		62.79±14.67		69.67±13.53		**0.016***
Gender	Male	28	71.79 %	38	56.72 %	0.122+
	Female	11	28.21 %	29	43.28 %	
Total leukocyte Count ($\times10^3$/L)		10.42±6.27		13.01±6.57		**0.049***
Hemoglobin (g/dL)		11.3±2.52		10.19±2.26		**0.022***
Hematocrit (%)		35.29±7.87		32.99±6.53		0.109*
Platelet Count ($\times10^3$/L)		316.51±167.47		258.69±108.75		**0.033***
Red blood Cell Count ($\times10^{12}$/L)		4.09±1.00		3.8±0.81		0.100*
Plateletcrit (%)		0.33±0.17		0.28±0.11		0.100*
Platelet Distribution Width (%)		11.86±2.11		13.09±2.63		**0.016***
Red Cell Distribution Width (%)		14.59±2.04		15.72±2.78		**0.03***
Mean Corpuscular Hemoglobin (pg)		27.82±1.96		27.35±2.71		0.343*
Mean Corpuscular Hemoglobin Concentration (MCHC)		32.04±1.67		31.26±2.00		**0.042***
Mean Corpuscular Volume (fL)		86.83±4.83		87.41±7.06		0.651*
Basophil Count ($\times10^3$/L)		0.03±0.02		0.04±0.04		0.468‡
Basophil (%)		0.28±0.20		0.28±0.24		0.602‡
Eosinophil Count ($\times10^3$/L)		0.09±0.12		0.07±0.12		0.423‡
Eosinophil (%)		1.02±1.5		0.56±0.92		0.243‡
Lymphocyte Count ($\times10^3$/L)		0.98±0.49		0.93±0.75		0.145‡
Lymphocyte (%)		11.7±8.28		8.36±5.91		**0.008‡**
Monocyte Count ($\times10^3$/L)		0.6±0.39		0.64±0.47		0.801‡
Monocyte (%)		5.88±2.36		4.99±2.15		**0.04‡**
Mean platelet Volume (fL)		10.3±0.89		10.92±1.12		**0.005***
Neutrophil Count ($\times10^3$/L)		8.73±5.95		11.32±5.99		**0.007‡**
Neutrophil (%)		81.25±10.49		85.81±7.04		**0009***

*Independent t test ‡Mann Whitney U test +chi-square test.

Table 2: Biochemistry Characteristics of Critical Patients with COVID-19.

	Survivor (n:39)	**Non-Survivors (n:67)**	**p**
Interleukin -6 (pg/mL)	132.95±303.9	588.81±1106.12	**0.0001‡**
C-reactive Protein (mg/L)	104.98±85.3	162.56±105.84	**0.004‡**
Ferritin (ml/ng)	599.16±684.37	598.78±457.23	0.337‡
Fibrinogen (mg/dL)	502.27±168.09	491.09±154.3	0.792*
D-dimer (ng/mL)	3.1±7.63	2.64±2.35	0.063‡
Procalcitonin (ng/mL)	1.52±4.67	5.89±19.92	**0.003‡**
Neutrophil/Lymphocyte Ratio (NLR)	11.29±9.6	17.79±16.33	**0.007‡**
Mean Platelet Volume/Platelet Count Ratio (MPV/Plt)	0.04±0.02	0.05±0.03	**0.049***
Fibrinogen / Platelet Count Ratio	1.86±1.32	2.22±1.13	0.102‡
Neutrophil Count/Albumin Ratio (NAR)	28.63±6.85	33.91±6.52	**0.0001***
Serum Urea (mg/dL)	50.88±34.72	86.13±57	**0.001***
Serum Creatinine (mg/dL)	1.02±0.81	1.57±1.73	0.053‡
Serum Uric Acid (mg/dL)	4.76±2.85	5.44±3.18	0.306‡
Direct Bilirubin (mg/dL)	0.17±0.11	0.45±1.51	**0.016‡**
Total Bilirubin (mg/dL)	0.58±0.28	0.94±2.59	0.902‡
Serum Total Protein (g/dL)	5.99±0.75	5.78±0.73	0.160*
Serum Albumin (g/dL)	2.94±0.51	2.6±0.39	**0.0001***
Serum Sodium (Na) (mEq/L)	139.59±6.51	139.69±8.06	0.949*
Serum Calcium (Ca) (mg/dL)	8.24±0.62	7.93±0.55	**0.008***
Serum Chlor (Cl) (mmol/L)	102.54±6.5	102.01±7.17	0.708*
Serum Potassium (K) (mEq/L)	43±0.81	4.29±0.7	0.960*
Serum LDH (U/L)	500.79±328.18	595.07±509.97	0.177‡
Serum Pro-BNP (pg/ml)	6405.72±11473.39	11397.89±12469.74	**0.002‡**

*Independent t test ‡Mann Whitney U test.

4. Discussion

While the death rates caused by the Coronavirus strains were respectively 10% [19] in Severe Acute Respiratory Syndrome (SARS) and 34 % (20) in Middle East Respiratory Syndrome (MERS), the initially reported death rate for COVID-19 was around 2 %. Studies conducted over time have shown that the mortality of COVID-19 was to be ranging from 4 % to 28 % (21-23). In their study with hospitalized patients, Mikami et al. found the mortality rate among COVID-19 patients to be 25.9 % (24). In a meta-analysis by Jia et al., patients with more severe illnesses were found to yield a much higher mortality rate than those with mild or moderate illnesses (25). Thus, it proves favorable to concentrate on those who are more probable to develop serious diseases at the time of diagnosis so as to reduce mortality. It is possible to obtain an early prognosis with fewer resources during the early stages, as well as to effectively manage the treatment with various hemogram and inflammatory parameters to prevent complications, and thus, reduce the mortality rate in the recurring waves of the COVID-19 pandemic.

Many studies showed that the age (24, 26) and the levels of CRP were taken as high-sensitivity predictors for COVID-19 mortality (27) that might prove useful in identifying the major COVID-19 cases (28, 29).

Gemin's study showed that CRP levels were related to severe COVID-19 pneumonia and mortality (30). Initial laboratory findings of non-survivors exhibited higher levels of CRP along with Procalcitonin in Mikami's study (24). Consistent with the literature, our study also shows that mortality increases with aging and increased CRP-Procalcitonin levels.

Leukocytosis, neutrophil count, and neutrophil ratio % (24) were all related to severe COVID-19 pneumonia and mortality in Gemin's study (30). Some of the studies showed that neutrophils count and NLR were significant predictors of critical illnesses (31, 32). In a multivariate logistic regression analysis by Yan et al., high levels of NLR were found to be associated with death being an independent risk factor to predict in-hospital mortality among COVID-19 cases (33). This study, instead of including a specific group of patients, includes all groups either with patients who died, patients with ongoing treatments, or patients discharged. The study was conducted with patients in the ICU, a more specific group found to be associated with NLR mortality. In Varim et al.'s study conducted, on 144 patients, results suggest that COVID-19 patients with high NAR values be closely followed and be treated in ICU due to the close relation with early mortality (34).

Table 3: Mortality associated risk factors for critical patients with COVID-19.

	B	S.E.	p	OR	95 % OR Lower limit	Upper limit
Age (Year)	0.02	0.03	0.593	1.02	0.96	1.08
Interleukin-6 (pg/mL)	0.01	0.01	0.07	1.01	1.00	1.02
C-reactive Protein (mg/L)	0.01	0.01	**0.045**	1.01	1.00	1.02
Procalcitonin (ng/mL)	0.29	0.13	**0.025**	0.75	0.58	0.96
Total Leukocyte Count ($\times 10^3$/L)	-0.23	0.22	0.285	0.79	0.52	1.21
Hemoglobin (g/dL)	0.03	0.24	0.901	1.03	0.64	1.66
Platelet Count ($\times 10^3$/L)	-0.02	0.01	**0.041**	0.98	0.96	1.00
Platelet Distribution Width (%)	0.21	0.25	0.397	1.23	0.76	2.00
Red Cell Distribution Width (%)	-0.42	0.30	0.163	0.66	0.36	1.19
Mean Corpuscular Hemoglobin Concentration (MCHC)	-0.64	0.38	0.097	0.53	0.25	1.12
Neutrophil / Lymphocyte Ratio (NLR)	0.08	0.05	0.147	1.08	0.97	1.19
Mean platelet volume/Platelet Count Ratio (MPV/Plt)	-1.78	1.44	**0.027**	0.00	0.00	0.00
Monocyte (%)	2.10	2.76	0.446	8.18	0.04	1.99
Serum Urea (mg/dL)	0.02	0.02	0.125	1.02	0.99	1.05
Direct Bilirubin (mg/dL)	0.74	0.47	0.385	1.14	0.00	2.20
Serum Albumin (g/dL)	-1.02	2.40	0.670	0.36	0.00	3.54
Serum Pro-BNP (pg/ml)	0.00	0.00	**0.017**	1.00	1.00	1.00
Neutrophil Count/Albumin Ratio (NAR)	0.03	0.20	0.874	0.97	0.65	1.44

In our study, a significant correlation was found between increased leukocytosis, which was consistent with other studies (24), neutrophil count, neutrophil ratio (%), NLR and NAR, and decreased lymphocyte count and mortality.

Wenhua et al.'s study showed that direct bilirubin and LDH were significant predictors of critical illnesses in COVID-19 patients (31). In our study, a significant relationship was found between mortality and high levels of direct bilirubin, but no significant relationship could be found with LDH. Earlier research also suggested the relation between the increasing LDH levels and the high mortality risk of COVID-19 (31). According to the observation of Ji et al., COVID-19 patients with serum levels of LDH higher than 500 U/L exhibited a dramatic disease advance in a multivariate Cox analysis, in comparison to the group with LDH levels < 250 U/L ([35). Even though no significant relationship was spotted between the LDH levels and the mortality rates in our study, the median LDH values of our living and deceased patients were found to be 500 and 595, respectively. Because both survivors and non-survivors were in the ICU having serious respiratory failure, they had hypoxia in tissue level, leading to increased LDH.

Mikami et al's study showed that preliminary findings of non-survivors show higher IL-6 levels and increased IL-6 levels as an independent prognostic risk factor, adding to even higher levels among survivors. (24). Liang et al.'s study showed that serum IL-6 and IL-10 levels in COVID-19 cases proved to be significantly higher in the critical group in proportion to the moderate and severe disease groups. Accordingly, this showed that cytokine storms in higher amounts are related to the development of a more serious disease, and thus can be used as a predictor for swift diagnosis for patients. Given the high cytokine levels caused by SARS-CoV-2, it may be critical in the early treatment stage to reduce cytokine-related lung damage in the group with high levels of IL-6 (36). A statistically significant relationship was found between IL-6 levels and mortality rate among our patients in the critical group.

In Sundas et al.'s study, they concluded that RDW is an important parameter that can greatly assist clinicians in different stages of COVID-19 patient evaluation, such as identifying PCR false negatives at initial triage, at the referral centers, and in the community, as well as predicting complications, and monitoring progression (37). According to the findings of Gong et al., RDW-long with other parameters- was associated with severe COVID-19 patients (38). Likewise, Wang et al., in another research, also suggested that NLR and RDW, when combined together, accurately diagnose with an accuracy ratio of 85.7 % and can be useful to predict patients' staging, thus enabling to administer the necessary treatment promptly on time (39). In our study, a significant relationship was also found between the increased RDW values and mortality rates in accordance with the literature.

High levels of Platelet Distribution Width (PDW) indicate the destruction of the newly produced immature platelets and parallel size variations (40). In COVID-19, PDW is expected to be found high as a result of increasing cytokine release and platelet production, and the destruction mechanism affected by inflammation (41). In a study by Ozcelik et al., COVID-19 groups were compared to influenza

pneumonia groups, and PDW proved to be significantly higher among COVID-19 cases compared to influenza cases (42). In accordance with the literature, increased PDW was found to be associated with mortality in our study. Although high rates of venous thromboembolism and the evidence of endothelial dysfunction caused by COVID-19 have been reported, the precise etiology of the increased thrombotic risk associated with COVID-19 infection is not fully elucidated. Thrombocytosis was related to disease activity in SARS and was considered to be secondary to the direct effect of the virus or the inflammatory cytokines (43). A previous study with primates revealed that IL-6 induced thrombocytosis in a dose-dependent response (46). The incoherence between high IL-6 levels and deficiency of thrombocytosis in the deceased could be related to the harm of endothelium and latter platelet expenditure by viral infection, reduced platelet release from megakaryocytes in the lung, or direct damage of hematopoiesis (45) which might indicate that the lack of reactive thrombocytosis might mark a weak reaction to COVID-19 (24).

An increase in MPV values is a sensitive and prognostic marker for platelet activity and thromboinflammation (46), and also is associated with some specific viral infections (47). Therefore, in Comer et al.'s research, in which they evaluated clinical platelet parameters and circulating platelet activity in severe (intensive care) and non-severe (non-intensive care) COVID-19 patients, it was shown that the increased MPV among patients with severe and mild COVID-19 disease is associated with the disease severity in COVID-19 (48). MPV and MPR proved to be significantly lower in the COVID-19 group than in the influenza group, in a study by Özçelik et al. (42). In Zhong et al.'s research with 85 cases, high MPR levels were spotted to be an independent risk factor for severe pneumonia in COVID-19 cases (18). Guclu et al. found that the decreased MPV values were associated with mortality in the follow-up of the COVID-19 cases (49), in contrast to the other studies supporting a significant relationship between high MPV and mortality in patients hospitalized in the ICU (50). In a mortality study with 100 patients, it was found that MPR is not significantly associated with mortality (29). Survivors had a more significant decrease in hemoglobin levels and platelet counts. (24). In our study with the critically conditioned COVID-19 patients hospitalized in the ICU, it has been found that there is a significant relationship between increased MPV and MPR and decreased hemoglobin and platelet and mortality.

The major drawbacks of the present study are its retrospective design and low sample size limiting the researchers to reach a more precise conclusion. Additionally, the patient group was selected from patients at the first 6 months of the epidemic when the variant of SARS-CoV-2 was yet to be identified.

5. Conclusion

MPV, MPR, and NLR are easily available hematologic parameters that can be used to evaluate the risk of the disease progression. Even in limited laboratical environments, these parameters can help physicians to classify and identify COVID-19 patients with a higher risk of a severe inflammatory state.

Limitations of the Study

Low number of patients included in the study.

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Conflict of Interests

The authors declared no conflict of interest.

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This study received no financial support.

Author Contributions

ES, AEA, SK designed the research. Collected the data ES, CY, CCB. Analysis and interpretation of data SOS, AEA. Wrote the manuscript read and approved final script ES, SOS.

Ethical Approval

The study was approved by the Ethics Committee of the Bagcilar Training and Research Hospital (Date: May 29, 2020; IRB Number: 2020.05.2.05.061)

Data sharing statement

All data relevant to the study are included in the article.

Informed Consent

Informed consent was obtained from all participants included in the study.

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Evaluation of the Perceptions About the Covid-19 Pandemic of Patients with the Diagnosis of Generalized Anxiety Disorder: A Qualitative Study

Fatma Tuygar Okutucu[1*], Hacer Akgul Ceyhun[1]

[1]Department of Psychiatry, Medical Faculty, Ataturk University, Erzurum, Turkey

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[*]***Corresponding Author***
Fatma Tuygar Okutucu
Department of Psychiatry
Medical Faculty
Ataturk University
Erzurum, Turkey
Phone:+905058401678
E-mail: fokutucu.tuygar@atauni.edu.tr

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Authors' ORCIDs
Fatma Tuygar Okutucu
http://orcid.org/0000-0001-7706-6751
Hacer Akgül Ceyhun
http://orcid.org/0000-0002-1831-7634

Abstract: Covid-19 pandemic has raised concerns about how to deal with the psychiatric aspects of the pandemic and it was a matter of curiosity how the perceptions of individuals who already had a psychiatric diagnosis would be, especially a vulnerable diagnosis of Generalized Anxiety Disorder (GAD). We aimed to explore the perceptions about Covid-19 pandemic in a sample of individuals with the diagnosis of GAD. Qualitative data was obtained using a semi-structured interview questionnaire by a psychiatrist with 33 individuals diagnosed with GAD. 14 (42.4 %) of the participants were male, 19 (57.6 %) were female. Average age was 34.7. 7 (21.2 %) participants had Covid-19 infection. Three main themes were determined as "learning from the pandemic process", "impact of the pandemic process on changes in personal development", "effects on the psychiatric treatment process". Sub-themes; 'value of health', 'patience', 'importance of cleanliness and hygiene', 'changing world', 'difficulties of staying at home', 'necessity of preparing yourself for the worst', 'people are no good for anyone but themselves', 'things happen to happen' 'learning to be happy with little things', 'being able to read more books', 'uncertainty', 'spending time with children', 'enduring', 'discipline', 'negativities added to health', 'no changes', 'fires again, me again', and 'worsening with stress factor', ' awareness ', 'experience', 'helped-it, it was good' have been detected. People with the diagnosis of GAD may be particularly vulnerable to the psychological effects of the COVID-19 pandemic. But their positive and negative appraisals and coping behaviours could prevent or ameliorate future problems. © 2022 NTMS.

Keywords: Covid-19; Generalized Anxiety Disorder; Impact of Stressor; Perceptions About the Pandemic.

1. Introduction

After the new coronavirus (COVID-19) outbreak was declared a pandemic by the World Health Organization (1) in March 2020, extraordinary precautions were taken, such as quarantining all countries. The rapid spread of viral infection has raised concerns about how to deal with the psychiatric aspects of the pandemic in people with a diagnosis of psychiatric disorder and the impact of the COVID-19 pandemic on psychiatric disorders has been an important research topic for scientists.

An important aspect of mental health that may be particularly affected by the coronavirus pandemic is generalized anxiety disorder. Anxiety is a normal feeling of worry, fear, nervousness, or apprehension that is experienced when facing or anticipating a perceived or real threat. Anxiety disorders, on the other

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hand, are characterized by persistent, overwhelming worry and fear that interferes with normal functioning and DSM-5 includes nine types of anxiety disorders (2). The types are highly comorbid and all are characterized by anxiety, fear, and related behavioral disturbances. Generalized anxiety disorder (GAD) is the most common type of anxiety disorder (3). The current prevalence of the disorder is between 1.6 % to 5.0 % in the general population (4).

Interestingly, individuals with 'high health anxiety' (possibly with generalized anxiety disorder) are more likely to misinterpret harmless bodily symptoms and emotions as evidence of dangerous illness (for example, benign muscle aches or coughing as a descriptive sign of being infected with COVID-19). This can increase their anxiety and distress and affect their behavior and decision-making capacity (5, 6).

In general, individuals have difficulty coping with uncertainty and this creates anxiety (7). COVID-19 has taken this uncertainty to a high level (8). Traumatic events like this can reduce people's sense of security, remind them of the reality of death, and have negative effects on their mental health. Questions that do not have a definite answer, such as when the epidemic will end and treatment methods; constant exposure to the information about the pandemic and its effects; reduction in social relationships due to the pandemic; and suggestions/prohibitions such as staying at home as much as possible may adversely affect the mental health of individuals (9).

Fear, anger, anxiety and panic about possible worse outcomes can precipitate boredom and feelings of loneliness and guilt about not being with family. In a person with a pre-existing psychiatric disorder, all these problems may reappear (10). Having a history of pre-existing psychiatric illness may cause people to experience anxiety and anger even 4-6 months after leaving quarantine (11).

Especially vulnerable individuals who already have a diagnosis of generalized anxiety disorder may be more vulnerable to public panic and anxiety triggered by the pandemic, which can worsen existing anxiety disorder symptoms. Due to the sudden outbreak and lack of experience with COVID-19, most mental health services were not ready and able to reach psychiatric patients during quarantine. As a result, the needs of psychiatric patients are neglected during the pandemic. Although there are many studies known to date on the impact of the COVID-19 pandemic on the mental health of the general population (12-14), on COVID-19 patients (15), and on healthcare professionals (16, 17), little research is available on the psychological impact on pre-existing psychiatric patients living in the community during the COVID-19 pandemic. Although most of them are quantitative, very little are qualitative studies (18).

Therefore, this study aimed to analyze the impact of fear, anxiety and distress related to COVID-19 on people suffering from generalized anxiety disorder and their perceptions of social isolation, prevention behaviors and emotional impact of COVID-19 social conditions. We assumed that these individuals experienced a high psychological burden.

2. Material and Methods

This research is a qualitative study using semi-structured interview and document analysis methods. The study protocol was accepted by the ethical committees of the Non-Interventional Studies Ethics Committee at Ataturk University (2021-5/23).

2.1. Study population

Patients who applied to the psychiatry outpatient clinic of our hospital for 1 month and were diagnosed with Generalized Anxiety Disorder (GAD) according to DSM-5 diagnostic criteria with SCID-5 semi-structured interview form and clinical interview constituted the sample of our study.

Qualitative data was obtained by using a semi-structured interview form about the perceptions of the patients about the Covid-19 pandemic.

Informed consent was obtained from the patients for participation in the study, and the files and interview forms of those who were interviewed and gave consent were evaluated.

Those with mental retardation, hearing impairment, illiteracy and those who did not give consent to participate in the study were excluded.

2.2. Data Collection

The participants' age, gender, marital status, educational status, occupation, economic status, previous medical conditions, past or present psychiatric disorders, and medications they used were learned from digital hospital records and paper documents of interview notes kept confidential in envelope files by the interviewer.

The interviewer had permission to access the internal documents.

2.3. Evaluating perceptions about the Covid-19 pandemic process

It was prepared as a form asking them to indicate their perceptions by filling in the blanks. The contents were evaluated.

Qualitative data was obtained with a descriptive approach by making direct quotations from what the participants said (with their original formats), what was written and the contents of the documents.

2.4. Statistical Analysis

In the descriptive statistics of the quantitative findings of the study, number, percentage distribution and mean, standard deviations were used.

Nvivo12 program was used in the analysis of the qualitative data. The data was coded, codes were found from the events and facts that were frequently repeated or emphasized by the participants, themes were found

from the codes, and the codes of the data were interpreted according to the themes.

3. Results

Our sample consisted of patients between the ages of 18-65 years who applied to the psychiatry outpatient clinic between 01.07.2021 and 30.07.2021 due to anxiety symptoms and were diagnosed with Generalized Anxiety Disorder (GAD) according to DSM-5 diagnostic criteria.

1 person with hearing impairment and 3 people who did not consent to participate in the study were excluded from the study, and a total of 33 people were included.

14 (42.4 %) of the participants were male, 19 (57.6 %) were female. Average age was 34.7. 7 (21.2 %) participants had Covid infection.

When the patients were evaluated according to their past psychiatric histories; it was found that 18 (54.5 %) had a pre-existing diagnosis of generalized anxiety disorder, and 15 (45.5 %) were diagnosed with generalized anxiety disorder for the first time. Among those with a previous diagnosis of anxiety disorder 11 (61.1 %) participants described increase, 5 (15.2 %) participants described no change, 2 participants described decrease in anxiety symptoms during the pandemic process. Sociodemographic characteristics are illustrated in Table 1.

Table 1: Sociodemographic characteristics of the patients.

Patient	Age	Gender	Education	Occupation	Economical Situation	Pre-existing Anxiety Diagnosis	Had Covid-19 Infection	Change in Anxiety Symptoms
P1	27	F	University	House-wife	Modarate	-	-	
P2	23	F	University	Student	High	Yes	-	Increase
P3	61	M	University	Officer	Modarate	Yes	-	No change
P4	18	F	Primary school	No work	Modarate	-	-	
P5	49	M	High school	Self-employement	High	-	-	
P6	32	M	University	No work	Low	-	Yes	
P7	21	F	University	Student	Modarate	-	-	
P8	39	M	University	Officer	Modarate	-	-	
P9	32	F	University	Self-employement	High	Yes	-	Increase
P10	41	M	University	Officer	Modarate	Yes	-	Increase
P11	21	M	University	Student	Modarate	Yes	-	Increase
P12	30	M	Primary school	Self-employement	High	-	-	
P13	29	F	University	Officer	High	Yes	-	Increase
P14	30	F	Primary school	House-wife	Modarate	-	-	
P15	23	F	University	Student	Modarate	-	Yes	
P16	24	F	University	Self-employement	High	Yes	-	No change
P17	19	F	High school	Student	Modarate	Yes	-	No change
P18	47	F	Primary school	House-wife	Modarate	Yes	-	Decrease
P19	42	F	High school	House-wife	Modarate	Yes	-	Increase
P20	55	M	Primary school	Self-employement	Low	-	-	
P21	24	M	University	Student	Modarate	Yes	-	Increase
P22	30	M	Primary school	Self-employement	Modarate	-	-	
P23	36	F	High school	House-wife	Modarate	Yes	-	Increase
P24	56	M	Primary school	Self-employement	Modarate	Yes	Yes	Increase
P25	26	F	University	Officer	Low	Yes	Yes	Decrease
P26	20	F	University	Student	Modarate	Yes	Yes	Increase
P27	45	M	University	Officer	High	Yes	Yes	No change
P28	48	M	High school	Self-employement	Low	-	-	
P29	20	F	High school	Student	Modarate	-	yes	
P30	23	F	University	Student	High	Yes	-	Increase
P31	31	F	Primary school	House-wife	Modarate	-	-	
P32	58	M	Primary school	Self-employement	Modarate	-	-	
P33	65	F	Primary school	House-wife	Modarate	Yes	-	No change

After the data obtained from the qualitative analysis of the interviews were coded, three general themes were established: “learning from the pandemic process”, “the impact of the pandemic process on changes in their personal development”, “the effects of the pandemic process on the psychiatric treatment” (Table 2).

Table 2: Theme categories and sub-themes.

1-Learning From the Pandemic Process
a. Value of health
b. Patience
c. Importance of cleanliness and hygiene
d. Changing world
e. Difficulties of staying at home
f. Necessity of preparing yourself for the worst
g. People are no good for anyone but themselves
h. Things happen to happen
2- Impact of the Pandemic Process on Changes in Their Personal Development
a. Learning to be happy with little things
b. Being able to read more books
c. Uncertainty
d. Spending time with children
e. Enduring
f. Discipline
g. Negativities added to health
h. No change
3- **The Effects of Pandemic Process on the Psychiatric Treatment**
a. “Fires again, me again" and worsening with stress factor
b. Awareness
c. Experience
d. Helped-it, it was good

The first category of the theme “learning from the pandemic process” was to recognize the ‘value of health’. It included the expressions of people who had constant anxiety in their daily work, saying that health was more important than anything else.

“My husband and I used to argue all the time because of money, now I don’t think it matters, I only think about our health but nothing else’’ (P18).

“I have many diseases, diabetes, high blood pressure and others, I only think not to catch this infection and can’t see anything else” (P20).

“I have my own business and closed it immediately because of my fear, I say we can make a living somehow, just don’t get the virüs” (P28).

While trying to comply with quarantine and precautions ‘patience’ was another category.

“In order not to go to the market, I even started to make my own bread at home, we will somehow endure this situation” (P14).

“While we were always together at home, we started to argue with my husband. I try to be patient with everything” (P13).

Besides some were satisfied with the cleaning and hygiene efforts, some of them complained.

“I understood the importance of cleanliness once again, I used to hardly wash my hands before, now I pay attention to the cleanliness of everything” (P22).

“I wasn’t as meticulous as now before, nowadays I spray everything with disinfectant, I even wash the fruits with soap, my hands don’t come out of the water, I’m tired” (P19).

Some stated their perceptions about the changing world in addition to those who stated the difficulties of staying at home.

“Everything has changed in an instant, all the balances in the world have changed and even the most powerful countries cannot cope with it” (P2).

“I can’t believe that the giants of the world are fighting for the mask, it’s like the world has turned upside down” (P21).

“Normally I like to spend time at home, but when it was forbidden, I felt trapped.” (P26).

“It used to be difficult to go to work before, but now I miss and say it was a blessing” (P3).

Some constantly thought about the worst scenarios and some stated that the anxiety of thinking that no one can help themselves when infected was the most distressing situation. There were also those who said that they could not believe that the most unexpected and what could be said about things happened.

“The situation of the patients in the intensive care unit keeps coming to my mind, I say that one day I can be like that, one of my relatives died and we couldn’t even go to his funeral, I can’t get it out of my mind” (P24).

"If I get infected, no one can even come close to me, I will be alone, I think I can't even ask for water from anyone, there is no benefit from anyone in this life" (P27).
"It's unbelievable, I wouldn't believe these things if I saw them in my dreams. What else do I say to what happens every day?" (P10).
In the theme of the "impact of the pandemic process on changes in their personal development", the category 'learning to be happy with the little things' was in the first sequence.
"I couldn't believe that watching movies at home would make me so happy. I think I'm happier now, because everything outside is left outside" (P1).
There were also those who reported an increase in their activities such as "I started to read the books that I got out because of boredom at home, I missed it actually, I was far away after school." (P16).
There were also those who reported that it was good for them to spend time with the children at home.
"In fact, no one at home could see each other's faces, we were always outside, being a working mother distanced me from my children, this process made me more comfortable" (P25).
"We cook meals that children like and have fun together, actually, we haven't done anything like this for a long time" (P31).
Uncertainty category included codes related to the diagnosis of the virus infection, its outcomes, fear of death, status of relatives, and intolerance to the unknown about the future.
"I wonder if I will catch this disease, what will be the result, or if I die, it is a frightening process" (P33).
"If it infects me or one of my family, if it is serious, if we stay in the hospital, or if one of us dies, we won't even be able to see each other, no one will even come to our funeral, I don't even want to think about being buried in a bag and like that anymore" (P5).
Despite the uncertainty of the disease, some patients insisted on going to the hospital and getting tested even if it was not considered risky, while others avoided knowing whether they were ill and being stigmatized.
"My son's nose was running, I said if it is positive, if it infects all of us, just in case, I took him for a test right away, but it came out negative" (P23).
"I was sick but I didn't want to go to the hospital, if they test and comes out positive, how will everyone look at me, they will put a big quarantine sign on our door, I said to myself, I used herbal medicines, it passed" (P8).
There were also those who stated that the constant obligation to obey the rules was a hard disciplinary process to endure.
"As if I'm in the military, rules, rules, rules, discipline is overwhelming" (P12).
Negative effects added to health due to the lack of access to adequate health services or because of contamination were also reported.
"My hypertension medication was over, I couldn't print, they weren't taking patients other than covid, two days later my blood pressure was up, I was in an emergency service" (28).
"I went to the emergency service for my stomach ache, I wish I hadn't gone, I think it infected me there, and all family got the infection, we were devastated, we were in quarantine for 14 days" (P6).
There were also those who said that it had no effect on their life.
"It had no effect" (P33).
In the theme of "the effects of the pandemic process on the psychiatric treatment", 'worsening in such a stressful situation' was the first category.
"What happened, of course, fires again, me again, my palpitations have increased, I can't sleep at all, I'm very bad" (P9).
"I don't even watch TV anymore, I don't want to response the calls, I'm afraid to get news that it has infected someone in my family immediately, my treatment was going well for a long time, everything turned upside down" (P11).
There were those who said it was good, it provided experience and improvement, and there were also those who gave simple answers as it contributed a lot to my treatment or it never effected.
"I said, "I think this is the worst thing what happens in life, it's been an experience for me, I couldn't think of other bad things for a while" (P18).
"It actually helped, it was nice, I learned to overcome my fears, albeit a little bit" (P17).
There were also simple responses that were thought to be related to their socio-cultural status.
"It has effected a lot" (P32).
"It did not effect" (P24).

4. Discussion

In our qualitative study, we aimed to understand the perceptions, feelings, experiences and reactions about Covid-19 pandemic of patients with generalized anxiety disorder (GAD).
On the theme of what they learned from the pandemic process; the value of health was at the first place and showed that they could put other concerns about daily functions in the back. Patience was sometimes a coping attitude shown in this unexpected panic situation, but sometimes it was an attitude that caused angry behaviors too (19).
The importance of cleanliness and hygiene was prioritized among the requirements of daily life and that they showed harmonious and motivated but sometimes exaggerated behaviors in complying with cleaning and

hygiene rules. Fear of contamination and getting the infection may be a reason of anxiety for these patients (20).

Besides perceptions such as ‘the changing world and the necessity of being prepared and open to changes and preparing oneself for the worst’ may be some part of constant anxiety and generalized anxiety symptoms (5). In addition, the symptoms of anxiety caused by quarantine and isolation related to the difficulties of staying at home were shared, and perceptions such as " things happen to happen " and the sense of decrease in trust have been reached regarding the belief that ‘one has no good for anyone but himself’ were existed with a review examining the effects of isolation on patients' mental well-being and behaviors which determined negative effects such as increased anger and anxiety scores in isolated patients too (21).

As the impact of the pandemic process on changes in their personal development positive contributions such as 'learning to be happy with little things', 'reading more books', 'spending time with children' were determined suggesting that people may experience positive quarantine experiences, including an increased sense of freedom, joy, and calmness due to increased leisure time and slower pace of life. With people being encouraged to work from home and some taking leave, leisure has increased and more opportunities to spend time with family have emerged, which may have led to increased well-being (22).

Negative statements such as 'discipline', 'endurance', and 'uncertainty' were detected too and these seem to be related with anxiety and anger. It was especially seen that anxiety increased about uncertainty and it could be said that it was significant for the increase in anxiety symptoms. The category of 'uncertainty' was associated with fear of death and anxiety and the role of uncertainty in anxiety is known (7) and this uncertainty regarding the COVID-19 pandemic is at its peak.

In our interviews, some patients felt threatened by the virus while others did not. Some reported problems about obtaining hospital care and negative effects on health. Due to the health anxiety triggered by this process, these individuals may apply to physicians and hospitals frequently. Conversely, individuals with high anxiety may also be reluctant to seek medical attention because of the concern that hospitals are source of contamination. Also, the fact that individuals with psychiatric symptoms have difficulty in getting medical help due to reasons such as citizens being asked to stay at home during the pandemic, may also have adversely affected these processes (23, 24).

When we look at the effect of the pandemic process on the psychiatric treatment; increase in symptoms were related may be due to worsening with stress factors that one said ‘fires again me again’ and not being able to go to the hospital but sub-themes such as ‘experience’ and ‘helpful- it was nice’ including some dealing and coping attitudes were detected too. It may also have provided a practice for exposure and respond preventing therapies suggested in anxiety therapy (25). Another qualitative study evaluating perceptions of COVID-19 found that positive and negative emotions often coexist, but negative emotions predominate in the early stages of the epidemic and positive emotions emerge much more slowly, and stated that it is related to psychological or lifestyle adjustments (i.e. daily writing, awareness, exercise, distraction, humor and rationalization) and personal development (26). Although we have no data on lifestyle changes of participants in our study but the attitude towards personal development and coping strategies supports this.

Participants stated resilience, adaptability, and coping as well as negative experiences. Previous studies also showed that psychological resilience and flexibility are associated with greater well-being during quarantine (27, 28). Although people with existing psychiatric diagnoses are particularly vulnerable to the psychological impact of the COVID-19 pandemic, their positive and negative assessments and coping behaviors can prevent or ameliorate some problems. Our results seem to be consistent with another qualitative study carried out with patients with existing psychiatric diagnoses (22). Resilience may be related to know how to respond to challenges and feeling in control (29). An important aspect of this process that facilitates coping may be the availability of technology, opportunities such as social media, video communication, and being able to work and get education remotely.

In our study; some experiences confirmed the findings of previous studies, such as negative emotions and exacerbation of symptoms (10, 30). Others cited positive experiences such as resilience, adaptation, and coping. Emotional responses varied between individuals, and this seems in line with the literature suggesting that people experience a multitude of different emotions (10).

Our study showed that in a vulnerable group of generalized anxiety disorder, besides the negative consequences of the pandemic process, they could also exhibit good coping attitudes and resilience and produce positive perceptions.

5. Conclusions

Although people with existing psychiatric diagnoses are particularly vulnerable to the psychological impact of the COVID-19 pandemic, their positive and negative assessments and coping behaviors can prevent or ameliorate some problems.

Limitations of the Study

The interviews were conducted face to face and the results of the complaints and experiences of the participants of their own applications were evaluated. It is possible to mention that it is a realistic qualitative

evaluation. Since it is a retrospective evaluation, a comparison could not be made regarding conditions such as individual characteristics, lifestyle changes, supports and access to technology.

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Conflict of Interests
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Author Contributions
Creating the design of the study: FTO, HAC, Determining the working method: FTO, HAC Conducting the study and collecting data: FTO, HAC, Analysis and statistical evaluation of data: HAC

Ethical Approval
The study protocol was accepted by the ethical committees of the Non-Interventional Studies Ethics Committee at Atatürk University (2021-5/23).

Data sharing statement
It can be shared if requested from the author.

Informed Consent
All the participants had signed the informed consent form prepared by researcher.

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Strategy for Successful Urine Sample Preparation for LC-MS/MS Device at Drug Verification Laboratory

Esin Eren[1], Asuman Göncü Sürü[1], Necat Yılmaz[1*], Cemile Oz[1]

[1]Department of Medical Biochemistry and LC/MS-MS Laboratory, Antalya Training and Research Hospital, Health Sciences University, Antalya, Turkey

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[*]***Corresponding Author***
Necat Yılmaz
Department of Medical Biochemistry
and LC/MS-MS Laboratory
Antalya Training and Research Hospital
Health Sciences University
Antalya, Turkey
Phone: +90 2423578305
E-mail: necatyilmaz@hotmail.com

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Authors' ORCIDs
Esin Eren
http://orcid.org/0000-0001-9780-2437
Asuman Göncü Sürü
http://orcid.org/0000-0001-9330-5601
Necat Yılmaz
http://orcid.org/0000-0002-3865-9156
Cemile Oz
http://orcid.org/0000-0001-7835-7454

Abstract: The use of liquid chromatography with tandem mass spectrometry (LC-MS/MS) device has increased significantly in toxicology validation laboratories in recent years. The maintenance cost of this expensive equipment is high as well as the cost of consumables. Reduction of the matrix effect and preparation more clear samples are very important for the validation of methods in clinical laboratories. The primary goal is to create a cleaner urine sample preparation technique to reduce the cost of maintenance of the LC-MS/MS device without affecting test results. We prepared the patients' urine in two different ways; routine urine preparation method and used our centrifuged method (14000 rpm, 10 minutes) for routine illicit substance use. The standard material used to determine whether there was a statistical difference in the urine sample with both different methods was added to both urine samples. Our findings showed that there was no statistical difference between the results of both methods for detection of illicit substance use. There was no difference between the high and low quantities of the 14 illicit substances measured and the centrifuged method and routine urine preparation methods ($p>0.05$). However, the urine sample obtained by our newly developed centrifuged method was cleaner, lucid and homogeneous. This preliminary study shows that the centrifugation method, although time consuming, can be reliable as it does not have statistically different results from routine practice. Long-term use of the centrifuge method may potentially reduce device maintenance, repair and consumption costs. According to these initial findings, positive effects of using centrifuge method for a long time on column costs and replacement processes can be expected in future studies © 2022 NTMS.

Keyword: LC-MS/MS; Urine; Toxicology; Morphine; Screening; Validation.

1. Introduction

Although mass spectrometry (MS) technique has high selectivity, other molecules included in the same urine matrix is very difficult to separate. The MS technique is a system designed to separate the substances depending on the m/z (mass/charge) ratios. MS, when combined with the separating liquid chromatography (LC) system (LC-MS), have unique features as both systems are combined Tandem Mass Spectrometry

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(LC-MS/MS) system has been designed and started to be used in routine practice (1, 2).

LC-MS/MS system has been frequently preferred in many advanced hospital laboratories on recent times due to its superior features such as sensitivity, speed and selectivity for detection and identification of toxic/non-toxic molecules in urine. Undoubtedly, urine sample preparation for LC-MS/MS is very important because the toxic or non-toxic analytes that are intended to be measured must be accurately targeted and they must be of the appropriate amount. Huge matrix effects in urine result in ion suppression (loss of signal) or ion enhancement (gain in signal). Also, matrix effects have a negative impact on the accuracy, precision, and robustness of the method (2, 3). As well as, a good illicit drugs verification method needs to be selective, accurate, sensitive, easy to use and automated.

However, the routine urine sample preparation techniques that are widely used today may not be appropriate for LC-MS/MS because, there are only a few simple urine sample preparation methods described in literature for urine screening with LC-MS/MS (4). Therefore, the most difficult and time-consuming step is the routine urine sample preparation phase. When routine minimal sample preparation procedures are combined with short analysis times, large amounts of endogenous species can potentially coexist with the target analyte in urine. To date, limited reports on routine urine sample preparation for LC-MS/MS have been published (1-4).

Moreover, when clear and good samples are not used, these shorten the column life, increases device downtime times, and the costs of manpower and equipment maintenance. Today, when preparing urine sample for drug analysis with LC-MS/MS, direct dilution of the urine sample or expensive extraction procedures are applied (1-4).

For example, some of the alternative methods for routine urine sample preparation are complex procedures such as; urine dilution with different solvents, protein precipitation and filtration with several precipitating agents, liquid-liquid extraction (LLE) and solid-phase extraction (SPE) respectively. Of course, concentrating the urine analyte helps to increase sensitivity and thus reach lower detection limits (5, 6). Moreover, these complex concentering procedures removes the interfering strong matrix elements which alter the peak measurement or elute together with the target analyte (phospholipids, salts, proteins, nucleic acids, sugars, etc.) (4). However, there is not a complete consensus on the preparation of concentrated urine in the validation laboratory in previously published articles. Liquid-liquid extraction has a long history and, although other techniques are available, this technique is still accepted. More recently, liquid-solid extraction or, as it is more often called, SPE, has gained more importance. As expected, the role of routine urine sample preparation is to remove interferences from sample matrix and improve analytical system performance for LC-MS/MS (5-6). However, as mentioned above, there is no study in the literature demonstrating the effect of precipitation by centrifugation prior to injecting urine sample.

Therefore, in this study, it is aimed to determine whether the interfering substances removed from the urine by the long-term centrifuge procedure in the routine urine preparation in the validation laboratory have an effect on the results of the illegal drug use measured in the LC-MS/MS. Firstly, we aimed to reduce the effect of urine matrix with this method. We hypothesized that the results would be more cost-effective and reliable, even if the laboratory workload and analysis time increased as expected.

2. Material and Methods

2.1. Subjects and Sample Preparation

The routine administration samples of negative 50 patients who were sent to our laboratory to determine the use of illegal drugs were used to compare two different sample preparation methods (A and B). The samples were stored for maximum 30 days (-80 °C) and then analyzed. Illicit drug analytes were prepared with low and high concentrations of standards and studied with method A and B. High and low concentration illicit drug standards added equal amounts (1ppm) to the urine and the matrix effect of urine samples was determined.

The routine illicit drug tests were added in urine samples; 6 Acetyl Morphine Hydrochloride, MDMA, MDEA, MDA, Benzoylecgonine, Codein Hydrochloride, DL-Amphetamine free base, DL-Metamphetamine free base, Lorazepam, Nordiazepam, Oxazepam, Temazepam, Morphine Monohydrate, Dihydrocodeine Hydrogen. The relevant expert laboratory personnel were responsible for the performance of this analysis. The study was conducted in accordance with the declaration of Helsinki.

Routine LC-MS/MS procedures include four main steps: sample preparation, chromatographic separation, MS detection and data ratings. For the preparation of routine urine samples in LC-MS/MS measurement in our laboratory, the sample preparation method, which was generally accepted and which was explained in the user manual of the device was preferred. In this study, the samples were divided into two parts. The first part was measured using the routine administration preparation procedure with urine-A method (Figure 1). Sample A was then injected directly into the LC-MS/MS device as shown in Figure 1. In method B, long- time centrifugation (14000 g,10 minutes) was performed on the routine urine samples. The samples were taken from the upper part of the clean urine sample and the procedures in Figure 1were performed. The B method results were compared with method A

administration samples. As a result, the results obtained in both methods were carefully recorded and analyzed.

2.2. LC-MS / MS Methods

We used the Thermo Scientific LC-MS/MS to identify illegal drug use in the verification laboratory. The device was verified according to the thermo instructions and the original column and other materials were used. LC-MS/MS analysis, Thermo Scientific Dionex Ultimate 3000 pump and Ultimate Open automatic sampler, Thermo Scientific TSQ ENDURA is done by three-stage four-pole mass spectrometer. Thermo Scientific Hypersil Gold analytical column was used at ambient temperature. The measurement parameters and the LC-MS/MS procedure were as explained in LC Conditions; Thermo Scientific Hypersil Gold Column which is used (50×2.1 mm ×1.9 µm particle size). The auto sample receiver temperature was set to 15 °C, the column was set to +40°C in oven. The autosampler needle was rinsed before and after sample injection to avoid carry over. The mobile phase consists of 2 mM ammonium acetate and 0.2 % formic acid, 250 mL water with and 2 mM ammonium acetate and 0.2 % formic acid with 250 mL methanol.

HPLC Conditions; the same column and two mobile phase combinations were used in all samples (Table 1 and 2). LC gradient and mobile phase transitions are shown in Figure 2.

MS /MS Terms; the mass spectrometer was operated with heated electrospray ionization in both positive and negative ionization modes (HESI-II). For MS, all the conditions are shown in Figure 2.

2.3. Statistical analysis

Urines analyzed for illicit substances were measured twice using two different methods. Obtained test results were evaluated by statistical analysis. The statistical analysis was performed using MedCalc© Statistical Software version 15.8 (MedCalc Software® bvba, Ostend, Belgium; https://www.medcalc.org; 2018). The Kolmogorov-Smirnov for normal distribution and paired sample test was used to assess the distribution of constant variables. A P-value of <0.05 was considered statistically significant.

3. Results

Screening of a wide range of compounds from various matrices, such as urine, is challenging, but LC-MS/MS has proven to be suitable for such applications. Briefly both urine preparation methods (A and B) used in this study met our analytical standard criteria. Therefore, no significant statistical difference was found between sample A and Sample B preparation among the illicit substance measurements in urine (Table 3, p>0.05). In both methods the sensitivity and linear dynamic ranges were may be appropriate for clinical use to monitor drug use in urine. However, the duration of the urine preparation was 20 minutes longer in the sample B compared to sample A, as expected. In both standard sample preparation methods (sample A, sample B), the accuracy and dilution integrity of the methods were acceptable for the quantitative urine drug tests (Figure 3and 4).

Moreover, the analytes were stable under the conditions specified in the stored samples and did not show a significant difference over a month. The measurements of both samples stored for one month were not different in all parameters compared to fresh urine results. Analytes were stable during sample preparation and storage under the stated conditions (data not show). The centrifugation of the samples allowed to obtain a clearer urine sample, but the analysis time was longer for at least 20 minutes in method B.

Table 1: Chemicals used for analysis, certified and unmarked certified standards and brand and origin of the column used.

Chemical	Brand	Country
Acetonitrile	Carlo Erba	France
Propanol	Carlo Erba	France
Ammonium Acetate	Carlo Erba	Germany
Formic Acid	Carlo Erba	Germany
Methanol	Carlo Erba	France
Beta-glucronidase enzyme	Covachem	Germany
Internal Standard (CRM-marked)	Chiron	Norvey
Internal Standard (CRM-un-marked)	Chiron	Norvey

ISTD: Marked Standard.

Table 2: Certified and unmarked reference materials used during analysis.

Labeled Internal Standards (1ppm)	Standard (1ppm)
DL-Amphetamine-d5 Hydrochloride	DL-Amphetamine
Morphine-d3 Hydrochloride	Morphine
Benzoylecgonine-d3	Benzoylecgonine
MDA-d5 Hydrochloride	MDA
Lorazepam-13C6(7-chlorobenzo- 13C6-d5)	Lorazepam
Nordiazepam-d5(phenyl-d5)	Nordiazepam
Oxazepam-d5(phenyl-d5)	Oxazepam
Temazepam-d5(phenyl-d5)	Temazepam
Codeine-N-Methyl-d3 Hydrochloride	Codeine
Methamphetamine-d5 HCl	Methamphetamine
MDMA-d5 HCl	MDMA
(+,-) -MDEA-D5 Hydrochloride(Ethyl d5)	(+,-) –MDEA
6-Acetylmorphine-d3 HCl	6-Acetylmorphine
(-) - Trans-delta 9 - THC-d3 (pentyl-5,5,5-d3)	(+-) - Trans-11 Nor-9-carboxy delta 9 THC

Table 3: The results of the illicit substance measurements in sample A and Sample B.

Standard analytes added to urine 1ppb	Non-centrifuge Low Concentration	Centrifuge Low Concentration	Non-centrifuge High Concentration	Centrifuge High Concentration
DL-Amphetamine Free Base(ng/ml)	185,24±4.42	187,60±4.64	312,78±3,69	314,27±3,85
DL-Metamphetamine Free Base(ng/ml)	181,74±3.72	187,78±4.94	312,30±4.02	315,49±4.27
MDA(ng/ml)	186,25±4.38	187,72±4.86	312,93±5.03	314,75±5.72
MDMA(ng/ml)	191,10±3,85	187,69±3,05	312,33±4,65	316,71±5,57
MDEA(ng/ml)	183,78±4,76	187,87±4,23	312,59±4,69	312,09±4,36
Lorazepam(ng/ml)	153,02±2,57	156,07±2,73	249,87±3,75	234,37±3,84
Nordiazepam(ng/ml)	151,31±2,94	150,62±2,83	250,15±3,65	248,88±3,49
Oxazepam(ng/ml)	153,00±2,58	150,61±2,42	250,15±4,03	257,90±3,05
Temazepam(ng/ml)	150,45±2,63	150,89±2,01	250,22±3,14	241,02±3,35
11 nor THC-COOH(ng/ml)	11,18±0,38	11,17±0,32	18,41±0,54	18,04±0,73
Benzoylecgonine(ng/ml)	76,98±1,35	75,07±1,43	126,38±1,74	125,06±1,52
6 Acetyl Morphine Hydrochloride(ng/ml)	7,86±0,12	7,69±0,51	12,39±0,78	12,60±0,82
Codeine Hydrochloride(ng/ml)	227,08±1,42	224,97±1,48	373,83±0,39	374,39±0,48
Morphine Monohydrate(ng/ml)	226,21±0,48	225,64±0,51	366,47±1,12	379,31±10

* Kolmogorov-Smirnov: all parameters accepted normal distribution, Mean± standart deviation(SD), Paired sample test p>0.05 for all paraters.

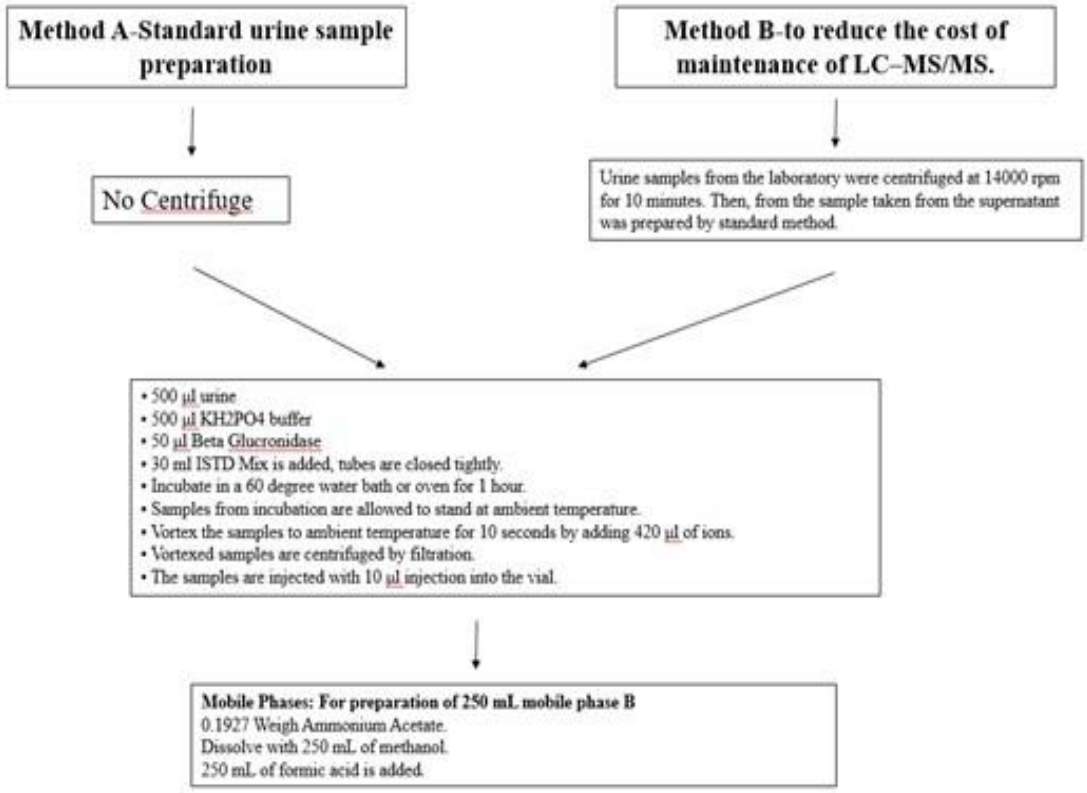

Figure 1: : Preparation of Samples.

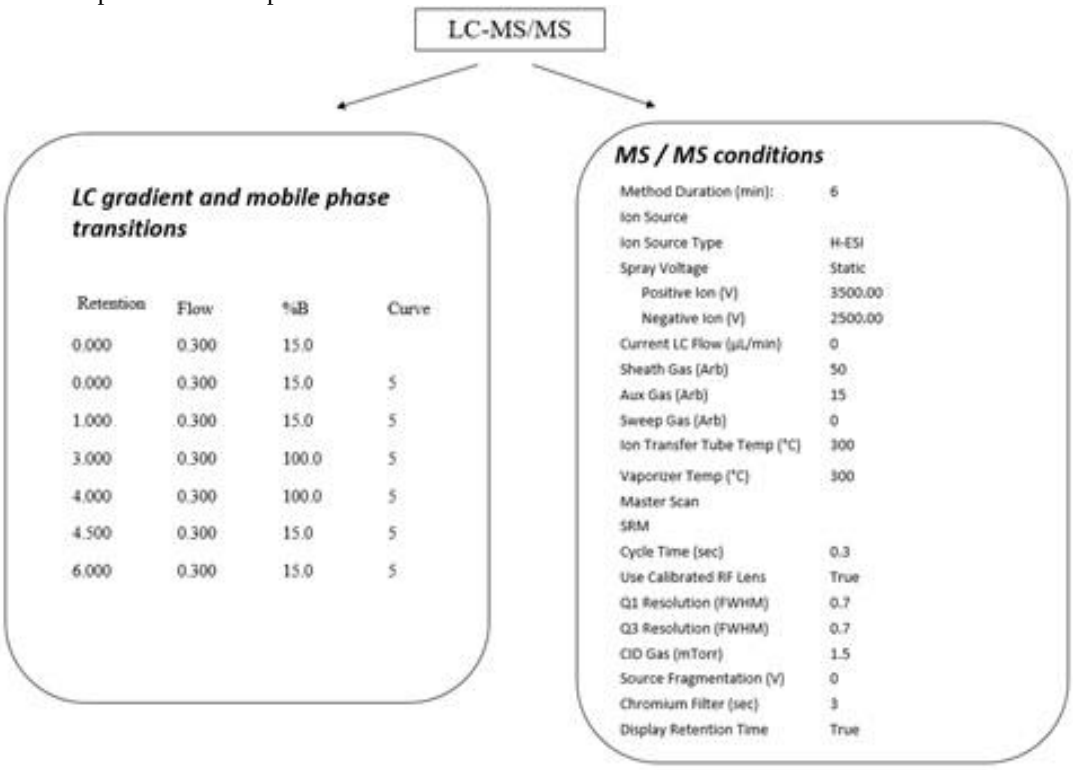

Figure 2: LC gradient and mobile phase transitions and MS/MS conditions.

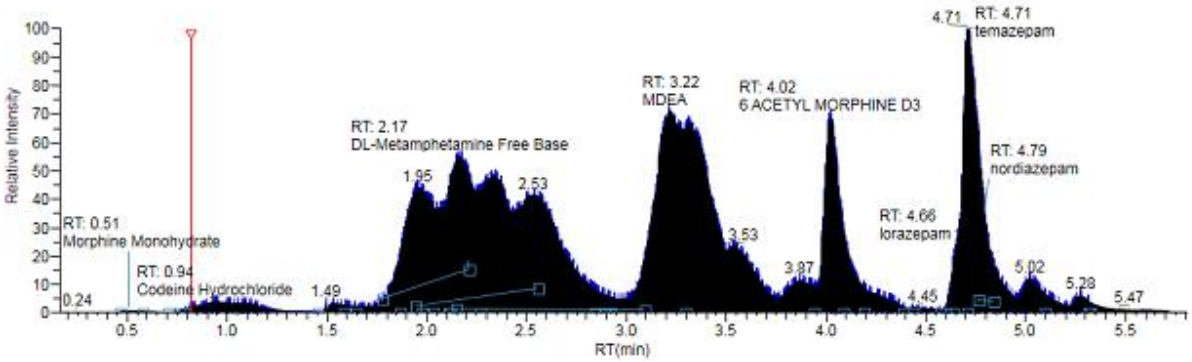

Figure 3: Method A example chromatogram.

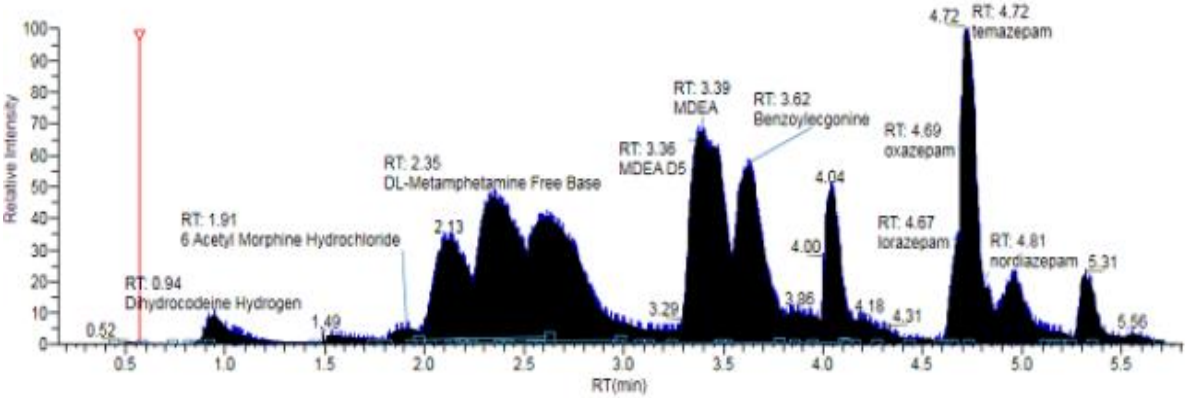

Figure 4: *Method B example chromatogram*.

4. Discussion

Our results show that urine pre-centrifuges have no statistical effect on the test results obtained. In laboratories, it is important to provide timely and rapid results, but another important feature is to reduce the equipment maintenance costs. We could not determine whether the equipment maintenance costs decreased, because the urine preparation method B was not used for a long time in our laboratory. Regarding this preliminary study, we can conclude that the long centrifugation procedure for urine (Method-B) used in our validation laboratory can reduce the urinary matrix effect when compared to the initial findings. Moreover, more clear urine samples did not change the results of toxicological analysis. Unfortunately, the commonly used complex urine preparation methods cause challenges in practice in terms of filtration and chemical separation methods and these are expensive (7-9).

Routine immunological tests are widely used to screen illicit drug use. In case of a positive result, sometimes an additional selective verification analysis may be required. Immunoassay tests are simple and fast, but not precise. Furthermore, immunological screening assays are not very selective. In addition to the compounds in a group, other structural compounds may result in an incorrect positive test due to cross-reactivity (4, 10).

We could not obtain data for long-term use of this new method for in terms of device maintenance, repair and consumption costs. However, it can be determined in more comprehensive studies can be conducted to see whether the LC-MS/MS device will reduce the maintenance costs or not (4, 8-11). In this study, we showed that a simple but time-consuming urine preparation method for the first time in urine preparation did not influence the results. Our hypothesis is that the cost of replacing the very expensive parts as columns and LC-MS/MS device will be reduced by the use of clean and urine samples. However, the initial findings suggest that column costs and replacement processes can be positively affected. In this way, we may confirm this hypothesis in the future by using the method-centrifuge in routine urine analysis for a certain period of time in our routine practices (12). However, the urine sample obtained by our newly developed method is clean, clear and homogeneous. The results of these preliminary studies indicate that although the new method is time-consuming, it can be reliable.

5. Conclusions

Consequently, this study is a pilot study and we can say that long-term pre-centrifugation does not have a negative effect on routine LC-MS/MS toxicological test measurements.

Limitations of the Study

This study is on the preliminary and includes the development and comparison of purely technical methods. The effectiveness of different urine purification techniques should be supported by clinical studies.

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Conflict of Interests

None of the authors have a conflict of interest.

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Author Contributions

Study conception and design: E.E; Data collection: E.E, A.G; Analysis and interpretation of results: N.Y, E.E; Draft manuscript preparation: N.Y. C.O. All authors reviewed the results and approved the final version of the manuscript.

Ethical Approval

A Methodical Tecnical study, no ethics committee approval was received for this study from the ethics committee of Antalya Training and Research Hospital.

Data sharing statement

All data relevant to the study are included in the article.

Consent to participate

Methodical Technical study patient material and informed consent were not obtained.

Informed Consent

A Methodical Technical study patient material and informed consent were not obtained.

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Assessment of Serum Cystatin C Level Under Levetiracetam Monotherapy in Patients with Epilepsy

Fatma Şimşek[1], Seda Aşkın[2]

[1]Department of Neurology, Faculty of Medicine, Atatürk University, Erzurum, Turkey
[2]Department of Biochemistry, Faculty of Medicine, Atatürk University, Erzurum, Turkey

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****Corresponding Author***
Fatma Şimşek
Department of Neurology
Faculty of Medicine
Atatürk University
Erzurum, Turkey
Phone:+904423447099
E-mail: klamaks@hotmail.com

DOI: 10.56766/ntms.1108819

Authors' ORCIDs
Fatma Şimşek
http://orcid.org/0000-0003-1662-5534
Seda Aşkın
http://orcid.org/0000-0001-6133-9065

Abstract: Cystatin C is a cysteine protease inhibitor that has been shown to have antiviral, antibacterial and neuroprotective efficacy. The aim of this study is to assess the effect of levetiracetam monotherapy on serum cystatin C levels in patients with epilepsy. 30 patients, who were diagnosed with epilepsy for the first time and subsequently started on levetiracetam monotherapy, were included in the study as the study group, whereas 30 healthy volunteers were included in the study as the control group. Serum cystatin C and creatinine levels of patients were measured twice, once before they were started on the levetiracetam treatment and once after the completion of six months of treatment levetiracetam, whereas the serum cystatin C and creatinine levels of the healthy control subjects were measured once. Both the pre-treatment and post-treatment creatinine levels of epilepsy patients were found to be statistically significantly higher compared to the creatinine levels of the healthy control subjects. The pre-treatment and post-treatment serum cystatin C levels of epilepsy patients were found to be lower compared to the serum cystatin C levels of the healthy control subjects, albeit not statistically significantly. Additionally, serum cystatin C levels of epilepsy patients were found to have increased after the completion of the levetiracetam treatment, even though not statistically significantly. The observed increase in the levels of cystatin C, a neuroprotective agent, following completion of levetiracetam treatment in epileptic patients may suggest a neuroprotective effect of levetiracetam.© 2022 NTMS.
Keywords: Levetiracetam; Epilepsy; Cystatin C.

1. Introduction

The majority of seizures suffered by adults are focal seizures. The first choice of preference in the treatment of epilepsy is medical therapy, and levetiracetam is one of the most frequently used types of antiepileptic drugs in the treatment of focal seizures. It is usually possible to control the seizures in a vast majority of patients with the administration of right medication. The mechanisms of action of antiepileptics are different, and levetiracetam binds to synaptic vesicular protein 2A and acts by inhibiting Ca^{2+} release (1, 2). Levetiracetam easily crosses the blood-brain barrier, and its cerebrospinal fluid (CSF) half-life is 3 times longer than its plasma half-life (3). Most of the levetiracetam is metabolized through non-hepatic hydrolysis and the rest is excreted by the kidneys without any change.

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Cystatins are proteinase inhibitors that are expressed in many regions in the mammalian body. Cystatins protect the organism against endogenous proteases released from lysosomes. Cystatin C (Cys C) is widely distributed in body fluids such as CSF, saliva, blood plasma, and urine (4). Cys C has been identified as an alternative endogenous marker indicating the renal functions and is more sensitive than creatinine (5, 6). It has been shown that the Cys C level in serum is strongly dependent on the glomerular filtration rate (7). Cys C has many biological functions and has growth-supporting, inflammation-reducing, antiviral and antibacterial properties (8). It has been shown that serum CysC level is decreased in Alzheimer's disease, and CysC expression is found in experimental animal models of induction of transient forebrain ischemia and epilepsy (9-11). In an experimental study conducted with animals, in which an electrically induced status epilepticus model was created, Cys C protein levels were shown to have elevated and remain elevated for a few months (12). In another study, it was shown that exogenously applied Cys C protects neuronal cells from death in a concentration-dependent manner, i.e., that it has a neuroprotective effect (13).

Due to its neuroprotective effect, Cys C may be beneficial in terms of controlling neurodegenerative diseases, such as epilepsy, and the development of new treatment strategies to that effect. Accordingly, in this study, it is aimed to assess the effect of levetiracetam on Cys C.

2. Material and Methods

This study was carried out as a prospective clinical study. The ethics committee approval required to conduct the study was obtained from the local ethics committee of Atatürk University Faculty of Medicine (04/19/ 30.05.2019). Informed consents were obtained from all patients and healthy control subjects who participated in the study.

2.1. Patient Selection

The study was conducted with 30 patients who were diagnosed with epilepsy for the first time, at Atatürk University neurology outpatient clinic, between June 2019 and May 2020. The 1981 International Classification of Epileptic Seizures of the International League Against Epilepsy (ILAE) was used for diagnosis. Patients with focal epilepsy or secondary generalized tonic-clonic seizures were included in the study. Hypertension, diabetes mellitus, liver and kidney dysfunction, history of stroke, mental retardation, central nervous system disease such as multiple sclerosis, history of malignancy, history of depression, organic brain lesion on cranial magnetic resonance imaging, who presented with status epilepticus in the last two weeks patients with a history of infection and under 18 years of age were excluded. The demographic characteristics of the patients, types of the seizures the patients suffered, as well as the frequencies in which they suffered these seizures were recorded. Patients were started on levetiracetam (500 to 2000 mg/day) monotherapy following their diagnosis with epilepsy and were followed up for a minimum of 6 months thereafter.

2.2. Control Subjects

Control subjects were selected from among healthy volunteers of matching gender and of an an age group comparable to the patients.

2.3. Collection of serum samples

Venous blood samples were collected from the patient and control groups following a 12-hour fasting period. Blood samples were taken from the patients twice, that is once before they were started on the levetiracetam treatment and once after the completion of six months of treatment levetiracetam, provided that they were seizure-free for a period of at least 2 weeks before blood-sampling. On the other hand, blood samples were taken from the control group once. Blood samples taken were let rest at room temperature for half an hour, and were then centrifuged. Subsequently, centrifuged serum samples were placed in epandorf tubes, and preserved at -80 °C until they were studied.

2.4. Cystatin C test

Cystatin C levels were tested via the Enzyme-Linked Immunosorbent Assay (ELISA) method using a respective ELISA kit (SunRed, Lot: 201-12-1105, China). Test results were calculated in ng/ml in line with the manufacturer's instructions.

2.5. Statistical Analysis

All statistical tests were performed using the SPSS (IBM Statistical Package for the Social Sciences version 20) Software. Kolmogorov-Smirnov test was used to analyzed whether the research data conformed to the normal distribution. Categorical data were expressed as n (%), whereas numerical data were expressed as Mean±Standard Deviation (SD) in case of data that conformed to the normal distribution, and as median (minimum-maximum) in case of data that did not conform to the normal distribution. Student’s t-test was used for the analysis of numerical data that conformed to the normal distribution, whereas Mann-Whitney test was used for numerical data that did not conform to the normal distribution. Additionally, Wilcoxon test was used to analyze the data that did not conform to normal distribution in paired groups. Furthermore, Spearman's correlation analysis was used to determine the relationship between two numerical values that do not conform to the normal distribution. It has been accepted that the probability (p) values of ≤ 0.05 indicate statistical significance.

3. Results

There were 16 females and 14 males in both patient and control groups. The mean age of the patients group was calculated as 31.87±5.69 years, and the mean age of the control group was calculated as 31.47±5.78 years. The

mean age of the gender-matched groups was similar (p=0.790). The creatinine levels were found to be between 0.54 and 0.95 mg/dL, thus normal, in both the patient and control groups. The creatinine levels of the patient group both before and after the levetiracetam monotherapy were found to be statistically significantly higher than the creatinine levels of the control group (p<0.001, p=0.001, respectively). The post-treatment creatinine levels of the patient group were found to be lower than the pre-treatment creatinine levels of the patient group, albeit not statistically significantly (p=0.288). The pre-treatment and post-treatment serum Cys C levels of the patient group were found to be lower compared to the serum Cys C levels of the control group, albeit not statistically significantly (p=0.141, p=0.631, respectively). Additionally, serum Cys C levels of the patient group were found to have increased after the completion of the levetiracetam treatment, even though not statistically significantly (p=0.147) (Figure 1). The demographic characteristics and laboratory test results of the patient and control groups are given in Table 1.

Table 1: Clinical, demographic and laboratory data of the patient and control group.

	Patient	Control	p
Age±SD	31.87±5.69	31.47±5.79	0.79
Sex n(%)			
Female	16 (53.3)	16 (53.3)	1
Male	14 (46.7)	14 (46.7)	
Seizure type n(%)			
Focal Seizure	16 (53.3)	-	
Secondary Generalized Seizure	14 (46.7)	-	
Seizure frequency/6 month, med (min-max)	2 (1-6)	-	
LEV Drug Dose (mg/day), Med (min-max)	1000 (500-2000)	-	
Cystatin C (ng/mL), Med (min-max)			
Before LEV	3.281 (0.21-122.6)	6.38 (1.10-148.6)	0.141
After LEV	5.277 (0.25-155.51)		0.631
p	0.147		
Creatinine (mg/dL), Med (min-max)			
Before LEV	0.7 (0.5-0.9)	0.55 (0.4-0.8)	**<0.001***
After LEV	0.65 (0.4-0.9)		**0.001****
p	0.288		

LEV: Levetiracetam, SD: standard deviation, min: minimum, max: maximum, med: median, *It shows a significant difference in creatinine levels between the patient and control group before levetiracetam treatment, **It shows a significant difference in creatinine levels between the patient and control group after levetiracetam treatment.

Cystatin and creatinine levels before and after treatment according to seizure type are given in Table 2. There was no significant difference between the pre-treatment and post-treatment Cys C levels of the patient group with focal seizures and the patient group with secondary generalized seizures (p=0.983, p=0.114, respectively). Cys C levels of patients with focal seizures increased after treatment compared to pre-treatment, and there was a significant difference (p=0.049). There was no significant difference in Cys C levels before and after treatment in patients with secondary generalized seizures (p=0.975). Additionally, no significant relationship was found between the frequency of seizures and the serum creatinine and Cys C levels (r=-0.004, p=0.982; r=0.016, p=0.931, respectively). There was no significant correlation between the pre-treatment and post-treatment serum Cys C and creatinine levels (r=-0.066, p= 0.618; r= 0.009, p= 0.945, respectively). Dosage of the drug used in the patient group following the levetiracetam treatment was found to be correlated with the serum Cys C and creatinine levels in the negative direction, albeit not statistically significantly (r=-0.349, p=0.059; r=-0.199, p=0.292, respectively) (Figure 2). A statistically significantly positive correlation was found between the drug dosage and the frequency of seizures (r=0.541, p=0.002).

Table 2: Cystatin and creatinine levels before and after treatment by seizure type.

	Focal Seizure (n=16)	Secondary Generalized Seizure (n=14)	p
Before LEV, med (min-max)			
Cystatin (ng/mL)	2.83 (0.35-51.47)	3.72 (0.21-122.60)	0.983
Creatinine (mg/dL)	0.70 (0.50-0.90)	0.71 (0.50-0.90)	0.253
After LEV, med (min-max)			
Cystatin (ng/mL)	9.52 (0.25-155.51)	3.56 (0.44-39.83)	0.114
Creatinine (mg/dL)	0.67 (0.40-0.90)	0.65 (0.60-0.90	1

LEV: Levetiracetam, min: minimum, max: maximum, med: median.

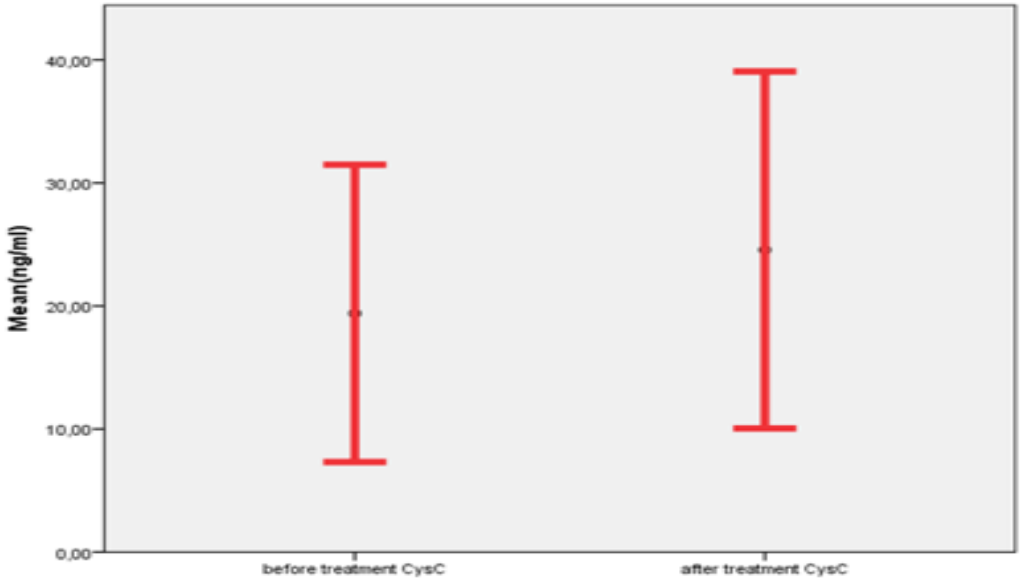

Figure 1. Mean serum cystatin C levels of patients before and after levetiracetam treatment.

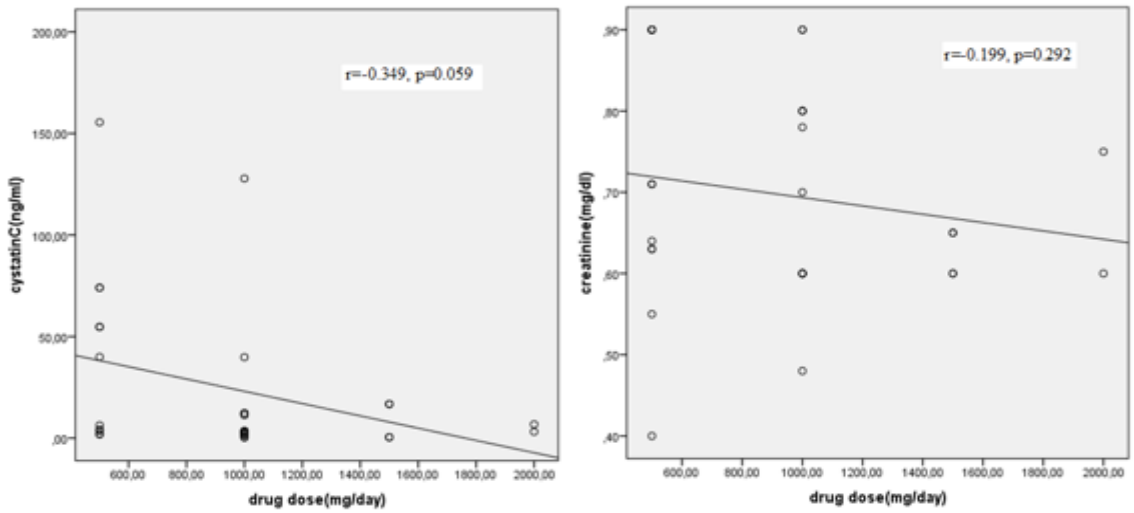

Figure 2. Levetiracetam drug dose correlation with serum cystatin C and creatinine levels. X-axis represents the levetiracetam drug dose in mg/day, whereas y-axis represents the serum cystatin C levels in ng/ml (left picture) and creatinine levels in mg/dl (right picture). Each point on the graph represents one patient. Some dots are darker because some patient values are the same. Dark dots represent more than one patient. There was a negative but insignificant relationship between the drug dosages and serum cystatin C and creatinine levels of the patients treated with levetiracetam (r=-0.349, p=0.059; r=-0.199, p=0.292, respectively).

4. Discussion

The creatinine levels of epilepsy patients both before and after the levetiracetam monotherapy were found to be statistically significantly higher than the creatinine levels of the healthy control subjects. Even though not statistically significant, a decrease was observed in the creatinine levels and an increase was observed in the Cys C levels, following the completion of the levetiracetam monotherapy. Levetiracetam was used at higher doses in patients with higher seizure frequency, and it was observed that as the dosage of the levetiracetam was increased, the Cys C levels decreased, however no statistically significant relationship was found between the two.

The seizures increase the consumption of adenosine triphosphate (ATP), since the energy need of the metabolism increases during seizures. Creatine is an important source of energy in that it accelerates ATP energy production by increasing the creatine phosphate pool to meet the high energy demand of the brain tissue during seizures (14, 15). Creatine phosphate contributes to the energy production by providing high energy phosphate molecules to adenosine diphosphate (ADP) during the production of ATP (Creatine phosphate+ ADP↔Creatinine+ATP). Thus, it is an expected outcome that the use of creatine phosphate during the above described ATP production would lead to a decrease in the creatine phosphate levels and an increase in the creatinine levels. Lee et al. reported to have observed a decrease in the creatine phosphate levels and an increase in the creatinine levels in their study conducted in the form of an experimental epilepsy model (16). In comparison, similar results were found in this study, since both the pre-treatment and post-treatment creatinine levels of the epilepsy patients were found to be statistically significantly higher than the creatinine levels of the healthy control subjects.

It was reported in various studies conducted in the form of experimental animal models available in the literature that the Cys C levels increased following status epilepticus and then decreased over time (11, 12). Such results suggest that the increases in Cys C levels occur not due to the neurodegenerative process, but as part of a cellular repair response during the acute period. In comparison, in this study, both the pre-treatment and post-treatment Cys C levels of the patients were found to be lower than the Cys C levels of the healthy control subjects. Additionally, Cys C levels of the patients were observed to have increased after treatment with levetiracetam, which were observed to have decreased however with increasing doses of levetiracetam. High doses of levetiracetam have been used in patients whose seizures cannot be controlled and who have more frequent seizures. The fact that a decrease was observed in the Cys C levels with higher doses of levetiracetam seems to be due to the increase in the frequency of seizures, and not to the increase in the drug dosage. In other words, the decrease in the Cys C levels suggests the neurodegenerative process. Along these lines, it has been shown in an experimental study that the exogenous Cys C protects neuronal cells under neurotoxic stimuli from death in a concentration-dependent manner (13). Accordingly, the increase in Cys C levels following levetiracetam treatment may suggest that levetiracetam has a neuroprotective effect. Cys C is the predominant cysteine protease inhibitor in the cerebrospinal fluid (CSF) and its levels in CSF are 5.5 times higher than in plasma (17). Accordingly, more meaningful results could have been obtained as a result of this study, were the Cys C levels to be checked in CSF instead of venous blood sample.

It has been demonstrated in various studies available in the literature that levetiracetam has less side effects on cognitive functions than the other antiepileptic drugs. In a study of healthy volunteers, carbamazepine had the greatest effect on neuropsychological tests and levetiracetam the least effect when compared to levetiracetam, carbamazepine or oxcarbazepine. In another study with healthy volunteers, levetiracetam was found to cause less cognitive deficits compared to carbamazepine (18, 19). The reason for levetiracetam having less side effects on cognitive functions compared to other antiepileptics may be attributed to the increase it causes in the level of Cys C, a neuroprotective agent. As a matter of fact, there are studies, in which Cys C levels were found to be decreased in patients with Alzheimer's disease (20). Findings indicating a decrease in Cys C levels in case of patients with diseases affecting cognitive functions, such as Alzheimer's disease, suggests that Cys C has a significant effect on cognitive functions.

It was reported in various studies that the risk of depressive symptoms were three times higher in individuals with renal impairment than in individuals without renal impairment (21). There are other studies, in which high serum Cys C levels and impaired kidney functions were found to be associated with depressive symptoms in older adults (22-24). In comparison, in this study, an increase was observed in Cys C levels following the levetiracetam treatment, which may explain the emergence of depressive symptoms observed in other studies available in the literature in relation to the use of levetiracetam following the treatment. Nonetheless, in this study, there were no patients with depressive symptoms both before and after the treatment.

It has been shown that creatinine levels vary depending on age, gender and body mass, unlike Cys C levels, which were not found to have been affected by these parameters (25). This result suggests that Cys C can be used with more ease and as a more reliable parameter in the follow-up of kidney functions of patients. The finding of lack of a significant difference between the post-treatment Cys C levels of the patients and the Cys

C levels of the healthy control subjects in this study supports the hypothesis that levetiracetam has no serious side effects on renal functions.

This study had some limitations, such as the relatively small number of the patients included in the study, the fact that it was based on a relatively shorter follow-up period and that different doses of levetiracetam were administered to each patient.

5. Conclusions

The fact that Cys C levels increased after treatment compared to pretreatment in epilepsy patients suggests that levetiracetam may have a neuroprotective effect. Levetiracetam, which increases Cys C levels, may have positive effects such as protecting cognitive functions and slowing down the neurodegenerative process, as well as negative effects such as increasing depressive symptoms. In order to provide more meaningful data on the relationship between changes in Cys C levels and drug doses, studies with larger numbers of patients and including higher drug doses are needed.

Limitations of the Study

The limited number of cases the lack of seizure control in all patients and the different seizures frequencies are the limitations of the study.

Acknowledgement

None

Conflict of Interests

The authors declare no conflict of interest.

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Author Contributions

Conceived and designed the analysis: F, SA. Contributed data or analysis tools: F, SA. Performed the analysis: F, SA. Wrote the paper: F, SA.

Ethical Approval

Ethics committee aproval was received fort his study from the study from the ethics committee of Atatürk University.

Data sharing statement

All data relevant to the study are included in the article.

Informed Consent

Written informed consent was obtained from all patients at the of the study.

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Hazardous Effects of Lumbar Somatosensitive Dorsal Root Ganglion Ischemia on Abdominal Skin Following Spinal Subarachnoid Hemorrhage: The First Experimental Study

Abdulkerim Olgun[1*], Mehmet Hakan Sahin[2], Mete Zeynal[2], Mehmet Kursat Karadag[2], Rabia Demirtas[3], Ozgur Caglar[4], Mehmet Dumlu Aydin[2]

[1]Department of Plastic Reconstructive and Aesthetic Surgery, Faculty of Medicine, Atatürk University, Erzurum, Turkey
[2]Department of Neurosurgery, Faculty of Medicine, Atatürk University, Erzurum, Turkey
[3]Department of Pathology, Faculty of Medicine, Atatürk University, Erzurum, Turkey
[4]Department of Pediatric Surgery, Dr. Sami Ulus Maternity and Children's Training and Research Hospital, Ankara/Turkey

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****Corresponding Author***
Abdulkerim Olgun
Department of Plastic Reconstructive and Aesthetic Surgery
Faculty of Medicine
Atatürk University
Erzurum, Turkey
Phone: +90 5335418665
E-mail: abdulkerimolgun@gmail.com

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Authors' ORCIDs
Abdulkerim Olgun
http://orcid.org/0000-0002-1493-5152
Mehmet Hakan Sahin
http://orcid.org/0000-0002-5309-4165
Mete Zeynal
http://orcid.org/0000-0002-7398-443X
Mehmet Kursat Karadag
http://orcid.org/0000-0001-9123-0597
Rabia Demirtas
http://orcid.org/0000-0001-8743-1847
Ozgur Caglar
http://orcid.org/0000-0003-4000-4308
Mehmet Dumlu Aydın
http://orcid.org/0000-0002-0383-9739

Abstract: Somatosensitive innervation of lower abdominal region skin region (LARS) is mainly managed by somatosensitive fibers of lumbar spinal dorsal root ganglions (DRG). We investigated if there is a relationship between the degenerated neuron densities (DND) of the first lumbar DRG (L1) and LARS' total tissue viability score (TVS) following subarachnoid hemorrhage (SAH). Study was conducted on 20 rabbits. All animals divided in three groups as Control group (n=5), SHAM group (n=5) and study group (n=10). For SHAM group 0,5 cc saline solution injected into lumbar spinal subarachnoid space and for study group, 0.5cc autolog blood injected into lumbar spinal subarachnoid space under general anesthesia. All animals were followed up three weeks and sacrificed. DND of L1 DRG (n/mm3) and the histopathological changes of LARS examined histopathologically. A value determined for hair loss per square millimeter (n): 1 point(P); if n<5, 2P if 10>n>5; 3P if n>10. For skin thickness as micrometer (T□m), it was scored as 1P if T>700□m, 2P if 600□m <T>400□m, and 3P if T<400□m. Evaluation was made on their total 12-points tissue viability score (TVS). If TVS>10, the skin is considered as normal; If 10<TVS>6, the moderately damaged; if TVS < 7, the was severely damaged. DND and TVS values analyzed by Mann Witney U test. The mean DND of LI DRG (n/mm3) and TVS values were determined as 13±3/11±1 in control; 34±8/8±2 in SHAM and 263±44/<6 in the study group. Statistical values were: p<0.05 (Control/SHAM), p<0.0005 (SHAM/Study) and p<0.0001 (Control/Study). Somatosensitive innervation deficiency resulting from DRG ischemia following SAH may lead to deterioration of LARS which may be important in reconstructive surgery. © 2022 NTMS.

Keywords: Abdominal Skin; Dorsal Root Ganglion Ischemia; Spinal Subarachnoid Hemorrhage.

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1. Introduction

The LARS is innervated by somatosensitive spinal nerves, sacral parasympathetic and thoracolumbar sympathetic nerves. Tissue viability of LARS depends on the normal formation of the intercostal, superior epigastric, external and internal epigastric arteries feeding these areas (1). Abdominal wall blood supply is maintained by internal thoracic, internal iliac, external iliac arteries and some branches of aorta (2). Experimental and clinical studies shown that histoanatomical characteristic of recipient and donor sites' viabilities are important for surgical outcome. Or else, tissue reject/necrosis or dehiscence in surgical area could be inevitable. Vasodilators have wound healing potential (3). It is well known that DRG neurons secrete vasodilators and play major roles on wound healing (4). SAH can lead decreased spermatogenesis (5), Hirschsprung-like diseases (6), second motor neuron degeneration (7), urinary retention (8), descending colon dilatation (9). These experiments have shown that spinal subarachnoid hemorrhages can cause cutaneous ischemia and necrosis in related dermatomes with their degenerative effects in dorsal root ganglia, in addition to chronic complications in visceral organs as well as paraparesis-paraplegia. The pearl of our study is that denervation injury cause memory loss in tissue and causes of organ or flap rejection and even dehiscence. In current plastic surgery principles, it is known that adequate vascular flow is generally considered in flap design for the area to be reconstructed. However, the neglected importance of the neural network is that it also innervates the vessels and that its neovascularization originates from the arteria nervorum rather than the arteries, and it seems to open a new horizon for all surgeons in this field.

2. Material and Methods

This experimental study was performed with 20 rabbits. Ethical approval obtained from The Ethical Committee of Ataturk University. Five rabbits (n=5) were used to determine the normal structure of the L1DRG and LARS. The remainder animals (n=15) were anaesthetized by 25 mg/kg ketamine hydrochloride, 15 mg/kg lidocain hydrochloride and 1mg/kg acepromasine combination. After required thoracolumbar cleaning, SHAM group (n=5) received 0,5 cc saline and 0.5 cc autolog blood injected into lumbar spinal subarachnoid space of study group (n=10). After followed-up three weeks, they were sacrificed under general anesthesia following intracardiac formaline injection. Their L1DRG and rots complexes at the level of L1 and LARS were removed and preserved in 10 % formalin solution for four days. Tissues were stained with hematoxylene and eosin (H&E) and analysed by Stereological and cavaliery methods. Histopathologically, condensed cytoplasmic, shrinkaged nucleus, angulated neurons and halo formation covered cytoplasm and regressed cytoplasm accepted as neuro-degeneration criteria of dorsal root ganglion neurons. Physical dissector method was used to evaluate the numbers of neurons in DRG. A value determined for deformed hair follicles numbers per square millimeter (n): 1 point(P); if n<5, 2P if 10>n>5; 3P if n>10. For skin thickness as micrometer (Tμm), it was scored as 1P if T>700 μm, 2P if 600 μm <T>400 μm, and 3P if T<400 μm. Evaluation was made on their total 12-points tissue viability score (TVS). If TVS>10, the skin is considered as normal; If 10<TVS>6, the moderately damaged; if TVS<7, the was severely damaged. DND and TVS values analyzed by Mann Witney U test.

2.1. Statistical Analysis

All values are expressed as the mean±SD. The differences between the TVS and the DND compared statistically. Mann-Whitney U nonparametric test used for statistical comparisons. Differences were considered to be significant at $p<0.05$.

3. Results

Two animals dead with surgery and SAH complications within the first week. Intestinal and bladder distenions, paraparesia, spastic or flask gait disturbances an limitations of tail movements were observed in some surviving animals of study grup. Histopathologicaly, notified severe vasospasm of Adamkiewicz artery branches and arteria nervorums, neuronal degeneration was observed in the Onuf's nucleus and dorsal root ganglia of pudendal nerves in SAH group. Macroscopical appearances of spinal cords shown subaracnhnoid hemorrhage induced spinal cord edema, clot formation in subarachnoid spaces and nerve roots in the study group.

3.1. Histopathological Results

Histopathological appearances of spinal cord and L1 DRG, DRG with normal and degenerated neurons in a SHAM and study animal (Figure 1). Histopathological appearances of spinal cord and L1 DRG with normal and degenerated neurons, spinal cord and nerve roots are seen in a study animal (Figure 2). Histopathological appearances of skiin and normal and degenerated hair follicles with normal skin in a normal, DH and thinned skin in a SHAM and many numbers of DH and very tinned and fragmented skiin in a study animal (Figure 3).

3.2 Numerical Results

The mean DND of LI DRG (n/mm3) and TVS values were determined as 13±3/11±1 in control; 34±8/8±2 in SHAM and 263±44/<6 in the study group. Statistical values were: $p<0.05$ (Control/SHAM), $p<0.0005$ (SHAM/Study) and $p<0.0001$ (Control/Study).

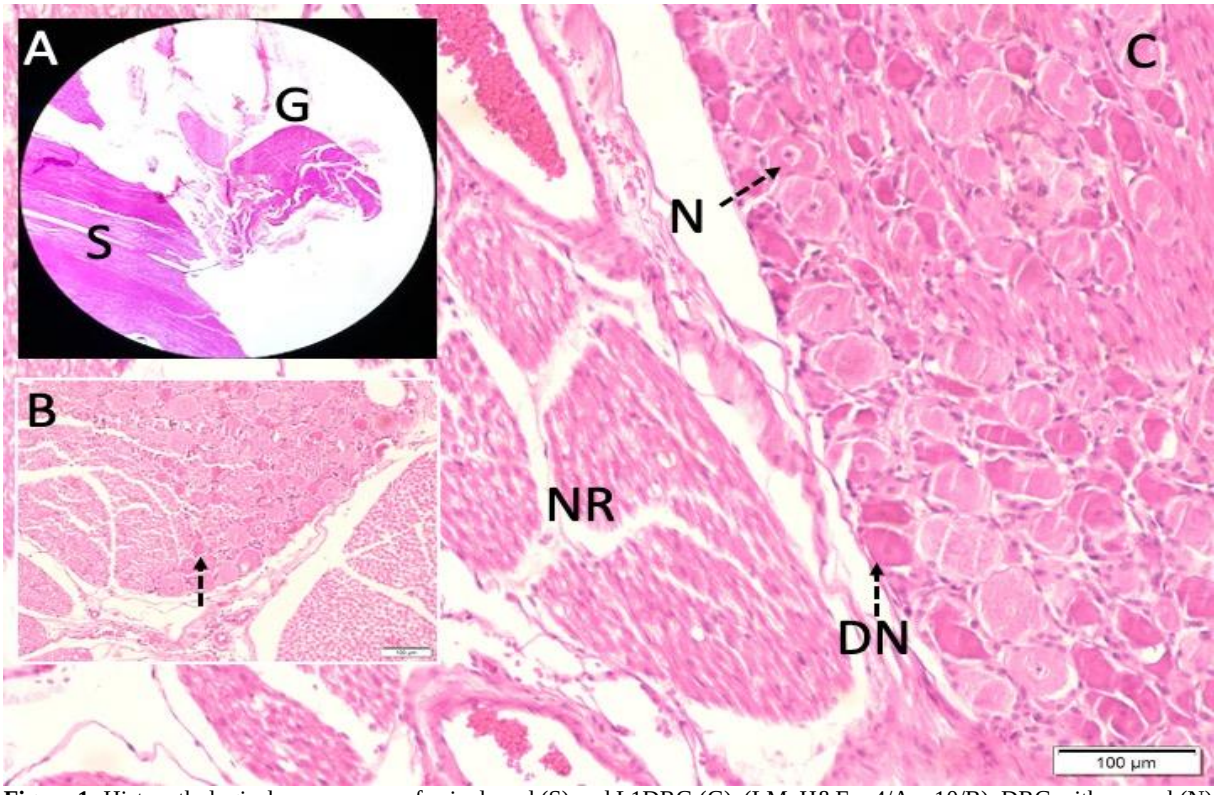

Figure 1: Histopathological appearances of spinal cord (S) and L1DRG (G) (LM, H&E, x4/A; x10/B), DRG with normal (N) and degenerated neurons (DN) in a SHAM (A,B) and study animal (C) are seen.

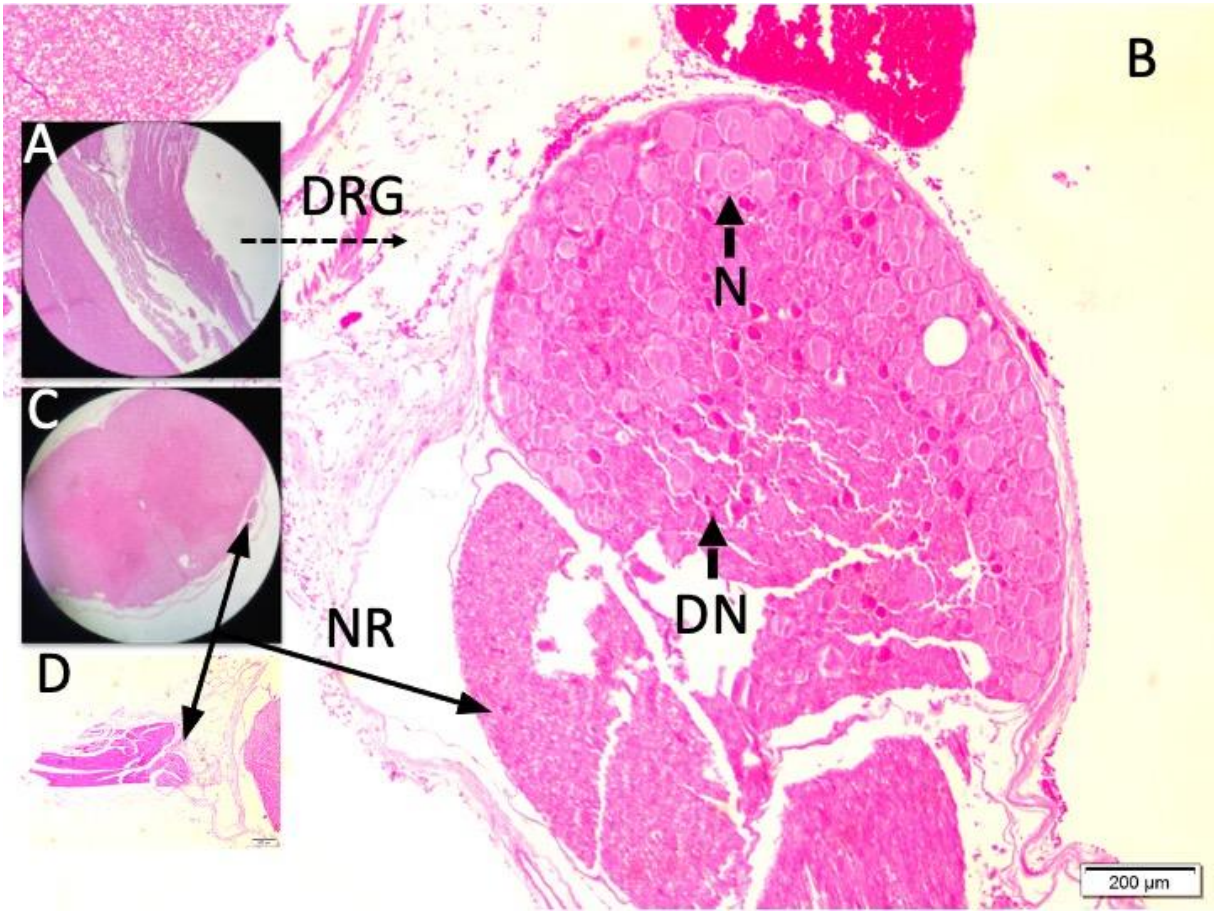

Figure 2: Histopathological appearances of spinal cord and L1DRG with normal (N) and degenerated neurons (DN) (LM, H&E, x4/B), spinal cord (LM, H&E, x4/C) and nerve roots are seen in a study animal are seen (LM, H&E, x4/B,C.D).

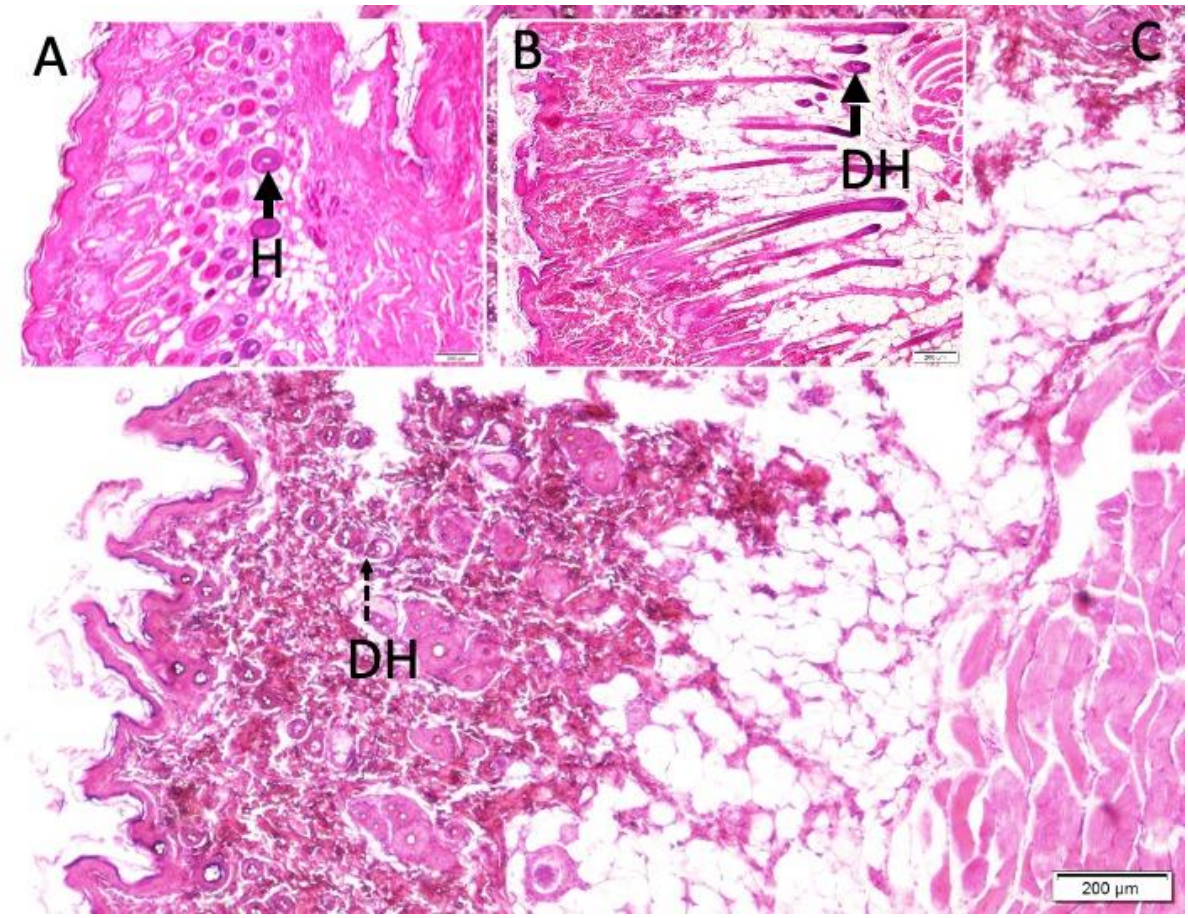

Figure 3: Histopathological appearances of skiin and normal (H) and degenerated hair follicles (DH) with normal skiin in a normal (A), DH and thinned skiin in a SHAM (B) and many numbers of DH and very tinned and fragmented skiin in a study animal (LM, H&E, x20).

4. Discussion

The vitality of the histological units in the abdominal skin and surrounding organs are related to the somatosensitive spinal nerves innervating these areas, the parasympathetic fibers originating from Onuf's nucleus and thoracolumbar sympathetic nerves. It also depends on the normal circulation of the intercostal, superior epigastric, external and internal epigastric arteries of LARS. Any damage of neurovascular network impairs the health of the abdominal structures. Especially neurovascular network diasabilities lead to poor prognosis following reconstructive surgical interventions. Here, we examined the neurodegenerative changes in the L1 root of the lumbar somatosensitive nerve network induced by spinal SAH and the microanatomical changes in the LARS innervated by this roots.

4.1. Innervation of Lower Abdominal Skin

Lower abdomen is inervated by L1-S1 spinal nerve roots, sacral parasympathetics and abdominopelvic sympathetic nerves (1). It has been reported that examining of afferent cutaneal sensory discharges are important indicator for tissue strength and viability. Because tissue sensation measurement is required for not only donor site but also recipient site preoperatively. Altough doppler USG examination is essential for determination of tissue viability, but it not adequately be alone. It should not be forgotten that neurophysiological studies of related nerves certainly required (10).

Abdominal wall weakness occurs due to decreased strength of muscles (11). So, DRG degeneration may be rely on decreased strength of muscles. Cutaneous microcirculatory regulation is maintained by vibration sensing mechanoreceptors innervated by somatosensitive fibers arisig from segmental DRG (12). Because the skin innervating nerves of abdominal wall travel around of epigastric arteries; injury of that neurovascular tree associated with hair loss (1). For obtaining of excellent result, neural-vascular and related tissues should be transferred together (13).

4.2. Arteries of The Lower Abdominal Skin

Abdominal wall blood supply is maintained by internal iliac artery and it’s branches such as umbilical artery, ductus deferentis artery, superior vesical artey, prostatic artery, superior gluteal artery, iliolumbal

artery, obturatory artery and inferior gluteal artery. The other main arterial tree is external iliac artery and it's branches such as inferior epigastric artery and deep circumflex iliac artery and superior epigastric artery branch of internal thoracic artery (2).

Epidemiological studies have suggested that histoanatomical architectures of recipient and donor site-supplying arteries should be normal range (14). Or else, tissue reject/necrosis could be inevitable. Nitric oxide-like vasodilators producing nerves have wound healing potential (3). It is well known that DRG neurons secrete copious amounts vasodilators. For example, nitric oxide is a vasodilatory neurotransmitter in the mesenteric artery (4).

It is known that long vein grafts used to maintain flow continuity in the flap pedicle will have negative consequences on flap viability (15). In our opinion, although long vein grafts are considered appropriate in terms of vascular anatomy, segmental neural innervation of such a long flap will be unsuccessful due to insufficient innervation because it is made from more than one root. As a new idea, we can say that although the hardware of the vascular structures are in-house, the software comes from the neural tissues. In summary, a graft tissue that is used without its neural components has a memory loss due to neural software insufficiency. As a team, we have studies on this subject that are still in the publication stage. Memory loss in tissue may be one of the most important causes of organ or flap rejection and even dehiscence.

4.3. Spinal SAH Induced Somatovisceral Disorders

Lumbosacral pathologies can lead to infertility due to decreased sperm number following spinal SAH induced by testiculary artey spasm due to parasympathetic innervation deficiency (5). Onuf's nucleus insults could rely on Hirschsprung-like diseases and vascular insufficiency (6). Gray matter ischemia may lead to second motor neron degeneration (7). Adamkiewicz artery spasm lead to dorsal root ganglion degeneration (16), Onuf's nucleus-pudendal nerve ganglia insult and urinary retention (8), descending colon dilatation (9). In summary, the sacral parasympathetics, the parasympathetic innervaters of the arteries supplying the lower abdomen, arise from Onuf's nucleus and supply the Adamkiewicz artery. Ischemic damage to the Adamkiewicz artery after SAH invites all the somatic and autonomic pathologies mentioned in this section.

5. Conclusion

These experiments have revealed the importance of DRGs in maintaining skin viability, which has not received much attention in clinical practice. The spinal cord takes the necessary precautions about the source that sends the signal by transmitting the decisions it makes about the skin according to the mechanosensitive signals it receives from the skin through DRGs, both to the skin and to the entire nervous system. Interruption of this information in pathological conditions may be an overlooked cause of non-healing wounds and decubitus ulcers.

Future Insight

DRGs, each containing neurons, a neurochip yet to be identified, are actually a miniature memory store that records signals from tissue. Damage to these neurochips may impair tissue memory, leading to a loss of identity in the tissue. The solution of this extremely dangerous darkness with digital logic may herald great revolutions that cannot be imagined yet in the illumination of unknown medical problems, especially the rejection process seen in reconstruction and transplantation surgery.

Limitations of the Study

This study doesn't include electrophysiological studies.

Acknowledgement

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Conflict of Interests

There is no conflict of interest.

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Author Contributions

Conceived and designed the analysis: AO, MHS, MZ, MKK, RD, OC, MDA Collected the data: AO, MHS, MZ, RD. Contributed data or analysis tools: AO, MHS, MKK, RD, MDA. Performed the analysis: OEY, YK. Wrote the paper: AO, MHS, MZ, MKK, RD, OC, MDA

Ethical Approval

Ethics committee approval was received for this study from the ethics committee of Atatürk University

Data sharing statement

All data relevant to the study are included in the article.

Consent to participate

None

Informed Consent

None

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The Impact of the Covid-19 Pandemic on Medical Students' Attitudes of Professionalism: A Cross-Sectional Study

Esra Çınar Tanrıverdi[1*], Erol Gürpınar[2]

[1]Department of Medical Education, Faculty of Medicine, Atatürk University, Erzurum, Turkey
[2]Department of Medical Education, Faculty of Medicine, Akdeniz University, Antalya, Turkey

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****Corresponding Author***
Esra Çınar Tanrıverdi
Department of Medical Education
Faculty of Medicine
Atatürk University
Erzurum, Turkey
Phone: +90 533323347
E-mail: esracinart@yahoo.com

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Authors' ORCIDs
Esra Çınar Tanrıverdi
http://orcid.org/0000-0001-8857-3986
Erol Gürpınar
http://orcid.org/0000-0002-5544-7040

Abstract: In this study, it is aimed to investigate the effect of the pandemic on the professionalism attitudes of medical students. This cross-sectional study was conducted with 329 medical students. The data was collected with sociodemographic information form and Pennsylvania State University Faculty of Medicine Professionalism Attitude Scale. Data were collected via an online survey. SPSS 20 package program was used for statistical analysis. The statistical significance level was $p<0.05$. The mean age of the students was 21.32±2.21, and 50.5 % (n=166) were female. Professionalism attitude scores were 30.36±4.26 for accountability, 25.84±3.53 for enrichment, 18.23±2.39 for honor and integrity 36.39±4.21, for altruism 13.04±1.94, for assignment 22.23±2.87 and for respect dimension 8.95±1.34. Compared to the pre-pandemic period, there was a significant increase in enrichment scores ($p<0.001$). There was no change in the other dimension scores ($p>0.05$). Gender, program, and presence of a doctor in the family had no significant effect on attitude scores ($p>0.05$). Students who stated that they preferred medical school because they had an ideal/dream and to help people had significantly higher attitude scores than students who preferred it for other reasons ($p<0.001$). The attitudes of the third-year medical students towards medical professionalism are positive and the attitude scores are satisfactory. Enrichment scores increased significantly during the Covid-19 pandemic period compared to the pre-pandemic period. © 2022 NTMS.

Keywords: Medical Education; Medical Student; Medical Professionalism; Atitude; COVID-19.

1. Introduction

Medical professionalism is the whole of the attitudes and behaviors that form the basis of the public's trust in doctors (1, 2). It is a very broad and inclusive concept and is a collection of good qualities (3, 4).

Leading organizations engaged in medical education have listed the key elements of professionalism. These are sacrifice, respect, compassion, honesty, accountability, duty, honor, commitment, and respect for others. The well-being of the patient, autonomy, and social justice are the basic principles of professionalism (5-7). Professionalism requires that the interests of the patient be held above the physician's own interests (5). Medical professionalism is one of the graduate qualifications and accreditation criteria of medical

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faculties (8). In our country, it has been determined as an accreditation standard by the National Medical Education Accreditation Board (UTEAK). Professionalism is also one of the physician competencies (9).

In the COVID-19 Pandemic, education has been one of the most affected areas along with health. Since the date of the first COVID-19 case in Turkey (11.03.2020), education at Atatürk University Faculty of Medicine has been completely suspended as it is throughout the country. Then rapidly transitioned to online education (10, 11). In the pre-clinical phase, theoretical courses, laboratory applications, clinical and communication skills training, and exams were carried out entirely on online platforms (12). At the time of the study, students had been studying online for three semesters.

The pandemic has brought several dilemmas with it in health practices. All over the world, practices such as mask and distance applications, restrictions, and quarantines were made to prevent the spread of the disease. In the fight against this new and unknown disease, physicians from every branch were assigned to COVID-19 services. Especially in the early stages of the pandemic, some of the health care providers had to live apart from their homes and families. The health workers' permits were revoked, and their resignations were not accepted.

The COVID-19 pandemic has been one of the periods when altruism and altruistic behaviors are best felt. Doctors cared for patients at risk of transmission of the infection (13). It was the doctors who felt ill and lost their lives. Especially in the early stages of the outbreak, outpatient clinic services other than emergencies were canceled. To cope with the number of patients, all hospitals were converted into pandemic hospitals, elective surgeries were postponed. Later, some of the hospitals were converted into pandemic hospitals, while others accepted routine patients. This situation has caused an inexorable intensity in hospitals that do not have pandemic hospitals, as well as in pandemic hospitals.

While these negative conditions are hand-in-hand, it has been seen that there are very important developments in ethical behavior, empathy and professional approach to patients, solidarity between colleagues, and selfless professional tasks. In the process, it was necessary to refocus on the importance of professional values and professional behaviors and to rebuild the importance of values such as autonomy, non-harm, and social justice (14).

The pandemic has been seen to increase cooperation and solidarity between doctors and health workers. Thus, the conditions created by the pandemic are seen as an opportunity to rebuild professional values. Doctors and scientists from all over the world have struggled to develop vaccines and medicines against COVID-19 while fighting the disease. The development of many COVID-19 vaccines at the same time is an example of this cooperation process (15).

During the pandemic, doctors faced both positive and negative discrimination. While they applauded doctors and other health workers to show their respect and gratitude, on the other hand, they also exhibited stigmatizing and discriminatory behaviors such as trying to stay away from health workers in public areas such as markets and elevators for the fear that they could carry and transmit diseases (16, 17).

There has been some controversy regarding health service delivery. The large number of patients and the limited number of intensive care beds and respiratory support devices led to speculations that priority patient applications (such as giving priority to young and vaccinated patients) will be made in intensive care units (18). Inequalities in access to medicines and vaccines in various parts of the world were highlighted (19). Especially in the early stages of the pandemic, there has been extensive debate on issues such as personal protective equipment indications and triage, due to the lack of an effective vaccine and drug and the unknowns about the disease (14).

Medical education was also seriously affected by this process. The move of education to the online platform has been criticized for causing inequalities of opportunity in terms of technological devices or internet access in terms of connecting to online courses (20).

It is likely that all of this has affected the attitudes of professionalism of medical students, who are the doctors of the future. In recent years, it is seen that interest in research on professionalism has increased. However, these are more concerned with students' perceptions and opinions towards professionalism (21-23). Some researchs are aimed to develop and adapt scales to assess professionalism (24, 25).

During the pandemic period, we could not reach a study investigating the professionalism attitudes of medical students. In this study, it is aimed to investigate the effect of the COVID-19 pandemic on the professionalism attitudes of medical students.

2. Material and Methods

2.1. Ethical Consent

Ethical permission for the study has been obtained from Atatürk University Faculty of Medicine Clinical Research Ethics Committee (Date:28.04.2021 Number:187). The research was conducted in accordance with the principles of the Helsinki Declaration. The informed consent of the participants was obtained.

2.2. Study Design, Universe and Sample

This study is a cross-sectional study, conducted with Atatürk University Faculty of Medicine third- year students.

2.3. Training Program

Professionalism is one of the graduate qualifications of Atatürk University Faculty of Medicine. During the clinical period, there are practices for professionalism such as the white coat ceremony, good medical practices, community-based medical practices and social sensitivity projects, access to information, evidence-based medicine, self-life and lifelong learning, continuous professional development, hospital orientations, medical history and ethics courses, communication skills, simulated patient practices.

2.4. Participants

In the 2020-2021 academic year, 365 students studying in the third-grade were included in the study. Students were informed about the purpose and scope of the study and invited to participate in the survey. Since it was aimed to reach all of the students, the sample was not calculated, and all of the students who were studying and volunteering in the third-grade were included in the study. Thirty-six of the students did not return the call to participate. The data of 329 students who voluntarily participated in the survey and filled out the form completely were evaluated. Thus, 90 % of the universe has been reached. In order to assess the effects of the COVID-19 pandemic on students' attitudes, the results were compared with the results of another study conducted with third-year students of Atatürk University Faculty of Medicine in the pre- COVID period (26).

The data were collected between 01.06.2021-15.06.2021 through an online questionnaire. Due to pandemic conditions, there were no face-to-face meetings with the students, and no printed materials were used. A questionnaire form was created by the researchers by using the literature through Google forms. Through the Class Whatsapp group, students were informed about the purpose and scope of the study and invited to participate. Then the link of questionnaire was shared with the students. The first question of the survey was written as "I voluntarily agree to participate in the study". Those who did not consent to this statement could not answer the other questions. Thus, the online consent of the participants was obtained. In the questionnaire, the students were not asked any questions that indicates their identity. The data was collected anonymously. Each student was given the right to take the survey only once. Students were given two weeks to respond to the questionnaire, during the time in which they were sent three reminders. The questionnaire took about 10-15 minutes to answer. Students who did not volunteer and did not study in the third year were excluded from the study.

2.5. Data Collection Tools

The questionnaire was created in two parts. In the first part, sociodemographic questions such as age, gender, nationality, medical program they studied, the reason for preferring medical school, whether there was a loss of years in medical school were included. The second part was the Pennsylvania State University School of Medicine Professionalism Scale-Student Form (PSCOM-SF).

2.5.1. Pennsylvania State University School of Medicine Professionalism Scale-Student Form

It is a scale developed by the Pennsylvania State University School of Medicine (2007) to evaluate the professionalism attitudes of medical students. There are a total of 36 items in 7 dimensions on the scale. The scale dimensions are: 1) Accountability, 2) Enrichment, 3) Equity, 4) Honor and Integrity, 5) Altruism, 6) Duty and 7) Respect. The internal consistency of the scale was found to be 0.51-0.78 (24). The adaptation of the scale to Turkish was made by Demirören et al. (2015) and the internal consistency was found to be 0.46-0.76 (25).

In the current study, the internal consistency of the scale was found to be between 0.69-0.90.

The answering and scoring of the scale is answered according to the 5-point likert system: Never (1), a little (2), sometimes (3), often (4), mostly (5). There is no reverse scored item in the scale. By summing the scores of the items in each dimension, the score for that dimension is obtained. The total attitude score can be obtained by also summing the scores of all dimensions. The higher the score, the more positive the professional attitudes are considered.

2.6. Statistical Analysis

The data were analyzed in SPSS 20.0 (SPSS Inc., Chicago, IL, USA) statistical program. The suitability of the data to the normal distribution was checked by Kolmogorov Smirnov test. Categorical variables were presented as numbers and percentages, numerical variables were presented as mean, standard deviation, median, minimum, maximum. Mann-Whitney U test was used to compare the data that did not fit the normal distribution. Cronbach's alpha value was used for the internal consistency of the scale. The statistical significance level was accepted as $p<0.05$.

3. Results

3.1. Sociodemographic Characteristics

The average age of the students was 21.32±2.21, and 50.5 % were female. The sociodemographic characteristics of the students are presented in Table 1.

Table 1: Sociodemographic characteristics of students.

Variable		Count (n)	Percent (%)
Sex	Female	166	50.5
	Male	163	49.5
Program	Turkish medicine	248	75.4
	English medicine	81	24.6
Reasons to Preference Medical School	İdeal, the desire to help people	203	61.7
	Family/teacher referral	61	18.5
	Dignity	32	9.7
	The impact of role models	5	1.5
	Ekonomic	28	8.5
The Presence of a Doctor in the Family (First Degree Relatives)	Yes	49	14.9
	No	280	85.1

3.2. Professionalism Attitude Scores

The average scores taken by the students for each scale item was at least four or above four over five. Students' professionalism attitude scores are given in Table 2.

3.3. Comparison of Attitude Scores

A comparison of students' professionalism attitude scores according to various variables is given in Table 3. There was no significant difference in attitude scores according to gender, medical program, and presence of a doctor in the family ($p>0.05$). According to the reasons for preferring the faculty, there was a significant difference in attitude scores. Students who chose medical school because it was their ideal, and to help people had significantly higher scores than those who chose the medical school for other reasons ($p<0.001$ for all dimensions). There was no statistically significant difference between the attitude scores of students with and without year loss in the medical school except for the respect dimension ($p>0.05$). The respect dimension scores of the students who had lost a year because they failed the class were significantly lower than the students who did not failed. ($p<0.05$, Table 3)**.**

3.4. Comparison of Attitude Scores with Pre-Covid-19 Period

While there was a significant increase in the enrichment dimension ($p<0.001$), there was no significant change in the other dimensions scores ($p>0.05$, Table 4).

Table 2: The professionalism attitude scores of the students.

Scale dimensions	Mean±SD	Median (min-maks)	Cronbach alpha
Accountability	30.36±4.26	31.00 (10-35)	0.90
Enrichment	25.84±3.53	26.00 (13-30)	0.87
Equity	18.23±2.39	19.00 (6-20)	0.89
Honor and integrity	36.39±4.21	38.00 (16-40)	0.89
Altruism	13.04±1.94	13.00 (5-15)	0.83
Duty	22.23±2.87	23.00 (8-25)	0.86
Respect	8.95±1.34	10.00 (4-10)	0.69

Table 3: Comparison of students' professionalism attitude scores according to various variables.

Sex	**Female**		**Male**			
	Mean±SD	Median (Min-Max)	Mean±SD	Median (Min-Max)	Z	p
Accountability	30.67±3.68	31 (14-35)	30.04±4.78	31 (10-13)	-.757	.449
Enrichment	25.86±3.40	26 (15-30)	25.82±3.66	26 (13-30)	-.088	.930
Equity	18.3 ±2.31	20 (10-20)	18.14±2.47	19 (6-20)	-.929	.353
Honor and Integrity	36.41 ±4.20	38 (22-40)	36.37±4.24	37 (16-40)	-.352	.725
Altruism	12.98 ±1.81	13 (8-15)	13.10±2.06	14 (5-15)	-1,224	.221
Duty	22.37±2.70	23 (15-25)	22.09±3.03	23 (8-25)	-.740	.459
Respect	9.05 ±1.23	10 (5-10)	8.85±1.45	9 (4-10)	-1.042	.297
Program	**Turkish Medicine**		**English Medicine**			
	Mean±SD	Median (Min-Max)	Mean±SD	Median (Min-Max)	Z	p
Accountability	30.47±3.95	31 (10-35)	30.04±5.11	31 (12-35)	-.072	.943
Enrichment	25.88±3.47	26 (13-30)	25.70±3.70	26 (17-30)	-.297	.766
Equity	18.25±2.35	19 (6-20)	18.17±2.52	20 (10-20)	-.102	.919
Honor and Integrity	36.46±4.03	38 (16-40)	36.17±4.76	38 (22-40)	-.367	.714
Altruism	13.02±1.92	13 (5-15)	13.09±1.99	13 (7-15)	-.510	.610
Duty	22.22±2.88	23 (8-25)	22.26±2.86	23 (15-25)	-.195	.845
Respect	8.98±1.33	10 (4-10)	8.85±1.40	9 (5-10)	-.680	.497
Reason for preference	**Ideal/Request for Help to People**		**Other**			
	Mean±SD	Median (Min-Max)	Mean±SD	Median (Min-Max)	Z	p
Accountability	31.06±3.95	32 (12-35)	29.23±4.50	29 (10-35)	-4.057	**.000**
Enrichment	26,48±3.30	27 (15-30)	24.79±3.64	25 (13-30)	-4.070	**.000**
Equity	18.68±1.98	20 (10-20)	17.50±2.79	18 (6-20)	-4.414	**.000**
Honor and Integrity	37.06±3.55	38 (22-40)	35.30±4.93	36 (16-40)	-3.507	**.000**
Altruism	13.42±1.73	14 (9-15)	12.43±2.09	12 (5-15)	-4.408	**.000**
Duty	22.79±2.57	24 (12-25)	21.33±3.10	22 (8-25)	-4.647	**.000**
Respect	9.28±1.05	10 (5-10)	8.42±1.58	9 (4-10)	-5.296	**.000**
Doctor in the Family	**Yes**		**No**			
	Mean±SD	Median (Min-Max)	Mean±SD	Median (Min-Max)	Z	p
Accountability	29.65±5.08	30 (12-35)	30.49±4.10	31 (10-35)	-.810	.418
Enrichment	25.76±3.38	26(17-30)	25.85±3.56	26 (13-30)	-.267	.790
Equity	18.06±2.60	19 (10-20)	18.26±2.36	19 (6-20)	-.270	.787
Honor and Integrity	36.55±4.47	38 (22-40)	36.36±4.17	37 (16-40)	-.798	.425
Altruism	13.18±2.01	14 (8-15)	13.01±1.93	13 (5-15)	-.819	.413
Duty	22.24±3.09	23 (14-25)	22.23±2.83	23 (8-25)	-.422	.673
Respect	9.06±1.30	10 (5-10)	8.93±1.35	9 (4-10)	-.686	.493
Fail in Class	**Yes**		**No**			
	Mean±SD	Median (Min-Max)	Mean±SD	Median (Min-Max)	Z	p
Accountability	28.82±5.40	29 (12-35)	30.57±4.05	31 (10-35)	-1.805	.071
Enrichment	25.46±4.28	26 (17-30)	25.89±3.42	26 (13-30)	-.309	.757
Equity	17.92±2.82	19 (10-20)	18.27±2.33	19 (6-20)	-.282	.778
Honor and Integrity	35.72±5.17	37 (24-40)	36.48±4.07	38 (16-40)	-.244	.807
Altruism	12.87±2.19	13 (8-15)	13.06±1.90	13 (5-15)	-.290	.772
Duty	22.08±3.26	23 (15-25)	22.25±2.82	23 (8-25)	-.116	.908
Respect	8.51±1.55	9 (5-10)	9.01±1.30	9 (4-10)	-1.982	**.047**

Table 4: Comparison of students' professionalism attitude scores before COVID-19 and during the COVID-19 pandemic period.

Scale Dimensions	Before COVID-19 Mean ±SD	During COVID-19 Mean ±SD	t	p
Accountability	30.29 ± 4.19	30,36±4,26	0.212	0.831
Enrichment	24.88 ± 3.78	25,84±3,53	3.371	**0.000**
Equity	18.26 ± 2.12	18,23±2,39	0.170	0.864
Honor and Integrity	36.37 ± 3.87	36,39±4,21	0.063	0.949
Altruism	13.11 ±1.90	13,04±1,94	0.639	0.070
Duty	21.92 ± 2.80	22,23±2,87	1,404	0.160
Respect	9.05 ± 1.25	8,95±1,34	0.99	0.321

SD Standard Deviation.

4. Discussion

The results of this study, which was conducted to investigate the impact of the COVID -19 pandemic on the professional attitudes of medical students, showed a significant increase in enrichment dimension scores. To the best of our knowledge, this study is the first to investigate medical students' attitudes towards professionalism during the COVID-19 pandemic.

Health and education are among the areas most affected by the COVID -19 pandemic. It has often been brought up that the pandemic causes inequalities in both areas. Inequalities in access to medicines and vaccines among the countries of the world, in the utilization of health care systems, in education are some of them.

During the pandemic period, various factors such as doctors taking care of patients, the risk of getting sick or even death while healing patients, fears of infecting their families, and the need to work collaboratively with colleagues and team members have brought professional values to the forefront more than ever.

There have been many dilemmas, the doctors' own physical and mental health has also been affected by the situation, and those who have fallen ill or even lost their lives. While all this was happening, the medical school students followed the situation as the doctors of the future and were naturally affected. Studies have reported that in the COVID-19 pandemic, medical students question their branch preferences and even their career preferences (27, 28).

The COVID-19 pandemic has been one of the periods when doctors and other health workers have been collaborating the most. Humane values such as social benefit, prioritizing patient benefit, altruism, altruism, compassionate care, compassion, empathy, which are at the core of professionalism, have come to the fore. Especially at the beginning of the pandemic, due to the fact that there is a lot of information about the disease, the responsibility of the necessary care and attention to the patients has been completely undertaken by health professionals instead of family members or relatives. A scientific committee was formed by the Ministry of Health to regularly assess the situation and inform the public. In the fight against the disease, initially all health units were transformed into COVID-19 units, and physicians, regardless of specialty, are assigned to COVID-19 services. Health workers have carried out their duties extremely selflessly and have been appreciated by society all over the world. Medical students have witnessed this whole process mentioned. During the pandemic period, professional trainings could not be held face-to-face. Although we predicted that this situation may have led to a decrease in the attitude scores, our results did not confirm it. Physician roles in the community may also have had a positive impact during the pandemic. On the other hand, it can be considered that online education can be as successful as face-to-face education. All of these situations may have had an impact on our research results. Further research to understand the impact of each factor would be beneficial.

The results of our research have shown that students' attitudes towards professionalism are positive. The average scores for all items of the country are close to the highest possible scores. Apart from the study in which the comparison was made, three studies were conducted in our country in the pre-pandemic period, evaluating the professional attitudes of medical students and using PSCOM-SF (25, 29, 30). One of them is the adaptation of the PSCOM-SF scale into Turkish by Demirören et al. In this study, students' attitude scores were found to be between 5.32-19.64 (25). The second study is the study conducted by Şenol et al. (2016-2017) with medical students at Akdeniz University. In this study, students' attitude scores were found to be between 5.5-19.6 (30). In the third study which is conducted by Demirören and Şenol with the students of two medical faculties (Ankara and Akdeniz), attitude scores were found between 6.35-23.57 (29). The scores obtained in a study conducted by Akhund et al. with medical students in Pakistan are similar to the results of a study conducted in our country (21). The scores obtained in our study were between 8.9-30.3 and were higher than the scores in the mentioned studies. In addition to the fact that there is a complex structure of professionalism, past experiences, personal and environmental factors such as society and culture, the difference in education programs may also have been effective in this difference (24, 31). The fact

that the attitude scores were higher in our research and that the clinical semester students were not included in the study may have also had an effect.

In the current study, it was seen that the enrichment dimension scores increased significantly compared to the pre-pandemic period results. During the pandemic period, health workers all over the world acted together in the fight against the disease, and the interests of society were prioritized. On the other hand, vaccine and drug development studies were continued. We think that enrichment dimension scores may have been increased due to the cooperation between colleagues and interprofessional and working together for the same purpose during the COVID-19 period.

There was no significant change in the scores of the other dimensions. The high professionalism attitude scores of our students may also have been effective in these results. It was also pleasing that there was no decrease in the attitude scores of the students during the process.

In our study, there was no significant difference between the sexes. Some studies have reported that women's attitude scores are higher (25, 26, 30).

Most of the students did not have a doctor in their families. Although we thought that students with a doctor in their family might be more familiar with professional values and have higher professionalism attitude scores, our study results did not confirm this. There was no significant difference between the attitude scores of the students who had a doctor in their family and those who did not.

While not the focus of the research, our results have shown that preference for medical school has an impact on student's attitudes toward professionalism. The professionalism attitude scores of the students who choice because they had dreams and ideals, and with the desire to help people were found to be significantly higher than the students who choice for other reasons such as economic return, respectability, family and teacher orientation. Another study with third-graders before the pandemic shown similar results (26). These findings suggest that conscious medical career preference has a significant predictive effect on medical professionalism. Medical education is a long and challenging process. The profession of medicine requires dedication and lifelong learning. Therefore, intentional and conscious choices are very important. The high number of students who willingly made choices in our research group may have had an impact on the high professionalism attitude scores.

Similar to the respect dimension, some students score low on all dimensions of the scale. Professionalism is a set of values that develop over time. To be scalitized and have positive attitudes, these students with low scores should pay more attention and effort, and the attitudes related to professionalism should be evaluated. Although each student has some attitudes stemming from their previous experiences, it will be useful to start teaching professionalism in medical education at the earliest stage, to monitor and evaluate it regularly.

Our research results show that although face-to-face education could not be carried out during the pandemic period, student's attitudes toward professionalism did not decrease, and even there was a significant increase in the enrichment dimension

In conclusion, these findings suggest the following possibilities: 1). Exemplary doctor role models may have had a positive impact in the fight against COVID-19, 2). Online classes may have been as effective as face-to-face classes in professionalism education, 3). During the pandemic process, the perception of physician identity in the society and the professionalism attitudes of the students may have been strengthened. However, there is insufficient evidence for these assumptions.

5. Conclusion

We think that it would be beneficial to plan professional training to include online programs for possible pandemics. Given that medical professionalism is an area of competence, it can be said that more comprehensive research is needed, including regular follow-up of students and comparing the effects of time and training models.

Limitations of the Study

Our study has some limitations. First, the study is a cross-sectional study conducted with third-year students of a single medical school. Therefore, it is not possible to generalize the results for medical students. Secondly, the measurement tool used is a self-assessment tool and the results are based on the self-reports of the students. Finally, it should be considered that although there is a relationship between attitudes and behaviors, it may not always be consistent. A single assessment of students' attitudes toward their professionalism traits will not confirm that these attitudes will translate into behaviors in the future. The strength of our study is that it is the first study conducted in our country to evaluate the effects of the COVID-19 pandemic on the professionalism attitudes of medical students.

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Conflict of Interests

The authors declared no conflict of interest.

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Author Contributions

ECT and EG designed the research. ECT participated in data collection. ECT and EG wrote the manuscript, read and approved the final script.

Ethical Approval

Ethical permission for the study has been obtained from Atatürk University Faculty of Medicine Clinical Research Ethics Committee (Date:28.04.2021 Number:187). The research was conducted in accordance with the principles of the Helsinki Declaration.

Data sharing statement

None.

Consent to participate

Informed consent was obtained from all participants.

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The Effect of the Port Numbers Used in Video-assisted Thoracoscopic Surgery on the Success of Ultrasound-Guided Erector Spinae Plane Block; A Single Center Retrospective Study

Gokhan Sertcakacilar[1,2*], Gunes Ozlem Yildiz[2], İpek Bostancı[2], Zafer Cukurova[2], Gülsüm Oya Hergünsel[2]

[1]Department of Outcomes Research, Anesthesiology Institute, Cleveland Clinic, Cleveland, OH, USA
[2]Department of Anesthesiology and Reanimation, University of Health Science, Bakırköy Dr. Sadi Konuk Education and Research Hospital, Istanbul, Turkey

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[*]***Corresponding Author***
Gokhan Sertcakacilar
Department of Outcomes Research
Anesthesiology Institute
Cleveland Clinic
Cleveland, OH, USA
Phone: +90 5053130818
E-mail: drgokhansertcakacilar@gmail.com

DOI: 10.56766/ntms.1143709

Authors' ORCIDs
Gokhan Sertcakacilar
http://orcid.org/0000-0002-4574-0147
Gunes Ozlem Yildiz
http://orcid.org/0000-0002-4557-9517
İpek Bostancı
http://orcid.org/0000-0001-9766-453X
Zafer Cukurova
http://orcid.org/0000-0002-8893-3977
Gülsüm Oya Hergünsel
http://orcid.org/0000-0003-3218-0029

Abstract: Video-assisted thoracoscopic surgery (VATS) is a minimally invasive procedure with lower pain scores and less morbidity than thoracotomy. However, it is necessary to provide adequate pain control in VATS. For this purpose, blocking visceral and somatic nerve fibers results in successful pain management. This retrospective study evaluated the effect of using different numbers of ports on the success of erector spinae plane block (ESPB) for postoperative analgesia management in patients undergoing VATS. We conducted a retrospective, single-center study between Sep 2020 and Aug 2021. According to the number of ports used, seventy-three patients were assigned to three groups (single port, dual port, and three-port). Preoperative ultrasound-guided ESPB was performed on all patients. The primary outcome was the numerical rating scale (NRS) score assessed for pain severity at different periods after surgery. The secondary outcome was the postoperative cumulative opioid consumption, and the exploratory outcomes were to determine the length of hospital stay and the incidence of opioid-related side effects. Static and dynamic NRS scores were significantly higher in the three-port group in the first 12-hour postoperative period after admission to the post-anesthesia care unit ($p<0.05$). After the first 12-hour period after surgery, static and dynamic NRS scores did not differ significantly between the groups ($p=0.158$ and $p=0.125$, respectively). Cumulative opioid consumption in the first 24 hours postoperatively was considerably higher in the three-port group than in the other groups (33.04±21.35 mg, $p=0.001$). Rescue analgesia consumption was similar between all groups ($p=0.341$). Preoperative single-shot ESPB injection may be associated with better analgesia and less opioid consumption in the first 12 hours postoperatively in patients undergoing single or two-port VATS compared to the multi-port technique. © 2022 NTMS.

Keywords: Video-Assisted Thoracoscopic Surgery; Erector Spinae Plane Block; Multiport; Single Port; Ultrasound.

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1. Introduction

Video-assisted thoracoscopic surgery (VATS) is increasingly used for primary lung cancer surgery and helps to reduce postoperative pain (1, 2). VATS is also accepted as a standard technique for many types of lung surgery. The main advantages of the procedure can be summarized as reducing the postoperative pain and incidence of pulmonary dysfunction, shorter chest tube duration, and reduced hospital stay (3). However, it is a fact that the pain can be severe and long-lasting after VATS. Therefore, it is essential to apply multimodal analgesic methods in postoperative pain control. Rocco et al. first reported single-port video-assisted thoracoscopic surgery in 2004 after wedge resection (4). Studies have reported that the single-port VATS technique results in less postoperative pain. However, the data obtained belong to the studies that compared the surgical technique regardless of the analgesic method applied. No study in the literature compares the success of thoracic wall blocks according to the number of ports.

Different drugs and doses have been studied and defined for regional analgesia methods, patient-controlled analgesia, and regional techniques, regardless of the number of ports in studies conducted regarding analgesic efficacy after VATS. Thoracic epidural analgesia (TEA) is a classic regional method with proven effectiveness in reducing postoperative pain following VATS surgery (5). Similarly, the thoracic paravertebral block (TPVB) is the first described chest wall block widely used in thoracic surgery (6, 7). Erector spinae plane block (ESPB) was first described by Forero et al. in 2016 (8). ESPB is achieved by injecting LA into the fascial plane between the erector spinae muscle and the transverse process. Therefore, it is far from the pleura and neuraxial structures. However, ESPB penetrates the paravertebral space with intertransverse connective tissues. Thus, in addition to the dorsal and ventral branches of the spinal nerve, it also blocks the lateral cutaneous branches of the intercostal nerves (9).

Some clinical research studies have reported that ESPB can provide adequate analgesia in the thoracic region after thoracotomy and VATS in thoracic surgery (10, 11). However, the effect of the number of ports used in VATS on the success of ESPB has not been evaluated until now. Therefore, we think that the number of ports used in VATS may create different results in the success of ESPB for postoperative analgesia management.

2. Material and Methods

2.1. Patients and Study Design

This retrospective study was conducted by the Declaration of Helsinki (as revised in 2013) and was approved by the institutional ethics committee (Bakırköy Dr. Sadi Konuk Training and Research Hospital, Institutional Review Board, Istanbul, Republic of Turkey; approval number: 2019-10-34/2019-251). The files of patients aged between 18-80 years, with American Society of Anesthesiologists (ASA) physical status 1-3, and who had undergone unilateral lobectomy and segmentectomy with VATS technique with different numbers of trocar ports for lung cancer between 1 Sep 2020 and 31 Aug 2021 were retrospectively reviewed. Patients with abnormal coagulation hemostatic test results, receiving anticoagulant therapy, a history of allergy to local anesthetic agents, using chronic opioids (at least three months), undergoing thoracotomy, and obese (body mass index >35 kg/m^2) were excluded from the study. A total of 73 patients were enrolled in this study. The patients were known to the anesthesiologist since the applied block was performed by a single clinician (GS). However, the persons on duty at the statistical and writing stage did not know which patients belonged to which group. Likewise, post-anesthesia care unit nurses tasked with evaluating the results, such as postoperative pain severity, were independent of the study and were blinded by the port numbers used. In addition, separate from the study, each patient was informed about the procedure to be done, and their consent was obtained.

2.2 Ultrasound-Guided ESPB Block

ESPB was performed with the patient in the lateral decubitus position before induction of general anesthesia under standard monitoring (Figure 1A). Before the procedure, the patient was sedated with midazolam at a dose of 0.03-0.05 mg/kg, and the back area was sterilized with 10% povidone-iodine. The 5–12 MHz linear ultrasound transducer probe (Esaote MyLabSeven / Esaote S.p.A, Genoa-Italy) was covered in a sterile sheath. Ultrasound-guided ESPB was performed at the T5 vertebral level. First, musculus (m) trapezius, m. rhomboid major, and m. erector spinae were visualized by moving the probe approximately 2-3 cm lateral to the midline with the in-plane technique. Next, a 20 gauge 100 mm peripheral nerve block needle (Stimuplex Ultra 360 30 ° - BRA-04892510-01 / B. Braun Melsungen AG, Japan) was advanced towards the interfascial plane between the erector spinae muscle and the transverse process of the vertebra. After confirming the location of the interfascial plane with the hydrodissection method using 3 mL of physiological saline solution, a paramedian longitudinal block was performed by injecting 20 mL of 0.25% bupivacaine (Marcaine 0.5 %, 5 mg/mL) (Figure 1B).

2.3. General Anesthesia

General anesthesia was provided with propofol (2-3 mg/kg) and fentanyl (1-2 μg/kg) in both groups. Before tracheal intubation with a double-lumen tube, the procedure was facilitated by providing muscle relaxation with rocuronium (0.6-1 mg/kg). Anesthesia was maintained with sevoflurane (1-2 %) and remifentanil infusion (0.05-0.1 mcg/kg/min). Hemodynamic data such as electrocardiography, peripheral oxygen saturation (SpO_2), invasive arterial pressure, exhaled CO_2 (end-tidal capnography), and

body temperature were monitored in the perioperative period. At the end of the surgery, anesthetic agents were discontinued, and the muscle relaxant effect was reversed with sugammadex (1-2 mg/kg). In addition, 8 mg ondansetron as a prophylactic antiemetic and 20 mg tenoxicam, and 100 mg tramadol hydrochloride as an analgesic were given. All patients were transferred to the post-anesthesia care unit (PACU) for close hemodynamic monitoring for the first 24 hours after extubation.

2.4. Postoperative Pain Management

Postoperative pain management was performed in all three groups according to our clinical protocol. During the postoperative period, intravenous patient-controlled analgesia (IV PCA) device (CADD-Legacy PCA Ambulatory Infusion Pump, Model 6300/Smiths Medical/USA) was connected to the patient, and morphine infusion was started. Morphine solution (0.5 mg/mL) was prepared in 100 mL isotonic saline; the PCA was adjusted as 1 mg bolus, lockout interval of 10 minutes, 1-hour limit dose of 4 mg, and no basal infusion. In addition, 25 mg meperidine was administered intravenously as rescue analgesia to patients with an NRS score of 4 and above. The evaluation of postoperative of pain and opioid consumption was done by a nurse on the PACU team who was independent of the study. Numerical rating scale (NRS) scores of all patients at 1-6-12-24 hours were recorded in the pain follow-up form. Furthermore, side effects such as nausea, vomiting, itching, sedation, urinary retention, and constipation due to postoperative opioid consumption were recorded.

2.5. Outcome Measurements

The primary outcome included NRS scores for pain at rest and coughing to assess the quality of analgesia at 1, 6, 12, and 24 hours post-surgery, while the secondary outcome included cumulative opioid consumption in the first 24 hours postoperatively. Additionally, intraoperative opioid requirement, postoperative rescue analgesic requirement, and postoperative adverse effects related to opioid consumption were evaluated.

2.6. Statistical Analysis

Based on our preliminary retrospective study, sample size was calculated based on the NRS mean difference between the 3 ESPB-treated groups. Data were collected retrospectively from 73 consecutive cases. We estimated that 73 subjects per group would be needed to provide a type I error of 0.05, a power of 90 %, and an estimated dropout rate of 20 % to detect a difference of 1 point between the 3 groups considered clinically significant.

The G*Power 3.1.9.2 program was used to calculate the sample size of the study. Data were analyzed using SPSS 22 for Windows (IBM Corp., Armonk, NY, USA). The normal distribution of the data was evaluated using the Kolmogorov-Smirnov test. The normally distributed variables were presented as the mean ± standard deviation, while the non- normally distributed variables were presented as the median. Categorical variables were presented as numbers and percentages. ANOVA test (post-hoc: Bonferroni and Dunnett Test) was used for the group comparison of the normally and homogeneous distributed variables and Welch test was used for non-homogeneous group comparisons of numerical variables. Kruskall Wallis Test (post-hoc: Mann Whitney U Test) was used for the intergroup comparison of the non-normally distributed variables. The Chi Square Test was used for the intergroup comparison of the categorical variables.

3. Results

After exclusion criteria, data from seventy-three patients for the study were collected and analyzed retrospectively. When the demographic data of the patients and the duration of surgery and anesthesia were compared, no statistically significant difference was found between the groups (Table 1). Repeated measurements of NRS scores at rest and during coughing revealed that static and dynamic NRS scores were significantly higher in the three-port group until the first 12 hours postoperatively ($p<0.05$). There was no significant difference between the single port and two port groups in terms of pain scores.

In the postoperative period after 12 hours, it was determined that static and dynamic NRS scores did not differ between the groups ($p=0.158$ and $p=0.125$, respectively) (Figure 2). PCA demand dose, delivered dose, and cumulative opioid consumption (mg) were significantly higher in the three port group in the first 24 hours postoperatively ($p=0.010$, $p=0.034$, and $p=0.001$, respectively). The need for postoperative rescue analgesia was similar between the groups ($p=0.341$). There was no significant difference between all three groups regarding the length of stay in PACU and hospital ($p=1.000$ and $p=0.269$, respectively) (Table 2). While vomiting developed in the patients in the single port group and the three port group in the postoperative period, no such complication was found in the two port group ($p=0.347$) (Table 3). All three groups had no complications related to the block procedure such as pneumothorax, local anesthetic systemic toxicity, or hematoma.

Table 1: Comparison of the demographical and surgical data between groups.

	Single Port Group (*n*=24)	Two Port Group (*n*=24)	Three Port Group (*n*=25)	*P* Value
Age	30.08±14.16	35.70±17.49	31.00±12.80	0.379
Gender				
Male	17 (70.8 %)	21 (87.5 %)	23 (92.0 %)	0.111
Female	7 (29.2 %)	3 (12.5 %)	2 (8.0 %)	
Height	174.08±11.85	175.58±7.01	174.16±8.46	0.821
Weight	64.45±9.23	68.91±9.23	62.04±10.87	0.053
BMI	21.45±3.99	22.42±3.40	20.27±2.91	0.100
ASA				
I	8 (33.3 %)	1 (4.2 %)	4 (16 %)	0.077
II	16 (66.7 %)	21 (87.5 %)	19 (76 %)	
III	0 (0.0 %)	2 (8.3 %)	2 (8 %)	
Surgery type				
Lobectomy	24 (100.0 %)	22 (91.7 %)	23 (92.0 %)	0.354
Segmentectomy	0 (0.0 %)	2 (8.3 %)	2 (8.0 %)	
Duration of Anesthesia	100.79±40.06	103.7±28.77	110.6±58.52	0.675
Duration of Surgery	63.04±33.92	73.25±28.22	64.40±44.61	0.111

Data are presented as mean±standard deviation (SD) or number (%).
BMI: body mass index, ASA: American Society of Anesthesiologists.

Table 2: Comparison of perioperative data between groups.

	Single Port Group (*n*=24)	Two Port Group (*n*=24)	Three Port Group (*n*=25)	*p* Value
Mean Remifentanil Use (μg/kg/min)	0.033±0.016	0.036±0.014	0.039±0.025	0.533
Total Remifentanil Use (mcg)	211.1±138.7	186.3±111.7	325.5±360.3	0.132
Intraoperative Hemodynamic Parameters and Complications				
HR	71.95±9.65	74.25±12.28	69.68±10.78	0.350
MAP	72.54±9.54	74.75±10.01	69.16±12.70	0.200
Bradycardia (Y/N)	1/23	1/23	2/23	0.792
Tachycardia (Y/N)	5/19	4/20	5/20	0.927
Hypotension (Y/N)	18/6	19/5	19/6	0.938
Hypertension (Y/N)	3/21	5/19	7/18	0.406
Chest tube Removal (day)	3.42±1.31	4.28±1.59	5.00±4.29	**0.048**
Length of PACU Stay (day)	1 (100.0%)	1 (100.0%)	1 (100.0%)	1.000
Length of Hospital Stay (day)	4.79±1.44	5.75±2.67	5.20±1.80	0.269

Data are presented as Mean±Standard Deviation (SD) or number (%).
PONV: postoperative nausea and vomiting, PPC: postoperative pulmonary complication, PACU: post-anesthesia care unit.

Table 3: Comparison of morphine consumptions and NRS scores between groups.

	Single Port Group (*n*=24)	**Two Port Group (*n*=24)**	**Three Port Group (*n*=25)**	***P* Value**
Postoperative Pain Scores at Rest				
PO 1 h	2.5 (0-6)	2 (0-5)	4 (0-5)	**0.002**
PO 6 h	2 (0-5)	2 (0-4)	3 (0-5)	**0.005**
PO 12 h	2 (0-6)	3 (0-5)	4 (0-6)	**0.023**
PO 24 h	2 (0-5)	2 (0-4)	3 (0-7)	0.158
Postoperative Pain Scores While Coughing				
PO 1 h	2.5 (1-6)	3 (1-4)	4 (1-8)	**0.028**
PO 6 h	3 (2-6)	3 (2-5)	5 (2-7)	**0.001**
PO 12 h	4 (0-7)	3 (1-7)	5 (2-7)	**0.003**
PO 24 h	3 (1-7)	4 (1-6)	5 (1-7)	0.125
PCA Demand Dose	16.75±18.43	18.20±9.84	33.04±21.35	**0.010**
PCA Delivered Dose	9.08±6.15	10.88±6.11	13.75±6.13	**0.034**
Cumulative Opioid Consumption (mg)	15.18±10.03	17.75±13.5	28.74±13.62	**0.001**
Rescue Analgesia Requirement	5 (20.8%)	7 (29.2%)	10 (40.0%)	0.341
Opioid Related Complications				
Nausea (Y/N)	6/18	6/18	4/21	0.678
Vomiting (Y/N)	2/22	0/24	1/24	0.347
Itchiness (Y/N)	0/24	1/23	0/25	0.355
Sedation (Y/N)	2/22	3/21	2/23	0.839
Constipation (Y/N)	0/24	1/23	1/24	0.604
Urinary retention (Y/N)	0/24	0/24	0/25	N/A

Data are presented as mean±standard deviation (SD).
PCA: patient-controlled analgesia, PO: postoperative, S-NRS: static numerical rating scale, D-NRS: dynamic numerical rating scale, h: hour.

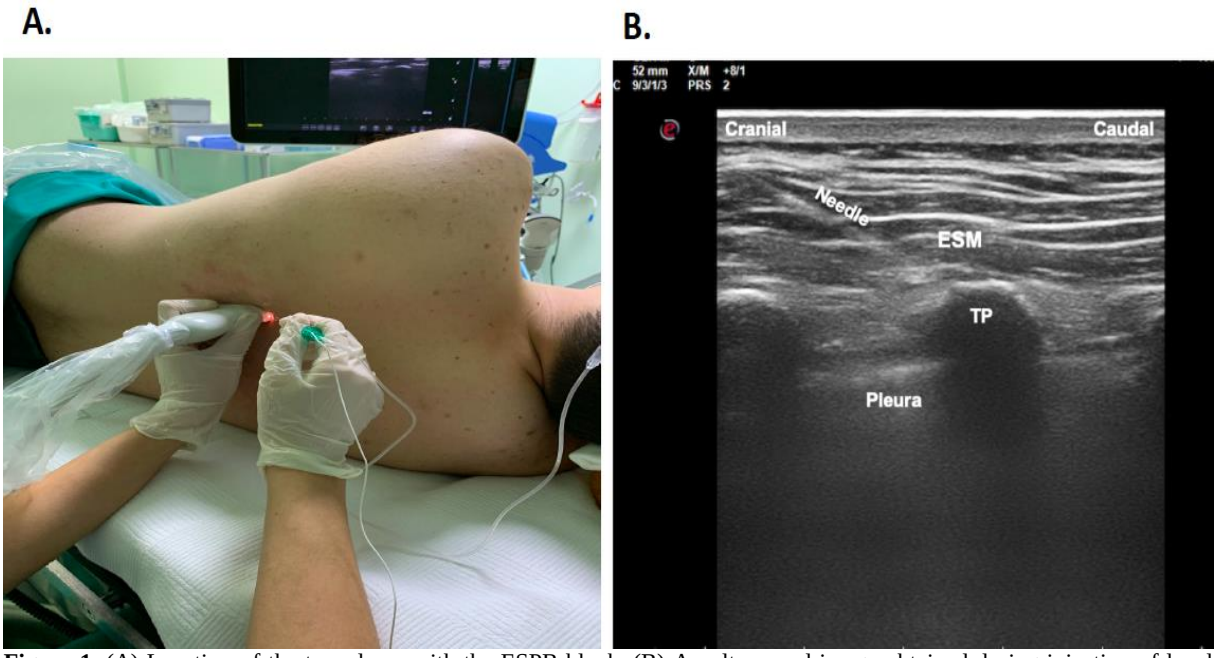

Figure 1. (A) Location of the transducer with the ESPB block, (B) An ultrasound image obtained during injection of local anesthetics. ESPB: Erector spinae plane block, ESM: erector spinae muscle, TP: transverse process, LA: local anesthetics.

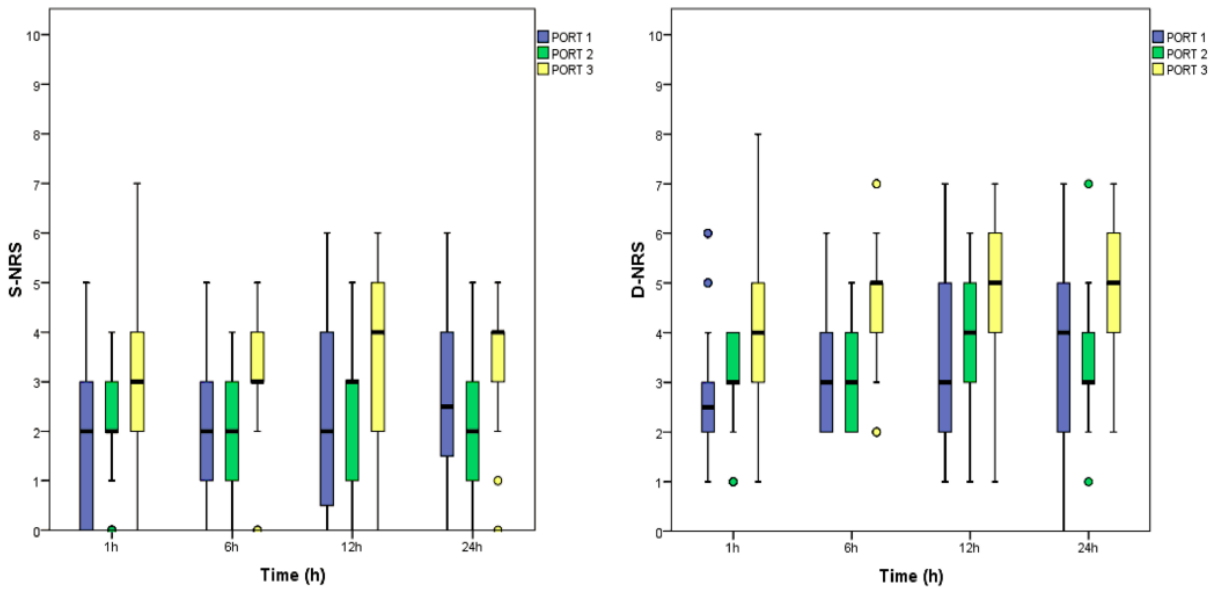

Figure 2. Postoperative static and dynamic NRS scores at the 1, 6, 12 and 24 hour follow-ups. NRS: Numerical rating scale.

4. Discussion

Our study showed that ultrasound-guided single-shot ESPB performed before anesthesia induction in VATS patients significantly reduced NRS scores and opioid consumption in the first 12 hours postoperatively in the single and two-port groups compared to the three-port group. ESPB similarly helped reduce pain and opioid consumption in patients in the single and dual port groups after 12 hours. Although it was stated in previous studies that reducing opioid consumption in the first 24 hours postoperatively could reduce the length of hospital stay and the development of postoperative pulmonary complications, we did not reach such a result in our study (12).

TEA or TPVB has been used for many years in pain management after thoracic surgery. However, with the widespread use of ultrasound in daily anesthesia practice, thoracic wall blocks have become more preferred. ESPB is primarily used as an alternative to TPVB and is considered to be safer due to the area in which it is applied (13). Because the TPVB application area is close to the pleura and the epidural distance, complications such as pneumothorax, widespread epidural spread, and total spinal block can be seen (14). Furthermore, clinical and cadaveric studies have shown that ESPB can spread to the epidural and intercostal areas at the T5-T9 level (15, 16). In addition, clinical research studies have reported that ESPB can reduce somatic and visceral pain in the chest region (10, 17, 18). Therefore, analgesia obtained with ESPB may also play a role in the relief of visceral pain originating from port entry sites.

In different clinical studies comparing ESPB with the control group and other truncal blocks, its effectiveness in reducing postoperative pain has been demonstrated (14, 19). Apart from VATS, studies in thoracotomy have also reported that ESPB effectively reduces pain and opioid consumption. (20, 21). In addition, different studies in the literature report that the single-port technique causes less postoperative pain, regardless of the analgesic method applied after VATS surgery (22, 23).

In these studies, shorter hospital stays and fewer pulmonary complications were determined as advantages of the single port technique. However, these studies only examined the results of the applied surgical procedure on pain. Therefore, the data obtained are independent of analgesic methods. However, regional analgesic techniques may have different effects depending on the number of ports. Unfortunately, there is no study in the literature examining the relationship between any regional technique applied for analgesia and the number of ports.

Although previous clinical studies reported that patients experienced less pain after single-port VATS, the analgesic method applied in most of the studies was not specified (22-24). However, different analgesic techniques may produce different results. In our study, results supporting this hypothesis were obtained. There was a significant difference between the groups after ESPB in both NRS scores and opioid consumption in the first 12 hours. As the reason for this, as stated in previous studies, it was assumed that the local anesthetic could act in an area up to three levels below the application point. Another finding is that when using three ports, there is a distance of four costa distance between the ports according to the placement technique, and a local anesthetic volume of 20 ml may have been insufficient for the spread over this distance. In the present study, it can be understood that the applied local anesthetic dose was inadequate after the postoperative 12th hour, with the disappearance of the statistical difference in NRS scores between the groups. If a longer duration of analgesia is preferred, local anesthetic infusion with catheterization may be considered.

The study has some limitations. The most important limitation of retrospective analysis is its ability to

identify associations without being able to assess causality. Furthermore, since it was a retrospective study, randomization could not be performed, and the patients were included in the study consecutively according to the admission order. In addition, the results of this study may not be generalizable to patients treated in healthcare centers with different dosing protocols. In addition, dermatomal evaluation could not be performed after the blocks. Still, the spread of local anesthetic to the correct area was confirmed by ultrasound guidance.

5. Conclusion

Ultrasound-guided ESPB may be associated with better analgesic efficacy and less opioid consumption, especially in the first 12 hours postoperatively for the single and two-port VATS technique. However, due to the decrease in the analgesic effect of single shot ESPB after 12 hours, patients' pain scores may be similar regardless of port numbers.

Limitations of the Study

The main limitations of this study are that it is a retrospective study and the number of patients included in the groups are small.

Acknowledgement

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Conflict of Interests

The authors declare that there is no conflict of interest regarding the publication of this article. The authors have no sources of funding to declare for this manuscript.

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Author Contributions

All authors contributed to the study conception and design. Conception and design, Provision of study materials, Data collection or management, Manuscript writing, Critical Review, Literature Review, Final approval: GS, Study design, Manuscript preparation, Critical Review, Literature Review, Statistical analysis: GOY. Study design, Manuscript preparation, Language editing, Critical Review, Literature Review,: İB. Study design, Manuscript preparation, Language editing, Statistical analysis, Final approval: ZÇ. Conception and design, Manuscript preparation, Language editing, Statistical analysis, Final approval: GOH. All the authors contributed to the interpretation of the results and the proof reading of the manuscript.

Ethical Approval

The trial was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of the Bakirkoy Dr. Sadi Konuk Training and Research Hospital. (approval number: 2019-251, approval date: 20/05/2019).

Data sharing statement

Data and statistical analysis plan will be shared if requested.

Consent to participate

Consent was obtained from all patients for the use of data and photographs under ethical conditions.

Informed Consent

Informed consent forms were obtained from all patients that preoperatively the patient data could be used in the retrospective studies.

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Evaluation of Analgesic Effectivity of Infra Orbital Nerve Block in Open Septorhinoplasty Surgery: A Retrospective Study

Nihal Efe Atila[1*], İrem Ateş[1]

[1]Department of Otorhinolaryngology, Erzurum Regional Training and Research Hospital, Health Science University, Erzurum, Turkey
[2]Department of Anesthesiology and Reanimation, Faculty of Medicine, Ataturk University, Erzurum, Turkey

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[*]***Corresponding Author***
Nihal Efe Atila
Department of Otorhinolaryngology
Erzurum Regional Training and
Research Hospital
Health Science University
Erzurum, Turkey
Phone:+90 4424321010
E-mail: nihalefe24@hotmail.com

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Authors' ORCIDs
Nihal Efe Atilla
http://orcid.org/0000-0002-3239-4142
İpek Ateş
http://orcid.org/0000-0001-9867-5011

Abstract: The effect of local anesthesia for pain characteristics is still a challenge for septorhinoplasty surgery. The aim of this study is to demonstrate the clinical benefit of infraorbital nevre block for open septorhinolasty. After ethical board approval, 60 patient saged 18-45 years, ASA group I-II were randomly assigned two groups; Group B received 15 mg bupivacaine hydrochloride to infraorbital foramen for each side. Group C did not received any local anesthesia. Data from patient files on postoperative 12-hour pain scores (Visual Analog Scales) and analgesic consumption of the patients were analyzed. Group B had lower pain scores at 1 hour postoperatively compared to group C. It was observed that the effective postoperative analgesia continued consistently at the 6th and 12th hours in the patients in group B. At the same time, the need for IV analgesics at the postoperative 12 hours was significantly lower in group B compared to group C.Intraoperative infraorbital regional bupivacaine anesthesia is an effective procedure that reduces postoperative pain levels in septorhinoplasty operations. © 2022 NTMS.

Keywords: Septorhinoplasty; Pain Control; Bupivacaine; Regional Anesthesia.

1. Introduction

Septorhinoplasty is one of the operations performed quite often by ear nose throat and plastic surgery specialists. If adequate analgesia is not provided due to bone manipulation and periosteal irritation in septorhinoplasty surgery, the postoperative period can be quite painful for the patient (1).

Postoperative pain is acute inflammatory pain. It begins with surgery trauma and ends with tissue healing. In combination with catecholamine discharge, pain can lead to cardiovascular events, neuroendocrine or metabolically undesirable changes, thromboembolic events, pulmonary complications, and prolonged hospitalization (2).

Successful analgesia management increases patient comfort and shortens hospital stay length (3).

Important research and clinical observations show that local anesthesia can reduce pain in nose surgery (4, 5). The application of local anesthesia in combination with general anesthesia is widely used in head and neck surgery. In particular, infraorbital nerve block anesthesia added to general anesthesia can provide excellent pain control during and after surgery in septorhinoplasty surgery (6). Infraorbital nerve block and other peripheral nerve blocks have advantages such as reducing tissue edema in the operation area, widening the anesthesia area and reducing pain due to infiltration anesthesia (7). The infraorbital nerve can

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be easily block eddue to its constant association with the infraorbital foramen, an easily palpable bony landmark (8).

In this study, the effect of intraoperative infra orbital nerve block anesthesia on postoperative analgesia have been evaluated retrospectively in patients who underwent open septorhinoplasty.

2. Material and Methods

Approval was obtained from the ethics committee of Training and Research Hospital in eastern of Turkey. This retrospective study includes data from hospital management software and patient documentations files, which includes the postoperative 12-hour pain scores and analgesic consumption of 60 (33 Female, 27 Male) participants aged 18-45 years who underwent open septorhinoplasty, group I-II of the American Society of Anesthesiologists (ASA), and was conducted between 01.11.2021 and 30.06.2022.

Individuals with a known chronic disease, a history of gastrointestinal bleeding, a known allergy to bupivacaine, drug addiction, and pregnant women were excluded from the study.

Anesthesia induction was performed in the same way for all participants. During anesthesia induction, approximately 2.5–3.5 mg/kg propofol, 0.6 mg/kg rocuronium, and 50 mcg fentanyl were administered. Anesthesia was maintained with sevoflurane in a 40-60 % O_2-air mixture with minimal age-adjusted alveolar concentration (MAC). Remifentanil was administered as an intraoperative analgesic at 0.125 µg/kg/min. Approximately 30 minutes before the end of the operation, a 2 mg/kg tramadol loading was performed. At the end of the operation, a single dose of 40 mg esomeprazole was administered intravenously. The patients were divided into two groups;

The control group [Group C, n:30 (17 Female, 13 Male)] included 17 female and 13 male participants who underwent open septorhinoplasty and were not given infra-orbital regional anesthesia.

In the bupivacaine group [Group B, n = 30 (16 Female, 14 Men)], patients who underwent open septorhinoplasty and were administered intraoperatively 3 mL (15 mg) bupivacaine hydrochloride regional anesthesia to both infraorbital foramen were included.

Paindegrees of thepatientswereevaluatedwiththe Visual Analog Scale (VAS) at 1.6.12 hourspostoperatively. A 1 g paracetamol (Parol; Atabay Turkey®) flacon was administered to the patients for post operative pain control. And IV 50 mg dexketoprofentrometamol (Arveles) ampoule was administered to the patients as "rescue" analgesia.

2.1. Infra-Orbital Nerve Block Technique

After induction of anesthesia and tracheal intubation, a infra orbital nerve block procedure was performed. The index finger was placed in the infra-orbitalforamen. A 10 mL syringe with a 25 gauge and 2.54 cm needle was inserted through the fossa canine 1 cm outside the nostril. The needle tip was advanced towards the infraorbital foramen. Appropriate needle placement was confirmed when the needle tip was palpated close to the infraorbital foramen, and no blood was drawn in the aspiration (9). Then, 3 cc 0.5 % bupivacaine hydrochloride (Marcaine; Aztrezeneca, Turkey®) was injected, and the swelling was palpated. The same procedure was performed for the contralateral side (10).

2.1. Statistical Analyses

SPSS 18.0 statistical package program (SPSS Inc.; Chicago, IL, USA) was used in the analysis of the data. For the descriptive data in statistical analysis, Kolmogorov–Smirnov was used for the number, mean, ±standard deviation, compatibility of the groups to a normal distribution, Kruskal-Wallis was used. Gender distribution of B and C groups was evaluated with chisquare test. Student's t test was used to compare normally distributed continuous variables. For distributions other than normal, a Mann-Whitney U test was applied. A p level of <0.05 was accepted as statistically significant.

3. Results

The ASA and demographic variables of the cases in Group B (study group with bupivacaine infra orbital nerve block) and Group C (control group without infra orbital nerve block) are similar to each other (Table 1).

Table 1: Demographic variables of patients and ASA.

	Group B n:30	Group C n:30	P
Gender (Female/Male)	16/14	17/130.576	
ASA (I /II)	18/12	20/10	0,482
Age (Years)	30.5 ± 4.8	32.1 ±5.3	0.524

ASA: American Society of Anesthesiologist values are given as number or Mean±SD.

The comparison of VAS scores in the first 12 hours postoperatively between group B and group C is shown in Table 2.

Table 2. Visual Analog Scale (VAS) painscores at 1, 6 and 12 h post surgery in 60 patients.

VAS Scores	Group B (n=30)	Group B (n=30)	P
1 h Post Surgery	2.22 ± 1.03	4.8 ± 0.71	0.0012
6 h Post Surgery	2.05 ± 0.83	4.3 ± 0.58	0.0015
12 h Post Surgery	2.01 ± 0.42	3.7 ± 0.67	0.0138

Values are expressed Mean ± SD, h; hour.

In group B, compared to group C, VAS decreased significantly at the postoperative 1st hour, and

adequate postoperative analgesia was consistently maintained at the 6th and 12th hours. The rate of consumption of IV analgesics during the postoperative 12 hours is shown in table 3 for both groups. The need for IV analgesics was significantly lower in group B compared to group C, 21 and 11 patients, respectively, needed postoperative analgesic drug use (p:0.013).

Table3. Requirement for post-surgical analgesics in 60 patients.

Analgesic Drug Consumption	Group (n=30)	Group C (n=30)
Nil Additional Analgesic	19	9
I.V Analgesic Requirement		
Parasetamol 1 gr		
1 Flacon	7	10
2 Flacon	3	7
Deksketoprofen Trometamol 50 mg		
1 Flacon	1	4

In summary, bupivacaine infra orbital nerve block provides effective analgesia in postoperative 12 hours and significantly reduces analgesic consumption.

4. Discussion

Our results show that intra operative infraorbital nevre block with bupivacaine provides significant analgesic effect in the first 12 hours postoperatively and reduces the need for analgesic medication.

Patients complain of severe pain in the postoperative period after septorhinoplasty operations (11). In addition, the area of the operation is also rich in sensitive nerves, so much so that the pain may be more pronounced due to compression caused by leaks and blockages (1).

An effective analgesic treatment is necessary to prevent pain and pain-related morbidities and to reduce the length of hospital stay. For this reason, many analgesic drugs or interventions are administered to patients before, during, or after the operation (12).

Preoperative administration of certain analgesic drugs (preemptive analgesia) facilitates pain control by blocking central sensitization in the postoperative period (3).In the literature, there are studies showing the effectiveness of different groups of drugs (tramadol, ibuprofen, levobupivacaine, etc.) applied systemically and locally as preemptive analgesics (2, 3).

The nasal branches of the infraorbital nerve (V2 branch of the trigeminal nerve) innervate the outer nasal walls and the nasal septum (13). Therefore, local anesthesia of the infraorbital nerve provides anesthesia in the nasal structures. This procedure has a potential analgesic effect and minimal risk.

Edward R. et al., in their study, reported that intraoperative bilateral infraorbital block, added to standard general anesthesia in nasal surgery, is useful in pain management in the postoperative period (10).

Cekic et al., reported that infraorbital nevre block, created by adding 50 mg of tramadol to 0.25 % levobupivacaine, provides effective postoperative analgesia in patients under going outpatient nasal surgery (14).

Higashizawa et al. Reported that infraorbital nevre block with 0.25 % bupivacaine in endoscopic sinus surgery under general anesthesia reduces the consumption of inhaled anesthetic as it reduces the intraoperative pain stimulus (15).

Demiraran et al. (16) reported that local injection with levobupivacaine in nasal surgery compared with 0.25 % lidocaine +2 % epinephrine 1/100,000 in patients who received levobupivacaine infiltration significantly decreased VAS scores with analgesic consumption in the postoperative period. In parallel with this study, Yılmaz et al. reported that local injection with levobupivacaine in patients who underwent septoplasty significantly reduced VAS scores in the first 4 hours postoperatively compared to local injection with lidocaine (17).

5. Conclusions

In our study, we observed that intraoperative bupivacaine infraorbital nerve block provided statistically significant benefit in pain control in the postoperative period in septorhinoplasty operations, and the need for analgesic agents decreased in the first 12 hours. Intraoperative bupivacaine infraorbital nerve block application is an effective procedure to reduce postoperative pain levels in septorhinoplasty operations, easy to perform.

Limitations of the Study

It is our limitations that it is a retrospective study, the number of cases is low.

Conflict of Interests

The authors declare no conflict of interest.

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Author Contributions

Efe Atila N. contributed to the creation of the research idea, statistical analysis and article writing. Ateş İ. Data collection contributed to interpretation.

Ethical Approval

The study was approved by Erzurum BEAH KAEK with the decision numbered 2022/11-127.

Data sharing statement

None.

Informed Consent

None.

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