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Book

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Chapter in a book

Meltzer PS, Kallioniemi A, Trent JM. Chromosome alterations in human solid tumors. I n: Vogelstein B, Kinzler KW, editors. The genetic basis of human cancer. New York: McGraw-Hill; 2002. p. 93113.

Journal article on the Internet

Abood S. Quality improvement initiative in nursing homes: The ANA acts in an advisory role. Am J Nurs [serial on the Internet] 2002 [cited 12 Aug 2002]; 102. Available from: www.nursingworld.org/AJN/2002/june/wawatch.htm

Website

Cancer-pain.org [homepage on the Internet]. New York: Association of Cancer Online Resources [updated 16 May 2002; cited 9 Jul 2002]. Available from: www.cancer-pain.org

An organization as an author

The Intensive Care Society of Australia and New Zealand. Mechanical ventilation strategy in ARDS: Guidelines. Int Care J Aust 1996;164:282-4.

Acknowledgements

The source of financial grants and the contribution of colleagues or institutions should be acknowledged.

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Abbreviations that are used should be defined in parenthesis where the full word is first mentioned. Some common abbreviations can be used, such as iv, im, po, and sc.

Drugs should be referred to by their generic names, rather than brand names.

Editorial Correspondence

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ERRATUM

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Original Article / Orijinal Araştırma

Evaluation of the Effect of the Combination of Boron Compounds on Chronic Liver Disease

Bor Bileşikleri Kombinasyonunun Kronik Karaciğer Hastalığı Üzerindeki Etkisinin Değerlendirilmesi

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Abstract

Aim: *Enterococcus faecalis* has surface adhesion proteins that enable it to attach to human intestinal and vaginal tissue cells with antibiotic-resistant strains in patients. Due to these properties, boron and its derivatives are preferred as therapeutic agents due to their antibacterial, antifungal, antiparasitic and anti-inflammatory activities. In this study, we aimed to evaluate the synergistic effect of boron compounds and their effect on biofilms in an infection model created with *Enterococcus faecalis* 29212 on the HepG2 liver cell line.

Material and Method: Synergistic of boron and boron compounds and biofilms minimum inhibitor concentration, fractional inhibitor HepG2 cell analyzes with concentration and biofilm studies was made and evaluated.

Results: It was determined that sodium perborate monohydrate+zinc borate had the lowest values as a result of the minimum inhibitory concentration and fractional inhibitor concentration studies. It has also been shown that these doses reduce cytotoxic effects. In addition, 32 µg/ml Etidote+256 µg/ml Sodium perborate monohydrate showed the highest biofilm effect.

Conclusion: Biofilm formation of Enterococcus faecalis by boron compounds effectively reduce and cause the death of bacteria we showed.

Keywords: Biofilm, boron compounds, fractional inhibition concentration, cell culture, synergistic effect

Öz

Amaç: *Enterococcus faecalis*, hastalarda antibiyotiğe dirençli suşlarla insan bağırsak ve vajinal doku hücrelerine bağlanmasını sağlayan yüzey yapışma proteinlerine sahiptir. Bu özelliklerinden dolayı bor ve türevleri antibakteriyel, antifungal, antiparaziter ve antifungal aktivitelerinden dolayı terapötik ajanlar olarak tercih edilmektedir. Bu çalışmada, HepG2 karaciğer hücre hattı üzerinde *Enterococcus faecalis* 29212 ile oluşturulan bir enfeksiyon modelinde bor bileşiklerinin sinerjistik etkisini ve biyofilmler üzerindeki etkisini değerlendirmesi amaçlandı.

Gereç ve Yöntem: Bor ve bor bileşiklerinin sinerjistik ve biofilmler üzerine etkisi minimum inhibitör konsantrasyonu, fraksiyonel inhibitör konsantrasyonu ve biyofilm çalışmaları ile HepG2 hücre analizleri yapılarak değerlendirildi.

Bulgular: Minimum inhibitör konsantrasyonu ve fraksiyonel inhibitör konsantrasyonu çalışmaları sonucunda sodyum perborat monohidrat+çinko boratın en düşük değerlere sahip olduğu belirlendi. Ayrıca bu dozların sitotoksik etkileri azalttığı da gösterilmiştir. Ayrıca en yüksek biyofilm etkisini 32 µg/ml Etidot+256 µg/ml Sodyum Perborat Monohidrat gözlendi.

Sonuç: Bor bileşiklerinin *Enterococcus faecalis*'in biyofilm oluşumunu etkili bir şekilde azalttığı ve bakterilerin ölümüne neden olduğunu gösterdik.

Anahtar Kelimeler: Biyofilm, bor bileşikleri, fraksiyonel inhibisyon konsantrasyonu, hücre kültürü, sinerjik etki

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INTRODUCTION

Enterococcus faecalis is known to be abundant in the microbiota of patients with chronic liver disease associated with hepatitis C virus. *Enterococcus faecalis* is a gram-positive pathogen that forms biofilms and shows resistance to many antibiotics. It causes infections that are difficult to treat after liver transplantation in patients with chronic liver failure.[1] In addition, antibiotic-resistant *Enterococcus faecalis* has acquired intrinsic resistance to antimicrobial agents such as beta-lactams and aminoglycosides and resistance to glycopeptides, quinolones, tetracyclines, macrolides and streptogramin through the horizontal transfer of elements such as transposons and plasmids or resistance genes.[2] The pathogenic properties of *Enterococcus faecalis* often include biofilm formation with an increasing burden of antimicrobial resistance among the most highly pathogenic nosocomial infections. provides structural integrity.[3] Bacterial biofilms support the survival and persistence of infecting microbes as they facilitate fate defense against the host immune response.[4] *Enterococcus faecalis* encodes several factors that contribute to biofilm formation, including 2 sortase enzymes, SrtC and SrtA, which polymerize and attach the pili associated with endocarditis and biofilm formation.[5-7] This pili assists the adhesion of *Enterococcus faecalis* to surfaces, which is essential during the early stages of biofilm formation in vitro and in vivo during cathepsin-associated urinary tract infection.[8] Other biofilm-associated factors that bind to the cell wall by SrtA include Ace, aggregation agent, and Esp. *Enterococcus faecalis* must also defeat host defenses to establish infection. *Enterococcus faecalis* can modulate and evade the host immune response in a number of settings. Biofilm formation, along with the expression of the SrtA substrate aggregation agent, can promote the survival of *Enterococcus faecalis* within macrophages and neutrophils. The multipeptide resistance factor (MprF) protein of *Enterococcus faecalis* confers resistance to antimicrobial peptides via electrostatic repulsion and is important for both neutrophil-mediated clearance and survival in epithelial cells and macrophages in a variety of gram-positive bacteria.[9-11] The inadequacy of antibiotics used in the treatment against bacterial resistance and biofilms has led to a need for better alternatives. The unique electronic properties of boron that allow it to act as a transition state mimetic for tetrahedral intermediate peptide bond cleavage observed in proteolytic enzymes have attracted increased attention over the past few years as potential drugs. In addition to boronic acids discussed by numerous researchers, benzoxaborols, a class of compounds in which the boron atom is incorporated into a heteroaromatic ring system, have provided a number of interesting anti-inflammatory, antifungal, antiparasitic and antibacterial drug candidates.[12-14] In light of this information, we aimed to evaluate the synergistic effect of boron compounds and their effect on biofilms in the infection model created with *Enterococcus faecalis* 29212 on the HepG2 liver cell line.

MATERIAL AND METHOD

Reagents

Etidote (disodium octaborate tetrahydrate), sodium perborate monohydrate, zinc borate, Mueller Hinton broth, tryptic soy broth, Dulbecco's modified Eagle's medium (DMEM), phosphate buffer solution (PBS), fetal calf serum (FCS), antibiotic antimitotic solution (100×), L glutamine, trypsin–EDTA, paraformaldehyde and ethanol were obtained from Sigma Aldrich (St. Louis, MO, USA).

Bacterial Strain

Enterococcus faecalis 29212 was used in our study. The isolate was identified by conventional methods and an automated system (Phoenix, Becton Dickinson, USA). Suspension equivalent to a strain of 0.5 McFarland turbidity were prepared.

Bacterial Production

The bacterial stock of *Enterococcus faecalis* 29212 was added to 100 µl of tryptic soy broth (TSB) medium, and its production was carried out after 24 hours of incubation at 37°C and 150 rpm. Then, 200 µl of the growth medium was taken and inoculated into fresh TSB, and the stock medium was made ready for the study.

Minimum Inhibition Concentration Values

The MIC values of sodium perborate metahydrate (SPM), zinc borate (ZB), and Etidote compounds against *Enterococcus faecalis* 29212 were determined using the microdilution method. The dose range was determined to be 1024-0.97 µg/ml. Müeller Hinton Broth (MHB) medium was inoculated into 96-well plates to which 180 µl of each dilution was added. Then, 20 µl of *Enterococcus faecalis* 29212 (10^6 CFU/ml) was added to each well and incubated at 37°C. After 24 hours, TTC water-soluble salt solution, a biological indicator, was added to each well (5 mg/ml), and the plates were incubated for 2-3 hours.[3]

Biofilm Analysis

A total of 180 µl of the compounds whose MIC value was determined, prepared with TSB medium, was inoculated into a flat-bottomed 96-well plate. Glucose-enriched TSB medium was used as a negative control, and the *Enterococcus faecalis* 29212 strain was used as a positive control. Then, 20 µl (10^6 CFU/ml) of the *Enterococcus faecalis* 29212 strain was inoculated into each well except the negative well. The cells were incubated at 37°C for 48 hours. Biofilm analysis was performed in 3 repetitions.[3]

Combination Application of *Enterococcus faecalis* 29212 with SPM, ZB, Etidote Compounds

The most effective MIC concentrations of SPM, ZB, and Etidote compounds were prepared in combination with each other. In the analysis performed similar to the biofilm evaluation test principle, the *Enterococcus faecalis* 29212 strain was inoculated into MHB medium enriched with glucose and incubated at 37°C for 48 hours. Bacterial growth

was expected. In addition, the medium was made fresh by adding TSB medium to the plates at 24-36 hour intervals. After 48 hours, the liquid in the plates was evacuated. Then, 200 µl of glucose-enriched culture medium containing TTC (5 mg/ml) was added to each well and incubated at 37°C for 3-4 hours. The intensity of the red color at the end of the resulting test was considered an indicator of viable cell number and was measured at 490 nm. The results were compared with controls. The test was applied as 3 repetitions.[3]

Microdilution panels

The solutions were prepared by calculating the final concentrations of SPM, ZB, and Etidote compounds on the prepared panels. Intermediate dilutions with a concentration of four times the final concentration desired in the well were prepared. Then, 100 µl of TSB medium was dispensed into all wells. First, 100 µl of SPM was diluted in half and dispersed, and then 100 µl was added to the wells, which were diluted sequentially with ZB, Etidote 1000 µg/ml. Medium was prepared as a negative control, and bacterial wells were prepared as a positive control. Except for the negative control well, antimicrobial agents (5 µl) were dispensed into the plates. This process was repeated for the other ZB and Etidote and applied as 3 repetitions.[3]

Fractional Inhibitor Concentration Index-Combination (FIC)

It was applied according to the FIC index formula used to determine the effectiveness of the combinations. The results were determined according to the formula.

A: Antimicrobial 1 used in combination
B: Antimicrobial 2 used in combination
Calculation of the FIC index:
FIC A: MIC numerical value of An in the presence of B/MIC numerical value of A alone
FIC B: MIC numerical value of B in the presence of A/MIC numerical value of B alone
Σ FIC index FIC A+FIC B
Σ FIC index ≤ 0.5: synergy
Σ FIC index >0.5 and <1: additive
Σ FIC index ≥1 and 4 ≤: ineffective (indifference)
Σ FIC index >4: antagonism was accepted as.

Cell cultures

For our study, HepG2 cell (HB-8065 ATCC) cultures were obtained from the Department of Medical Pharmacology of Atatürk University (Erzurum, Turkey).

Briefly, the cells were resuspended in fresh medium (Dulbecco's modified Eagle's medium, DMEM), 10% fetal bovine serum (FBS) and 1% antibiotic (penicillin, streptomycin and amphotericin B). Then, the cells were seeded in 24-well plates (Corning, USA) and stored in an incubator (5% CO_2; 37°C).[15] After gaining an 85% confluence ratio, the model was established by using a 100 µl yellow pipet tip; according to the McFarland 0.5 scale, a bacterial suspension was then added to the cell culture. After 30 min of treatment with the HepG2 cell line, SPM 62.5 µg/ml+Etidote 125 µg/ml, SPM 62.5 µg/ml+ZB 31.25 µg/ml and ZB 31.25 µg/ml+Etidote 125 µg/ml were applied for 24 h.

MTT Assay

At the end of the two-part experiment (after 24 h of treatment with boric acid and potassium metaborate), 10 µL of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution was added to each well plate, and the samples were incubated for 4 h; 100 µL of DMSO solution was incorporated into all wells to dissolve formazan crystals. The optical density of the solutions was read at 570 nm using a Multiskan™ GO Microplate Spectrophotometer reader.[16]

Immunofluorescence

Cells cultivated in cell culture were incubated for 30 minutes in paraformaldehyde solution for 30 minutes. The cells were then incubated in 3% H_2O_2 for 5 minutes. A 0.1% Triton-X solution was dripped onto the cells washed with PBS and left for 15 minutes. After the incubation period, protein blocks were dripped onto the cells and kept in the dark for 5 minutes. Then, the primary antibody (8-OHdG cat no: sc-66036, dilution ratio: 1/100 US) was dropped and incubated in accordance with the instructions for use. Immunofluorescence secondary antibody was used as a secondary marker (FITC Cat No: ab6785 Diluent Ratio: 1/500, UK) and incubated in the dark for 45 minutes. Then, the second primary antibody (H2A. X Cat No: I 0856-1, Dilution Ratio: 1/100, US) was dripped onto the tissues and incubated in accordance with the instructions for use. An immunofluorescence secondary antibody was used as a secondary marker (Texas Red Cat No: ab6787 Diluent Ratio: 1/1000 UK) and incubated in the dark for 45 minutes. Then, DAPI with mounting medium (Cat no: D1306 Dilution Rate: 1/200 UK) was dripped onto the sections and kept in the dark for 5 minutes, and the sections were closed with a coverslip. The stained sections were examined under a fluorescence microscope (Zeiss AXIO GERMANY).

RESULTS

Microbiology Analysis

Minimal inhibition concentrations (MICs) were determined at concentrations of SPM 31.25 µg/ml+Etidote 125 µg/ml, SPM 31.25 µg/ml+ZB 62.5 µg/ml and ZB 62.5 µg/ml+Etidote 125 µg/ml. **Figure 1A**, Etidote 32 µg/ml+SPM 512 µg/ml, Etidote 32 µg/ml+SPM 128 µg/ml, Etidote 64 µg/ml+SPM 64 µg/ml, Etidote 32 µg/ml+SPM 64 µg/ml in A, Synergistic effect ≤ 0.5: detected at concentrations of Etidote 64 µg/ml+SPM 32 µg/ml and Etidote 32 µg/ml+SPM 32 µg/ml. If additive effect (>0.5 and <1); Etidote 128 µg/ml+SPM 512 µg/ml, Etidote 64 µg/ml+SPM 512 µg/ml, Etidote 64 µg/ml+SPM 256 µg/ml, Etidote 32 µg/ml+SPM 256 µg/ml, Etidote 64 µg/ml+SPM 128 µg/ml, Etidote 128 µg/ml+SPM 64 µg/ml, Etidote 256 µg/ml+SPM 32 µg/ml, Etidote 128 µg/ml+SPM 32 µg/ml were detected in doses, while the others were found to be ineffective.

In **Figure 1B**, Etidote 32 µg/ml+ZB 512 µg/ml, Etidote 64 µg/ml+ZB 256 µg/ml, Etidote 128 µg/ml+ZB 128 µg/ml, Etidote 64 µg/ml+ZB 128 µg/ml, Etidote 32 µg/ml+ZB 128 µg/ml, Etidote 64 µg/ml+ZB 64 µg/ml, Etidote 32 µg/ml+ZB 64 µg/ml and Etidote 32 µg/ml+ZB 32 µg/ml concentrations Synergistic effect ≤ 0.5: detected. Etidote 64 µg/ml+ZB 1024 µg/ml, Etidote 32 µg/ml+ZB 1024 µg/ml, Etidote 128 µg/ml+ZB 512 µg/ml, Etidote 64 µg/ml+ZB 512 µg/ml, Etidote 256 µg/ml+ZB 256 µg/ml, Etidote 128 µg/ml+ZB 256 µg/ml, Etidote 128 µg/ml+ZB 64 µg/ml, Etidote 512 µg/ml+ZB 32 µg/ml, Etidote 256 µg/ml+ZB 32 µg/ml, Etidote It showed Additive effect (>0.5 and <1) at 128 µg/ml+ZB 32 µg/ml and Etidote 64 µg/ml+ZB 32 µg/ml doses. Our other concentrations were found to be ineffective in terms of FIC value.

In **Figure 1C**, ZB 32 µg/ml+SPM 128 µg/ml, ZB 64 µg/ml+SPM 64 µg/ml, ZB 32 µg/ml+SPM 64 µg/ml, ZB 64 µg/ml+SPM 32 µg/ml and ZB 32 µg/ml+SPM 32 µg/ml Synergistic effect ≤ 0.5: detected at concentrations. If additive effect (>0.5 and <1); ZB 64 µg/ml+SPM 1024 µg/ml, ZB 512 µg/ml+SPM 512 µg/ml, ZB 64 µg/ml+SPM 256 µg/ml, ZB 32 µg/ml+SPM 256 µg/ml, ZB 128 µg/ml+SPM 128 µg/ml, ZB 64 µg/ml+SPM 128 µg/ml, ZB 256 µg/ml+SPM 64 µg/ml, ZB 128 µg/ml+SPM 64 µg/ml, ZB 256 µg/ml+SPM 32 µg/ml, ZB 128 µg/ml+SPM 32 µg/ml was determined at rates. Other concentrations were found to be ineffective.

The optical density (570/OD) results of the combinations made with the microdilution plate method are summarized in **Figure 2**. In **Figure 2A**, The highest effect on biofilm formation was detected at 32 µg/ml Etidote+256 µg/ml SPM. In **Figure 2B**, The highest effect on biofilm formation was detected at the concentration of Etidote 128 µg/ml+ZB 1024 µg/ml. In **Figure 2C**, The highest effect on biofilm was detected at the concentration of ZB 1024 µg/ml+SPM 256 µg/ml.

MTT Assay

We evaluated the toxicological effects of ZB, SPM and etidote on the HepG2 cell line. In our study, according to our results, ZB+Etidote effectively protected HepG2 cells against *Enterococcus faecalis* ($p>0.05$). Additionally, SPM+ZB protect cell viability but near to 22% cell lost vas evaluated. In addition, SPM+ZB did not protect cell viability ($p<0.05$) (**Figure 3**).

Immunohistochemical Evaluation

In H2A. X and nuclear DNA, 8-hydroxy-2'-deoxyguanosine (8-OHdG) is the predominant form of free radical-induced oxidative lesions. In line with the previous findings, H2A. X and 8-OHdG fluorescent signals were not observed in the control group. Several 8-OHdG fluorescence-cent signals were observed in the SPM+Etidote group, moderate signals were detected in the SPM+ZB group, and light signals were easily observed in the ZB+Etidote group (**Table 1**) (**Figure 4**).

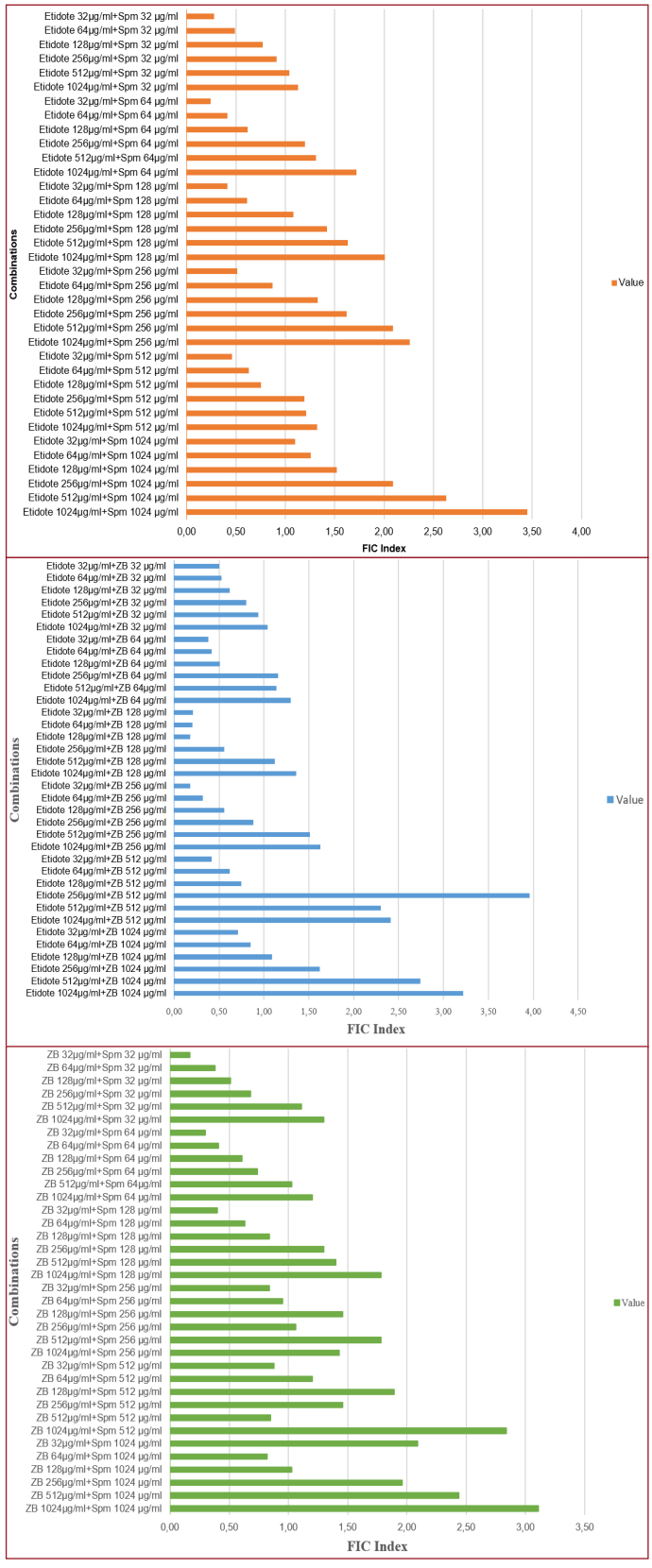

Figure 1. Boron compound FIC index results. A) Etidote+SPM combination FIC index, B) Etidote+ZB combination fix index, C) ZB+SPM combination fix index. Value ranges of boron combinations corresponding to Σ FIC index ≤ 0.5: synergy, >0.5 and <1: additive and ≥1 and 4 ≤: ineffective (indifference).

Table 1. Statistical analysis of immunofluorescent staining results.

	8-OHdG	H2A.X
Control	20.45±5.38[a]	18.68±5.37[a]
SPM+Etidote	61.36±5.91[b]	59.29±3.27[b]
SPM+ZB	39.84±5.61[c]	33.28±3.88[c]
ZB+Etidote	31.18±4.62[c]	29.75±2.59[c]

*a, b, c: different letters in the same column were considered statistically significant differences. ($p<0.05$)

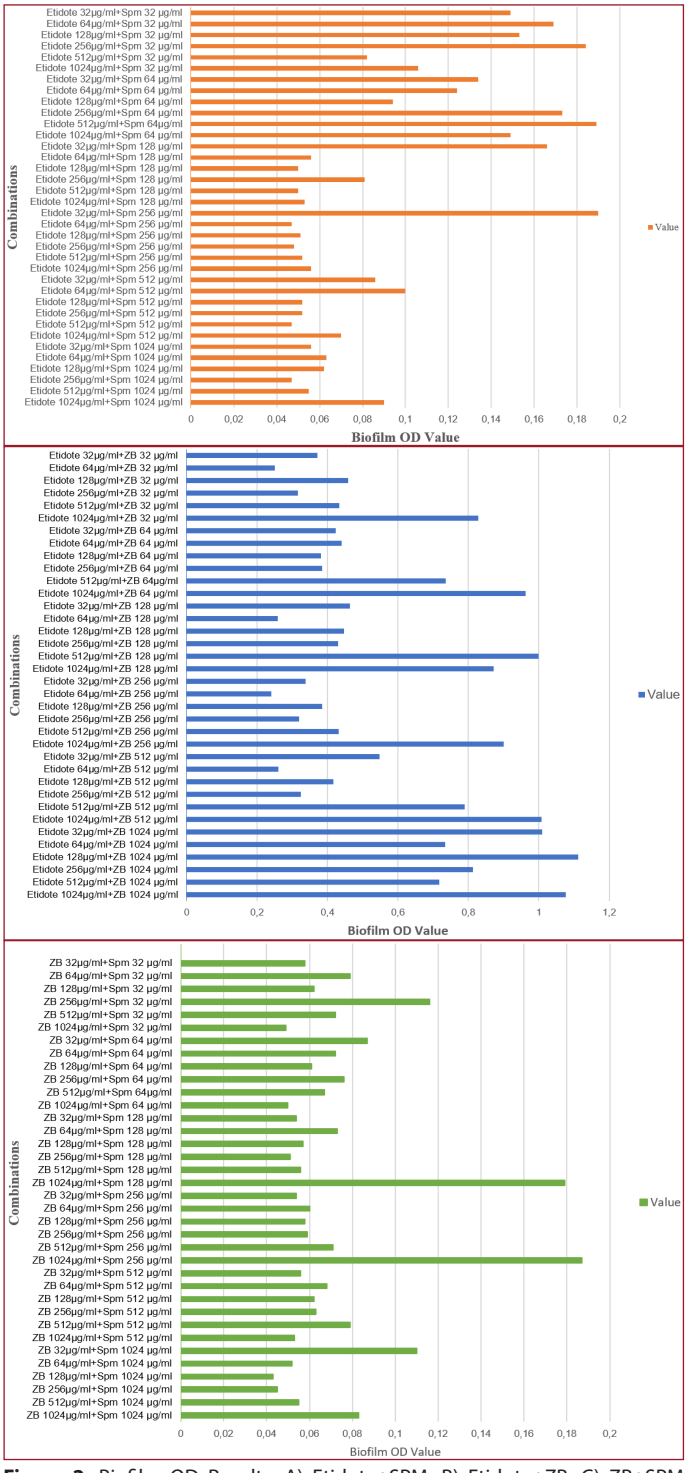

Figure 2. Biofilm OD Results. A) Etidote+SPM, B) Etidote+ZB, C) ZB+SPM biofilm OD values. The minimum and maximum OD values of Etidote+SPM, Etidote+ZB, and ZB+SPM biofilms ranged at 570 OD.

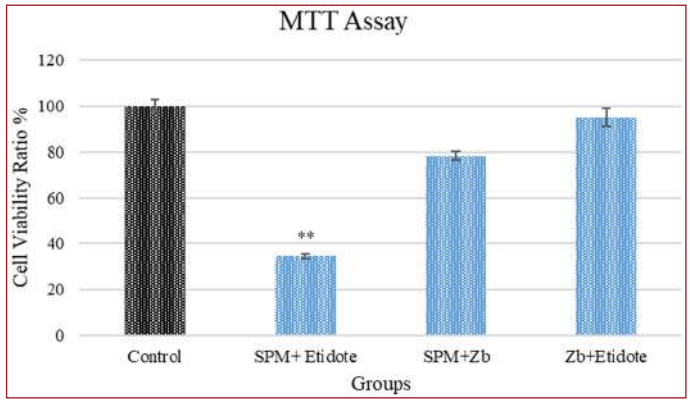

Figure 3. MTT assay results for the HepG2 cell line, control group (received only medium), *Enterococcus faecalis* bacteria cocultured for 24 h with SPM 62,5 µg/ml+Etidote 125 µg/ml, SPM 62,5 µg/ml+ZB 31,25 µg/ml and ZB 31,25 µg/ml+Etidote 125 µg/ml. (*$p<0.05$ compared to the control group).

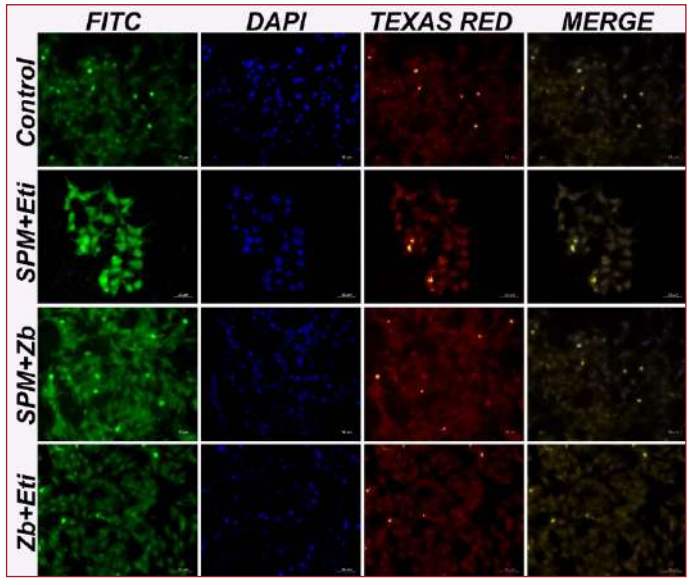

Figure 4. Cell lines, 8-OHdG expression (FITC) and H2A. X expression (Texas Red), IF, Bar: 50 µm.

Statistical Analysis

The results were calculated as the mean±standard error. Statistical comparisons between groups were calculated using one-way ANOVA and Tukey's LSD method. For statistical analyses, all calculations were performed using SPSS 20 software, and $p<0.05$ was considered to be a significant difference in all tests.

DISCUSSION

Enterococcus faecalis, which causes significant infection after liver transplantation, is difficult to treat due to its antimicrobial resistance and biofilm mechanism. We aimed to evaluate the synergistic effect of boron compounds and their effect on biofilms in an infection model created with *Enterococcus faecalis* 29212 on the HepG2 liver cell line to facilitate treatment with alternative treatment methods. *E. faecalis* is a factor that has started to pose a danger in hospital infections.

Resistance to ampicillin, which is routinely used, has been reported at a rate of 1.8% recently. The use of vancomycin is increasing to prevent this resistance from spreading and spreading to community-acquired infections.[17,18] However, hospital-acquired infections (HAIs) caused by vancomycin-resistant enterococci (VREs) are emerging as an additional burden on patients and healthcare systems globally, leaving limited therapeutic options.[19,20] The presence of its core detection system contributes to the spread of this resistance. In this respect, an alternative strategy, such as the degradation of the biofilm layer, is among the methods used to combat multidrug-resistant bacteria.[21] Antimicrobial studies targeting Fsr and cytolysin quorum-sensing systems have been carried out in vitro and in vivo, but it has been reported that more information is still needed about the mechanisms. [22-25] Investigation of synergistic effects with antimicrobial photodynamic therapy (aPDT) models in biofilm eradication[26], application of antimicrobials depending on antimicrobial lock therapy (ALT) and MIC values[27], and methods applied using antimicrobial peptides (AMPs).[28] Prevention of biofilm layer with electrical methods It is promising that resistance to antimicrobials does not develop and that it is not toxic. [29] Medical devices associated with biofilm formation are among other alternative searches in studies conducted with the method of coating with antibiofilm layers to prevent microorganisms from adhering to surfaces.[30] Boron, one of the alternative molecules in all these searches, has recently taken its place in the literature.[31,32] In studies on boron, information on cytotoxic activities in cell culture is scarce.[33] In a study using antibiofilm analysis, boric acid and etidote MICs were found to be between 0.77-3.09 mg/ml and 0.644-10.312 mg/ml, respectively.[34] In our study, Etidote 32 µg/ml+Spm 256 µg/ml had the highest effect on biofilms, Etidote 128 µg/ml+ZB 1024 µg/ml had the highest effect on biofilms, and ZB 1024 µg/ml+SPM 256 µg/ml had the highest effect on biofilms. detected at the highest rate. This effect, determined at lower concentrations, is promising. In another study, in which the cytotoxicity of tetra acetyl ethylen diamine-sodium perborate and sodium hypochlorite was compared, the cytotoxicity of the substances used in the study was examined at doses ranging from 0.0025% to 0.5%. In our study, it was determined that ZB+Etidote preserved a vitality rate of 95%. Similar results were found in the IF experiment, and DNA damage was shown to be minimal. However, our findings showed that the SPM+ZB group did not provide any protection against bacteria in the HepG2 cell line in the cellular environment.

CONCLUSION

The study is promising in terms of containing new information in terms of cell culture content, immunohistochemical analysis results and FIC concentrations. However, more comprehensive in vivo studies and determination of their effects at the molecular level are needed to determine the activities of these alternative compounds from living things.

ETHICAL DECLARATIONS

Ethics Committee Approval: The standard strain was used in this study. No ethical approval is required.

Informed Consent: Since this study was not conducted on patients, a consent form is not required.

Referee Evaluation Process: Externally peer-reviewed.

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

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article / Orijinal Araştırma

The Contribution of Diffusion Tensor Imaging to Conventional Magnetic Resonance Imaging in the Diagnosis of Multiple Sclerosis Patients

Multipl Skleroz Hastalarının Tanısında Difüzyon Tensör Görüntülemenin Konvansiyonel Manyetik Rezonans Görüntülemeye Katkısı

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Abstract

Aim: The aim of this study is to investigate whether anisotropic diffusion is superior to conventional magnetic resonance imaging for understanding the pathophysiology of multiple sclerosis (MS) disease by Fractional anisotropy (FA) measurements.

Material and Method: In our study, FA measurements were made from the plaque, the peri-plaque area, the normal appeared white matter contralateral to the plaque and normal appearing white matter areas in MS patients and from the normal white matter in the control group. 3D tractography maps were made in all MS patients and it was evaluated whether white pathways were affected by MS disease.

Results: When the degree of anisotropy was compared to the control group, the degree of plaques was found lowest. Increase was observed in peri-plaque, the normal appeared white matter contralateral to the plaque and normal appearing white matter, respectively. The active plaque FA value was found to be lower than the chronic plaque FA value, and the chronic plaque FA was found to be lower than the normal white matter FA value. It has been shown that plaques traced along axonal pathways in MS patients cause interruption in axonal pathways.

Conclusion: Progressive decrease in anisotropy from normal appearing white matter to peri-plaque white matter and plaque level indicates myelin damage. This suggests that the white matter that appears normal on T2 images on conventional MR is not actually normal. Based on these results, it was thought that diffusion tensor imaging would be useful in evaluating the burden of disease in MS patients.

Keywords: Multiple sclerosis, diffusion tensor imaging, fractional anisotropy, magnetic resonance imaging

Öz

Amaç: Bu çalışmanın amacı Fraksiyonel anizotropi (FA) ölçümleri ile multipl skleroz (MS) hastalığının patofizyolojisini anlamada anizotropik difüzyonun konvansiyonel manyetik rezonans görüntülemeye üstün olup olmadığını araştırmaktır.

Gereç ve Yöntem: Çalışmamızda MS hastalarında plak, periplak , plağın karşısında normal görünen beyaz cevher ve normal görünen beyaz cevher alanlarından, kontrol grubunda ise normal beyaz cevherden FA ölçümleri yapıldı. Tüm MS hastalarında 3 boyutlu traktografi haritaları yapıldı ve beyaz yolakların MS hastalığından etkilenip etkilenmediği değerlendirildi.

Bulgular: Anizotropinin derecesi kontrol grubu ile karşılaştırıldığında en düşük değer plakta saptanırken; periplak, plağın karşısında normal görünen beyaz cevher ve normal görünen beyaz cevherde anizotropinin sırasıyla arttığı izlenmiştir. Aktif plak FA, kronik plak FA'dan; kronik plak FA, normal beyaz cevherde FA'dan düşük saptanmıştır. MS hastalarında aksonal yollar boyunca izlenen plakların aksonal yollarda kesintiye neden olduğu gösterilmiştir.

Sonuç: Normal görünen beyaz cevherden plak düzeyine doğru anizotropideki progresif artış myelin hasarını göstermektedir. Bu da konvansiyonel MR'da T2 görüntülerde normal görünen beyaz cevherin aslında normal olmadığını düşündürmektedir. Bu sonuçlara dayanarak MS hastalarında hastalık yükünün değerlendirilmesinde Difüzyon tensör görüntülemenin faydalı olacağı düşünülmüştür.

Anahtar kelimeler: Multipl skleroz, difüzyon tensör görüntüleme, fraksiyonel anizotropi, manyetik rezonans görüntüleme

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INTRODUCTION

Multiple sclerosis (MS) is the most common chronic inflammatory demyelinating disease of the central nervous system (CNS) in young adults. Inflammation, demyelination and axonal damage are responsible for the pathology of the disease. Accordingly, the most prominent pathological finding of MS is cerebral or spinal plaques containing demyelination.[1] Cortex and deep gray matter are also affected in MS. Irreversible white matter damage and severe demyelination as a result of axonal loss is the main determinant of long-term disability in multiple sclerosis.[2,3] Magnetic resonance imaging (MRI) is a sensitive imaging modality for identifying plaques critical to the diagnosis of MS and evaluation of treatment response.[4] MRI helps in the diagnosis of MS by detecting demyelinating plaques in the periventricular, juxtacortical, and infratentorial areas. Until now, these demyelinating plaques were thought to be responsible for MS pathology. However, this technology is limited due to a lack of pathologic specificity and a poor correlation with disability. Studies performed with diffusion tensor imaging (DTI) in the postmortem period, it has been determined that tissue damage is not only in plaques, but also in white and gray matter in MS patients.[5,6] Therefore, it can be thought that conventional MRI has a limited role in the diagnosis and follow-up of MS when compared with DTI. Diffusion tensor imaging (DTI) is an advanced imaging method that can quantitatively show changes in brain tissue, unlike MRI, which has been increasingly used in MS recently.[7]

The movement of free water protons in the brain along the applied gradient is measured with diffusion-weighted imaging. In isotropic tissues where diffusion is independent of tissue alignment, information about all diffusion properties of the tissue can be obtained with a single Apparent diffusion coefficient (ADC) measurement. However, ADC measurement is insufficient in anisotropic tissues where diffusion is dependent on tissue alignment and myelin is dense.[8,9] It is important to determine the diffusion size with DTI, since the places where the anisotropic diffusion is greater indicate the white matter pathways. While diffusion-weighted MR is the method that shows the information of the diffusion rate of molecules in a single direction; Diffusion tensor MR imaging provides information about the direction of molecules as well as their velocity. However, it does not give any information about the rate. In addition, features such as density of axons, average axon diameter, myelin sheath thickness and directions of pathways in white matter pathways in DTI affect diffusion in that tissue and provide important information about the structure of pathways.

In this study; It was aimed to quantitatively demonstrate diffusion abnormalities of the plaque, the peri-plaque area, the normal appeared white matter contralateral to the plaque and normal appearing white matter areas that in MS patients with FA measurements with DTI. Determine the location of DTI imaging in terms of active and chronic plaque separation by evaluating FA measurements in active and chronic plaques in MS patients. In addition, to determine whether anisotropic diffusion is superior to conventional MR imaging and whether axonal pathways are affected by obtaining 3D tractography maps from DTI images in MS patients.

MATERIAL AND METHOD

The study is a prospective study conducted at Ankara Hospital of Başkent University between February 2012 and August 2013. Patients diagnosed with MS using clinical and laboratory methods using Mc- Donald criterias in neurology department and referred to our clinic for brain MRI were included in the patient group. People who were found to be neurologically healthy as a result of the examination performed by the Neurology department were included as the control group. The patient group was between the ages of 18-72 (mean 38±12); a total of 54 patients, 39 women and 15 men, and a total of 56 healthy individuals in the control group, 32 women and 24 men, aged 20-56 years (mean 31±7) were included.

MRI was performed in all patients with a 1.5 Tesla MR device (Siemens, Germany, Avanto) using a head coil in the supine position. Fluid attenuated inversion-recovery (FLAIR) in sagittal and transverse planes, TSE T2-weighted sequences in transverse and coronal planes and axial TSE T1-weighted sequences were taken from MS patients and control group. Postcontrast sagittal and transverse TSE T1-weighted and coronal fat-suppressed T1-weighted sections were obtained only from MS patients. DTI was administered to all MS patients and the control group. Images in DTI were acquired using the (EPI) sequence 30 directions were used to obtain tensor images.

In our study, FA measurements were made from plaque, peri-plaque, normal-appearing white matter (NAWM) and the normal appeared white matter contralateral to the plaque (CP-NAWM) in MS patients. The adjacent white matter, where the plate ends, was accepted as the peri-plaque area and the measurement was made. CP-NAWM was measured as the white matter area corresponding to the symmetry of the plaque in the opposite cerebral hemisphere. In MS patients, contrast retaining plaques considered active plaque, and non-contrast retaining plaques were considered as chronic plaques. FA measurements were made from active and chronic plaques. In the control group, FA measurements were made from normal white matter (NWM). After determining the location of the lesion in T2 or FLAIR-weighted sequence in MS patients, measurements were made manually using region of interest (ROI). The measurement we made from the MS patients included in our study is shown in **Figure 1**.

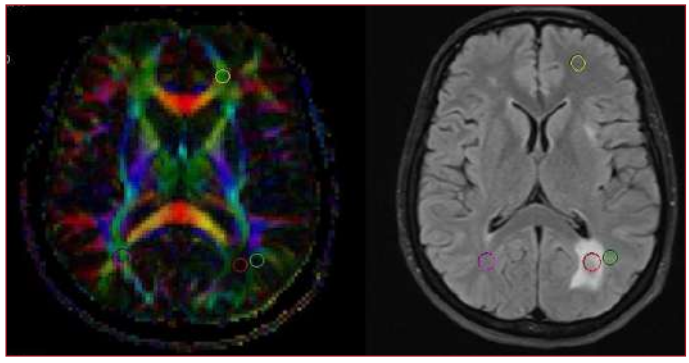


Figure 1: Demonstration of FA measurement in MS patients

A: Color FA map, B: Axial FLAIR images show the measurements we made using ROI. FA measurements from the plate adjacent to the left lateral ventricle (red ROI), periplaque area (green ROI), CP-NAWM area (pink ROI) and NAWM (yellow ROI) are shown

Since quantitative measurements will be made in DTI, plaques below 5 mm were not included in the study in order to avoid artifact and partial volume effects. In addition, if the white matter area that we will evaluate across the plate is not normal, no measurement was made from this area. Measurements made in MS patients were statistically compared among themselves and with the control group.

In MS patients, 158 chronic plaques (54.5%), 23 active plaques (7.9%), a total of 181 plaques (32%), 54 NAWM (9%), 171 peri plaques (30%), 150 CP-NAWM (27%) were evaluated. 56 NWM were evaluated in the control group.

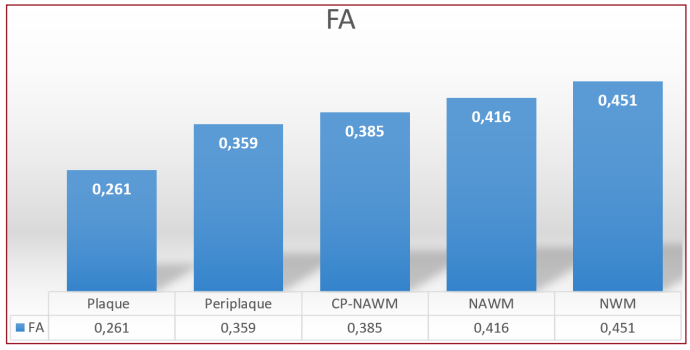
Our study was approved as a thesis project by the Ethics Committee of the Faculty of Medicine, Non-Interventional Clinical Research Ethics Committee of Başkent University in 2012, with the decision of the ethics committee numbered KA13/43.

Statistical analysis

As descriptive statistics, mean ± standard deviation in numerical variables and number values in categorical variables are given. Kolmogorov Smirnov test was used to examine whether the numerical data were normally distributed. For the comparison of two independent groups in terms of numerical variables, the "significance test of the difference between the two means" was applied, for the comparison of more than two groups, "One-way Analysis of Variance (ANOVA) if the variances were homogeneous," and if the variances were not homogeneous, "Welch Analysis of Variance" was applied. Spearman rho correlation coefficient was used to examine the amount of correlation between variables. It was considered statistically significant when P<0.05. All analyzes were performed in IBM SPSS (Statistical Package for the Social Sciences) Statistics 21 program.

RESULTS

In MS patients, 158 chronic plaques (54.5%), 23 active plaques (7.9%), a total of 181 plaques (32%), 54 NAWM (9%), 171 peri plaques (30%), 150 CP-NAWM (27%) were evaluated. 56 NWM were evaluated in the control group. When plaque, peri plaque, CP-NAWM, NAWM and control group NWM FA were compared in MS patients; value of the plaque FA (0.261±0.113) was the lowest. Other FA values increased in the following order: peri plaque (0.359±0.115.), CP-NAWM (mean FA 0.385±0.120), NAWM (mean FA 0.416±0.082), and NWM (mean FA 0.451±0.098). The difference between the mean FA values of plaque, periplaque, CP-NAWM, NAWM and control group NWM in MS patients is shown graphically (**Graph 1**). In our study, the difference between plaque (p<0.001), peri-plaque (p<0.001), CP-NAWM (p<0.001), NAWM (p<0.001) and NWM FA (p<0.001) values was statistically significant (**Table 1**).



Graph 1: Graph of mean FA values measured in MS patients and control group

Table 1: FA values in different localizations of MS patients and statistical comparison of FA values measured among themselves and with the control group

	FA	± SD	P
Plaque	0,261	0,113	p<0.001
Peri-plaque	0,359	0,115	p<0.001
CP-NAWM	0,385	0,120	p<0.001
NAWM	0,416	0,082	p<0.001
NWM	0,451	0,098	p=0,047

NWM: Normal white matter, NAWM: Normal-appearing white matter, CP-NAWM Normal-appearing white matter opposite the plaque, p< 0.05 statistically significant

Although active plaque FA value was lower than chronic plaque, it was not statistically significant (p=0.165) (**Table 2**). While 21 of MS patients were clinically active, 34 were not clinically active. While there was active plaque in the MRI of 7 of the clinically active patients, no active plaque was detected in 14 of them. FA was measured from 44 chronic plaques of 14 clinically active patients whose active plaque was not detected by conventional MR imaging. While the FA value of 24 of 44 chronic plaques was lower than the mean FA value (FA value of 0.230), the FA value of 20 chronic plaques was found to be higher than the mean FA value. Based on this result, we cannot comment on the activity of MS disease by evaluating the chronic plaques of patients who are clinically active and could not detect active plaque in conventional MRI.

Table 2: Mean and p values of active and chronic plaque FA measurements in MS patients

	Active Plaque	Chronic Plaque	p
FA	0,230 ± 0,090	0,265 ± 0,115	0,165

FA: Fractional anisotropy, p< 0.05 statistically significant

In all MS patients, the starting point of the mesencephalon was determined and 3D tractography maps were made. It was evaluated whether the white matter pathways of the patients whose tractography maps were obtained were affected by MS disease. It has been shown that plaques traced along axonal pathways in MS patients cause interruption in axonal pathways (**Figure 2**). However, it has been shown that in some patients the plaques are not during the drawn tractography pathways and therefore do not cause a significant pathology in the axonal pathways.

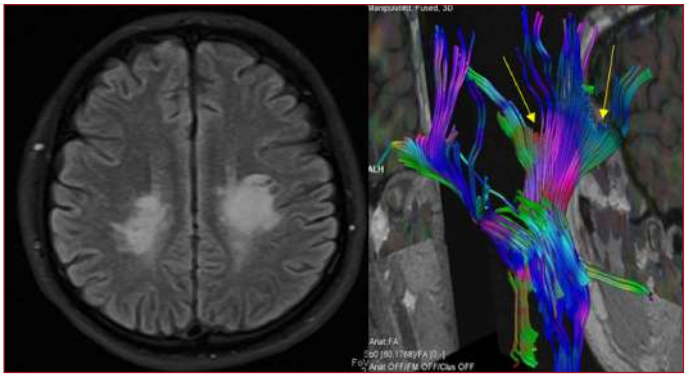


Figure 2: Demonstration of MS lesions by tractography

A: Axial FLAIR, B:3D tractography images of a 22-year-old male MS patient is shown. Large MS plaques observed in both periventricular white matter in the axial and sagittal FLAIR sequence have been shown to cause interruption of the axonal pathways in tractography images (yellow arrows)

DISCUSSION

Multiple Sclerosis (MS) is the most common chronic inflammatory, demyelinating disease of the central nervous system. It is a disease characterized by demyelinating plaques in the white matter.[1] Axonal injury in the NAWM is thought to be responsible for the pathology of MS disease, as well as demyelinating plaques.[3] Conventional MRI is the most commonly used imaging method in the diagnosis and follow-up of MS today. Although conventional MRI is very sensitive to show macroscopic lesions, it is insufficient to show hidden microscopic changes in NAWM. Inconsistency between the distribution of lesions and clinical findings in conventional MRI may be an indicator of this. DTI is an advanced MRI application that can focus on plaques and NAWM, which are thought to be responsible for MS pathology, with ROI analysis, and can evaluate tissue pathology quantitatively and objectively.[10]

In our study, when plaque, peri-plaque, NAWM, CP-NAWM and control group FA measurements were compared in MS patients; plaque FA was found to be lower than the others. Peri-plaque, CP-NAWM, NAWM and NWM FA measurements were also observed to increase in this order. Rachel E. Maia de Andrade.[11] measured plaque, peri-plaque, CP-NAWM, NAWM and NWM FA and plaque FA was the lowest; Peri-plaque, CP-NAWM, NAWM and NWM FA measurements were also found to increase in the same order. Alexande Guo et al.[12] obtained similar results in their study, but they did not evaluate CP-NAWM. In another study by Alexande Guo et al.[13], they showed that plaque FA was the lowest and peri-plaque and CP-NAWM measurements increased in this order, but they did not compare the results with the control group and NAWM. Sijens PE et al.[14] compared only plaque FA value with the control group and showed that plaque FA was lower than the control group NWM FA. Bing Hu et al.[15] compared plaque with NAWM and NWM FA values and obtained results similar to our study.

In our study, it was determined that the FA measurements obtained from the peri-plaque area, CP-NAWM and NAWM, which were normal in conventional MRI, were higher than the plaque but lower than the control group, and there was a statistically significant difference between them. Rachel E. Maia de Andrade.[11] and Alexande Guo et al. in two separate studies.[12,13] found that peri-plaque FA was higher than plaque FA and lower than the measurements made from white matter and the control group. In the literature, there are studies similar to our study showing that the NAWM FA value in MS patients is lower than the white matter of the control group and there is a statistical difference between them.[11,12,16-18]

In our study, the difference between FA values of plaque ($p<0.001$), peri-plaque ($p<0.001$), CP-NAWM ($p<0.001$), NAWM ($p=0.047$) and NWM ($p=0.047$) was found to be statistically significant. In the literature, Alexande Guo et al.[13] Rachel E. Maia de Andre et al.[11] Bing Hu et al.[15] and Sijens PE et al.[14] found similar findings.

The fact that the anisotropy at the plaque level was lower than in other localizations in our study indicates that plaques are mainly responsible for MS pathology. Detection of anisotropy in the peri-plaque area higher than plaque and lower than NAWM suggests that the peri-plaque area is affected, although not as much as plaque. There are many studies showing abnormalities in FA values of normal-appearing white matter in the peri-plaque area with Diffusion Tensor Imaging.[19-21] Therefore, FA is more sensitive than conventional MRI for the assessment of WM integrity in MS. Tievsk et al.[22] found a significant decrease in the ratio of N-acetylaspartate/creatine, which is considered as the marker of neuronal and axonal injury, in NAWM adjacent to the plaque in a different study they conducted with MR spectroscopy. This study supports the hypothesis that white matter that appears normal on conventional MRI with DTI is not normal. It also shows that the white matter area that appears normal on conventional MR is actually not normal and is affected.

When active and chronic plaque FA measurements are evaluated; although active plaque FA was observed to be lower than chronic plaque, no statistically significant difference was observed between active and chronic plaque FA ($p=0.165$) values. Filippi et al.[16], with 4728 chronic plaques and 128 active plaques, and Tievsk et al.[22] in another study, found the FA value in active plaque to be lower than in chronic plaque. However, unlike our study, they showed that

the difference between them was statistically significant. Lorenzo Testaverde et al.[23] in their study of 7 active plaques and 14 chronic plaques, they found the FA value of chronic plaque to be lower than that of active plaque, unlike our study and the literature. However, similar to the studies in the literature, they found the FA value of active and chronic plaque to be lower than NAWM and NWM, and they found a statistically significant difference between active, chronic plaque, NAWM and NWM FA values. The reason why the FA values between active and chronic plaques were not statistically significant in our study, unlike the literature, may be due to our small number of active plaque evaluations. However, DTI are not sufficient to distinguish between active and chronic plaques since there was no statistically significant difference between active plaque and chronic plaque FA values in our study.

Limitations

In our study, the normal-appearing white matter area opposite the plaque was evaluated. If this area is not normal in T2 signal, it was not included. In addition, since the ROI of small plates is limited to drawing, plates smaller than 8 voxels (78mm) were not included. The number of active plaques is less than chronic plaque; For this reason, it may cause inadequacy in statistical calculations.

CONCLUSION

In MS patients, abnormal findings were detected in FA values in plaque and all white matter areas. Progressive decrease in anisotropy from normal-appearing white matter to peri-plaque white matter and plaque level suggests myelin damage and white matter abnormalities extending beyond plaque. In addition, the decrease in anisotropy we detected in the white matter areas of MS patients compared to the control group indicates that the white matter that appears normal on T2 images in conventional MR is not actually normal but is affected by MS disease.

Unlike conventional MR, DTI provides more objective data by providing quantitative information about MS lesions and abnormalities in the white matter area. In addition, it gives information about the abnormalities in the white matter that appears normal on conventional MR and the areas other than the lesions observed in T2W, which gives an advantage in understanding MS pathologies. Based on these results, it was thought that DTI would provide more objective and quantitative information than conventional MR in the evaluation of disease burden in MS patients.

ETHICAL DECLARATIONS

Ethics Committee Approval: Our study was approved as a thesis project by the Ethics Committee of the Faculty of Medicine, Non-Interventional Clinical Research Ethics Committee of Başkent University in 2012, with the decision of the ethics committee numbered KA13/43.

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article / Orijinal Araştırma

A Bibliometric Analysis Study on Percutaneous Diskectomy

Perkütan Diskektomi Üzerine Bir Bibliyometrik Analiz Çalışması

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Abstract

Aim: Percutaneous discectomy is an important issue in the field of neurosurgery. However, the outputs of scientific publications on this subject are not known. The goal of this study was to add to the body of knowledge by performing a bibliometric analysis of the original scientific studies on percutaneous discectomy that have been published since 1970.

Material and Method: The literature review was done using the Web of Science database. All articles and citations related to percutaneous discectomy containing the keywords Mesh were searched in the "title" section of the search engine. The articles produced by the countries and their developments was analyzed. The Vosviewer program was utilized to map the coauthorship, keywords, etc. of the articles.

Results: There was 619 articles between 1983-2021. The first articles were published in 1983 (3 articles). Nearly 73% of the articles have been published since 2000. The leading country on percutaneous discectomy was the People's Republic of China (n=264, 42.649%). Corresponding authors from China, South Korea, the United States of America (USA), Japan and Germany were the most productive authors. The publications from China had 2237 citations (8.47 per article), the publications from South Korea had 3483 citations (34.49 per article). Wooridul Spine Hospital (South Korea) was the mostly publishing affiliation.

Conclusion: Future research on percutaneous discectomy will be able to benefit from the data collected in this bibliometric study. The majority of the publications originated from China, followed by South Korea and the USA. The number of publications from other countries around the world was very limited. These numbers need to be increased.

Keywords: Article, bibliometrics, percutaneous discectomy

Öz

Amaç: Perkütan diskektomi beyin cerrahisi alanında önemli bir konudur. Ancak bu konudaki bilimsel yayınların çıktıları bilinmemektedir. Bu çalışmanın amacı perkütan diskektomi ile ilgili 1970 yılından beri yayınlanan orijinal bilimsel çalışmaların bibliyometrik analizini yaparak bilgi birikimine katkıda bulunmaktır.

Gereç ve Yöntem: Literatür taraması Web of Science veri tabanı kullanılarak yapılmıştır. Perkütan diskektomi ile ilgili Mesh anahtar kelimelerini içeren tüm makaleler ve alıntıları, arama motorunun "başlık" bölümünden aratıldı. Ülkelerin ürettikleri makale sayısı ve gelişmişlik göstergeleri analiz edilmiştir. Makalelerin ortak yazarlığını, anahtar kelimelerini vb. Haritalamak için Vosviewer programından yararlanıldı.

Bulgular: 1983-2021 yılları arasında 619 makale bulundu. İlk makaleler 1983 yılında yayınlanmıştı (3 makale). Makalelerin yaklaşık %73'ü 2000 yılından beri yayınlanmıştı. Perkütan diskektomi konusunda lider ülke Çin Halk Cumhuriyeti idi (n=264, %42.649). Çin, Güney Kore, Amerika Birleşik Devletleri (USA), Japonya ve Almanya'dan gelen yazarlar en üretken yazarlar idi. Çin'den yayınlanan yayınlara 2237 (makale başına 8,47), Güney Kore'den yayınlanan yayınlara 3483 (makale başına 34,49) atıf yapılmıştı. Wooridul Spine Hospital (Güney Kore) en çok yayın yapan kuruluştu.

Sonuç: Perkütan diskektomi ile ilgili gelecekteki araştırmalar, bu bibliyometrik çalışmada toplanan verilerden yararlanabilecektir. Nöroşirürji alanındaki yayınların çeşitliliği, en gelişmiş ülkelerde coğrafi olarak artmaya devam etmiştir. Gelişmiş ve gelişmekte olan ülkeler arasındaki yayın oranlarındaki eşitsizlik aynı zamanda sabit kalmıştır.

Anahtar Kelimeler: Araştırma makalesi, bibliyometri, perkütan diskektomi

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INTRODUCTION

Vertebral discs hernias (VDH) especially lomber discs hernais (LDH) are a common cause of sciatica and back pain worldwide. Exploratory laminectomy has been replaced with percutaneous discectomy in the surgical treatment of VDH or prolapses.[1] Slighter invasive surgical methods, particularly for the treatment of LDHs, have been developed as a result of discussions about the outcomes of open spinal surgery, particularly on complications related to open surgery, such as post-discotomy syndrome. Percutaneous discectomy are based on a variety of intradiscal diagnostic and therapeutic approaches, including chemonucleolysis, retroperitoneal spinal disc fenestration, and discography.[2,3]

Since the 1970s, numerous techniques have been developed and put to use in clinical settings, including mechanical percutaneous nucleotomy, automated percutaneous nucleotomy, intradiscal laser procedures, and, to some extent, endoscopic intradiscal procedures. Depending on the author and study, the clinical outcomes range from 30-100% almost good/very good results. It is clear that there haven't been many prospective randomized studies with control groups receiving either conservative or surgical treatment. When compared to partially retrospective studies of a single method, which frequently have large case numbers but do not always meet the strict requirements for scientific study design.[2]

Yasargil performed the first removal of a herniated disc using the operating microscope in 1977.[4] However, it wasn't until the late 1980s that it started to be utilized more and more.[5] Many spinal surgeons gave up the traditional naked-eye discectomy procedure in the 1990s and switched to the common practice of microdiscectomy. The advantages of this method include the ability to remove any type of LDH with a limited laminoarthrectomy and a quick approach to the skin, fascia, and muscles. It is the procedure that the vast majority of spinal surgeons use and is regarded as the "gold standard" of surgical care for LDH.[6]

A methodological technique from the library sciences known as "bibliometric study" uses statistical analysis to estimate influence and impact by counting the number of times books, papers, and other publications have been cited. With this method reserchers can examine scientific literature by analyzing metrics at the author, article, and journal levels. Numerous bibliometric analyses have been conducted in a variety of specialities in medicine[7-18], including spine surgery.[19-23]

The objective of this study was to further knowledge by doing a bibliometric analysis of the original scientific studies on percutaneous discectomy that have been published since 1970.

MATERIAL AND METHOD

We looked through the Web of Science database for each article published between the dates of January 1, 1970, and December 31, 2021.

Mesh terms [Diskectomies, Percutaneous (Title) OR Percutaneous Diskectomies (Title) OR Percutaneous Diskectomy (Title) OR Discectomy, Percutaneous (Title) OR Discectomies, Percutaneous (Title) OR Percutaneous Discectomies (Title) OR Percutaneous Discectomy (Title) OR Nucleotomy, Percutaneous (Title) OR Nucleotomies, Percutaneous (Title) OR Percutaneous Nucleotomies (Title) OR Percutaneous Nucleotomy (Title)]

The overall number of articles, typical bibliometric measures like the H-index, and the absolute and average number of citations per article were also evaluated.

Also the Vosviewer program (VOSviewer 1.6.18 for Microsoft Windows systems) was utilized to map the coauthorship, keywords, etc. of the articles.

By using the visualization tool VOSviewer (created by the Science and Technology Research Center in Leiden, the Netherlands), it is possible to create visual network maps based on literature and, gain a thorough understanding of the scientific structure and dynamic development trend of a field.[24] VOSviewer can help for instance include journals, researchers, citations, bibliographic coupling, co-authorship relations, co-citation, etc. Additionally, text mining capabilities in VOSviewer may be used to create and display co-occurrence networks of significant terms taken from a corpus of scientific literature.[25]

RESULTS

There was 809 publications between 1970-2022 and 758 publications between 1983-2021. 619 of them articles. 93.053% of the articles published in Science Citation Index Expanded (SCI-EXPANDED) index and 94.507% of them in English language. These 619 articles cited 10,197 times in total and 16.47 times per article. The first articles were published in 1983 (3 articles). Nearly 73% of the articles published since 2000. The most productive year was 2020 with 76 published articles (**Figure 1**).

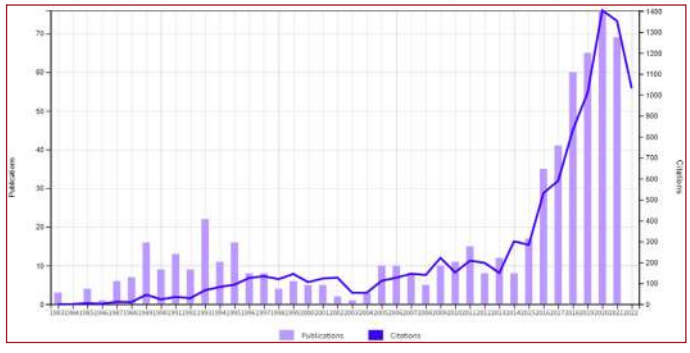

Figure 1. The quantity of percutaneous discectomy-related articles and citations between 1983 and 2021.

The leading country on percutaneous discectomy was the People's Republic of China (n=264, 42.649%). Corresponding authors from China, South Korea, the United States of America (USA), Japan and Germany were the most productive authors. The publications from China had 2237 citations (8.47 per article, H index:22), the publications from South Korea had 3483 citations (34.49 per article, H index: 35) (**Table 1**).

Table 1. Publication numbers of the most productive countries on percutaneous diskectomy

Countries/Regions	Record Count	% of 619
Peoples Rebuplic of China	264	42.649
South Korea	101	16.317
USA	83	13.409
Japan	36	5.816
Germany	31	5.008
France	19	3.069
Switzerland	12	1.939
Greece	9	1.454
Italy	9	1.454
Taiwan	9	1.454

*Showing 10 out of 38 countries; 2 records (0.323%) do not have data

A total of 1,958 authors contributed the publications on percutaneous diskectomy. Sang-Ho Lee from Wooridul Spine Hospital (South Korea) published most of the articles (**Figure 2**).

Figure 2. Mostly publishing authors

Wooridul Spine Hospital (South Korea) was the mostly publishing affiliation on percutaneous diskectomy. Tongji University (China), The Army Medical University (China), Chongqing Medical University (China) and Naval Medical University(China) were also mostly publishing affiliations.

Table 2. Publication numbers of the most productive countries on percutaneous diskectomy

Affiliations	n	% of 619
Wooridul Spine Hosp	37	5.977
Tongji University	29	4.685
Army Medical University	14	2.262
Chongqing Medical University	14	2.262
Naval Medical University	13	2.100
University of California System	13	2.100
Allegheny General Hospital	12	1.939
Seoul National University	12	1.939
Shanghai Jiao Tong University	12	1.939
Seoul National University Hospital	11	1.777
Sichuan University	11	1.777
Capital Medical University	10	1.616
Leon Wiltse Mem Hosp	10	1.616
Southern Medical University China	10	1.616
University of California San Francisco	10	1.616

National Natural Science Foundation Of China funded most of the articles (n=46).

Table 3. Main funding agencies on percutaneous diskectomy

Funding Agencies	n	% of 619
National Natural Science Foundation of China	46	7.431
Wooridul Spine Foundation	14	2.262
China Postdoctoral Science Foundation	3	0.485
Chinese Ministry of Health	3	0.485
Foundation for Leading Talent in Traditional Chinese Medicine of Jiangsu Province	3	0.485
Fundamental Research Funds for the Central Universities	3	0.485
Key Project of Medical Research of Chongqing Municipal Healthy Bureau	3	0.485
Korea Health Technology R D Project Through the Korea Health Industry Development Institute Khidi Ministry of Health Welfare Republic of Korea	3	0.485
National Key R D Program of China	3	0.485
Natural Science Foundation of Shandong Province	3	0.485

Showing 10 out of 196 entries; 447 record(s) (72.213%) do not contain data in the field being analyzed

The most of the articles (48.142%) were from Neurosciences/ Neurology research area (**Table 4**).

Table 4. Main research areas of the publications on percutaneous diskectomy

Research Areas	Record Count	% of 619
Neurosciences Neurology	298	48.142
Orthopedics	201	32.472
Surgery	193	31.179
General Internal Medicine	56	9.047
Radiology Nuclear Medicine Medical Imaging	56	9.047
Anesthesiology	50	8.078
Research Experimental Medicine	44	7.108
Biotechnology Applied Microbiology	15	2.423
Rheumatology	10	1.616
Cardiovascular System Cardiology	7	1.131

Showing 10 out of 31 entries

The most of the articles published in 'World Neurosurgery' journal. The mostly publishing journals on percutaneous discectomy listed in the **Table 5**.

Table 5. The list of the mostly publishing journals on percutaneous diskectomy.

Publication Titles	Record Count	% of 619
World Neurosurgery	48	7.754
Pain Physician	36	5.816
Spine	29	4.685
Medicine	22	3.554
Orthopaedic Surgery	18	2.908
Clinical Orthopaedics and Related Research	16	2.585
Biomed Research International	14	2.262
International Orthopaedics	14	2.262
Journal of Orthopaedic Surgery And Research	14	2.262
European Spine Journal	12	1.939
Zeitschrift Fur Orthopadie Und Ihre Grenzgebiete	11	1.777
Journal of Neurosurgery Spine	10	1.616
Bmc Musculoskeletal Disorders	9	1.454
Journal of Spinal Disorders	9	1.454
International Journal of Clinical and Experimental Medicine	8	1.292
Journal of Korean Neurosurgical Society	8	1.292
Journal of Neurological Surgery Part A Central European Neurosurgery	8	1.292
Korean Journal of Pain	8	1.292
Neuroradiology	8	1.292
Acta Neurochirurgica	7	1.131
Neurosurgery	7	1.131
Spine Journal	7	1.131
Acta Radiologica	6	0.969
Archives of Orthopaedic and Trauma Surgery	6	0.969
Journal of Spinal Disorders Techniques	6	0.969

*Showing 25 out of 178 entries

Mapping analysis (Co authorship analysis, Keyword analysis, Bibliographic coupling between countries and affiliations) with Vosviewer can be seen in **Figure 3-6**.

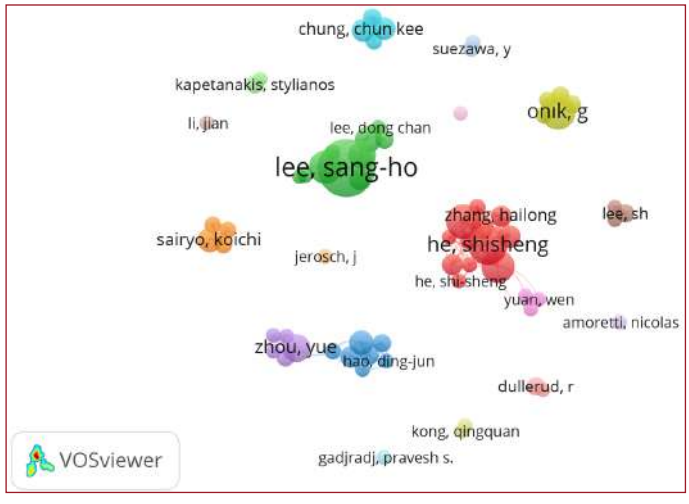

Figure 3. Co authorship analysis between authors with more than 5 articles

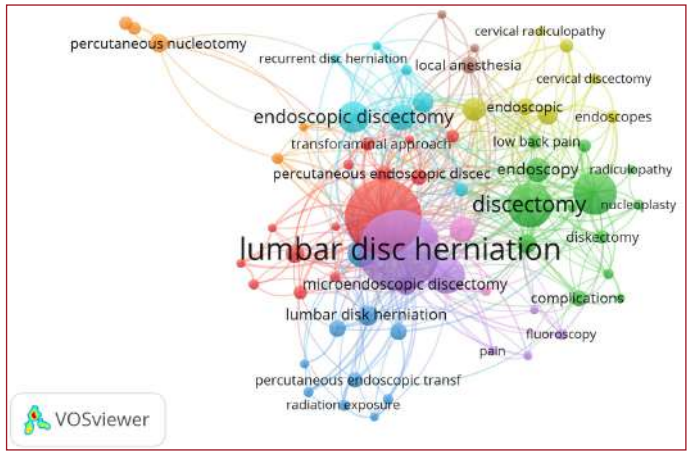

Figure 4. Keyword analysis

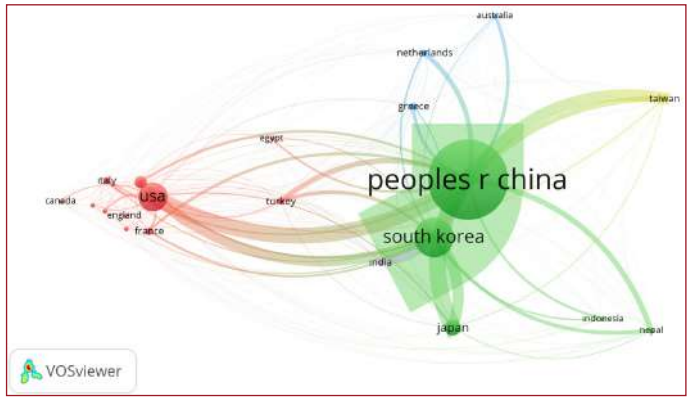

Figure 5. Bibliographic coupling between countries with minimum 3 articles

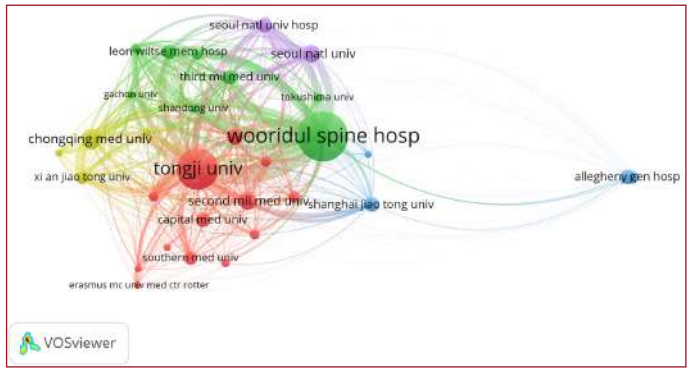

Figure 6. Bibliographic coupling between affiliations with minimum 3 articles

DISCUSSION

Neurosurgery is one example of a medical specialty that evolved later than other disciplines and is continually evolving due to new ideas and methods. For instance, in neurosurgery, bibliometrics was utilized to identify the top 100 referenced papers on carotid stenting, craniopharyngiomas, endovascular aneurysm therapy, pediatric neurosurgery, and skull base neurosurgery. It has been used to examine certain publications, databases of pediatric patients, funding and research from the US National Institutes of Health, as well as the publishing output of university neurosurgery departments and residency programs. To the best of our

knowledge, a thorough bibliometric analysis examination of the articles on percutaneous discectomy over the last 50 years has not yet been been out.[19-23] We tried to look into and determine regional publication trends (regional, national, and continental differences). Additionally, we looked at connections between authors, organizations, and countries.

This study's bibliometric analysis is based on the Web of Science Core Collection (WoSCC), whose high-quality and regularly updated literature can effectively guarantee the quality of literature analysis. For the purpose of visual analysis in this study, the literature obtained by WoSCC was imported into Microsoft Excel 2019 and VosViewer.

According to our analysis, the number of publications published has dramatically increased in recent years, particularly since 2000. Sang-Ho Lee from Wooridul Spine Hospital (South Korea) was the biggest contributor to the percutaneous discectomy literature.

38 countries made contributions articles, with China accounting for roughly half of them. China was also the majority of articles' corresponding author country of origin. The other most productive authors were those from South Korea, USA, Japan, and Germany. The publications from China had 2237 citations (8.47 per article, H index:22) and the publications from South Korea had 3483 citations (34.49 per article, H index: 35). In other words, although the number of articles originating from South Korea was less, the H index and the number of citations were considerably higher than those of China.

Universities and research institutes in the South Korea and China are the institutions with the most research and publications, according to the institutions' visual analysis. While there are still certain nations or areas that collaborate less with other nations, China has more research collaborations with South Korea and other developed European nations. It is advised that research organizations globally work aggressively together in the future to investigate the percutaneous discectomy.

The heart of a paper lies in its key words. In-depth study of keyword co-occurrence can more rapidly detect research hotspots and trends than keyword analysis, which represents the core and research emphasis of a document. A field's research hotspots and trends may be readily understood through the summary of key terms. A keyword co-occurrence map was created using VOSviewer to see and analyze all the terms.[26,27] The term cluster map created by VOSviewer reveals that it can be separated into four clusters based on various colors, including red, green, purple, and yellow. **Figure 4** displays the most frequent keywords and clusters.

Study Limitations

One of the study's drawbacks is that, in order to keep the research concise, we only looked at articles from journals that were indexed by WoS. Other significant publications that disseminate articles related to neurosurgery through other databases (Pubmed, Scopus, etc.) were not included.

CONCLUSIONS

Approximately half of the publications came from China, followed by South Korea, USA. There weren't many publications from other nations. Although upper- and lower-middle-income countries in Asia and America made significant contributions, there is still a sizable publication gap between industrialized and developing nations,they have not changed over the past 50 years.

ETHICAL DECLARATIONS

Ethics Committee Approval: As it is not a human or animal study there is no need for ethical approval.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

Financial Disclosure: The author declared that this study has received no financial support.

Author Contributions: The author declare that he has all participated in the design, execution, and analysis of the paper, and that he has approved the final version.

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Original Article / Orijinal Araştırma

The Utility of Glasgow Prognostic Score as an Indicator of Mortality after Transcatheter Aortic Valve Implantation

Transkateter Aort Kapak İmplantasyonu Yapılan Hastalarda Mortalite Öngördürücüsü Olarak Glasgow Prognostik Skorunun Kullanımı

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Abstract

Aim: The Glasgow Prognostic Score (GPS) is a scoring system obtained by using inflammatory (C-reactive protein) and nutritional (albumin) parameters together, and it has been shown to have prognostic value in various cardiac pathologies in previous studies. In this study, we aimed to investigate the utility of the Glasgow Prognostic Score (GPS) in predicting 1-year mortality in patients who underwent transcatheter aortic valve implantation (TAVI).

Material and Method: Patients who underwent TAVI with the diagnosis of severe, symptomatic aortic stenosis in our hospital between 2013 and 2017 were included in this single center retrospective study. Demographic, clinical and laboratory data were obtained by reviewing patient files. GPS value was calculated by using C-reactive protein and albumin values which was obtained on admission. Two groups were formed as survivors and non-survivors according to 1-year mortality data.

Results: A total of 170 patients were included in this retrospective study and 59 patients constituted the non-survivors group. History of chronic obstructive pulmonary disease and cerebrovascular disease were higher in non-survivors' group. STS-TAVR, Euro SCORE II and GPS levels were also higher in non-survivors group. High GPS value calculated with pre-procedural data was determined as a predictor of 1-year mortality.

Conclusion: The Glasgow Prognostic Score allows the evaluation of inflammation and nutritional status together, is a practical method that can be obtained from routine laboratory parameters. It can be used as a predictor of mortality in patients undergoing TAVI. It can guide clinicians in taking preventive measures to reduce mortality before the procedure.

Keywords: Albumin; C-reactive protein, glasgow prognostic score, transcatheter aortic valve replacement

Öz

Amaç: Glasgow Prognostik Skoru (GPS) inflamatuar (C reaktif protein) ve nutrisyonel (albümin) parametrelerin birlikte kullanımı ile elde edilen bir skorlama sistemidir ve daha önce yapılan çalışmalarda çeşitli kardiyak patolojilerde prognostik değeri olduğu gösterilmiştir. Bu çalışmada, Transkateter aort kapak replasmanı (TAVR) yapılan hastalarda 1-yıllık mortaliteyi öngörmede Glasgow Prognostik Skoru (GPS)'nun kullanılabilirliğinin araştırılması amaçlanmıştır.

Gereç ve Yöntem: Hastanemizde, 2013-2017 yılları arasında ciddi, semptomatik aort darlığı tanısı ile TAVR uygulanan hastalar geriye dönük olarak çalışmaya dahil edilmiştir. Demografik, klinik ve laboratuvar verileri hasta dosyaları incelenerek elde edilmiştir. İşlem öncesi C-reaktif protein ve albumin değerleri kullanılarak GPS değeri hesaplanmıştır. Hastaların 1-yıllık mortalite verisine göre mortalite (+) ve mortalite (-) olmak üzere 2 grup oluşturulmuştur.

Bulgular: Bu çalışmaya toplam 170 hasta dahil edilmiş ve 59 hasta mortalite (+) grubu oluşturmuştur. Demografik verilerden kronik obstruktif akciğer hastalığı, serebrovasküler hastalık öyküsü olması, yüksek STS-TAVR, Euro SCORE II ve GPS mortalite grubunda daha yüksek saptanmıştır. İşlem öncesi verilerle hesaplanan GPS değerinin yüksek olması 1-yıllık mortalitenin öngördürücüsü olarak belirlenmiştir.

Sonuç: İnflamasyon ve nutrisyonel durumun birlikte değerlendirilmesine olanak sağlayan Glasgow Prognostik Skoru, rutin laboratuar tetkiklerinden elde edilebilen pratik bir yöntemdir. TAVR uygulanan hastalarda mortalite öngördürücüsü olarak kullanılabilir. İşlem öncesi mortaliteyi azaltabilmek yönünde koruyucu önlemlerin alınmasında klinisyenlere yol gösterebilir.

Anahtar Kelimeler: Albümin, C-reaktif protein, glasgow prognostik skoru, transkateter aort kapak replasmanı

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INTRODUCTION

Aortic stenosis (AS) prevalence is increasing in developed countries and it is still the most common valvular disease.[1,2] Although the patients may stay asymptomatic, the prognosis is poor in symptomatic patients and need to be treated. Surgical or transcatheter aortic valve implantation (TAVI) are treatment options. TAVI is found non-inferior to surgical aortic valve replacement (SAVR) and superior to medical therapy in randomized clinical trials.[3-5] TAVI is especially recommended for older (≥75 years) and high-risk patients according to STS (Society of Thoracic Surgeons) and Euro SCORE (European System for Cardiac Operative Risk Evaluation>8%).[1] Although, STS and Euro SCORE include most of the comorbidities, they do not take into account the functional decline typical of elderly patients. Besides the benefit on mortality, quality of life and symptom status of the patients were demonstrated to be improved after TAVI.[6,7] Various studies have developed to define new parameters associated with increased early and late mortality rates.[8,9]

Aortic stenosis is defined as a degenerative process however, the role of inflammation and oxidative stress iin the progression of aortic stenosis progression is established.[10] The inflammatory process followed by endothelial dysfunction and lipid infiltration are the initiators for the progression of aortic stenosis.[11,12] Glasgow prognostic score (GPS), includes C-reactive protein (CRP) and albumin levels as variables and , is one of the validated prognostic risk scores in cancer patients. [13] Moreover, it has been studied in various fields of cardiac disorders and studies have indicated that GPS can be used as a prognostic tool for determining survival in heart failure as well as mortality in acute coronary syndromes.[14,15]

MATERIAL AND METHOD

This study was approved by Ethical Committee of Bağcılar Training and Research Hospital (Date: 05/07/2022 Number: 2022/07/01/001). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

The patients with severe symptomatic AS who were treated by TAVI between 2013 and 2017 were included in this retrospectively designed, single center study. The decision was based on the consensus of the heart team due to patients' high surgical risk.

Pre-, peri- and postoperative data were retrieved from hospital database and patients' files. Demographic, clinical, laboratory parameters and details of length of hospital stay were noted for each patient. Patiensts were evaluated according to the European System for Cardiac Operative Risk Evaluation II (Euro SCORE II) and Society of Thoracic Surgeons- Transcatheter Aortic Valve Replacement (STS-TAVR) scoring system.[16,17] Patients with preoperative serum creatinine >2 mg/dl albuminuria and chronic liver disease, albumin replacement therapy in past 6 months, malignancy, endocrinologic disorders (hypo/hyperthyroidism), previous diagnosis of systemic inflammatory, hematologic or autoimmune disease, active infection were excluded from the study. Also, those with unavailable serum CRP or albumin levels were excluded. Preoperative CRP and albumin levels were used for GPS calculation (https://www.mdcalc.com/glasgow-prognostic-score). An increased CRP value (>10 mg/L) or a low albumin value (<3.5 g/dL) were defined as 1 point each to define GPS. The patient had a score of 0 if both parameters were normal whereas, 1 if one of them was abnormal and 2 if both parameters were abnormal.[18]

Mortality data within 1-year follow-up was achieved using hospital records and national health database system. We sought to asses if GPS has a predictive value for mortality in patients undergoing TAVI.

Statistical Analyses

The Statistical Package for the Social Sciences 25.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. The normality of the data was analyzed by Kolmogorov-Smirnov test. Categorical data was stated as percentages and continuous data are stated as mean ± SD. The differences in categorical variables between groups was tested by Chi-square test. Student's t- or Mann Whitney U test was used to compare unpaired samples as needed. Independent variables of 30-day and 1-year mortality were identified by binary logistic regression analysis. The diagnostic accuracy of GPS for TAVI mortality was evaluated by receiver operating characteristic (ROC) curve analyses. A 2-sided $p<0.05$ was regarded as significant.

RESULTS

A total of 170 patients (84 male, 86 female) were included. Mean age of all included patients was 78.4±7.1. Two groups generated according to 1-year mortality and 59 patients were formed non-survivor group. There was no difference between groups regarding body mass index, age, gender, history of malignancy, diabetes mellitus (DM), hyperlipidemia (HL), coronary artery disease, hypertension (HT), smoking status and atrial fibrillation. Chronic obstructive pulmonary disease (72.8% vs. 49.5%, p=0.003), peripheral artery disease (45.7% vs. 31.5%, p=0.048), history of previous cerebrovascular accident (22% vs. 3.6%, p<0.0001) were found to be higher in non-survivor group. Moreover, patients were in advanced NYHA class in non-survivors when compared to survivors' group (64.4% vs. 32.4%, p<0.0001). Urea (50.8±19.8 vs 60.9±36.8; p=0.021), STS TAVR score [8.4 (7.4-11.0) vs 14.5 (9.7-17.2-10.1); p<0.0001], and Euro SCORE II [13.4 (6.2-15.1) vs 16.1(7.9-27.9); p<0.0001] were significantly higher in non-survivors. There were 100 patients in low (GPS=0) and 70 patients in high GPS groups (GPS≥1). The non-survivor patients had significantly higher GPS when compared to survivors' group (p=0.021). Remarkably, the non-survivor patients

had lower left ventricular ejection fraction than survivors (49.6±11.7 vs 54.4±9.5; p=0.004). There were no significant differences in terms of left ventricular end-diastolic and left atrial diameters and pulmonary artery pressure between groups. Preoperative medication such as statin, ACEİ/ARB, β blockers, antiaggregant and anti-coagulant usage were similar between groups, whereas calcium channel blockers usage was more common in survivors' group. All demographic, clinical and laboratory variables of patients are presented in detail in **Table 1**.

Binary regression analysis was performed to determine independent risk factors for 1-year mortality and STS TAVR and history of previous CVA [p<0.0001; β: 2.708, OR (95% CI): 1.547-4.740) and p=0.014; β: 0.048, OR (95% CI): 0.004-0.537), respectively] were found as independent risk factors for 1-year mortality.

Table 1. Comparison of demographic, clinical and laboratory parameters between groups according to 1-year mortality.

Variables	All (n=170)	Survivors (111)	Non-survivors (59)	p
Age (years)	78.4±7.1	77.9±7.1	79.5±7	0.169
Gender Male, n (%) Female, n (%)	 84 (49.4) 86 (50.6)	 49 (44.1) 62 (55.8)	 35 (59.3) 24 (40.6)	0.76
NYHA III/IV	74 (43.5)	36 (32.4)	38 (64.4)	<0.0001
Body Mass Index	26.7 (24.5-30.6)	26.9 (24.4-30.2)	26.7 (25.227.8)	0.232
Coronary Artery Disease, n (%)	137 (80.6)	85 (62)	52 (88.1)	0.51
Hypertension, n (%)	126 (74.1)	84 (75.6)	42 (71.1)	0.323
Chronic obstructive pulmonary disease, n (%)	98 (57.6)	55 (49.5)	43 (72.8)	0.003
Diabetes mellitus, n (%)	80 (47.1)	50 (45)	30 (50.8)	0.288
Peripheric Artery Disease, n (%)	62 (36.5)	35 (31.5)	27 (45.7)	0.048
Hyperlipidemia, n (%)	119 (70)	79 (71.2)	40 (67.8)	0.726
Cerebrovascular accident, n (%)	17 (10)	4 (3.6)	13 (22)	<0.0001
Malignancy, n(%)	29 (17.1)	19 (17.1)	10 (16.9)	0.579
Smoking, n (%)	86 (50,6)	52 (47.3)	34 (57.6)	0.259
Atrial fibrillation, n (%)	32 (18.8)	17 (15.7)	15 (25.4)	0.152
Urea, (mg/dl)	54.3±27.3	50.8±19.8	60.9±36.8	0.021
Creatinine, (mg/dl)	1.07±0.6	1.02±0.6	1.17±0.5	0.113
Sodium (mEq/L)	139.9±3.7	139.2±3.6	138.5±3.9	0.256
Glomerular Filtration Rate (ml/min/1.73m2)	63.1±19.5	66.1±18.3	57.6±20.6	0.007
Hemoglobin, (g/dl)	11.5±1.9	11.6±2.02	11.4±1.8	0.382
Hematocrit, (%)	36.2±5.5	34.4±5.5	35.8±5.4	0.470
White Blood Cell Count (103 /µl)	7.15 (3.37-15.2)	7.3 (3.5-8.0)	7.1 (3.4-15.2)	0.326
Platelet (103/L)	231.27±81.9	237±86	220±73	0.211
STS-TAVR	8.7 (7.4-17.2)	8.4 (7.4-11.0)	14.5 (9.7-17.2)	0.0001
Euro SCORE II	14.3 (6.2-27.9)	13.4 (6.2-15.1)	16.1 (7.9-27.9)	0.0001
Glasgow Prognostic Score GPS=0 GPS≥1	 100 (58.8) 70 (41.2)	 72 (64.9) 39 (35.1)	 28 (47.5) 31 (52.5)	0.021
Intensive care unit stay (days)	4.8±4.2	2.4±1.5	5.8±3.2	0.007
Left Ventricular Ejection Fraction (%)	52.7±10.5	54.4±9.5	49.6±11.7	0.004
Left Ventricular End-Diastolic Diameter (mm)	55±4.8	53±5.5	59±7.2	0.495
Left atrial diameter (mm)	4.3±0.5	4.2±0.46	4.3±0.61	0.167
Aortic valve area (mm2)	0.75±0.13	0.79±0.12	0.73±0.12	0.035
Maximum Aortic gradient (mmHg)	81.9±18.9	81.9±18.9	81.8±19	0.981
Mean Aortic Gradient (mmHg)	48.1±10.8	48.0±10.9	48.4±10.5	0.837
Pulmonary artery Pressure (Systolic) (mmHg)	39.6±12.9	38.7±11.8	41±14.9	0.283
Balloon valvuloplasty (pre-TAVR), n(%)	99 (58.2)	65 (58.6)	34 (57.6)	0.517
Balloon valvuloplasty (post-TAVR), n(%)	24 (14.1)	16 (14.4)	8 (13.6)	0.538
Preoperative treatment, n (%) Statins ß-blockers Calcium channel blocker ACEI/ARB Anti-coagulant Anti-aggregant	 71 (41.8) 113 (66.5) 52 (30.6) 124 (72.9) 21 (12.4) 131 (77.1)	 47 (42.3) 74 (66.7) 40 (36) 79 (71.2) 14 (12.6) 91 (81.9)	 24 (40.7) 39 (66.1) 12 (20.3) 45 (76.2) 7 (11.8) 40 (67.8)	 0.871 0.536 0.037 0.587 0.549 0.054

ACEI: Angiotensin converting enzyme inhibitor; ARB: Angiotensin receptor blocker; Euro SCORE: European System for Cardiac Operative Risk Evaluation; NYHA: New York Heart Association; STS: The Society of Thorasic Surgeons; TAVR: Transcatheter Aortic Valve Replacement

DISCUSSION

In this retrospective single center study, rate of in-hospital mortality was 8.8%, 30-day mortality was 11.8% and 1-year mortality was 34.1%. Higher pre-procedural GPS values were found to be associated with an increase in 1-year mortality, moreover high STS-TAVR score and history of previous CVA were found as independent predictors related with mortality.

Previous researchs have shown the role of Inflammation and endothelial injury in the pathophysiology and progression of aortic stenosis.[19,20] Also, studies revealed the mechanism at the cellular level and defined pathways which exhibited to contribute to the pathophysiology. The aortic valve cells were shown to become involved in the inflammatory environment by producing osteogenic protein.[21] The imbalance between pro- and anti-inflammatory status end-up with degradation of valvular structure and may fasten the progression of valvular pathology.

The scoring systems consist more than one variable to evaluate and concomitant use of these variables improve the diagnostic capacity in clinical practice. GPS, is a validated inflammatory risk score especially in malignancy and is reflecting both inflammatory and nutritional status. Its prognostic role has been studied in heart failure and acute coronary syndromes.[13-15] Prognostic nutritional index (PNI) is a scoring system consist of lymphocyte and albumin levels and is reflecting the inflammatory and nutritional status of patients similar to GPS. Higher PNI values were found to be related with higher short-term survival and lower complications after TAVI.[22] In our study, 30-day mortality was observed in 11.8% of patients and the rate of vascular surgery, acute heart failure and cerebrovascular accident was 15%, 18% and 1.7% respectively in mortality group. However, we couldn't detect a correlation between immune nutritional status as stated by the GPS score and 30-day mortality and rate of complications. In this cohort, patients with acute or chronic inflammatory diseases which may have affect CRP and albumin levels were excluded. Although, higher pre-procedural GPS values were found to be associated with an increase in 1-year mortality in our data, it is not identified as an independent predictor of 1-year mortality.

The validated risk scores for risk stratifying for TAVI are STS TAVR and Euro SCORE-II, which do not include variables such as nutritional status, frailty and inflammation. In patients who underwent TAVI with a diagnosis of severe symptomatic aortic stenosis, frailty is related with worse outcomes.[8] Hypoalbuminemia, is an important manifestation of frailty and shown to be linked with higher mortality rates in TAVI patients, as well.[23] In our study STS TAVR, Euro SCORE-II and GPS have been found to be associated with 1-year mortality. However, only the STS TAVR score was found as an independent predictor of 1-year mortality.

Small sample size, single-center experience and retrospective design are the limitations of this study. Since the use of TAVI is getting more common, the valve technology is improving. Our cohort consist of earlier periods of TAVI procedure including higher risk patients, this may be a reason for high rate of 1-year mortality.

CONCLUSION

Since TAVI is recommended for intermediate risk patients as well as high risk patients, managing complications and mortality is getting more precious. GPS value, which is a noninvasive, user-friendly score may support additive information to validated risk scores and may be used to determine the prognosis in this patient group. Further prospective studies in larger patient population may give more comprehensive information.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Bağcılar Training and Research Hospital Non-interventional Clinical Researches Ethics Committee (Date: 05/07/2022, Decision No: 2022/07/01/001).

Informed Consent: All patients signed the free and informed consent form.

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Original Article / Orijinal Araştırma

Diagnostic Value Of Systemic Immune–Inflammation Index (SIII) in Acute Ischemic Stroke

Akut İskemik İnmede Sistemik İmmün-İnflamasyon Endeksinin (SIII) Tanısal Değerliliği

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Abstract

Aim: Calculated based on platelet, neutrophil and lymphocyte counts, the systemic immune-inflammation index is thought to be associated with many malignancies in the literature. Despite the existing investigations on its diagnostic value, there have been no clear results reported regarding its diagnostic value in stroke patients. The current study is therefore intended to demonstrate the diagnostic value of the systemic immune-inflammation index and its prognostic value in cases of acute ischemic stroke.

Material and Method: A total of 150 cases of acute stroke and a control group of 150 individuals were retrospectively examined. The data recorded for each case included age, gender, history, vital findings, NIHSS, SIII, and outcome.

Results: In the current study, the group of stroke patients had significantly higher SIII than the control group. According to the diagnostic examinations, in stroke, the diagnostic value of SIII was greater than that of neutrophil-to-lymphocyte ratio at a statistically significant level. The present study also found that, compared to the SIII, the (Lymphocyte x Platelet)/Neutrophil ratio (called the reverse SIII) had a higher statistical significance in diagnosing the stroke and predicting early hospital mortality.

Conclusion: The SIII can be a good marker for both diagnostic evaluation and for predicting early hospital mortality in stroke cases. Additionally, it is approved to be a useful index since it can be calculated inexpensively and easily.

Keywords: Ischemic stroke, systemic immune-inflammation index, emergency department, prognosis

Öz

Amaç: Trombosit, nötrofil ve lenfosit sayılarına göre hesaplanan sistemik immün-enflamasyon indeksinin literatürde birçok malignite ile ilişkili olduğu düşünülmektedir. Tanısal değeri ile ilgili mevcut araştırmalara rağmen inme hastalarında tanısal değeri ile ilgili net sonuçlar bildirilmemiştir. Bu nedenle mevcut çalışma, akut iskemik inme vakalarında sistemik immün-enflamasyon indeksinin tanısal değerini ve bunun prognostik değerini göstermeyi amaçlamaktadır.

Gereç ve Yöntem: Toplam 150 akut inme olgusu ve 150 kişilik kontrol grubu retrospektif olarak incelendi. Her vaka için kaydedilen veriler yaş, cinsiyet, öykü, hayati bulgular, NIHSS, SIII ve sonucu içeriyordu.

Bulgular: Mevcut çalışmada, inmeli hasta grubunda kontrol grubuna göre anlamlı olarak daha yüksek SIII vardı. Tanısal incelemelere göre inmede SIII'ün tanısal değeri nötrofil/lenfosit oranından istatistiksel olarak anlamlı düzeyde daha yüksekti. Bu çalışma ayrıca, SIII ile karşılaştırıldığında, (Lenfosit x Trombosit)/Nötrofil oranının (yeni SIII olarak adlandırılır) inme tanısında ve erken hastane mortalitesini tahmin etmede daha yüksek bir istatistiksel öneme sahip olduğunu bulmuştur.

Sonuç: SIII, inme vakalarında hem tanısal değerlendirme hem de erken hastane mortalitesini öngörmek için iyi bir belirteç olabilir. Ayrıca ucuz ve kolay hesaplanabilmesi nedeniyle faydalı bir parametre olduğunu düşünmekteyiz.

Anahtar Kelimeler: İskemik inme, sistemik immün-inflamasyon indeksi, acil servis, prognoz

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INTRODUCTION

The World Health Organization (WHO) defines stroke as "rapidly developed clinical signs of focal or global deficit, lasting 24 hours or longer or leading to death, with no apparent cause other than of vascular origin".[1] Acute ischemic stroke (AIS) remains one of the leading causes of mortality and morbidity worldwide.[2]

In the diagnosis of AIS, must suspect in the clinical evaluation, must conduct the physical examinations, and support the diagnosis with imaging tests or make a differential diagnosis. In cases of stroke, the computed tomography of the brain is performed to differentiate between hemorrhagic stroke and ischemic stroke, while the diffusion-weighted magnetic resonance imaging (MRI) is used to confirm AIS.[3,4] Besides the radiation exposure of patients in CT, the infeasibility of diffusion-weighted MRI in patients with metal implants can also render the diagnosis of AIS difficult.[5] Considering these difficulties, it is understood that there is a need for new diagnostic markers or indices to support the diagnosis in the diagnosis of AIS.

Modulation of the inflammatory cell function plays a role in repairing brain damage after ischemia. There is data in the literature reporting that systemic inflammatory response may be involved in the prognosis of AIS.[6,7]

Recently developed based on platelet, neutrophil and lymphocyte counts to examine patients simultaneously for immune status and inflammatory response, the systemic immune-inflammation index (SIII) was reported to be associated with poor prognosis in patients with malignancy in the literature.[8-10]

The current study investigates the SIII as a marker that facilitates easier diagnosis of AIS and analyzes it to establish whether it was a useful marker for clinicians for help to diagnosis AIS.

MATERIAL AND METHOD

Study Setting

The present study was retrospectively conducted between September 1, 2021 and December 1, 2021. The study included a study group of 150 patients with confirmed diagnosis of AIS and a control group of 150 individuals with non-AIS diagnoses.

Study Population

Among of study patients, those patients under the age of 18, pregnant patients and patients with missing data were excluded from the study. Patients who could not have a diffusion-weighted MRI since they had metal prosthesis implanted in their body, as well as those patients whose outcomes could not be followed up and whose medical histories were unknown were excluded from the study. And also included patients with any diagnosis of malignancies in their medical history, patients with history of hematologic disease, as well as septic patients with elevated laboratory values presenting with obvious infectious symptoms were excluded from the study.

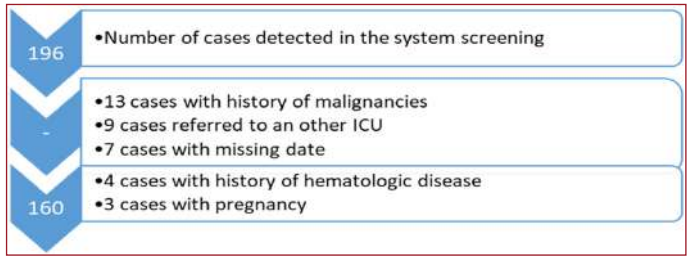
Patients aged 18 years and older, who came with a pre-diagnosis of AIS, whose medical history was known, and who did not have a history of any hematologic disease were included in the study. Patients who were in shock and whose vitals were unstable were not included in the study.

Data collection

To conduct the study, the file and automation system (Hospital Information Management System (HIMS)) was screened for patients to be included. The ICD10 diagnostic codes "I63.0-9, I64.0-9, I65.0-9, I66.0-9, I67.0-9 and I68.0-9" were used to screen for patients with AIS on the system. 196 patients were detected in the system screening. Of the 196 cases identified, 13 patients were excluded since they had history of malignancies, 9 were excluded as they were referred to another healthcare center due to lack of space in the intensive care unit (ICU), 7 were excluded because of missing data, 4 were excluded since they had history of hematological disease, and 3 were excluded due to pregnancy. Of the remaining 160 cases, 150 were randomized according to the date and time of hospital admission.

The control group was randomized in accordance with the inclusion and exclusion criteria of the study and in line with mean age of the patients. The control group included 150 patients who presented with "headache" but were not diagnosed with acute ischemic or hemorrhagic stroke. These patients were volunteers with known medical history.

Study Group



Data Calculation

In the present study, the laboratory results obtained in each case were used to calculate SIII ((NeutrophilxPlatelet)/ Lymphocyte), NLR (Neutrophil-to-Lymphocyte Ratio), PLR (Platelet-to-Lymphocyte Ratio), PNR (Platelet-to--Neutrophil Ratio), LNR (Lymphocyte-to-Neutrophil Ratio), and NSIII (is calculated Lymphocyte x Platelet) / Neutrophil). For those who were regularly smoking, it was allowed to smoke 1/2 package/ day. Mortality was based on the mortality of the patients during hospital stay. Due to the retrospective nature of the study, the patients were not followed up for their mortality status and causes after discharge.

Statistical Analyses

The SPSS 23.0 for Windows® statistics program (IBM Inc. Chicago, IL, USA) was used for the statistical analyses. The descriptive data were presented as number, percentage, mean, standard deviation, median, minimum, and maximum values. The distribution normality of the data was analyzed using the Kolmogorov-Smirnov Test. Pearson's chi-squared test and Fisher's Exact test were used to compare the categorical data. T Test was used to compare two independent groups of

numerical data and Kruskal Walles Test was used to compare three groups of numerical data. The obtained results were considered to be statistically significant at p<0.05, with a 95% confidence interval.

Ethical Considerations

Ethics committee approval was obtained from the ethics committee of our tertiary hospital (Ethics Committee no: 2021.04.34).

RESULTS

In our study, the data included demographic data of both the study and control groups, as well as their vascular risk factors, vitals at the time of admission, laboratory findings and SIII, NLR, PLR, PNR, LNR and reverse SIII values. According to these results, the rates of HT, CAD and smokers were found to be higher in the group of patients diagnosed with AIS. Similarly, the mean values of systolic and diastolic blood pressure were significantly higher in the study group. The group of patients with AIS had significantly higher neutrophil counts than the control group, while there was no difference between the two groups in terms of mean values of lymphocyte count and platelet count. The NLR and SIII calculated in the study group were significantly higher than in the control group and the PNR, LNR and NSIII were significantly lower in the study group than in the control group, while there was no difference between the two groups in terms of PLR (**Table 1**).

Table 1. Comparison of the demographic and clinical data of the study and control groups

Parameters	Study Groups (n=150) Mean±sd	Control Groups (n=150) Mean±sd	p
Demographic Data			
Male n (%)	90 (60.0)	85 (56.7)	0.558
Age (year)	65.49±12.89	65.21±12.29	0.848
Vascular Risk Factor			
Hypertension n (%)	87 (58.0)	62 (41.3)	0.004
Diabetes Mellitus n (%)	41 (27.3)	39 (26.0)	0.794
Coronary Artery Disease n (%)	56 (37.3)	36 (24.0)	0.012
Smoking n (%)	75 (50.0)	55 (36.7)	0.020
Vital Parameters			
Systolic BP (mmHg)	147.31±26.15	132.28±19.74	0.036
Diastolic BP (mmHg)	88.73±16.66	82.46±13.28	0.042
Pulse (beats/min.)	91.11±23.01	88.11±20.76	0.184
Laboratory Tests			
Neutrophil (x109/L)	5.50±5.43	4.11±4.84	0.020
Lymphocyte (x109/L)	1.87±0.95	2.03±0.94	0.129
Platelet (x109/L)	248.96±77.45	254.28±66.46	0.524
Ratings			
NLR	5.50±5.43	4.11±4.84	0.020
PLR	176.48±127.98	157.09±12.84	0.165
PNR	40.62±17.55	51.63±24.11	<0.001
LNR	0.31±0.20	0.42±0.25	<0.001
SIII	1367.96±1475.73	981.43±1031.34	0.009
NSIII	79.89±57.49	109.61±72.68	<0.001

sd: standard deviation; Smoking was considered positive for >1/2 pack/day use. BP: blood pressure; NLR: Neutrophil-lymphocyte ratio; PLR: Platelet lymphocyte ratio; PNR: Platelet neutrophil ratio; LNR: Lymphocyte neutrophil ratio; SIII: Systemic immune-inflammatory index; NSIII: The Novel systemic immune-inflammatory index

In the data analysis based on mortality status in the study group, mortality was found to be significantly higher in male cases. In addition, mortality was found to be higher in patients with history of HT and CAD. The mean value of neutrophil count of the cases with mortality was higher than that of the discharged group. The NIHSS score calculated based on the examination of the study group was significantly higher in the subgroup of patients with mortality (12.57±4.49 & 7.22±4.55; p<0.001). The rate of infarcts occurring in the MCA (Middle Cerebral Artery) and PCA (Posterior Cerebral Artery) watershed was significantly greater in the mortality subgroup compared to the subgroup of discharged patients, while the rate of infarcts in the ACA (Anterior Cerebral Artery) watershed was significantly lower in the mortality subgroup. The treatments administered were not associated with any significant difference in mortality. To compare the two subgroups of mortality and discharged patients, the NLR, PLR and SIII were statistically significantly higher and the PNR, LNR and NSIII were significantly lower in the mortality subgroup than in the subgroup of discharged patients. As expected, the rate of mortality was high in patients admitted to the intensive care unit. Again, the durations of hospital stay were found to be significantly higher in the subgroup of mortality (**Table 2**).

The cases were also studied for the time from the onset of the complaints until their admission by the emergency department, which was set and grouped as up to 4.5 hours and longer than 4.5 hours. Lymphocyte counts were significantly higher in patients admitted within the first 4.5 hours than in those admitted after 4.5 hours, while platelet counts were found to be significantly higher in patients admitted within the first 4.5 hours. We also found that PLR was significantly lower in the group of patients diagnosed with AIS (**Table 3**).

Table 3. Analysis of the demographic and clinical data by time until hospital admission

Parameters	Patient group admitted in the first 4.5 hours (n=69) Mean±sd	Patient group admitted after 4.5 hours (n=81) Mean±sd	p
Age (year)	64.23±13.60	66.57±12.24	0.270
Systolic BP (mmHg)	149.43±28.51	145.49±23.98	0.359
Diastolic BP (mmHg)	91.29±16.05	86.56±16.96	0.083
Pulse (beats/min.)	92.78±22.80	89.69±23.21	0.414
Neutrophil (x109/L)	7.26±3.82	6.93±2.80	0.545
Lymphocyte (x109/L)	2.06±1.01	1.70±0.88	0.021
Platelet (x109/L)	234.44±72.53	261.32±79.78	0.034
NLR	4.86±4.44	6.04±6.12	0.187
PLR	143.41±89.57	204.66±148.12	0.003
PNR	38.45±17.03	42.47±17.87	0.163
LNR	0.34±0.21	0.29±0.19	0.112
SIII	1093.91±1036.11	1601.40±1738.87	0.035
NSIII	83.57±59.19	76.76±56.19	0.472

Sd standard deviation; BP: blood pressure; NLR: Neutrophil-lymphocyte ratio; PLR: Platelet lymphocyte ratio; PNR: Platelet neutrophil ratio; LNR: Lymphocyte neutrophil ratio; SIII: Systemic immune-inflammatory index; NSIII: The Novel systemic immune-inflammatory index

Table 2. Comparison of demographic and disease data of discharged and exitus cases

Parameters	Exitus (n=19) Mean±sd	Discharge (n=131) Mean±sd	p
Demographic data			
Male n (%)	82 (64.6)	8 (34.8)	0.007
Age (year)	68.78±16.10	64.90±12.20	0.185
Vascular risk factor			
Hypertension n (%)	68 (53.5)	19 (82.6)	0.009
Diabetes Mellitus n (%)	36 (28.3)	5 (21.7)	0.513
Coronary artery disease n (%)	43 (33.9)	13 (56.5)	0.039
Smoking n (%)	62 (48.8)	13 (56.5)	0.497
Vital parameters			
Systolic BP (mmHg)	146.78±28.44	147.40±25.83	0.917
Diastolic BP (mmHg)	90.22±15.44	88.46±16.92	0.644
Pulse (beats/min.)	100.87±26.71	89.35±21.91	0.027
Laboratory tests			
Neutrophil (x10^9/L)	9.15±4.96	6.70±2.77	0.001
Lymphocyte (x10^9/L)	1.75±1.27	1.89±0.89	0.527
Platelet (x10^9/L)	239.78±82.26	250.62±76.77	0.539
Complaints start-hospital application time (hour)	4.83±3.39	11.10±17.40	0.088
NIHSS	12.57±4.49	7.22±4.55	<0.001
Ischemia area			
ACA	10 (7.9)	7 (30.4)	0.020
MCA	85 (66.9)	12 (52.2)	
PCA	32 (25.2)	4 (17.4)	
Treatment performed			
Medical	85 (66.9)	14 (60.9)	0.266
Thrombolytic reperfusion therapy	15 (11.8)	1 (4.3)	
Thrombectomy reperfusion treatment	27 (21.3)	8 (22.9)	
Rating			
NLR	8.24±6.80	5.00±5.01	0.008
PLR	226.19±198.41	167.48±109.37	0.043
PNR	31.59±14.53	42.25±17.60	0.007
LNR	0.22±0.19	0.33±0.20	0.014
SIII	1944.37±1955.37	1263.57±1355.02	0.041
NSIII	55.98±51.27	84.22±57.68	0.030
Place of hospitalization			
Service	3 (13.0)	78 (61.4)	<0.001
ICU	20 (87.0)	49 (38.6)	
Hospitalization period			
Service admission (day)	3.96±6.51	8.94±5.42	<0.001
ICU Hospitalization (day)	12.04±13.37	2.43±4.97	<0.001
Total Hospitalization (days)	16.00±13.82	11.37±7.17	0.017

sd: standard deviation; Smoking was considered positive for >1/2 pack/day use. BP: blood pressure; NLR: Neutrophil-lymphocyte ratio; PLR: Platelet lymphocyte ratio; PNR: Platelet neutrophil ratio; LNR: Lymphocyte neutrophil ratio; SIII: Systemic immune-inflammatory index; NSIII: The Novel systemic immune-inflammatory index

In study group, the factors that affected the diagnosis were analyzed and the logistic regression analysis was given in **Table 4**. HT, CAD, SIII, NLR, PNR and NSIII were found to be associated with increased suspicion for AIS diagnosis at a significant level.

Table 4. Evaluation of the results obtained in the multi-variate regression analysis in AIS diagnosis

Parameters	B	OR	%95 CI	p
Gender	-0.121	0.241	0.697-1.827	0.664
Smoking	0.421	2.69	0.397-1.085	0.101
Hypertension	-0.139	0.242	0.660-1.999	0.623
Diabetes Mellitus	0.649	6.73	0.320-0.753	0.009
Coronary Artery Disease	0.555	4.154	0.337-0.779	0.042
SII	0.410	1.326	1.026-1.874	0.008
NSII	0.294	1.246	1.140-1.728	0.034
NLR	0.368	1.392	0.896-1.644	0.038
PLR	0.260	3.35	0.998-1.055	0.165
PNR	0.304	1.425	1.096-1.848	0.028
LNR	0.221	1.164	1.002-1.774	0.053

Smoking was considered positive for >1/2 pack/day use; NLR: Neutrophil-lymphocyte ratio; PLR: Platelet lymphocyte ratio; PNR: Platelet neutrophil ratio; LNR: Lymphocyte neutrophil ratio; SIII: Systemic immune-inflammatory index; NSIII: The Novel systemic immune-inflammatory index

DISCUSSION

In a study, the human immune system is divided into two parts, innate and acquired immune system. The natural immune system constitutes the first line of defense against pathogens. The acquired immune system constitutes the secondary line of defense against inflammatory processes that are not caused by pathogens. It is one of the inflammatory processes that activates the immune system in AIS.[11]

In ischemic strokes, neutrophil migration occurs in the intra-parenchymal perivascular area within 6 to 24 hours following the ischemia, and neutrophils damage the blood-brain barrier with the cytokines they release into this area.[11] Lymphocytes migrate to the area 3 to 6 days after the stroke, and unlike neutrophils, undertake a regulatory function rather than acting with destructive impact. In this way, lymphocytes induce neuroprotection.[12,13] Apart from this regulatory effect of lymphocytes, the acquired immunity also leads to immunosuppression, which paves the way for opportunistic infections.[14]

In a study, platelets participate in immune response in addition to participating in hemostasis and thrombosis.[15] After stroke, activations begin in microglial cells, macrophages and mast cells, and proinflammatory cytokines are released as a result of these activations. With these released proinflammatory cytokines, activations begin in the hypothalamic–pituitary–adrenal axis and the release of catecholamines is induced by the activation of the sympathetic system.[16] As a result of all these activations, the stroke-induced inflammatory process begins. In the literature, Agus et al. reported that NLR, PNR and SIII calculated based on the blood cell counts were suitable for giving an insight about the balance between the innate and acquired immune systems.[17]

Our study investigates the diagnostic and prognostic values of NLR, LNR, PNR, PLR and NSIII, as well as of SIII, in patients admitted to the emergency department during the 4-month study period with suspected AIS and examined and then diagnosed with AIS by an experienced neurologist.

In our study, the mean NLR was found to be significantly higher in study group than in the control group. NLR was also found to be significantly higher in patients with mortality, compared to those surviving. Several underlying mechanisms are considered responsible for the poor progression in AIS that is attributed to NLR. In one of these mechanisms, after ischemic stroke, the NLR rate increases since the damaged brain tissue will produce a strong inflammatory response.[18] In some studies; in another mechanism, it is reported that circulating lymphocytes decrease due to the immunosuppression caused by catecholamines released from the sympathetic nervous system after the onset of stroke, and this mechanism is even reported to elevate the risk of infection after stroke.[19,20] This mechanism allows the use of neutrophils or lymphocytes alone to expose the imbalance between overactive inflammation and protective regulations.[21] In a study by Wang et al., the researchers reported that NLR was a successful index in predicting the risk of bleeding and predicting 3-month mortality in cases of AIS.[22]

In the studies available in the literature; the SIII, which combines platelet counts, neutrophil and lymphocyte clusters, represents the systemic immune response and has been found to be associated with poor outcome.[23] One of the major reasons here is the joint migration of leukocytes and platelets to the ischemia region and their interaction there. In an animal study, this interaction has been shown to control Cyclophilin D, a mediator of necrosis, and increase brain damage in the ischemic brain via this mediator.[24] In a study where Weng et al. included stroke cases that were performed intravenous thrombolytic, it is stated that the SIII is a good biomarker for predicting stroke severity and 3-month poor outcome.[25] Also, in a study where Zhou et al. included stroke patients, SIII is reported to be an independent predictor of negative outcomes of 3 months in patients. Additionally, the same study states that nomogram scoring with the SIII that is calculated in the next few hours after hospital admission can offer information for clinicians by predicting the likelihood of short-term negative outcomes in stroke cases.[26] In a study where Hu et al. focus on stroke, compared to other inflammatory markers, high SIII values are reported to be of a significantly higher value in determining post-stroke depression.[27] In a study of patients with aneurysmal subarachnoid hemorrhages, Yun et al. stated that SIII can be a useful indicator for poor prognosis.[28] In a study where Yi et al. included patients with AIS who underwent thrombectomy, high SIII is reported to be associated with poor results and may be a prognostic marker in AIS cases with large artery occlusion.[29] In our study, the mean SIII was found to be significantly higher in the cases of AIS than in the control group.

The SIII in the group of stroke patients admitted after 4.5 hours was found to be significantly higher than that of those admitted within the first 4.5 hours following the onset of symptoms. Similar to NLR, the mean SIII was significantly higher in the subgroup of mortality than in the subgroup of surviving cases. In addition, compared to NLR in terms of diagnostic value, the success of SIII in predicting AIS was significantly greater. In our study, we found that the SIII may be a utile biomarker for determining both diagnostic and early in-hospital mortality. Our data support the studies available in the literature.

In our study, we noticed that we had a noteworthy finding based on the data we obtained with the SIII. The (lymphocyte x platelet) / neutrophil ratio (i.e. the inversed version of neutrophil-to-lymphocyte ratio), which we called NSIII, had a higher statistical significance in AIS cases compared to the SIII that we calculated in our study ($p=0.009$ and $p<0.001$). In addition, this correlation continued in cases with mortality ($p=0.041$ and $p=0.030$). In addition, compared to the control group, LNR and PNR were statistically more significant than NLR and PLR in the group of AIS cases. However, this data needs to be verified with further studies to be done in the literature.

To conclude, in our study, the SIII was found to be significantly higher in the group of AIS cases than in the control group. The SIII was higher at a statistically significant level in the patients with early in-hospital mortality. We believe that this finding will serve as a warning to clinicians so they pay greater attention to the risk of mortality in cases with high SIII. Again, the group of stroke patients admitted after 4.5 hours had significantly higher SIII than those admitted within the first 4.5 hours. This result supports our opinion that SIII may be a guiding index in cases that requires a decision to be made to administer thrombolytic treatment, which may be further investigated by future studies.

Limitations of Study

There are several limitations in our study. The first of these limitations is the small number of participants included in both of the study and control group although the numbers foreseen by the power analysis data were used. The second limitation is that the study is limited to the data that was available to the clinicians although our study is retrospective and despite the proper recording of the data used here. Another limitation is that the time until hospital admission in each case was recorded as per the history reported by each individual patient. We do not think that the errors that will occur due to this data would be substantial to an extent where they could affect the results of the study.

CONCLUSION

According to many studies, compared to other ratios and indexes, the SIII is promising in demonstrating a systemic immune-inflammation response. Our study suggests that SIII is a good marker in diagnosing AIS, evaluating the response based on the time until hospital admission, and predicting early in-hospital mortality. We also found that the index called NSIII, which we created using the formula (lymphocyte x platelet)/ neutrophil, had a significantly higher diagnostic and predictive value in predicting early in-hospital mortality than the SIII had. However, this data needs to be supported by further studies to be conducted in the literature.

ETHICAL DECLARATIONS

Ethics Committee Approval: The Ethics committee approval was obtained for this study from Ministry of Healthy Başaksehir Çam and Sakura State Hospital Ethics Committee (Decision No: 2021.04.34)

Informed Consent: All participants signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article / Orijinal Araştırma

Prognostic Factors in Thyroid Papillary Microcarcinoma

Tiroid Papiller Mikrokarsinomda Prognostik Faktörler

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Abstract

Aim: Our aim in this retrospective study is to evaluate the factors that are important in the prognosis of thyroid papillary microcarcinoma.

Material and Method: This study is a retrospective study. In the study, 277 thyroid papillary microcarcinoma nodules belonging to 178 patients, diagnosed in Kütahya University of Health Sciences Hospital, between 2010 and 2020, were included. The prognostic significance of tumor diameter, unilaterality, bilaterality, multifocality, number of tumor foci, fibrosis, distance to the capsule and Gal-3, HBME-1 and CK19 staining were investigated retrospectively by comparing with negative prognostic factors such as lymphovascular invasion, lymph node metastasis, capsular invasion and extrathyroidal spread. In addition, the relationship between immunostains and fibrosis were examinated.

Results: The significant association were found between >0.5 tumor diameter and lymph node metastasis. Unilaterality, bilaterality, multifocality and number of tumor foci are associated with lymphovascular invasion and lymph node metastasis. Fibrosis is associated with capsular invasion and extrathyroidal spread. A correlation was found between the prevalence of Gal-3 and capsular invasion and extrathyroidal spread, and between the prevalence of CK-19 and lymph node metastasis. A positive correlation was found between fibrosis and Gal-3 and CK19, and negative correlations with HBME-1 intensity.

Conclusion: Negative prognostic markers are >0.5 tumor diameter, unilaterality, bilaterality, multifocality, number of tumor foci, fibrosis, Gal-3 and CK19 prevalence. A positive correlation was found between fibrosis and Gal-3 and CK19, and negative correlation with HBME-1 intensity.

Keywords: Thyroid papillary microcarcinoma, prognosis, Gal-3, HBME-1, CK19, fibrosis

Öz

Amaç: Bu retrospektif çalışmadaki amacımız, tiroid papiller mikrokarsinom (TPMC) prognozunda önemli olan faktörleri değerlendirmektir.

Gereç ve Yöntem: Bu çalışma retrospektif bir çalışmadır. Çalışmaya Kutahya Sağlık Bilimleri Üniversitesi Hastanesi'nde, 2010-2020 yılları arasında tanı konulan, 178 hastaya ait 277 tiroid papiller mikrokarsinom nodülü dahil edildi. Tümör çapı, unilateralite, bilateralite, multifokalite, tümör odak sayısı, fibrozis, kapsüle uzaklık ve Gal-3, HBME-1 ve CK19 boyanmasının prognostik önemi, lenfovasküler invazyon, lenf nodu metastazı, kapsül invazyonu ve tiroid dışı yayılım gibi negatif prognostik faktörlerle ile karşılaştırarak retrospektif olarak araştırıldı. Ayrıca immün boyalar ile fibrozis arasındaki ilişki incelendi.

Bulgular: Tümör çapının >0,5 olması ve lenf nodu metastazı arasında anlamlı ilişki bulundu. Unilateralite, bilateralite, multifokalite ve tümör odak sayısı, lenfovasküler invazyon ve lenf nodu metastazı ile ilişkiliydi. Fibrozis, kapsül invazyonu ve ekstratiroidal yayılım ile ilişkiliydi. Gal-3 prevalansı ile kapsül invazyonu ve tiroid dışı yayılım arasında, CK-19 prevalansı ve lenf nodu metastazı arasında ilişki bulundu. Fibrozis ile Gal-3 ve CK19 yoğunlukları arasında pozitif, HBME-1 ile negatif korelasyon bulundu.

Sonuç: Negatif prognostik belirteçler >0,5 tümör çapı, unilateralite, bilateralite, multifokalite, tümör odak sayısı, fibrozis, Gal-3 ve CK19 olacakprevalansıdır. Fibrozis ile Gal-3 ve CK19 yoğunlukları arasında pozitif, HBME-1 ile negatif korelasyon bulundu.

Anahtar Kelimeler: Tiroid papiller mikrokarsinom, prognoz, Gal-3, HBME-1, CK19, fibrozis

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INTRODUCTION

Although thyroid papillary microcarcinoma (TPMC) is generally known to be a tumor with a good prognosis,[1] there are publications reporting lymph node metastases (LNM),[2] distant metastasis (DM),[3,4] and even rare deaths.[1,3] Our aim was to determine the tumor diameter, unilaterality, bilaterality, multifocality, number of tumor foci (NTF), tumor fibrosis, distance to the capsule, and their relation with the occurrence of lymphovascular invasion (LVI), LNM, capsular invasion (CI), and extrathyroidal spread (ETS). Again, there are few studies in the literature investigating the prognostic effects of Gal-3, HBME-1 and CK19, and their association with fibrosis. There is no other publication in the literature that evaluates all parameters together.

MATERIAL AND METHOD

This retrospective study included a total of 277 TMPC nodules belonging to 178 patients presenting to Evliya Celebi Training and Research Hospital between 2010 and 2020. Hematoxylin-eosin slides of the patients diagnosed with TPMC were removed and re-examined retrospectively. Ethical approval was obtained (Date: 21.00.2021 , Decision No: 2021/14279). Tumor diameter, unilaterality, bilaterality, multifocality, NTF, tumor fibrosis, distance to the capsule, and prevalence of Gal-3, HBME-1 and CK19, were noted, and its relationship with negative prognostic factors such as LVI, LNM, CI, and ETS were investigated. The intensities of Gal-3, HBME-1 and CK19 stains were noted and the relationship between intensities and fibrosis were examinated. In multifocal cases, the highest value was taken into account when giving tumor size, fibrosis percentage, the prevalence and intensity of Gal-3, HBME-1 and CK19, while the lowest value was taken in capsule distance. Statistical comparison was made by dividing the tumor into 3 groups as the distance from the capsule <0.2, 0.2-0.5 and >0.5 cm, and the tumor diameter was divided into 2 groups as those smaller and larger than 0.5 cm. When assessing immunostaining, the staining intensity was graded as: 0=none; 1=weak; 2=medium; 3=strong. The percentage of cells staining positively (prevalence) with Gal-3, HBME-1 and CK19 was scored as follows: 0=0%, 1=1-25%, 2=26-50%, 3: 51-75%, 4=76-100%.

Statistical Analysis

Mean, standard deviation, median, minimum and maximum values were given as descriptive statistics for continuous data and percentage values were given for discrete data. The data were analyzed by SPSS version 16.0 (SPSS Co., Chicago, IL, USA) for Windows. The categorical variables were expressed as numbers and percentages. The associations between variables were tested using the chi-square ($\chi 2$) test. The relationship between tumor fibrosis values and staining was analyzed by Spearman Correlation coefficient. $P<0.05$ was accepted as the statistical significance limit.

RESULTS

Of the cases, 138 (77.5) were female and 40 (22.5%) were male. Tumor diameters (n=277) were median 0.5 (0.1-1), tumor diameter mean±SD 0.51±0.50. Distance to capsule is mean±SD 0.20±0.19 and median 0.2 (0-0.8). Lymphovascular invasion was observed in 5 (2.8%), CI in 15 (8.4%), ETS in 4 (2.2%), and lung metastasis in 1 (0.5%) case..Twenty-three (13%) of 178 patients also underwent lymph node dissection which 6 were metastatic. Since there was 1 case with lung metastasis, it was not included in the statistical evaluation. The number of associated TPMC nodules was 1-6, the percentage of tumor fibrosis was mean±SD 20.36±24.35, and the median was 5 (0-95). The tumor (n=277) was located in the right lobe in 151 (54.5%) cases, the left lobe in 101 (36.5%) cases, and the isthmus in 25 (9%) cases.

The associations between tumor characteristics and prognostic parameters of tumors are shown in **Table 1**.

Tumor Diameter

The significant associations were found between ≥ 0.5 tumor diameter and LNM ($\chi 2=5.425$, $P=0.02$).

Unilaterality, Bilaterality, Multifocality

The significant associations were found between unilaterality, bilaterality, multifocality and LVI and LNM ($\chi 2=9.417$, $P=0.002$, $\chi 2=6.668$, $P=0.01$; $\chi 2=4.362$, $P=0.04$, $\chi 2=13.692$, $P=0.000$; $\chi 2=4.477$, $P=0.03$, $\chi 2=6.241$, $P=0.01$, respectively).

Number of Tumor Focus (NTF)

The significant associations were found between NTF and LVI, LNM ($\chi 2=17.520$, $P=0.004$; $\chi 2=56.282$, $P=0.000$, respectively).

Fibrosis

There was a statistically significant relationship between CI, ETS and tumor fibrosis percentage ($\chi 2=35.195$, $P=0.000$; $\chi 2=14.796$, $P=0.002$). (**Figure 1**).

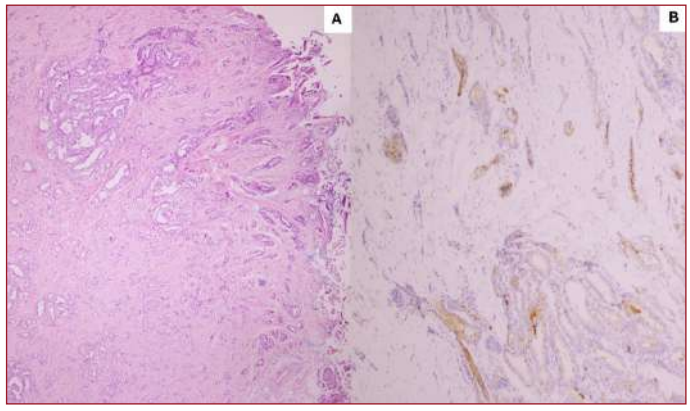

Figure 1-A-Capsule invasion and (x40) B-Gal-3 positivity in tumors with intense fibrosis (X100)

Tumor-Capsule Distance

There was a statistically significant relationship between CI and tumor capsule distance ($\chi 2=8.329$, $P=0.016$, respectively).

The Relationship between Tumor Characteristics and Prevalence of Gal-3, HBME-1 and CK19

The associations between tumor characteristics and prevalence of Gal-3, HBME-1 and CK19 are shown in **Table 2**. A relationship was found between prevalence of Gal-3 and CI and ETS (χ2=10.703, P=0.013; χ2=20.528, P=0.000) (**Figure 1**). There was no statistical significant associations prevalence of HBME-1 with tumor characteristics. There was a statistically significant relationship between prevalence of CK-19 and LNM (χ2=11.186, P=0.01).

Table 1. The associations between tumor characteristics and prognostic parameters of tumor (Each case was evaluated separately).

n=178 n (%)		Lymphovascular invasion		Lymph node metastasis		Capsular invasion		Extrathyroidal spread	
		-	+	-	+	-	+	-	+
Diameter	> 0.5	91 (95.8)	4 (4.2)	89 (93.6)	6 (6.4)	87 (91.6)	8 (8.4)	93 (97.9)	2 (2.1)
	≤ 0.5	82 (98.8)	1 (1.2)	83 (100)	0 (0)	76 (91.5)	7 (8.5)	81 (97.6)	2 (2.4)
X2 / p		1.466 / 0.226		5.425 / 0.02*		0.009 / 0.998		0.019 / 0.891	
Unilaterality -	-	136 (99.3)	1 (0.7)	135 (98.5)	2 (1.5)	128 (93.4)	9 (6.6)	135 (98.5)	2 (1.5)
	+	37 (90.2)	4 (9.8)	37 (90.2)	4 (9.8)	36 (85.7)	6 (14.3)	39 (95.1)	2 (4.9)
X2 / p		9.417 / 0.002*		6.668 / 0.01*		2.660 / 0.103		1.678 / 0.195	
Bilaterality -	-	137 (98.6)	2 (1.4)	138 (99.3)	1 (0.7)	129 (92.8)	10 (7.2)	136 (97.8)	3 (2.2)
	+	36 (92.3)	3 (7.7)	34 (87.2)	5 (12.8)	34 (87.2)	5 (12.8)	38 (97.4)	1 (2.6)
X2 / p		4.362 / 0.04*		13.692 / 0.000*		1.249 / 0.264		0.023 / 0.880	
Multifocality -	-	114 (99.1)	1 (0.9)	114 (99.1)	1 (0.9)	108 (93.9)	7 (6.1)	114 (99.1)	1 (0.9)
	+	59 (93.7)	4 (6.3)	58 (92.1)	5 (7.9)	55 (87.3)	8 (12.7)	60 (95.2)	3 (4.8)
X2 / p		4.477 / 0.03*		6.241 / 0.01*		2.306 / 0.129		2.807 / 0.094	
Number of tumor foci	1	118 (99.2)	1 (0.8)	118 (99.2)	1 (0.8)	109 (91.6)	10 (8.4)	117 (98.3)	2 (1.7)
	2	36 (97.3)	1 (2.7)	36 (97.3)	1 (2.7)	36 (97.3)	1 (2.7)	36 (97.3)	1 (2.7)
	3	11 (91.7)	1 (8.3)	12 (100)	0 (0)	10 (83.3)	2 (16.7)	12 (100)	0 (0)
	≥4	8 (80)	2 (20)	6 (66.7)	4 (33.3)	8 (80)	2 (20)	9 (90)	1 (10)
X2 / p		17.520 / 0.004*		56.282 / 0.000*		8.898 / 0.113		10.049 / 0.074	
Fibrosis	0-25%	116 (97.5)	3 (2.5)	114 (95.8)	5 (4.2)	117 (98.3)	2 (1.7)	119 (100)	0 (0)
	25-49%	31 (93.9)	2 (6.1)	32 (96.9)	1 (3.1)	29 (87.9)	4 (12.1)	32 (96.9)	1 (3.1)
	50-75%	13 (100)	0 (0)	13 (100)	0 (0)	10 (76.9)	3 (3.1)	12 (92.3)	1 (7.7)
	>75%	13 (86.7)	2 (13.3)	13 (100)	0 (0)	7 (53.8)	6 (46.2)	11 (84.6)	2 (5.3)
X2 / p		2.066 / 0.559		1.171 / 0.760		35.195 / 0.000*		14.796 / 0.002*	
Distance to the capsule	< 0.2	113 (95.7)	5 (4.3)	113 (95.7)	5 (4.3)	103 (87.3)	15 (2.7)	114 (96.6)	4 (3.4)
	0.2 - 0.5	45 (100)	0 (0)	44 (97.8)	1 (2.2)	45 (100)	0 (0)	45 (100)	0 (0)
	> 0.5	15 (100)	0 (0)	15 (100)	0 (0)	15 (100)	0 (0)	15 (100)	0 (0)
X2 / p		2.616 / 0.270		0.978 / 0.613		8.329 / 0.016*		2.081 / 0.353	

Table 2. The associations between tumor characteristics and prevalence of Gal-3, HBME-1 and CK19. (Each nodule was evaluated separately).

Prevalences			Lymphovascular invasion		Lymph node metastasis		Capsular invasion		Extrathyroidal spread	
			-	+	-	+	-	+	-	+
Gal-3	1	n (%)	65 (100)	0 (0)	63 (97)	2 (3)	62 (92.5)	5 (7.5)	65 (97)	2 (3)
	2	n (%)	7 (100)	0 (0)	7 (100)	0 (0)	7 (100)	0 (0)	7 (100)	0 (0)
	3	n (%)	7 (87.5)	1 (12.5)	7 (87.5)	1 (12.5)	5 (62.5)	3 (37.5)	6 (75)	2 (25)
	4	n (%)	94 (95.9)	4 (4.1)	95 (96.9)	3 (3.1)	89 (90.8)	9 (9.1)	96 (97.8)	2 (2.2)
X2 / p			5.414 / 0.144		2.337 / 0.505		10.703 / 0.013*		20.528 / 0.000*	
HBME-1	1	n (%)	20 100)	0 (0)	20 (100)	0 (0)	20 (95)	1 (5)	20 (100)	0 (0)
	2	n (%)	5 (100)	0 (0)	5 (100)	0 (0)	5 (100)	0 (0)	5 (100)	0 (0)
	3	n (%)	13 (100)	0 (0)	12 (92)	1 (8)	11 (84.6)	2 (5.3)	13 (100)	0 (0)
	4	n (%)	135 (96)	5 (4)	135 (96)	5 (4)	127 (0.7)	13 (9.3)	136 (97.1)	4 (2.9)
X2 / p			1.396 / 0.706		1.635 / 0.652		3.250 / 0.355		1.111 / 0.774	
CK19	1	n (%)	27 (100)	0 (0)	27 (100)	0 (0)	27 (100)	0 (0)	27 (100)	0 (0)
	2	n (%)	8 (89)	1 (11)	7 (78)	2 (22)	8 (88.8)	1 (11.2)	9 (90)	0 (0)
	3	n (%)	11 (100)	0 (0)	11 (100)	0 (0)	9 (81.8)	2 (18.2)	11 (100)	0 (0)
	4	n (%)	127 (97)	4 (3)	127 (97)	4 (3)	119 90.8)	12 (9.2)	127 (97)	4 (3)
X2 / p			3.399 / 0.334		11.186 / 0.01*		4.016 / 0.260		1.468 / 0.403	

Abbreviations: χ2: Chi-square values, Gal-3: Galectin-3, HBME-1: Human bone marrow endothelial cell marker-1, CK-19: Cytokeratin 19, , P: Probability of difference, . A P value < 0.05 was considered statistically significant.

The Relationship between Fibrosis and Gal-3, HBME-1 and CK19 (correlation)

The associations between fibrosis and intensity of Gal-3, HBME-1 and CK19 (Each nodule was evaluated separately) are shown in **Table 3** and **Figure 2**. A positive correlation was found between tumor fibrosis and Gal-3 and CK19 intensity (r=0.179 p<0.052; r=0.202 p<0.025, respectively). A negative correlation was found between tumor fibrosis and HBME-1 intensity of the cases (r=-0.155 p<0.048).

Table 3- The associations between fibrosis and immunohistochemical staining intensity (Each nodule was evaluated separately).

n=277		Fibrosis n (%)				r*	p
İntensity		0-25 %	26-50 %	51-75 %	>75 %		
Gal-3	1	174 (69)	35 (14)	16 (6)	27 (11)	0.179	0.052
	2	11 (61)	5 (28)	0 (0)	2 (11)		
	3	5 (71)	0 (0)	1 (14.5)	1 (14.5)		
HBME-1	1	22 (58)	8 (21)	2 (5)	6 (16)	-0.155	0.048
	2	121 (66)	32 (19)	10 (5)	19 (10)		
	3	46 (81)	7 (12)	2 (3.5)	2 (23.5)		
CK19	1	32 (68)	5 (11)	4 (8)	6 (13)	0.202	0.025
	2	31 (72)	2 (5)	5 (11.5)	5 (11.5)		
	3	119 (64)	38 (20)	11 (6)	19 (10)		

*Spearman's Correlation Coefficient.

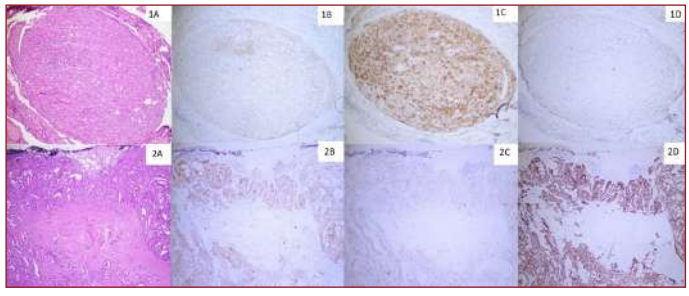

Figure 2- 1-Fibrosis negative group (X40) 2- Fibrosis positive group B-Gal-3 C-HBME-1 D-CK19 (X40).

DISCUSSION

Thyroid papillary microcarcinomas are thyroid papillary carcinomas of ≤1 cm by definition, and mortality is very low, less than 1%,[1] and mostly found incidentally.[3]

Tumor Diameter: Presence of LNM is one of the most important factors associated with local recurrence and distant metastasis.[5,6] There are publications stating that tumors larger than 0.5 mm progress more aggressively.[2,7,8] LNM ratio for tumors greater than 0.5 and less than 0.5 as follows: Goran et al. 45.71% vs. 24.34%, Lee et al. 29.1% vs. 18.2%.[2,9] This rate was also statistically significant in our study (%100 vs. %0) (p=0.02). There are also studies in the literature that do not have any difference in LNM formation.[10]

The rate of CI was higher in tumors larger than 0.5 in most studies.[2,9] This rate was 45.5 % vs. 59.8 % in Lee HS et al.'s study and 11.8% vs. 33.3% in Goran's study, and this rate was statistically significant in both studies (p<0.01).[2,9] However, in our study this rate was 8.5% vs 8.4% and do not have a statistically significant difference (χ2=0.009, P=0.998). In the group of patients with large tumors, predictor for LNM was only CI in Goran et al study.[2]

The rate of ETS in patients with TPMC varies from 2% to 53%.[10] In the study of Kim et al with 205 cases and in the study of Chow et al. with 203 case , only the rate of ETS was higher, and no difference was found in other comparisons (42.6% vs 22.2%; 29.3 % vs 4.3%).[10,11] Friguglietti et al. did not find any difference, in terms of ETS.[12] In our study, ETS was observed in 4 (2.24%) cases, and no difference was found in tumors greater than 0.5 and less than 0.5 cm group. (χ2=0.324, P=0.569)

Unilaterality, Bilaterality and Multifocality: In studies including large numbers of TPMC patients, the rate of multifocality ranges from 9.2 to 32% and that of bilaterality from 8.1 to 25.6%.[13] In our study, the multifocality rate was 35.3% and the bilaterality rate was 21.9%. Unilaterality rate was 14.7% in Goran's study, 3% in Apostol's study, and 23% in our study.[2,6] In the study of Apostol, multifocality and bilaterality were found to be associated with CI and ETS, but no significant relationship could be shown with LNM.[6] In our study, the relationship of unilaterality, bilaterality and multifocality with LVİ and LNM was statistically significant (χ2=9.417, P=0.002, χ2=6.668, P=0.01; χ2=4.362, P=0.04, χ2=13.692, P=0.000; χ2=4.477, P=0.03, χ2=6.241, P=0.01). There are publications that do not find a relationship between multifocality and LNM.[14] However, many studies have shown a relationship with LNM, as in our study.[13]

Number of tumor foci (NTF): Kim et al. reported that an increase in the NTF was strongly associated with cervical LNM, and advanced TNM stage of PTC.[11] The significant associations were found between NTF and LVI, LNM (χ2=17.520, P=0.004; χ2=56.282, P=0.000, respectively) in our study. Again, in the study of Guo et al., they suggested that LNM increased as the NTF increased in multifocal cases, and therefore they recommended more radical treatment in multifocal patients.[15]

Fibrosis: There are few publications investigating the relationship between thyroid fibrosis and prognosis. Firstly, Isarangkul et al. suggested that extensive fibrosis may be important for the diagnosis of PTC.[16] In the 511 case study of Liu et al., a relationship was found between fibrosis and LNM [17] In our study, no relationship was found between fibrosis and LNM, but a statistical difference was found between fibrosis and CI and ETS (χ2=35.195, P=0.000; χ2=14.796, P=0.002).

İmmunochemistry: Gal-3, HBME-1 and CK19, are widely used in the diagnosis of TPMC.[18] Gal-3 has been suggested to have such an effect on cancer metastasis.[19] However, a relationship was found between prevalence of Gal-3 and CI and ETS, in our study (χ2=10.703, P=0.013; χ2=20.528, P=0.000). No publication related to the prognostic significance of HBME-1 has been found in the literature. In our study, no significant relationship was found between HBME-1

with tumor characteristics. CK-19 is a poor prognostic factor whose aggressiveness is well known, mostly in liver tumors.[20] Dencic et al. found a statistically significant relationship between high CK19 expression and ETS.[21] There are studies that found a significant correlation between CK19 positivity and LNM, LVI.[22,23] In our study, a significant relationship was found between CK19 and LNM ($\chi 2=11.186$, $P=0.01$). In the Liu et al. study, IF was associated with increased Gal-3 and CK19 staining as in our study.[17] A negative correlation was found between fibrosis percentage HBME1 density ($p<0.05$). No other findings were found in the literature investigating the relationship between fibrosis and HBME-1 staining.

This study has several limitations. The first is that this is a retrospective study. The number of dissected lymph nodes is in the lower limits (13%) when compared to the literature. In the literature, this rate is 8-100%.[2] Follow-up and recurrence information were not included in the study.

CONCLUSIONS

Negative prognostic markers are >0.5 tumor diameter, unilaterality, bilaterality, multifocality, number of tumor foci, fibrosis, Gal-3 and CK19 prevalence. A positive correlation was found between fibrosis and Gal-3 and CK19, and negative correlation with HBME-1 intensity.

ETHICAL DECLARATIONS

Ethics Committee Approval: Ethical approval was obtained from Non-Interventional Clinical Research Ethics Committee of the Kutahya Health Science University Evliya Celebi Research and Training Hospital, prior to the initiation of the research work (Date: 21.00.2021 , Decision No: 2021/14279).

Informed Consent: All participants signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article / Orijinal Araştırma

Evaluation of YouTube Videos as a Patient Education Source for Inguinal Hernias

Kasık Fıtıkları için Hasta Eğitim Kaynağı Olarak YouTube Videolarının Değerlendirilmesi

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Abstract

Aim: Patients frequently use YouTube to obtain information about their conditions and possible treatment options. Inguinal hernia is one of the most common surgical diseases among the general population. This study aims to evaluate the quality of videos about groin hernia on YouTube.

Material and Method: The videos are sorted according to the number of views after searching for "groin hernia" on YouTube on 8.12.2021. The study was performed on the videos selected from the top 50 most-watched videos. Two independent reviewers reviewed all videos for relevance and content. In addition, the descriptive characteristics of each video (upload date, number of views, likes and dislikes, and comments below the video) were recorded in the dataset. DISCERN, GQS, and JAMA rating scales were used to evaluate the quality of the videos.

Results: A statistically significant difference was found in DISCERN scores in the videos uploaded by doctors and non-physicians ($p<0.001$). Similarly, when the two groups were compared, the videos uploaded by the doctors were statistically higher in quality in JAMA and GQS scores ($p<0.001$, p:039, respectively).

Conclusion: The quality of information about groin hernia on YouTube is variable. Helpful and misleading videos have no difference in terms of views and popularity. It is more appropriate for patients to prefer videos uploaded by physicians as a source of information. It is essential to pay attention to the person who uploads the content rather than the popularity, duration, or number of comments of a video.

Keywords: YouTube, video, inguinal hernia

Öz

Giriş: Hastalar, durumları ve olası tedavi seçenekleri hakkında bilgi almak için sıklıkla YouTube'u kullanır. Kasık fıtığı, genel popülasyonda en yaygın cerrahi hastalıklardan biridir. Bu çalışma YouTube'da kasık fıtığı ile ilgili videoların kalitesini değerlendirmeyi amaçlamaktadır.

Gereç ve Yöntem: 8.12.2021 tarihinde YouTube'da "kasık fıtığı" araması yapıldıktan sonra videolar izlenme sayısına göre sıralanmıştır. Çalışma en çok izlenen ilk 50 video arasından seçilen videolar üzerinden yapılmıştır. İki bağımsız yorumcu, alaka düzeyi ve içerik açısından tüm videoları inceledi. Ayrıca her bir videonun tanımlayıcı özellikleri (yükleme tarihi, izlenme sayısı, beğenilenler ve beğenilmeyenler, videonun altına yapılan yorumlar) veri setine kaydedilmiştir. Videoların kalitesini değerlendirmek için DISCERN, GQS ve JAMA derecelendirme ölçekleri kullanıldı.

Bulgular: Doktor ve hekim olmayan kişiler tarafından yüklenen videolarda DISCERN puanlarında istatistiksel olarak anlamlı fark bulundu ($p<0.001$). Benzer şekilde iki grup karşılaştırıldığında, doktorların yüklediği videoların kalitesi JAMA ve GQS puanlarında istatistiksel olarak daha yüksekti (sırasıyla $p<0.001$, p:039).

Sonuç: YouTube'da kasık fıtığı ile ilgili bilgilerin kalitesi değişkendir. Yararlı ve yanıltıcı videoların izlenme ve popülerlik açısından hiçbir farkı yoktur. Hastaların bilgi kaynağı olarak hekimler tarafından yüklenen videoları tercih etmesi daha uygundur. Bir videonun popüleritesinden, süresinden veya yorum sayısından çok içeriği yükleyen kişiye dikkat etmek esastır.

Anahtar kelimeler: YouTube, video, inguinal herni

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INTRODUCTION

British scientist Tim Berners-Lee invented the World Wide Web (www) while working at CERN in 1989.[1] After the internet network was provided for the first time in 1987, the relationship of the world population with the Internet has increased rapidly until today. Today, approximately 65.6 percent of the world's population is thought to have access to the Internet. Between 2000-2021, internet access increased by about 1.331%, and it is evident that this increase will continue.[2]

Abdominal wall hernias are common, with a prevalence of 1.7% at any age and 4% over 45. Inguinal hernias, which make up 75% of abdominal wall hernias, carry a lifetime risk of 27% in men and 3% in women.[3] Inguinal hernias are the most common surgery performed by general surgeons in daily surgical practice. In recent years, it has been frequently preferred by both patients and surgeons for education and information purposes due to the Internet and especially YouTube videos rich in visual content. While doctors generally use youtube for educational purposes, patients use it for informational purposes.[4] Recently, with the popularity of laparoscopic and robotic surgeries, there has been a significant increase in the number and resolution quality of laparoscopic and robotic surgery videos on YouTube.[5,6] However, the accuracy of the content and the quality of the information are essential, and the lack of a mechanism to control the accuracy of the content creates the possibility of misleading the users.

After YouTube was founded on February 14, 2005, it has become a free and easily accessible video-sharing platform. It is thought that more than a quarter of the world's population uses this platform every month, and the number of daily active users is 122 million.[7] Therefore, it is inevitable that such a popular website is used in health-related searches and used as a source of information.

Due to the impact of the current pandemic period, difficulties in accessing health services, and their general social phobia, patients searched for information about their illness on the YouTube video platform. Unfortunately, because this platform is public and anyone can upload videos, data can often be misleading, deceptive, or incomplete, and many studies about this topic have shown that.

This study aims to evaluate the quality of videos about groin hernia on YouTube. To the best of our knowledge, this is the first study in the literature to investigate this issue with objective data.

MATERIALS AND METHODS

In this study, data from YouTube videos that are open and available to everyone were used. The study was inspired by a systematic review of similar research.[8-11] The videos are sorted according to the number of views after searching for "groin hernia" in the YouTube search bar on 8.12.2021. The study was performed on the videos selected from the top 50 most-watched videos. Repetitive videos, videos with non-English language, videos not related to inguinal hernia, videos shorter than one minute, and videos for advertising purposes were excluded from the study. Therefore, the work consisted of 50 videos with the most views and met the requirements.

Two independent reviewers reviewed all videos for relevance and content. In addition, the descriptive characteristics of each video (upload date, number of views, likes and dislikes, and comments below the video) were recorded in the dataset.

DISCERN, GQS, and JAMA rating scales were used to evaluate the quality of the videos. The DISCERN scoring system is an evaluation criterion consisting of two different groups with 16 questions.[5] According to this assessment, the first section is concerned with safety, while the second section focuses on the quality of information regarding treatment options. Grading for the sixteenth question is done independently of the rating given for the previous 15 questions. Accordingly, 16-26 points indicate extremely low quality, 27-38 points indicate low quality, 39-50 points indicate medium quality, 51-62 points indicate acceptable quality and 63-75 points indicate exceptional quality (**Table 1**).[12,13]

Table 1. DISCERN Scoring System

Section	Questions	No		Partly		Yes
Reliability	1. Explicit aims	1	2	3	4	5
	2. Aims achieved	1	2	3	4	5
	3. Relevance to patients	1	2	3	4	5
	4. Source of information	1	2	3	4	5
	5. Currency (date) of information	1	2	3	4	5
	6. Bias and balance	1	2	3	4	5
	7. Additional sources of information	1	2	3	4	5
	8. Reference to areas of uncertainty	1	2	3	4	5
Quality	9. How treatment works	1	2	3	4	5
	10. Benefits of treatment	1	2	3	4	5
	11. Risks of treatment	1	2	3	4	5
	12. No treatment options	1	2	3	4	5
	13. Quality of life	1	2	3	4	5
	14. Other treatment options	1	2	3	4	5
	15. Shared decision making	1	2	3	4	5
16. Based on the answers to all of these questions, rate the overall quality of the publication as a source of information about treatment choices		1	2	3	4	5

The overall quality of all videos reviewed was assessed using the global quality scale (GQS), a 5-point scale. This scale includes the accessibility of the information in the video, the quality of that information, the overall flow of information, and how practical the reviewer thinks the particular video will be to a patient (**Table 2**).[14]

Table 2. GQS

Score	Description
1	Poor quality, poor flow of the site, most information missing, not at all useful for patients
2	Generally poor quality and poor flow, some information listed but many important topics missing, of very limited use to patients
3	Moderate quality, suboptimal flow, some important information is adequately discussed but others poorly discussed, somewhat useful for patients
4	Good quality and generally good flow, most of the relevant information is listed, but some topics not covered, useful for patients
5	Excellent quality and excellent flow, very useful for patients

*GQS: Global quality score

Data were also evaluated using the Journal of the American Medical Association (JAMA) scoring system. This scoring system considers the quality of videos in terms of authorship, attribution, description, and validity. Each item is evaluated as 0 and 1 points. In the JAMA evaluation, 1 point represents insufficient knowledge, 2-3 points partially sufficient information, and 4 points quality information (**Table 3**).[15]

Table 3. JAMA Scoring System

Authorship	Authors and contributors, their affiliations, and relevant credentials should be provided
Attribution	References and sources for all content should be listed clearly, and all relevant copyright information should be noted
Disclosure	Website "ownership" should be prominently and fully disclosed, as should any sponsorship, advertising, underwriting, commercial funding arrangements or support, or potential conflicts of interest
Currency	Dates when content was posted and updated should be indicated

*JAMA: Journal of the American Medical Association

The popularity of the videos was evaluated by the video power index (VPI: like×100/[like + dislike]). In addition, view rate (total view/time since upload) was used to avoid the bias that a video on YouTube would get more views because it was uploaded earlier.[16,17]

The videos were divided into two groups according to whether the content producers were physicians or not. Video duration 5, 5-10,> 10 minutes, release date before five years (new videos) and after five years (old videos), first and second 25 videos as views, daily views, daily views below 177 and above, VPI below 93 VPI above 93 and comment/year > 50 and below 50 groups were also evaluated. Video quality and interaction between groups were assessed. The videos are grouped by who made them: doctor, medical, patient, and other. In addition, the videos are divided into categories according to whether they contain animation or not.

In March 2021, YouTube decided to hide the number of dislikes. We needed this to calculate the VPI score in our study. This information has been accessed with a program developer's "return youtube dislikes" program.

Institutional ethics review board approval was not required for the study.

Statistical analysis

IBM SPSS 22 for Windows program was used to analyze the data. Median, IQR, minimum-maximum values, and mean±standard deviation were used to describe the data. The conformity of all data to the normal distribution within the group was tested using the Shapiro-Wilk test. Relationships between variables were determined by Spearman correlation. Regression of quality indicators with data was done by multiple regression analysis. Mann-Whitney U test was used to see a significant difference between the groups. A p-value less than 0.05 was considered statistically significant.

RESULTS

When the first 50 videos with the most clicks were examined, there were 31,721,281 total clicks. The average video length was 595.04±464.64 seconds, with a minimum of 71 seconds and a maximum of 2266 seconds. The most-watched video was watched 3,064,908 times. While the number of daily views was 249/day at the most, the average daily viewing was 355.56±437.35. Other descriptive statistics are shown in **Table 4**. The mean VPI, DISCERN, JAMA, and QRS values between the videos were 92.66±3.96, 55.16±13.4, 2.4±0.8, and 2.7±1.05, respectively.

Table 4 . Data of 50 most clicked videos on the YouTube platform

	Mean±Std	Median [IQR(25-75)]	Min-Max
Video length (sec)	595.04±464.64	443 (263.75-726)	71-2266
View count	634425.62±741342.5	355719.5 (215715.75-611134.75)	174256-3064908
View count Daily	355.56±437.35	177.12 (105.33-432.74)	45.77-249.05
Like	3887.4±4617.63	2550 (80-251	324-22500
Dislike	225.46±218.33	136.5 (80-251)	25-872
Comment/year	128.19±343.43	27.5 (8.9-110.7)	0-2300
VPI	92.66±3.96	93 (90.1-95.7)	80-98.8
DISCERN	55.16±13.4	60 (43.5-65)	20 72
JAMA	2.4±0.8	3(2-3)	0 3
GQS	2.7±1.05	3(2-3)	1 5

*JAMA: Journal of the American Medical Association, GQS: global quality scale, VPI: Popularity power index of videos, IQR:Interquartile range (25 to 75)

While 31 of those who uploaded videos to the YouTube platform were doctors, 19 were not. According to the DISCERN score, 15 videos were of exceptional quality, while 18 videos were of acceptable quality. Eight videos were of medium quality, and nine were of poor quality. No videos uploaded by doctors were of poor quality, and all videos of exceptional quality were uploaded by doctors. A statistically significant difference was found in terms of DISCERN scores in the videos uploaded by doctors and non-physicians ($p<0.001$). Similarly, when the two groups were compared, the videos uploaded by the doctors were statistically higher in quality in JAMA and GQS scores ($p<0.001$, p:039, respectively).

There was no difference in quality scores regarding videos uploaded in 5 years or videos older than five years. Similarly, the videos in the top 25 and the videos in the last 25 were evaluated according to the number of views of the videos. While there was no difference in DISCERN and JAMA scores (p: 0.607, p: 0.461, respectively), the GQS scores of the top 25 videos were significantly higher (p: 0.04).

Daily viewing numbers were evaluated as 177 or more. While there was no difference in DISCERN and JAMA scores between the groups (p:0.387, p:0.149, respectively), the GQS scores of videos watched more than 177 per day were significantly higher (p:0.031). In addition, videos longer than 5 minutes had a higher DISCERN score than shorter ones, while JAMA and GQS scores were similar (p:0.38, p:0.344, respectively).

According to VPI values, there was no difference in quality between the videos below 93 and above and the annual comment number of videos below 25 and above (**Table 5**).

As a result of our study, a positive correlation was found between the quality scores (p<0.001) (**Table 6**). In linear regression analysis, VPI and the number of clicks did not affect DISCERN scores. (P: 0.447, p: 0.033). However, DISCERN scores increased as the video length and daily views increased (p<0.001, p:0.004). A negative correlation was found between the annual number of comments and DISCERN scores (p<0.001). There is a positive correlation between JAMA and the number of daily views and annual comments (p:0.008, p<0.001, respectively). There is a positive correlation between GQS and video duration only (p<0.001, respectively).

Table 6: Correlation Between quality scores

	DISCERN	JAMA	GQS
DISCERN	1	0.779	0.657
JAMA	0.779	1	0.530
GQS	0.657	0.530	1

DISCUSSION

The YouTube algorithm developed to reach quality and relevant videos among the 4 billion videos on YouTube works very complexly and personalized.[18] For this reason, when different users search for "groin hernia" the listed results will be different, so the top 50 most-watched videos related to our topic were examined.

In our research, JAMA, GQS, and DISCERN values were found to be high in the videos uploaded by the doctors. This result showed that YouTube might have accurate and reliable information about groin hernia, but only in videos uploaded by subject matter experts. Poor quality information accessed on YouTube can cause patients to access wrong information and make wrong decisions. It can also cause conflicts in the patient-physician relationship. Values other than this (Number of views, likes/dislikes, etc.) were not correlated with the video quality.

There is information pollution on the YouTube video platform as in the whole Internet. In our study, 62% of the videos were uploaded by doctors, and most of them were of high quality. However, this finding means that individuals or institutions uploaded the remaining 38% of the videos without medical expertise. This heterogeneous and uncontrolled information pollution on YouTube™ was previously reported by Roshan et al.[19] and Keelan et al.[20] In our study, videos uploaded by non-physicians were of poor quality, in line with the literature.[9,21]

After it was founded in 2005, the YouTube platform has continued to develop and has now become a source of information for both patients and doctors. In their study, Celentano et al.[22] concluded that most of the surgical residents watch the surgery videos on the YouTube platform. Unfortunately, algorithm-based search results are based on views and comments rather than quality.[23] Furthermore, YouTube's heterogeneous upload sources

Table 5. Relationship between seven categoric variables and videos quality

Video source	n	DISCERN [Median(IQR)]	p	JAMA [Median(IQR)]	p	GQS [Median(IQR)]	p
Physicians	31	62(60 to 62)	<0,001*	3(3 to 3)	<0,001*	3(4 to 2)	<0,001*
Non-Physicians	19	42(35 to 52)					
Old videos(>5 years)	23	56(42 to 62)	0,335*	3 (2 to 3)	0,519*	3(2 to 3)	0,447
New videos (≤5 years)	27	61(45 to 65)		3 (2 to 3)		3(2 to 4)	
View count first 25	25	61(43 to 64.5)	0,607*	3 (2 to 3)	0,461*	3(3 to 4)	0,04*
View count second 25	25	56 (43.5 to 66.5)		2 (2 to 3)		2(2 to 3)	
View count daily (>177)	24	61(44.5 to 65)	0,387*	3 (2 to 3)	0,149*	3 (2.25 to 3)	0,152*
View count daily (≤177)	26	56(40 to 63.5)		2 (2 to 3)		2 (2 to 3)	
Video length(>5 minutes)	22	62(52 to 69)	0.01*	3 (2 to 4)	0,38	3(2 to 3)	0,344
Video lenght(≤5 minute)	28	52(36 to 62)		3 (2 to 3)		2 (2 to 3)	
VPI (≤93)	25	56 (43 to 65)	0,58	3 (2 to 3)	0,803	3 (2 to 3)	0,571
VPI (>93)	25	61 (43.5 to 66.5)		3 (2 to 3)		2 (2 to 4)	
Comment/year(≤25)	25	59(49.5 to 63.5)	0,946	3(3 to 2)	0,437	2 (2 to 3)	0,086
Comment/year(>25)	25	61(42 to 67)		3(2 to 4)		3 (1.5 to 3)	

*Mann-Whitney U test; Statistically significant data are marked in bold.
JAMA: Journal of the American Medical Association, GQS: global quality scale, VPI: Popularity power index of videos; IQR:Interquartile range (25 to 75)

prevent standardization of video quality because of using Web 2.0 technology. In our study, no difference was found in DISCERN and JAMA scores between the first 25 most-watched videos and the last 25 videos. However, GQS was found to be higher in the first 25 videos.

It is a natural result that previously uploaded videos get more views over the years. Therefore, daily views were calculated to remove bias. There was no difference between the groups and quality scores regarding the number of daily viewings.

Although many studies have shown that the quality of videos uploaded by physicians is higher than those of non-physicians, the number of views was lower. This may be since patients may have difficulty understanding the physician's videos.[24,25] Although there were many surgical videos in the first 50 videos in our study, the number of views was high. However, there was no difference between VPI rates in terms of interaction. Although there are reports of poor-quality videos that are more popular than quality ones, such a result was not found in our study.[26]

The major limitation of our study is that there is no gold standard method for assessing the quality of YouTube videos. While JAMA, DISCERN, and GQS are not designed to evaluate the quality of youtube videos, they have been used in most studies.[27] These systems have often been found valuable in examining video quality.[15,16,28,29] Two different surgeons evaluated the scoring system. But two reviewers may be insufficient for validation. In addition, the YouTube platform is a platform where millions of videos are uploaded every day, and the evaluation may only be specific to the reviewed dates. Previously uploaded videos may have more views regardless of their quality. Therefore, the daily view count is calculated to eliminate this dilemma. Also, despite this fast rotation of uploaded content, the most popular videos list may not change that fast. Another limitation of the study can be considered the small number of included videos (n=50). Still, the total number of views is 31,721,281, which shows the effect of the videos and, therefore, the value of the study.

CONCLUSION

YouTube is the most popular website among doctors. Although the quality range of these videos is quite broad, the views of poor quality videos can be as high as quality videos. The quality of information about groin hernia on YouTube is variable. Helpful and misleading videos have no difference in terms of views and popularity. It is more appropriate for patients and doctors to prefer videos uploaded by doctors as a source of information. It is essential to pay attention to the person who uploads the content, rather than the popularity, duration, or number of comments of a video.

ETHICAL DECLARATIONS

Ethics Committee Approval: Institutional ethics review board approval was not required for the study.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article / Orijinal Araştırma

Survival Outcomes of Cervical Esophageal Cancer Treated with Definitive Chemoradiotherapy using Intensity-modulated or 3D Conformal Radiation Therapy: A Single Institute Experience

Yoğunluk Ayarlı veya 3 Boyutlu Konformal Radyasyon Tedavisi Kullanılarak Definitif Kemoradyoterapi ile Tedavi Edilen Servikal Özofagus Kanserinin Sağkalım Sonuçları: Tek Merkez Deneyimi

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Abstract

Aim: The aim of this study is to report the survival and treatment results of patients with cervical esophageal cancer treated with definitive chemoradiotherapy, whose incidence is very low in the population and there is not enough information about treatment and toxicity results in the literature.

Material and Method: Between 2013 and 2022, 10 patients (six males and four females) with cervical esophageal cancer treated with definitive chemoradiotherapy were included. Among these patients, seven had stage II disease, one stage III, and 2 stage IVA. All patients received radiotherapy at a median dose of 50.4 Gy and concurrent weekly chemotherapy.

Results: The median follow-up period was 18 months. The two-year and 5-year overall survival rates were 42.2% and 21.1%, respectively. The two-year and 5-year disease-free survival rates were 45.7% and 22.9%, respectively. Disease progression was noted in 3 out of 10 patients (30%). Three patients were still alive during analyze. Percutaneous enteral gastrostomy was performed in 3 of 10 patients. These requirement occured in 1 due to local progression, and in the remaining 2 patients due to the side effect of RT.

Conclusions: Overall survival rates were low, as similar findings appear in the literature. It was remarkable that the need for a percutaneous enteral gastrostomy was not observed in the IMRT group. All patients with complete remission were in stage 2.

Keywords: Cervical esophageal cancer, squamous cell carcinoma, definitive chemoradiotherapy, survival, toxicity

Öz

Amaç: Bu çalışmanın amacı, popülasyonda insidansı çok düşük olan ve literatürde tedavi ve toksisite sonuçları hakkında yeterli bilgi bulunmayan, definitif kemoradyoterapi ile tedavi edilen servikal özofagus kanserli hastaların sağkalım ve tedavi sonuçlarını bildirmektir.

Gereç ve Yöntem: 2013-2022 yılları arasında definitif kemoradyoterapi ile tedavi edilen servikal özofagus kanserli 10 hasta (altı erkek ve dört kadın) dahil edildi. Bu hastaların yedisinde evre II hastalık, birinde evre III ve ikisinde evre IVA hastalık vardı. Tüm hastalar medyan 50.4 Gy dozda radyoterapi ve eş zamanlı haftalık kemoterapi aldı.

Bulgular: Ortanca takip süresi 18 aydı. İki yıllık ve 5 yıllık genel sağkalım oranları sırasıyla %42.2 ve %21.1 idi. İki yıllık ve 5 yıllık hastalıksız sağkalım oranları sırasıyla %45.7 ve %22.9 idi. 10 hastanın 3'ünde (%30) hastalık progresyonu kaydedildi. Analiz sırasında üç hasta hala hayattaydı. 10 hastanın 3'üne perkütan enteral gastrostomi uygulandı. Bu gereksinim 1 hastada lokal progresyon nedeniyle, geri kalan 2 hastada RT'nin yan etkisi nedeniyle ortaya çıktı.

Sonuçlar: Literatürdekine benzer şekilde genel sağkalım oranları düşüktü. IMRT grubunda perkütan enteral gastrostomi ihtiyacının görülmemesi dikkat çekiciydi. Tam remisyona tespit edilen tüm hastalar evre 2'deydi. Çok merkezli randomize çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Servikal özofagus kanseri, skuamöz hücreli karsinom, definitif kemoradyoterapi, sağkalım, toksisite

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INTRODUCTION

Only 4.6% of all esophageal malignancies are cervical esophageal carcinoma (CEC).[1] According to retrospective trials in the past, it has been demonstrated that radiotherapy (RT) with concurrent chemotherapy (CT) for CEC has a similar survival rate to curative surgery.[2,3] A reduced rate of acute morbidity and the opportunity for laryngeal preservation make definitive chemoradiotherapy (dCRT) preferable to surgery.[2–4] In earlier research, the locoregional failure (LRF) rate ranged from 12 to 50%, while the 5-year overall survival (OS) rate following chemoradiotherapy (CRT) for CEC was between 18 and 54%.[4–8] The aim of this study is to analyze the survival outcome in patients with cervical esophageal cancer treated with chemoradiotherapy.

MATERIAL AND METHOD

The Case Recording System was authorized to track down the individuals who had a cervical esophageal cancer diagnosis and who were treated as dCRT at our faculty between January 2013 and January 2022. Patients who underwent surgery as the primary treatment, those who only received chemotherapy or radiotherapy, those who had another cancer diagnosis either before or after receiving dCRT, and those who already had distant metastases at the time of initial diagnosis were all excluded from the study. A total of ten patients diagnosed with CEC who received dCRT were identified and included in the study for survival analyses.

Statistical Method

Using the Kaplan-Meier method, the survival curve was calculated. Outpatient follow-up visits were normally conducted once every three months up until two years after the therapy and once every six months thereafter up to five years utilizing a blood test, esophagogastroduodenoscopy with biopsy, if necessary, a CT scan, and an FDG-PET, if necessary.

No signs of disease were detected by radiological and/or endoscopic biopsy results in post-treatment follow-ups; a complete response; detection of regression in the T or N stage with the presence of disease finding; a partial response; similar persistence of disease finding; stable disease; and progression of the disease from the pre-treatment stage to a more advanced stage was considered progression. Toxicity was evaluated considering the RTOG toxicity criteria.

Ethics Statement

All procedures performed involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was carried out with the permission of the Selçuk University Ethics Committee (Date: 21.06.2022, Decision No: 2022/132). All patients provided informed consent before their treatment started.

RESULTS

The clinical characteristics, treatment details, and results of this study are summarized in **Table 1**. The median age of the cohort was 67 (range, 37-69). There were six males and four females in the patient cohort. The histopathologic diagnosis of all patients in the study was squamous cell carcinoma. The pretreatment clinical stages were as following; stage II (7 patients), stage III (1 patient), and stage IVA (2 patients). The median follow-up period was 18 months (range, 5-111). The median irradiation dose of 50.4 Gy (45-50.4) was delivered. The estimated 2-year and 5-year overall survival (OS) rates were 42.2% and 21.1%, respectively. The estimated median value of OS was 25±8.6 months (95% CI 8-41.9) (**Figure 1**). The estimated 2-year and 5-year disease-free survival (DFS) rates were 45.7% and 22.9%, respectively. The estimated median value of DFS was 14±25.2 months (95% CI 0-63.3) (**Figure 2**).

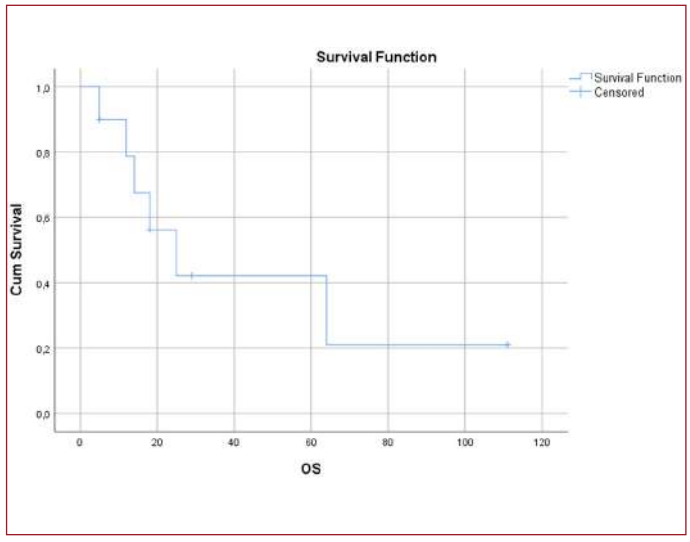

Figure 1. Overall survival (OS) curve

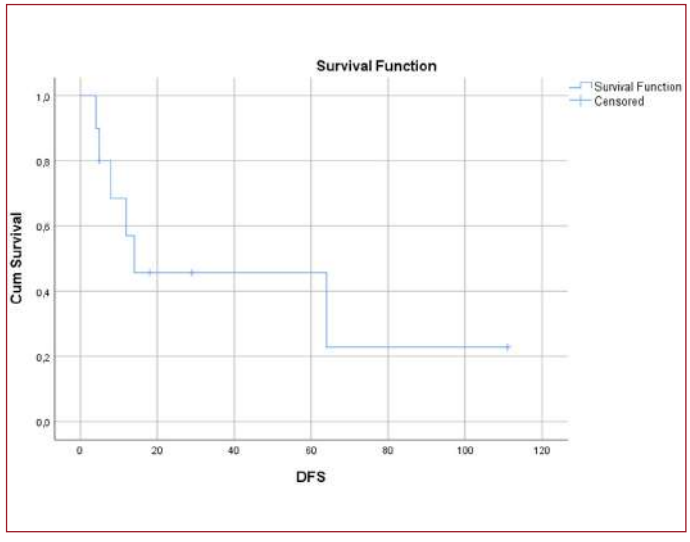

Figure 2. Disease-free survival (DFS) curve

Table 1. Patient demographics, disease features, treatment, side effects, and survival informations

Case No	Age	Gender	ECOG	Location	Tumor type	Clinical stage	Irradiation dose (Gy) and technic	Concurrent Chemo/No.	PEG	Response evaluation	Clinical course	OS (months)	DFS (months)
1	52	M	0	CeUt	SC	T2N0, stage II	45 Gy/ 25 fr 3D-CRT	Cisplatin (30- 40 mg/m²) / weekly/5	-	CR	CR continue	111	111
2	37	F	1	Ce	SC	T4N0, stage IVA	50.4 Gy/ 28 fr 3D-CRT	Cisplatin (30- 40 mg/m²) / weekly/5	+ (due to LR)	PR	Progression, salvage operation, recurrence, cancer death	14	8
3	44	F	1	CeUt	SC	T4N2, stage IVA	50.4 Gy/ 28 fr 3D-CRT	Paclitaxel (50 mg/m²) +Carboplatin AUC 2/ weekly/ 6	+ (due to RT toxicity)	PR	death of other disease (MI)	5	5
4	69	F	1	Ut	SC	T3N0, stage II	50.4 Gy/ 28 fr 3D-CRT	Paclitaxel (50 mg/m²) +Carboplatin AUC 2/ weekly/ 5	+ (due to RT toxicity)	CR	death of other disease (intestinal perforation)	64	64
5	47	F	0	Ut	SC	T2N2, stage III	50.4 Gy/ 28 fr 3D-CRT	Paclitaxel (50 mg/m²) +Carboplatin AUC 2/ weekly/ 6	-	CR	reccurred (bone), cancer death	25	4
6	61	M	0	Ut	SC	T3N0, stage II	50.4 Gy/ 28 fr IMRT	Paclitaxel (50 mg/m²) +Carboplatin AUC 2/ weekly/ 6	-	CR	CR continue	29	29
7	69	M	0	Ut	SC	T3N0, stage II	50.4 Gy/ 28 fr IMRT	Oxaliplatin (85 mg/m²) IV on Day 1, 15, and 29 for 3 doses, FU (180 mg/m²) IV on days 1, and 33	-	PR	peritoneal and omental metastatic implants. Loss of follow-up. Considered to be death.	18	14
8	67	M	0	Ut	SC	T3N0, stage II	50.4 Gy/ 28 fr IMRT	Oxaliplatin (85 mg/m²) IV on Day 1, 15, and 29 for 3 doses, FU (180 mg/m²) IV on days 1, and 33	-	Stable	death of other disease (MI)	12	12
9	67	M	1	Ut	SC	T3N0, stage II	50.4 Gy/ 28 fr IMRT	Paclitaxel (50 mg/m²) +Carboplatin AUC 2/ weekly/ 5	-	CR	CR continue	18	18
10	67	M	0	Ut	SC	T3N0, stage II	50.4 Gy/ 28 fr IMRT	Paclitaxel (50 mg/m²) +Carboplatin AUC 2/ weekly/ 5	-	PR	Loss of follow-up	5	5

PEG: percutaneous enteral gastrostomy, CR: complete response, PR: partial response, OS: overall survival, DFS: disease-free survival, ECOG: Eastern Cooperative Oncology Group performance scale, SC: squamous cell, Ce: cervical, Ut: upper thoracal, M: male, F: female, Gy: gray, fr: fraction, IMRT: intensity modulated radiotherapy, 3D-CRT: 3 dimensional conformal radiotherapy

DISCUSSION

Being an uncommon tumor with a poor oncological prognosis, CEC has mostly been treated with surgery as the primary option. Nevertheless, locally advanced CEC has been disallowed for definitive surgery. Additionally, patients frequently decline surgical approaches in order to maintain laryngeal function and prevent the procedure's significant risk of morbidity and mortality. For these particular patients, dCRT is regarded as a successful treatment with declared survival rates as similar with surgical excision. Owing to the low disease prevalence, comprehensive prospective studies are uncommon, and the majority of the available data is based on tiny retrospective series.[1-4]

Cervical esophageal cancer can be detected in a wide age range (18–87).[8] The age distribution of our study cohort was between 37 and 69 years. Men were the most commonly affected patient group in most studies as in ours (60%). In the study reported by Nakata et al., six of 10 patients were men.[9] Some studies have reported a much higher male gender predominance. In the study reported by Kim et al., which included 79 patients, almost all of the patients (n=75) were male.[10] Similarly, 6 of 10 patients were male in our study. Undoubtedly, it is not surprising that the male gender is dominant in this disease group, where smoking and alcohol consumption are the main risk factors.

A better dosage coverage and conformity to target volumes in their CEC have reportedly been made possible by advancements in RT techniques, which also permit less excessive doses to neighboring organs. However, because there are so few studies evaluating RT procedures in terms of survival and side effects, and because the sample sizes in the studies are so tiny and diverse, it is very challenging to draw a conclusive judgment about the clinical significance of this situation. The patient groups who got IMRT and 2D conventional RT were retrospectively assessed in the study reported by Cao et al., which included 101 patients with a diagnosis of CEC. In spite of the fact that there was no statistically significant difference in OS, regional failure-free survival, or local failure-free survival between the IMRT group and the 2D-RT group, the incidence of late toxicity decreased with IMRT (6.3 vs. 8.1%), which developed the beneficial ratio for CEC patients (4). In the study published in 2017 and reported by Ito et al., the clinical results of 80 patients were evaluated, Patients with a diagnosis of CEC who were treated with IMRT and 3D conformal RT were compared in terms of OS, failure patterns and toxicity. They achieved complete response in 24 of 32 patients (75%) in the IMRT group and in 33 of 48 patients (68.75%) in the 3D conformal RT group. These results did not create a statistically significant difference between the two groups in terms of complete responses. The median RT dose was 60 Gy (50–70.2). There was no significant difference in the incidence of late toxicities between the IMRT and 3D conformal RT groups. Esophageal stricture was one of the notable toxicities. Among patients those had esophageal stricture, in 9 patients with locally their tumor undercontrol in the esophagus (11%), comprising 5 (16%) in the IMRT group and 4 (8%) in the 3D conformal RT group. Four of those nine patients (44%) were diagnosed with T4 lesions. One

grade 4 pericardial effusion developed in the 3D conformal RT group. No grade 3 or higher pulmonary toxicity was observed.[11] Surgical treatment of CEC is associated with an early postoperative morbidity rate of up to 30–40%, such as anastomotic leakage, wound healing problems, fistula, and the need for reoperation, which negatively affects quality of life.[12,13] Unfortunately, both RT alone and CRT, which are alternatives to surgical treatment due to the high morbidity rates of surgery and have reported similar survival rates, are associated with relevant side effects that should not be ignored at all. Forsooth, severe acute and late toxicity have been reported in almost 20-30% of patients receiving RT or CRT.[6,14] In addition to the frequently seen side effects such as mucositis and cytopenia, more severe toxicities such as dysphagia or esophageal stenosis, which is the toxicity of RT or CRT treatments in both acute and chronic periods, may develop. This situation negatively affects the quality of life. In these cases, nutritional support can be provided parenterally or with a feeding tube. In our treatment outcome report, PEG was performed in 3 of 10 patients. While PEG requirement occured in 1 of these 3 patients due to local progression, this procedure was performed in the remaining 2 patients due to the side effects of RT. All 3 patients who underwent PEG were in the 3D CRT group. It was remarkable that none of the 5 patients treated with IMRT developed a need for PEG. Neither pulmonary nor hematological grade 3 or higher toxicity was observed. There are currently insufficient and heterogeneous reports on what the optimal radiotherapy dose should be, considering the balance related to oncological outcomes and toxicities. Although doses as high as 60–70 Gy are given to the tumor based on data with squamous cell cancer in the head and neck, it is not clear whether these contribute to local or overall survival. In a study evaluating 260 patients with any histological diagnosis and in any location of the esophagus, it was reported that the administration of 61.6 Gy to 50.4 Gy radiotherapy doses did not increase local control.[15] In our study, 9 patients received an irradiation dose of 50.4 Gy, while 1 patient received 45 Gy. A complete response was detected in 5 patients. Recurrence occurred in 2 of the 5 patients who developed a complete response. Three patients continue their lives with a complete response. It was quite remarkable that all of these patients were at stage II.

Despite major advances in drug production and technological improvements, the survival of patients with CEC is still not promising. In the meta-analysis published in 2022, in which 22 studies regarding definitive RT or CRT were applied to patients with CEC were analyzed, estimated pooled OS rates at 1, 3, and 5 years were 77.9% (73.9–82.2), 48.4% (43.2–54.3), and 35.3% (29.7–41.9), respectively. The median OS was 33.4 months (25.8–42.2).[8] In the present study, the estimated 2-year and 5-year OS rates were 42.2% and 21.1%, respectively, which were slightly lower than those mentioned in the meta-analysis.

According to statistics pertaining to the thoracic location, combining CT with RT has significantly improved outcomes in the treatment of esophageal cancer. However, because to a lack of data, an enhanced survival rate for the upper esophagus could not be proven. Several chemotherapy regimens have been suggested and modified by proven treatments for lower esophageal and hypopharyngeal squamous cell carcinoma. One of the current therapeutic options for CEC is high-dose cisplatin-based CT, frequently in conjunction with paclitaxel or 5-fluorouracil (5-FU). A limitation in the ability to draw any inferences is that the individual chemotherapeutic drugs utilized were heterogeneous, and no stratified data were presented. In our study, the patients those with who had concurrent RT and CT treatment selected for the trial. There were no standart CT scheme in analysed group.

The current study's mayor limitations were that it was designed at a single-centre, only covered a limited number of patients, and was retrospective in nature.

CONCLUSION

In the presented study, OS and DFS rates were low, consistent with the literature. It was remarkable that the need for PEG did not develop in those treated with IMRT. Side effects may be reduced with IMRT. All patients with complete remission were in stage 2. As a result of findings, there is a chance of cure with dCRT in early-stage patients without lymph node involvement.

Based on our trial results and the literature, there are important issues to be mentioned regarding CEC. Since the number of CEC patients is very small, information on this subject in the literature is so limited. Postoperative morbidity is very high. Currently, the primary recommended treatment is dCRT. Survival rates are very low. There are no strong recommendations regarding its treatments depending on randomized studies. There is a strong need for studies regarding chemotherapy schemes, the dose of radiotherapy, and the radiotherapy field.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of the Selçuk University Ethics Committee (Date: 21.06.2022, Decision No: 2022/132).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article / Orijinal Araştırma

Reorganizing as a COVID-Free Heart Center: Does It Really Matter for the Primary Percutaneous Coronary Intervention Endpoints During the COVID-19 Pandemic?

Covid'siz Bir Kalp Merkezi Olarak Yeniden Yapılanma: COVID-19 Pandemisi Primer Perkütan Koroner Girişimin Sonlanim Noktalari Için Gerçekten Önemli Mi?

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Abstract

Aim: Investigating the effects of the extraordinary environment produced by the COVID-19 pandemic on the angiographic endpoints of the primary percutaneous intervention is the main objective of the present study.

Material and Method: Data regarding the organizational arrangements as defining COVID-free heart centers during the first waves is scarce. 88 STEMI patients admitted between March 11, 2020, and June 11, 2020 (Group1) as well as the 79 Patients admitted in the same period of 2019 (group 2) were investigated. Two of the patients with positive COVID-PCR test results were transported to other centers. Analysis of the data from these admissions resulted in the enrollment of 70 patients for group 1 and 55 Patients for group 2. None of these cases had hospital acquired SARS CoV-2 infection during the follow-up. Therefore, no COVID-related morbidity or mortality was observed in this vulnerable group.

Results: When we analyzed the 88 primary percutaneous coronary intervention procedures of the non-COVID STEMI patients of the lockdown period and compared the 70 of them with the 50 STEMI patients of the previous year, the results were not that encouraging. Even our hospital was declared as a COVID-free cardiovascular center, there was a significant delay in the symptom-to-door time (SDT) during the pandemic (4.8 vs. 2.5 hours, respectively; P<0.001). Door-to-balloon time (DBT) for the lockdown period was not different than the prepandemic era. The main difference regarding the angiographic endpoints was in corrected TIMI frame counts (cTFC) which was significantly higher during the pandemic (32.9 vs. 27.3) (P<0.001). Furthermore, a powerful positive correlation between SDT and TFC was represented (R=0.731, p<0.001). Hospitalization duration was shortened during the pandemic (2.3 days in pandemic and 3.4 days in 2019, P<0.001). None of the patients had hospital-acquired infection and related morbidity. However, in-hospital mortality was significantly higher than the previous year's (11.4% vs. 1.8% respectively, P=0.039). TFC was found to be an independent predictor of in-hospital cardiac events (OR: 1.17, 95% CI: 1.05-1.31, P< 0.01).

Conclusion: These results suggest that, when we exclude morbidity and mortality resulting from hospital-acquired infection, reorganizing as a COVID-free cardiac center doesn't have satisfactory favorable impact on the adverse cardiovascular outcome during the pandemic, unless the public is well informed.

Keywords: COVID-19; percutaneous coronary intervention; STEMI

Öz

Amaç: COVID-19 pandemisinin oluşturduğu olağanüstü ortamın primer perkütan girişimin anjiyografik son noktalarına etkisinin araştırılması planlandı.

Gereç ve Yöntem: Pandemi döneminde COVID'siz kalp merkezlerini tanımlayan organizasyonel düzenlemelere ilişkin veriler azdır. 11 Mart 2020-11 Haziran 2020 tarihleri arasında başvuran 88 STEMI hastası (Grup 1) ve 2019 yılının aynı döneminde başvuran 79 hasta (Grup 2) incelendi. COVID-PCR testi pozitif çıkan hastalardan ikisi başka merkezlere sevk edildi. Ardından elde edilen verilerin analizi, grup 1'e 70 hastanın ve grup 2'ye 55 hastanın kaydıyla sonuçlandı. Bu vakaların hiçbirinde takip sırasında hastaneden edinilmiş SARS CoV-2 enfeksiyonu yoktu. Bu nedenle, bu hassas grupta COVID ile ilgili herhangi bir morbidite veya mortalite gözlenmedi.

Bulgular: Sokağa çıkma yasağı döneminde COVID olmayan STEMI hastalarının 88 birincil perkütan koroner girişim prosedürünü analiz ettiğimizde ve bunların 70'ini önceki yılın 50 STEMI hastasıyla karşılaştırdığımızda sonuçlar o kadar da iç açıcı değildi. Hastanemiz COVID'siz bir kardiyovasküler merkez olarak ilan edilse bile, pandemi sırasında semptomlardan kapıya kadar geçen sürede (SDT) önemli bir gecikme oldu (sırasıyla 4,8 - 2,5 saat; P<0,001). Karantina döneminde kapıdan balona geçen süre (DBT), pandemi öncesi dönemden farklı değildi. Anjiyografik sonlanım noktalarına ilişkin temel fark, pandemi sırasında önemli ölçüde daha yüksek olan düzeltilmiş TIMI frame count (cTFC) olmuştur (32,9 - 27,3, P<0,001). Ayrıca, SDT ile TFC arasında güçlü bir pozitif korelasyon gösterildi (R=0.731, p<0.001). Pandemi sırasında hastanede kalış süresi kısaldı (pandemide 2,3 gün ve 2019'da 3,4 gün, P<0,001). Hastaların hiçbirinde hastane kaynaklı enfeksiyon ve buna bağlı morbidite yoktu. Ancak, hastane içi ölüm oranı bir önceki yıla göre önemli ölçüde yüksekti (sırasıyla %11,4'e karşı %1,8, P=0,039). TFC'nin hastane içi kardiyak olayların bağımsız bir belirleyicisi olduğu bulundu (OR: 1.17, %95 GA: 1.05-1.31, P< 0.01).

Sonuçlar: Bu sonuçlar, hastane kaynaklı enfeksiyondan kaynaklanan morbidite ve mortaliteyi hariç tuttuğumuzda, COVID'siz bir kalp merkezi olarak yeniden yapılanmanın, halk iyi bilgilendirilmedikçe, pandemi sırasında olumsuz kardiyovasküler sonuç üzerinde tatmin edici olumlu bir etkiye sahip olmadığını göstermektedir.

Anahtar Kelimeler: COVID-19; perkütan koroner girişim; STEMI

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INTRODUCTION

The coronavirus disease 2019 (COVID-19) has caused repeated waves of outbreaks across the globe since early 2020.[1,2] Following the detection of highly transmissible variants of the virus s Omicron causing superspreading events recently, it has been proposed that COVID-19 vaccines may be less effective against the new variant(s) and there may be a rise in morbidity and mortality again, resulting in a huge extra burden that threatens to overwhelm the health services.[3] New measures in the management of cardiovascular diseases during such extraordinary public health problems may be necessary again in the future.[4] Multiple studies have shown that many patients suffering myocardial infarction did not receive proper medical care during the first wave of the pandemic.[1] Approximately 30% reduction in emergency ambulance calls for chest pain and a significant delay in primary percutaneous coronary intervention (PPCI) during ST segment elevation myocardial infarction (STEMI) was reported owing to prolonged symptom onset to door time (SDT) and door to balloon time (DBT). These unfavorable effects of the pandemic gave rise to an increase in both in-hospital and long-term mortality of STEMI.[2,5-7] In addition to the admission delay, screening and infection control procedures, lack of rapid testing for COVID-19, scarcity of protective equipment for hospital staff and organizational delay further exacerbated the delay for revascularization, resulting in a negative impact on patient prognosis as an increase in in-hospital mortality.[8] It was suggested that reorganization of some cardiovascular centers as COVID-free centers may help to overcome these issues.[9] Being reorganized as a COVID-free tertiary cardiovascular center during the first wave and thereafter, we aimed to present our primary PCI experience.

Revascularization during acute myocardial infarction restores blood flow in the target vessel resulting in epicardial reperfusion which is not equal to myocardial perfusion. Latter is shown to be adversely affected from both symptom onset to balloon time (SBT) and DBT.[4] Therefore, any delay before or after the hospital admission harms the myocardial reperfusion. SBT itself is an independent predictor of microvascular reperfusion failure.[10]

The TIMI frame count (TFC), is a method defined by Gibson CM et al to objectively evaluate an index of coronary flow as a continuous quantitative variable by counting the number of cineframes needed for contrast medium to reach a standard distal coronary landmark in the infarct-related artery. TFC facilitates comparisons of angiographic end points and also provides information about microvascular perfusion.[11] In this study, we aimed to explore the angiographic endpoints of the interventions for ST-Segment Elevation Myocardial Infarction (STEMI) during the outbreak period by using TIMI frame count as a standardized method.

MATERIAL AND METHOD

Our center, a cardiology center with a 24-hour primary PCI facility, was determined and organized as a COVID-free center during the pandemic. COVID patients were not treated in our center. Patients with COVID, or suspicion of it, were not brought to our center. Moreover, all patients were checked with COVID-PCR test soon after hospitalization and the patient was transported to other hospitals if the result was positive. Therefore, study population was selected from COVID-free patients who were admitted to our hospital during the outbreak. This study was approved by the local ethical committee and the study was conducted in accordance with the Helsinki Declaration. The study was carried out with the permission of İstanbul Üniversitesi Cerrahpaşa Faculty of Medicine Clinical Researches Ethics Committee (Date: 18.03.2021, Decision No: 55570).

Study Design and patient selection

The study was planned as a retrospective analysis of inpatient data by surveying the emergency admissions that were diagnosed and internalized with acute coronary syndrome in the periods of the COVID-19 outbreak and the same period of the previous year. All subjects had ischemic symptoms and elevated cardiac troponin-T levels with ST-segment elevation on ECG. Each patient with ongoing acute STEMI underwent emergency primary PCI, regardless of the symptom onset or admission time. Inclusion and exclusion criteria are presented within the consort flow diagram. (**Figure 1**) Only the patients with type 1 myocardial infarction according to the fourth universal definition of myocardial infarction by using hs-cTnT (Elecsys; Roche Diagnostics) were included in both groups. 88 STEMI patients admitted between March 11, 2020, and June 11, 2020 (Group1) as well as the 79 Patients admitted in the same period of 2019 (group 2) were investigated. Two of the patients with positive COVID-PCR test results were transported to other centers. These and other 5 patients with COVID-infection history were excluded. Analysis of the data from these admissions resulted in the enrollment of 70 patients for group 1 and 55 Patients for group 2.

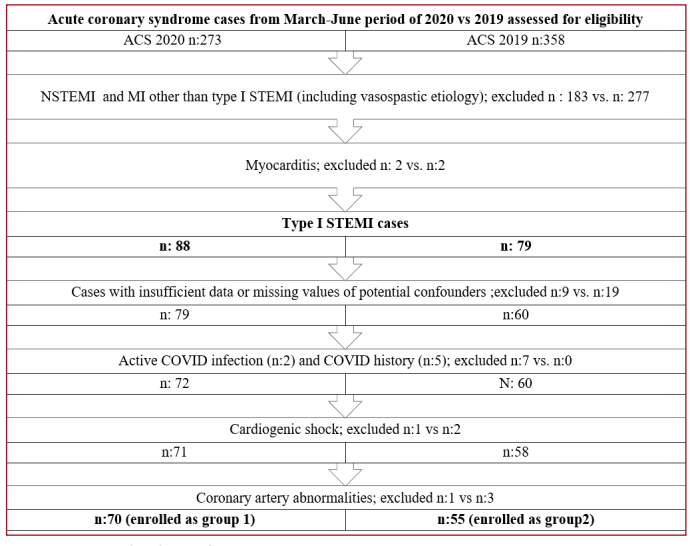

Figure 1. Study Flow Chart.
Abbreviations: STEMI: ST-segment-elevation myocardial infarction, NSTEMI: Non-ST-segment-elevation myocardial infarction

Data collection

Demographic characteristics of the patients were obtained from the medical record database of the hospital electronic system and symptom onset to admission time besides the first ECG recordings were received from patient files. Angiographic image recordings were reviewed and coronary flow was quantified by two experienced interventional cardiologists and controversial recordings were evaluated by a third senior interventional cardiologist. Three consecutive frames from the same phase of the cardiac cycle in the optimal projection that identified the stenosis in its greatest severity were selected for quantitative angiographic measurement. TFC was determined as the number of cineframes necessary for contrast to first reach a standard distal coronary landmark at a cinefilming rate of 30 frames per second. To objectively evaluate an index of coronary flow as a continuous quantitative variable, the number of cineframes required for contrast to first reach standardized distal coronary landmarks in the infarct-related artery (the TIMI frame count) was measured with a frame counter. The first frame used for TIMI frame counting was the first frame in which dye fully entered the artery. If the LAD was sub-selectively engaged and the LCx was the culprit vessel, the TIMI frame count began when dye first touched both borders at the origin of the LCx. The same rule was true for sub-selective engagement of the circumflex artery. In order to get the corrected TFC results, LAD counts were divided by 1.7 due to the longer length of LAD than RCA and LCx (11). In this sense, patients with coronary artery abnormalities were also excluded (Figure 1). Before the correction, normal TFC for LAD, LCx and RCA were regarded as 36±2.3, 22.2±3.8 and 21.7±2.8 respectively.

Statistical Analysis

Statistical analysis was performed by using SPSS for windows version 21 (Chicago, Illinois). Kolmogorov-Smirnov test was used to determine the distribution of continuous variables. Continuous variables with normal distribution were presented as mean±standard deviation by using Student's t-test. Non-normal distribution of independent samples was tested with Mann-Whitney U-test. Categorical data were tested with Pearson's chi-square test or Fisher's exact test and expressed as numbers and frequencies. Pearson correlation coefficient was used for the correlations between the continuous variables. Factors related to TFC were analyzed with binary logistic regression analysis. P<0.05 is accepted for statistical significance.

RESULTS

Baseline characteristics

Mean age of all study population was 58.8±10.3 and 21% of the subjects were female. Both COVID-19 period group and the control group were similar in terms of risk factors as smoking, diabetes, hypertension and hyperlipidemia. Baseline characteristics were depicted in **Table 1**. The blood test analyses of both groups also displayed no statistical difference except the admission troponin-T levels which was significantly higher in the patients of the COVID-19 period with 0.5±1.5 and 0.7±1.5 for the control and COVID-period groups respectively with a cut-off value of 0.014 (P <0.001) (**Table 1**).

Outcome measures

One of the main differences between two groups was obtained in the analysis of symptom-to-door time (SDT) defined as the length of the time period between the chest pain onset and emergency room admission. There was a significant delay in the presentations of the patients with myocardial infarction during the COVID-19 period when compared to the previous year (4.8±1.7 hours vs 2.5±1.4 hours, respectively; P<0.001). However, after hospital arrival, DBT was similar for both groups. (58.8±14 minutes vs. 62.1±12 minutes, P=0.09) (**Table 1**).

Table 1. Baseline Demographic Characteristics of Study Groups

	All Population (n:176)	Grup1 (n:70)	Grup 2 (n:55)	P value
Age (years)	58.8±10.3	57.6±11.5	60.3±8.5	0.161
Female (%)	21 (16.8%)	11 (15.7%)	10 (18.2%)	0.714
Smoker (%)	87 (69.6%)	48 (68.6%)	39 (70.9%)	0.778
DM (%)	63 (50.4%)	43 (61.4%)	20 (36.4%)	0.005
HT (%)	42 (33.6%)	25 (35.7%)	17 (30.9%)	0.572
HL (%)	54 (43.2%)	34 (48.6%)	20 (36.4%)	0.171
Anterior MI (%)	56 (44.8%)	27 (38.6%)	29 (52.7%)	0.114
SVD (%)	53 (42.4%)	28 (40.0%)	25 (45.5%)	0.306
LAD (%)	57%45.6%)	28 (40.0%)	29 (52.7%)	0.366
No-reflow (%)	15 (12.0%)	10 (14.3%)	5 (9.1%)	0.420
Hospitalization (days)	2.8±1.9	2.3±1.9	3.4±1.8	<0.001
Stent diameter	2.8±0.2	2.8±0.3	2.9±0.3	0.843
Stent length	24.9±7.6	24.8±7.3	25.1±8.1	0.911
SDT (hours)	3.8±1.9	4.8±1.7	2.5±1.4	<0.001
DBT (minutes)	60.6±13	62.1±12	58.8±14	0.09
TFC	30.5±8.2	32.9±7.4	27.3±8.3	<0.001
LVEF	42.8±8.1	42.2±8.2	43.6±7.9	0.342
First TnT	0.6±1.5	0.7±1.5	0.5±1.5	<0.001
In-hospital mortality (%)	9 (7.2%)	8 (11.4%)	1 (1.8%)	0.039
BUN	16.6±7.5	16.7±7.4	16.5±9.4	0.060
Creatinin	0.9±.4	0.9±0.2	1.0±0.6	0.990
GFR	91.3±25.2	90.2±24.0	92.8±26.7	0.570
Total cholesterol	177.4±51.5	181.6±52.8	172.1±49.7	0.309
LDL cholesterol	115.0±46.5	123.2±50.2	104.5±34.5	0.062
Triglyceride	166.4±119.4	164.4±82.2	168.8±155.2	0.341
WBC	11881.6±4594	11975.7±4729	11761.8±4458	0.877
Hemoglobin	14.5±1.6	14.6±1.3	14.4±1.9	0.519
Platelet	246±64	246±64	247±63	0.957
MPV	8.7±0.9	8.7±0.9	8.6±0.8	0.666

Values are expressed as mean±standard deviation (SD) or as median for continuous variables, or n (%) for categorical variables.
Abbreviations: BMI, body mass index; BUN, Blood urea nitrogen; CAG, coronary angiography; CABG, coronary artery bypass grafting; DBT, door-to-balloon time; DM, diabetes mellitus; GFR, glomerular filtration rate; HT, hypertension; HL, hyperlipidemia; LAD, left anterior descending artery (as the target vessel); LVEF, left ventricular ejection fraction; MI, myocardial infarction; MPV, mean platelet volume; PCI, percutaneous coronary intervention; SDT, symptom-to-door time; SVD, single vessel disease; TFC, TIMI frame count; TnT, Troponin T; WBC, white blood cell count.

Evaluation of the coronary angiogram and intervention recordings revealed 45.6% LAD target lesion without considerable difference between two groups. Contrary to the lesion location, the difference between the cTFC of the groups was significant (27.3±8.3 and 32.9±7.4 for control and COVID-19 period groups respectively; P<0.001). Frequency of no-reflow as angiographic end-point was approximate for both groups (9.1% vs 14.3% with a P value of 0.420) as well as the implanted stent size.

Length of hospital stay was 2.3±1.9 days for the patients who had myocardial infarction during the pandemic and this was significantly shorter than the 3.4±1.8 day hospitalization of the patients from the previous year (P<0.001). Contrary to the shorter length of hospitalization, in-hospital mortality of the COVID19 period patients was significantly higher than the patients of the control group (11.4% vs 1.8% respectively, P=0.039). The echocardiographic evaluation before discharge revealed no significant difference between both groups in terms of LVEF (43.6±7.9 vs 42.2±8.2, respectively; P= 0.342).

Among the variables of interest, correlation analysis displayed powerful positive correlation between the admission time and TFC with an R value of 0.731(P<0.001) and weaker positive correlation with initial troponin and DBT (R=0.201, P=0.025 and R=0.202, P=0.024 respectively) (**Table 2**).

Table 2: Spearman Correlation analysis of NT-proBNP and BDNF levels

	TFC	
	R (x2)	P value
SDT	731	<0,001
TnT	201	0,025
DBT	202	0,024

Abbreviations: DBT, door-to-balloon time; R: correlation coefficient; SDT, Symptom-to-door time; TnT, (Initial) troponin T level; TFC, TIMI frame count.

In order to determine the predictors of in-hospital cardiac event, defined as combined in hospital mortality and no-reflow phenomenon as an angiographic end point, DBT, SDT, TFC, LVEF and DM were analyzed by binary logistic regression. TFC was found to be the independent predictor of in-hospital cardiac events (OR: 1.17, 95% CI: 1.05-1.31, P< 0.01) (**Table 3**).

Table 3: Predictors of mortality and noreflow using binary logistic regression

	Event		
	OR	%95 CI	P-value
DBT	1,18	0,78-1,78	0,41
SDT	1,02	0,96-1,07	0,42
TFC	1,17	1,05-1,31	< 0,01
LVEF	0,99	0,92-1,06	0,85
DM	1,71	0,47-6,23	0,41

Abbreviations: DBT, door-to-balloon time; DM, diabetes mellitus; LVEF, ejection fraction; OR, Odds Ratio , Nagelkerke R2: 0.41; SDT, Symptom-to-door time; TFC, TIMI frame count

DISCUSSION

The main consequences of the present study can be summarized as:

No hospital acquired COVID infection was observed in our center. Hospital-acquired COVID infection may lead to increased mortality in patients with acute coronary syndrome (12). A prominent decrease ranging between 18 to 80% in the admissions with ST segment elevation myocardial infarction (STEMI) was reported all over the world during the pandemic (13). Fear of contagion was an important factor, and it wasn't such an unfounded fear. It was estimated that more than 11.3% of hospitalized patients with COVID-19 acquired the infection from hospitals during the outbreak and this proportion increased to more than 15% by the middle of May, 2020, months after the peak of admissions. During the same period of COVID, none of the 273 acute coronary syndrome patients, hospitalized and treated at our center, had hospital acquired COVID infection which might have been fatal in this vulnerable population. These data reveal the importance of COVID-free centers in reducing morbidity and mortality due to in-hospital transmission in a vulnerable patient group such as acute coronary syndrome.

We tried to explore the angiographic end points of the interventions for STEMI of the outbreak period by using TIMI frame count as a standardized method. Patients with Myocardial infarction arrived later to the emergency department of our center during the COVID-19 pandemic.

This result was in accordance with the results of the previous reports from all over the world suggesting a resistance of patients against utilizing health care system facilities globally. [1] COVID-19 pandemic resulted in increased morbidity and mortality of noninfectious medical emergencies which was related to an approximately 50% decline in emergency department admissions of patients including myocardial infarction cases. There was a dramatic increase in out of hospital cardiac arrests (OHCA) in March 2020 when compared to February 2020, proposing that patients had a tendency to wait too long to seek cardiac care. More than 20 % reduction in STEMI hospital admissions were reported in Europe during the lockdown period of the pandemic.[12-14] However, contrary to our results, time from symptom onset to admission in patients with STEMI was the same as before during the outbreak, and the interventional treatment of both STEMI and NSTEMI was also not affected in France. [14] De Rosa S et al presented from Italy that both patient- and system-related declared delays were substantially increased during the COVID-19 outbreak. In their multicenter nationwide study, time from symptom onset to coronary angiography for the patients with acute myocardial infarction was increased by 39.2% in 2020 compared with the previous year while the time from first medical contact to coronary revascularization was increased by 31.5%. They speculated that the possible reasons behind the delay might have been linked to both the fear of the virus contagion and also the occupation of the medical system with COVID-19 cases which might explain why the reduction in hospitalizations for STEMI (26.5%) was prominently less than with NSTEMI (65.1%). [15] SDT was longer for the STEMI patients in our study. The

occupation of the EMS with COVID patients which could cause a system related delay that might share the responsibility for the pre-hospital delay in our study however, this subject is multifactorial. Our center is located in the city center of Istanbul and the heavy traffic in Istanbul is a time consuming factor which may prolong SDT. However, the lock down period decreased the load of traffic in Istanbul impressively which eased the reach of the patients to our hospital. Moreover, our organization for 7/24 PCI did not change during the outbreak. Therefore, it was expected for a patient with STEMI to get the same or even faster interventional treatment after his or her call for EMS during the pandemic when compared to the period without the pandemic, but our results were contrary to this expectation. TURKMI-2 registry presented that there were no significant delays between the pre-pandemic and pandemic periods in terms of the EMS patient transport durations for STEMI patients after first contact with the patient.[9] Therefore we speculate the main reason of the delay as the fear of the contagion. A reflection of the admission delay, as expected, was the significantly higher troponin levels of this group in our study which was another clue about the meaningfulness of the aforementioned delay. Our results about the pre-hospital delay were also compatible with the previous results from our country. Karagoz A et al presented a significant pre-hospital delay in both self and ambulance transported patients in Istanbul Turkey which was also concordant with another, nationwide study that compared 1113 patients from 48 centers in Turkey who had myocardial infarction during COVID pandemic with 1872 patients from pre-pandemic period.[9,16]

The TFC of the patients with STEMI during the COVID pandemic was significantly higher than the patients of the previous year

Since our hospital was selected as a COVID free cardiology center, all of our patients were proven to be COVID negative and seasonal variations were excluded. The period between symptom onset and target vessel revascularization during STEMI is important for angiographic results. Lee CH et al found that DBT >90 minutes, compared with ≤90 minutes, was independently associated with increased cTFC (>28) owing to possible microvascular obstruction. STEMI patients with DBT >90 minutes also had higher 30-day mortality.[17] Our DBT was around 60 minutes without any significant change during COVID pandemic. Therefore, we supposed that the reason behind the increased TFC was probably the pre-hospital delay.

The data about the factors affecting coronary flow by using TFC as a measure of angiographic end point after primary PCI is scarce. Most of the studies are about no-reflow phenomenon which can be regarded as the end of deteriorated coronary flow spectrum. Age, STEMI, delayed presentation (longer SDT) and cardiogenic shock on admission as clinical predictors and complex, bifurcation or longer coronary lesion, need for a glycoprotein IIb/IIIa inhibitor during PCI, pre-procedural TIMI flow grade 0, plaque features (burden, necrotic core size, and cap thickness on IVU-S) as the angiographic predictors of no-reflow were defined by the previous studies.[18-20]

Failure to achieve normal coronary flow after PPCI was shown to be associated with some other factors as systolic blood pressure on admission, total stent length, and baseline TIMI flow.[13] Interestingly, smoking and previous PCI had paradoxical preventive effect from no-reflow. The reason for the former is not known however the latter is thought to be related to antiplatelet use.[4]

We didn't evaluate all angiographic predictors of no-reflow since their relationship with cTFC was not clear from the previous studies. However, we recorded and analyzed the stent sizes which were similar for both groups. Our groups were identical in terms of smoking habit however their previous medications as well as GPIIb-IIIa inhibitor use were not evaluated. In previous studies, failure to restore normal coronary flow was presented to result in worse short-term clinical outcomes during PPCI.[13] Patients with AMI who developed no-reflow had greater mortality, both in the catheterization laboratory and during the overall hospitalization. The largest study with 182,467 STEMI patients demonstrated 2.7% no-reflow ratio.[20] In our study no-reflow percentage among STEMI patients was higher than this study by Robert WH et al, like the other previous smaller scale studies concluding the incidence of no-reflow during PCI as 11% to 41% of patients.[20] However, the relationship between SDT and no-reflow couldn't reach statistical significance, although it was higher for the patients suffered myocardial infarction during the pandemic of COVID-19 (9.1% vs 14.3%, P=0.420). This result was interpreted as being related to relatively small sample size of our study.

Gibson et al presented in the TIMI studies that the corrected TIMI frame count was an independent predictor of in-hospital mortality in patients with myocardial infarction.[21] Compatible with this result, our study revealed a statistically significant increase in the in-hospital mortality of myocardial infarction patients of the pandemic period when compared to the previous year (11.4% vs 1.8% respectively, P=0.039) although mean hospital stay was significantly shorter during the pandemic (2.3±1.9 vs 3.4±1.8 days respectively, P<0.001). The shorter stay of the patients was mainly attributed to four factors: The first one was the effort of the hospital management to keep the beds as unoccupied as possible which was a part of the nationwide precautions, the second was the fear of the hospital staff from possible contagion of any patient; the third factor was the willingness of the patients to leave the hospital as soon as possible when they feel good enough to go home and fourth was the shortage at the hospital staff due to rearrangement for the first COVID-19 wave precautions . We contemplate that it would be reasonable to study the long term mortality of these somehow prematurely discharged patients of COVID-19 period since the difference in between may go even wider gradually after the discharge.

Limitations

Small sample size and retrospective design were two main limitations of our study. We needed to combine mortality and no-reflow data to define the event rate. And another limitation was related to previous medication analyses including antiplatelets which might have an effect on coronary flow.

CONCLUSION

This study shows that COVID-free centers may be useful to prevent hospital acquired infections in vulnerable patient groups with cardiovascular diseases. However, delayed SDT in STEMI patients during the pandemic was also relevant for our COVID-free tertiary cardiology center where the risk of contagion was very low. Correlated with this pre-hospital delay, cTFC was longer which was found to be an independent predictor of in-hospital events. Effect of COVID-19 pandemic on human life has gone beyond the disease's morbidity or mortality potential. The fear was sometimes more devastating than the danger itself. In such occasions, more effort and organizational arrangements may be required to ensure that informing does not turn into intimidation which may negatively affect cardiovascular disease management. Further studies are needed to demonstrate the necessity of COVID-free centers to reduce the secondary unfavorable effects of this and other disasters on cardiovascular morbidity and mortality.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of İstanbul Üniversitesi Cerrahpaşa Faculty of Medicine Clinical Researches Ethics Committee (Date: 18.03.2021, Decision No: 55570).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

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

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article / Orijinal Araştırma

Subthalamic Nucleus Degeneration As A Dark Cause of Parkinson's Disease After Subarachnoid Hemorrhage: A Preliminary Experimental Study

Subaraknoid Kanamaya Bağlı Parkinson Hastalığının Karanlık Bir Nedeni Olarak Subtalamik Çekirdek Dejenerasyonu: Deneysel Bir Ön Çalışma

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Abstract

Aim: Although the subtahalamic nucleus degeneration has been accused of Parkinson's disease, the obscure roles of subtalamic nucleus degeneration induced by subarachnoid hemorrhages has not been adequately studied. The aim of the study is to examine the histopathological changes in the subthalamic nucleus after subarachnoid hemorrhage.

Material and Method: Twenty-one wild male healthy rabbits were included in this study. The test subjects were divided as: control (GI, n=5); SHAM 1.2 cc of saline injected (GII, n=6) and 0.75 cc of autologous blood injection into cisterna magna (GIII, n=10). They followed up for three weeks and sacrificed under general anesthesia. Vasospasm index (VSI) was estimated by the circle surface estimation method, degenerated neuron densities of the subthalamic nucleus were estimated by Stereological methods and analyzed by Mann Witney U test.

Results: Two rabbits dead in the study group were represented by meningeal irritation signs and unconsciousness. Prolonged QT intervals, ST depressions, and low voltage QRSs were noticed in GIII animals. Numerical documents of heart-respiratory rates (n/min), VSI values, and degenerated neuron densities of the subthalamic nucleus (n/mm3) as follows: 1.05±0.03/ 219±324/21±4/8±3 in GI; 1.75±0.23/209±14/15±4/16±4 in GII; and 2.03±0.14/175±19/19±5/123±21 GIII. P values between the VSI values and degenerated neuron densities of the subthalamic nucleus were nearly eqund: p<0.005 in GI/GII; p<0.0005 in GII/GIII and p<0.00001 in GI/GIII.

Conclusion: Subarachnoid hemorrhage causes spasm of the arteries supplying the subthalamic nucleus, leading to ischemic injury, and hydrocephalus leading to mechanical stress injury.

Keywords: Subarachnoid hemorrhage, Subthalamic nucleus, Parkinson's disease, Neuronal degeneration

Öz

Amaç: Subtalamik çekirdek dejenerasyonu Parkinson hastalığı ile suçlanmış olsa da, subaraknoid kanamaların neden olduğu subtalamik çekirdek dejenerasyonunun belirsiz rolleri yeterince çalışılmamıştır. Bu çalışmanın amacı, subaraknoid kanama sonrası subtalamik çekirdekte meydana gelen histopatolojik değişiklikleri incelemektir.

Gereç ve Yöntem: Bu çalışmaya 21 adet yabani erkek sağlıklı tavşan dahil edildi. Denekler şu şekilde ayrıldı: kontrol (GI, n=5); SHAM 1.2 cc salin enjekte edildi (GII, n=6) ve sisterna magnaya 1.2 cc otolog kan enjeksiyonu (GIII, n=10). Üç hafta takip edildiler ve genel anestezi altında sakrifiye edildiler. Vazospazm indeksi (VSI) daire yüzey tahmin yöntemi ile, subtalamik çekirdeğin dejenere nöron yoğunlukları Stereolojik yöntemlerle tahmin edildi ve Mann Witney U testi ile analiz edildi.

Bulgular: Çalışma grubunda ölen iki tavşan meningeal iritasyon bulguları ve bilinç kaybı ile temsil edildi. GIII hayvanlarında uzamış QT aralıkları, ST çöküntüleri ve düşük voltajlı QRS'ler fark edildi. Kalp-solunum hızlarının (n/dak), VSI değerlerinin ve subtalamik çekirdeğin dejenere nöron yoğunluklarının (n/mm3) sayısal belgeleri aşağıdaki gibidir: GI'de 1,05±0,03/ 219±324/21±4/8±3; GII'de 1,75±0,23/209±14/15±4/16±4; ve 2,03±0,14/175±19/19±5/123±21 GIII. Subtalamik çekirdeğin VSI değerleri ile dejenere nöron yoğunlukları arasındaki P değerleri hemen hemen eşitti: GI/GII'de p<0.005; GII/GIII'de p<0,0005 ve GI/GIII'de p<0,00001.

Sonuç: Subaraknoid kanama, subtalamik çekirdeği besleyen arterlerin spazmına neden olarak iskemik yaralanmaya, hidrosefali ise mekanik stres yaralanmasına neden olur.

Anahtar Kelimeler: Subaraknoid kanama, Subtalamik çekirdek, Parkinson hastalığı, Nöronal dejenerasyon

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INTRODUCTION

Recently, the tendency towards cerebral ischemic pathologies, which should be investigated in the etiology of Parkinson's disease, has been increasing. The obscure importance of cerebral ischemic injuries and subarachnoid hemorrhage (SAH) in the etiology of Alzheimer's and Parkinson's disease is increasingly being clarified.[1] It is now well known that the movement disorders seen in Parkinson's disease are caused by traumatic and spontaneous deep brain center hematomas.[2,3] From this top it is interesting that the subthalamic nucleus has not been adequately studied in subarachnoid hemorrhages. According to current knowledges, anosmia and ageusia is the earliest and most common symptom of Parkinson's disease.[4] As a matter of fact, pathologies that are the cause of the anosmia and ageusia like findings are now included in the list of causes.[5] Parkinson's disease is accompanied by neurodegeneration, neuronal loss, reactive gliosis and synuclein (Lewy bodies) accumulation in the substantia nigra. Dying neurons undergo phagocytosis by microglia or neuronophagia.[6] Blood brain barrier disruption is an important stimulator on the development of Parkinson disease.[7] Magnetic resonance images detected gray matter, hippocampus, amygdala and basal ganglia atrophy.[8] The subthalamic nucleus is anatomically and functionally connected with important regions of brain such as cerebral cortex, basal ganglia, brainstem, limbic system and also spinal cord.[9-11] For the functonal connectivity of subtahalamic nucleus, deep brain stimulation and focused ultrasound applications directed to that nucleus are becoming widely accepted as a therapeutic option in Parkinson's disease. Because they allows closed focal blood-brain barrier opening.[12] The blood flow of subthalamic nucleus can be constructed because of subarachnoid hemorrhage induced various mechanisms and obliged to ishemic insult.[13,14] The deep mechanisms of ischemic pathologies, which will be investigated more effectively in the future. Increasing research on the loss of olfactory taste signals as the cause, not the result, and understanding the deep mechanisms of ischemic pathologies will create profound revolutions in the etiology and treatment of Parkinson's disease.

MATERIAL AND METHOD

Ethical approval for this study was given by Atatürk University Faculty of Medicine, HAYDEK Ethics Committee (Date: 09/11/2022, Decision No: E-42190979-050.01.04-2200370519). This study was conducted on twenty-one, aged wild rabbits collected from mountains nourished in a natural farmers. Vital signs, body measurements were recorded. The test subjects were divided into three groups as: control group (GI, n=5); SHAM group 0.75 cc of saline injected (n=6) and study (GIII, n=10) object to SAH with autologous 0.75 cc blood injected in tapering doses into their cisterna magna [15]. After 6 hours of non feeding period before surgery and sacrified after general anesthesia with isoflurane by a face mask, 0.2 mL/kg; KetamineHCL, 150 mg/1.5 mL; Xylazine HCL, 30 mg/1.5 mL; and distilled water, 1 mL. Craniectomy performed and cerebral tissues were resected just after intracardiac formaline injection and then fixed in 10% of formaline solution. Microsections of caudate nucleus were taken as parallel with axial plane to observe neuronal numbers. Twenty sections (5µm) of subthalamic nucleus examined to estimate degenerated neurons with stereological methods. The specimens were stained with hematoxylene-eozine (H&E) and glial fibrillary acidic protein (GFAP). Stereological methods were performed as described in our previous manuscripts.

All values are expressed as the mean±SD. The differences between the degenerated neuron densities of subthalamic nucleus each groups were compared statistically. A one-way analysis of variance (ANOVA) followed by Boneferroni's Post Hoc Test was used to determine significant differences between the groups for Differences were considered to be significant at $p< 0.05$.

Clinical Results

Two of the test subjects died in study group with a clinic of by meningeal irritation signs and unconsciousness. Prolonged QT intervals, ST depressions, and low voltage QRSs were noticed in GIII animals. Numerical documents of heart-respiratory rates (n/min), degenerated neuron densities of subthalamic nucleus (n/mm3) as follows: 219±324/21±4/8±3 in GI; 209±14/15±4/16±4 in GII; and 175±19/19±5/123±21 GIII. P values: $p<0.005$ in GI/GII; $p<0.0005$ in GII/GIII and $p<0.00001$ in GI/GIII.

Histopathological Results

Histologically, cellular angulation, nuclear shrinkage, cytoplasmic condensation and cellular darkening were accepted as criteria for neuronal degeneration. Severe vasospasm has been studied in ethers feeding the subthalamic nucleus, and subthalamic nucleus destruction has also been associated with spasm developing in this vessels arc. Blood brain barrier destruction fndings such as capillary vasospasm, endothelial injury, astrocytic foot fragmentation, narrowed perivasculary spaces and perivasculary inflammation was moderately in SHAM animals and seriously study animals (**Figure 1-3**).

Numerical Results

Three rabbits dead in study group represented by meningeal irritation signs and unconsciousness. Numerical documents about degenerated neuron densities of subthalamic nucleus (n/mm3) as follows: 8±3 in GI; 18±4 in GII; and 123±21 GIII.

Statistical Results

P values: $p<0.005$ in GI/GII; $p<0.0005$ in GII/GIII and $p<0.00001$ in GI/GIII.

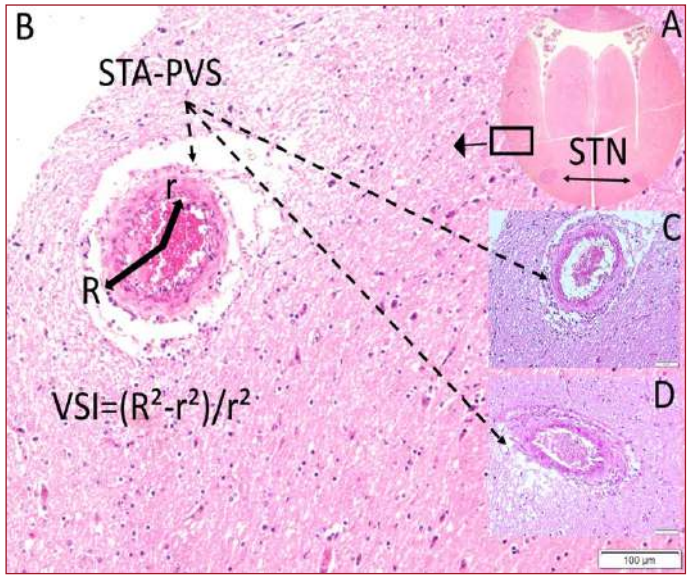

Figure 1: In a normal subject, subthalamic nucleus (STN) and the arterioles (STA) location (Square) feeding the subthalamic nucleus and the perivascular space (PVS) filled with cerebrospinal fluid are observed around it (A,B). A subject (C) belonging to the SHAM group has a moderately contracted arteriole with few blood elements and pial adhesions in its perivascular area. In a subject (D) belonging to the study group, an arteriole is significantly contracted and has many blood elements in its perivascular area and adhesions with pial thickening. At the same time, sponging and fluid increase in the brain parenchyma around the arteriole are clearly observed. Also, vasospasm index calculation method (VSI) is seen in figure B (LM, H&E; x4/A; x10/B-D).

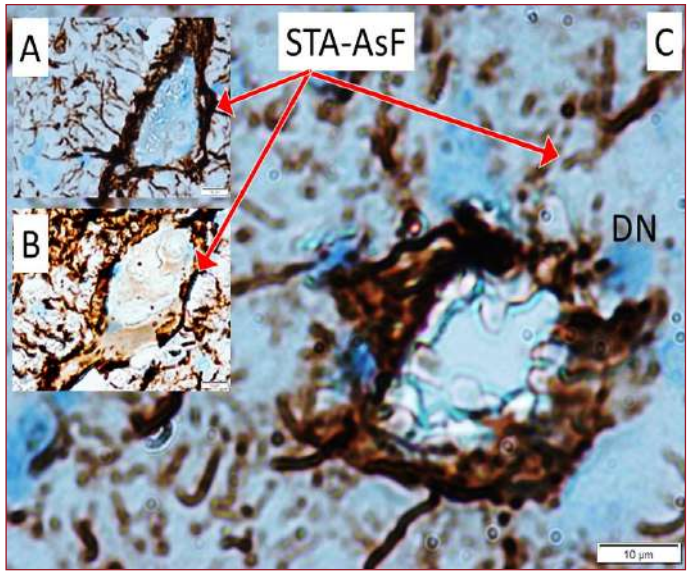

Figure 2: In a normal subject, arteriole (STA) feeding the subthalamic nucleus, astrocyte and astrocyte footplates (AsF) concentrated around the artery and perivascular space filled with cerebrospinal fluid are observed around it (A). In a subject (B) belonging to the SHAM group, a small number of astrocytes with reduced density around the artery and a narrowed perivascular space are observed alongside partially fragmented astrocyte astrocyte footpads (AsF). In a study subject (C), an arteriole is significantly contracted and has many blood elements and pial thickening and adhesions in its perivascular area. At the same time, degenerated neurons (DN) are clearly observed in addition to sponging and fluid increase in the brain parenchyma around the arteriole (LM, GFAP, x100/A-C).

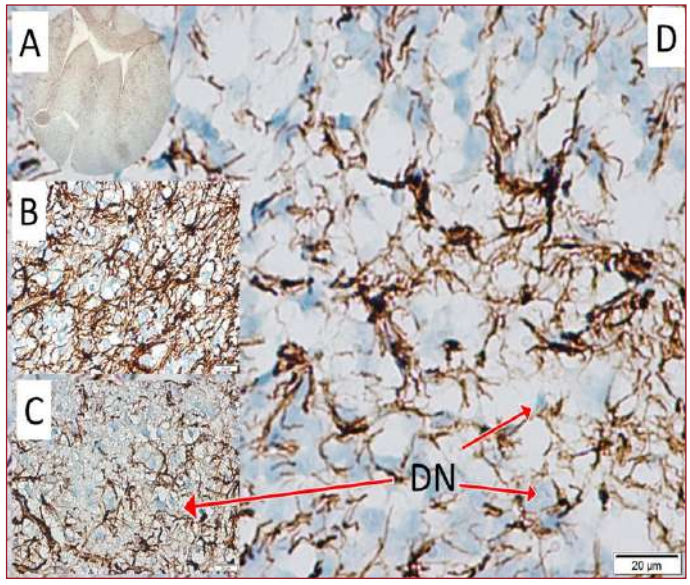

Figure 3: Localization of the subthalamic nucleus (STN) in a normal subject (A); Normal light blue neurons contained in the subthalamic nucleus and a large number of astrocytes with abundant pedicles surrounding them are observed (B). In a subject belonging to the SHAM group, a large number of slightly dark colored neurons and slightly dark blue neurons in slightly deformed condition and partially reduced branch and number astrocytes (C). In a subject belonging to the study group, in addition to the dark blue colored neurons (DN), which are considerably reduced and deformed, astrocytes with significantly reduced number and branches are observed.

DISCUSSION

The safest abstract findings such as anosmia and ageusia are considered as the most common and frequent nonmotor feature of Parkinson's disease.[4] However, the pathologies that are the cause of these findings are now on the list of causes.[5] As new studies on the etiology of Parkinson's disease imply that olfactory and taste disorders may be the initiating cause, not the finding; It will also reveal the responsibility of asymptomatic microischemic pathologies that disrupt the blood brain barrier. Although cerebral ischemic pathologies have not been taken into account sufficiently in the etiology of Parkinson's disease, recent studies have begun to focus on these pathologies. It has recently become clear that cerebral ischemic insults and subarachnoid hemorrhage should be considered as important etiological factors in the etiology of Alzheimer's and Parkinson's disease. It is well known that traumatic or spontaneous deep brain centers hematomas or ischemia can cause extrapyramidal sypmtoms.[1-3] It is very interesting that the subthalamic nucleus has not been adequately studied in subarachnoid hemorrhages. Anosmia and ageusia have been recognized as the earliest, most common, and most frequent non-motor symptoms of Parkinson's disease, and all theories are based on this idea.[4] However, it is observed that the views in the form of fault lines, which form the basis of these theories, are beginning to be shaken. Indeed, the pathologies that are the cause

of the findings are now included in the list of causes.[5] In the future, ischemic pathologies of the basal ganglia, which will be explained more effectively, will also take their deserved place in the etiology of Parkinson's disease. Indeed, disruption of the blood-brain barrier in the basal ganglia is an important initiating cause, which strengthens this theory.

Parkinson's disease is accompanied by neurodegeneration, neuronal loss, reactive gliosis and synuclein (Lewy bodies) accumulation in the substantia nigra. Dying neurons undergo phagocytosis by microglia or neuronophagia.[6] Blood brain barrier disruption is an important stimulator on the development of Parkinson disease.[7] Magnetic resonance images detected gray matter, hippocampus, amygdala and basal ganglia atrophy.[8] The subthalamic nucleus is anatomically and functionally connected with important regions of brain such as cerebral cortex, basal ganglia, brainstem, limbic system and also spinal cord.[9-11] For the functonal cönnectivity of subtahalamic nucleus, deep brain stimulation and focused ultrasound applications directed to that nucleus are becoming widely accepted as a therapeutic option in Parkinson's disease. Because they allows closed focal blood-brain barrier opening.[12]

The blood flow of subthalamic nucleus can be constructed because of subarachnoid hemorrhage induced various mechanisms and obliged to ishemic insult.[13,14] Because we think that the subthalamic nucleus is exposed to neurodegeneration in such events and loses its ability to work like the battery of the brain. Indeed, in a neurophysical sense, deep brain stimulation is actually nothing more than an electrical charge of the subthalamic nucleus. In this study, we discuss whether the subthalamic nucleus is exposed to any neurodegeneration in subarachnoid hemorrhages, which are important causes of movement disorders.

As well as considering the inability to smell and taste in the etiology of Parkinson's disease as a symptom rather than a cause; it is also very surprising that the ischemic damage of the basal ganglia, which we think has a serious role in the etiology, has not been adequately examined. As a team, we decided to illuminate this darkness as much as we could, and one of our first articles is this study. Although cerebral ischemic pathologies have not been taken into account sufficiently in the etiology of Parkinson's disease, recent studies have begun to focus on these pathologies. It has recently become clear that cerebral ischemic insults and subarachnoid hemorrhage should be considered as important etiological factors in the etiology of Alzheimer's and Parkinson's disease.[1] It is well known that traumatic or spontaneous deep brain centers hematomas or ischemia can cause extrapyramidal sypmtoms.[2,3] Parkinson's disease is accompanied by neurodegeneration, neuronal loss, reactive gliosis and synuclein (Lewy bodies) accumulation occurs in the substantia nigra. Dying neurons undergo phagocytosis by microglia or neuronophagia.[6] Blood barrier disruption is an important stimulator on the development of Parkinson disease.[7] Blood brain barrier dysruptions vascular inflammation are observed in the basal ganglia in Parkinson's disease.[16,17] Magnetic resonance images detected atrophy in gray matter, amygdala, hippocampus and basal ganglia.[8] Microscopic iron crystals which 3 to 8 nm in diameter can be found in degenerated brain regions. These electromagnetic field generator DADA-Black Holes, described for the first time in the literature, can delete neuronal information that has not been mentioned in the literature.[18] In this study, it was observed that subarachnoid hemorrhage caused both neuronal and glial cell loss in the subthalamic nucleus which hasnot been adequately investigated so far.

Iron mapping shows blood brain barrier dysruption in the basal ganglia in Parkinson's disease.[19] With age, iron accumulation in the basal ganglia increases and may contribute to the pathology of neurodegenerative diseases. Deterioration of the blood-brain barrier leads to iron accumulation, and an increase in iron in the basal ganglia increases the damage of the blood-brain barrier.[20]

Great goal in Parkinson's disease slowing down neurodegeneration. The goal of thermal lesions with focused ultrasound is to renormalize dopamine-driven basal ganglia abnormalities and temporarily open the blood-brain barrier.[21] The subthalamic nucleus is anatomically and functionally connected with some important regions such as zona incerta, brainstem reticular formation, cerebral cortex, substantia nigra, globus pallidus, amygdala, habenular nucleus, tegmental regions, limbic system, preoptic and periventricular area, stria terminalis, arcuate nucleus, mammillary nucleus, central gray, raphe and parabrachial nucleus, solitary tract nucleus cuneiform nucleus and nucleus locus ceruleus.[9-11] Focused ultrasound applications in low-intensity modality and neuromodulation, is becoming widely accepted as a therapeutic option, allows closed focal blood-brain barrier opening.[12] Buy, the peri-lead edema after bilateral deep brain stimulation is the result of severe microtrauma with blood-brain barrier disruption is an unwanted complication of deep brain stimulation.[22] On the other hand; mostly levodapa is used in the treatment of Parkinson's disease on behalf of dopamine because dopamine cannot cross the blood-brain barrier.[23] In patients using levodopa, the drug's lack of facility over time and the progression of the disease may be due to the fact that levodopa disrupts the blood-brain barrier. Because, it has been suggested that levodopa induced dyskinesia is associated with a disrupted blood-brain barrier.[24]

What Our Study Suggests About the Etiology of Parkinson's Disease

It is now certain that cerebral icemias disrupt the blood-brain barrier.[7] If so; disruption of the blood-brain barrier as well as the collapse of the intelligence, military and civil defense systems of the brain; it means that it has become vulnerable to internal and external attacks. The blood flow

of subthalamic nucleus is maintained by the perforforant branches of middle cerebral artery, posterior cerebral artery and anterior cerebral arteries in rats in order from most to least.[13,25,26] In this case, subthalamic nucleus ischemia due to subarachnoid hemorrhages also disrupts the blood-brain barrier in these structures. Because we have determined by immunohistochemical methods that structures similar to the blood-brain region in the subthalamic nuclei of the stones are damaged after subarachnoid hemorrhage. Subthalamic nucleus degeneration may be the source of psychological, psychiatric, mental disability, speech and comprehension disorders, as well as severe mental destructions and movement disorders that will soon be enlightened.

Limitations

This study does not include clinical data.

CONCLUSION

When we think of the subthalamic nucleus in terms of neurophysics, we conclude that this nucleus is an accumulator that specifically charges the basal ganglia. Because stimulating this core with a battery is actually a process to increase its weakened electrical potential. When we think of the subthalamic nucleus in terms of neurophysics, we conclude that this nucleus is an accumulator that specifically charges the basal ganglia. Because stimulating this core with a battery is actually a process to increase its weakened electrical potential. However, the fact that this procedure destroys the subthalamic nucleus over time weakens the popularity of the procedure.

Future Insights

By making more digital approaches to the software and hardware of the subthalamic nucleus in the initiation, continuation and termination of movements, more effective, easier and cheaper treatment methods will be developed with wirelwees signals.

ETHICAL DECLARATIONS

Ethics Committee Approval: Ethical approval for this study was given by Atatürk University Faculty of Medicine, HAYDEK Ethics Committee (Date: 09/11/2022, Decision No: E-42190979-050.01.04-2200370519).

Informed Consent: All participants signed the free and informed consent form.

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Original Article / Orijinal Araştırma

An Analysis of Thyroid Fine Needle Aspiration Biopsy Results According to the Bethesda System for Reporting Thyroid Cytopathology: A Cross-Sectional Retrospective Study

Tiroid İnce İğne Aspirasyon Biyopsi Sonuçlarının Tiroid Sitopatolojisini Raporlamak İçin Bethesda Sistemine Göre Analizi: Kesitsel Retrospektif Bir Çalışma

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Abstract

Aim: Thyroid fine needle aspiration biopsy (TFNAB) is an effective and convenient evaluation method. It is the gold standard in preoperative evaluation. The Bethesda System for Reporting Thyroid Cytopathology is a standardized evaluation system that has been widely used all over the world. In this study, we aimed to investigate the compatibility of the TFNAB results, which we evaluated according to the Bethesda System for Reporting Thyroid Cytopathology.

Material and Method: The data of 333(236 female, 97 male) patients, who underwent TFNAB between January 2020 and January 2022, were collected retrospectively. Their Bethesda categories, ages, and gender characteristics were recorded.

Results: The mean age for the 333 patients included in the study was 46.46±14.53 for the female patients and 50.19±10.06 for the male patients. When all patients were examined, B1 and B2 were the most common cytologies. While benign cytology was observed at an earlier age, the suspicion of malignancy or the incidence of malignant cytology increased as the mean age of the patients increased.

Conclusion: The Bethesda System for Reporting Thyroid Cytopathology is a widely accepted and reliable benchmark around the world in the evaluation phase of the cytopathological examination. In this study, we examined the cytopathological evaluation results of the patients who underwent thyroid FNAB at our center according to the Bethesda System for Reporting Thyroid Cytopathology. In terms of diagnostic cytology, we recommend a USG-guided biopsy performed by an experienced clinician.

Keywords: Thyroid fine needle aspiration biopsy, the Bethesda system, thyroid cytopathology

Öz

Amaç: Tiroidin ince iğne aspirasyon biyopsisi etklili ve kullanışl bir değerlendirme yöntemidir. Preoperatif değerlendirmede altın standarttır. Tiroid sitopatolojisini raporlamak için Bethesda Sistemi, tüm dünyada yaygın olarak kullanılan standartlaştırılmış bir değerlendirme sistemidir. Bu çalışmada Bethesda Sistemi' ne göre değerlendirdiğimiz ince iğne aspirasyon biyopsisi sonuçlarının uyumluluğunu araştırmayı amaçladık.

Gereç ve Yöntem: Ocak 2020-Ocak 2022 tarihleri arasında ince iğne aspirasyon biyopsisi uygulanan 333 (236 kadın, 97 erkek) hastanın verileri geriye dönük olarak toplandı. Bethesda kategorileri, yaşları ve cinsiyet özellikleri kaydedildi.

Bulgular: Çalışmaya alınan 333 hastanın yaş ortalaması kadın hastalarda 46,46±14,53, erkekhastalarda 50,19±10,06 idi. Tüm hastalar incelendiğinde Non diagnostic (B1), Benign (B2) en sık görülen sitolojilerdi. Benign sitoloji daha erken yaşlarda görülürken hastaların yaş ortalaması arttıkça malignite şüphesi veya malign sitoloji görülme sıklığı artmaktaydı.

Sonuç: Tiroid sitolojisini raporlamak için Bethesda Sistemi sitopatolojik incelemenin değerlendirme aşamasında dünya çapında yaygın olarak kabul gören güvenilir bir kriterdir. Bu çalışmada merkezimizde ince iğne aspirasyon biyopsisi uygulanan hastaların sitopatolojik değerlendirme sonuçlarını Bethesda Sistemi'göre inceledik. Tanısal sitoloji açısından deneyimli bir klinisyen tarafından alınan ultrasonografi eşliğinde biyopsi yapılmasını öneriyoruz.

Anahtar Kelimeler: Tiroid ince iğne aspirasyon biyopsisi, Bethesda sistemi, tiroid sitopatolojisi

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INTRODUCTION

The thyroid nodule is a common health problem today, and its frequency in adults has been reported to be 4–7% on average in cases detected through palpation and 67% in ultrasound scans.[1] Thyroid fine needle aspiration biopsy (TFNAB) is an effective and convenient evaluation method frequently used in the follow-up and evaluation of thyroid nodules due to its ease of application, low cost, and low number of complications.[2] It is the gold standard in preoperative evaluation.[3]

The Bethesda System for Reporting Thyroid Cytopathology is a standardized evaluation system that has been widely used all over the world since 2009 in the evaluation of TFNAB, and it has enabled a correlation between clinicians and pathologists.[4] The Bethesda System for Reporting Thyroid Cytopathology consists of 6 diagnostic categories, which are non-diagnostic/unsatisfactory (ND), benign cytology (BC), atypia of undetermined significance/follicular lesion (FLUS), follicular neoplasm or suspicious for follicular neoplasm (FN), cytology suspicious for malignancy (SFN), and malignant cytology (MC).[4]

In this study, we aimed to investigate the compatibility of the TFNAB results, which we evaluated according to the Bethesda System for Reporting Thyroid Cytopathology.

MATERIAL AND METHOD

Approval of the ethics committee was obtained from Mardin Artuklu University prior to the study (Date: 08/03/2022 - No:2022-6). The data of 333 patients, who underwent TFNAB between January 2020 and January 2022, were collected retrospectively. Their Bethesda categories, ages, and gender characteristics were recorded. Biopsy procedures on the patients were performed by a radiologist or endocrinologist with ultrasonography (USG) or by a general surgeon or an ENT specialist without USG. An evaluation of their biopsy procedures had been reported by the relevant department in the same hospital. Their biopsy results were classified according to Bethesda categories. The categories were as follows:

1. Nondiagnostic (B1),
2. Benign (B2),
3. Atypia of undetermined significance or follicular lesion of undetermined significance (B3),
4. Follicular neoplasm or suspicious for follicular neoplasm (B4),
5. Suspicious for malignancy (B5),
6. Malignant (B6).

Statistical Analysis

The IBM SPSS 21.0 for Windows statistical package software was used in the statistical evaluation of our research data. Measured variables were presented as mean±standard deviation and median value, and categorical variables were presented as numbers and percentages (%). The data were checked in terms of conforming to the normal distribution. Kruskal Wallis H Test was used to compare non-normally distributed groupings with multiple options. A Bonferroni correction was performed for post-hoc analysis. The Mann-Whitney U Test was used to compare the groupings with two options. Intergroup comparison of qualitative variables was performed using the Chi-square test. The hypotheses were bidirectional, and the results were considered statistically significant at $p<0.05$.

RESULTS

The mean age for the 333 patients (236 female, 97 male) included in the study was 46.46±14.53 for the female patients and 50.19±10.06 for the male patients. The B2, B1, and B3 were the most common cytologies among females, respectively. Among the male patients, the B1, B5, B2 and B3 were the most common cytology, respectively, and the B2 and B3 were observed in equal proportions (**Table 1**).

Table 1: Comparison of diagnosis groups in terms of gender variable according to Bethesda cytopathology classification

	Female (n)(%)	Male (n)(%)	Total	p
B1	72 (52.2%)	66 (47.8%)	138	
B2	88 (90.7%)	9 (9.3%)	97	
B3	45 (83.3%)	9 (16.7%)	54	
B4	12 (100%)	0 (0%)	12	<0.001
B5	13 (56.5%)	10 (43.5%)	23	
B6	6 (66.7%)	3 (33.3%)	9	
Total	236 (70.9%)	97 (29.1%)	333	

Nondiagnostic (B1), benign (B2), atypia of undetermined significance or follicular lesion of undetermined significance (B3), follicular neoplasm or suspicious for follicular neoplasm (B4), suspicious for malignancy (B5), malignant (B6) p: significance value of the Chi-Square test

When all patients were examined, B1, B2 and B3 were the most common cytologies (**Table 2**).

Table 2: Comparison of patient numbers according to Bethesda cytopathology classification

	B1	B2	B3	B4	B5	B6	P
N (%)	138 (41.4%)	97 (29.1%)	54 (16.2%)	12 (3.6%)	23 (6.9%)	9 (2.7%)	<0.001

N: Number, Nondiagnostic (B1), Benign (B2), Atypia of undetermined significance or follicular lesion of undetermined significance (B3), follicular neoplasm or suspicious for follicular neoplasm (B4), Suspicious for malignancy (B5), Malignant (B6) p: significance value of the Chi-square test

While benign cytology was observed at an earlier age, the suspicion of malignancy or the incidence of malignant cytology increased as the mean age of the patients increased (**Table 3**).

Table 3: Comparison of diagnosis groups in terms of age variable according to Bethesda cytopathology classification

	B1	B2	B3	B4	B5	B6	P
Age (median) (years)	47.00	39.00	55.50	45.00	71.00	56.00	<0.001 PB2-B3<0.001 PB2-B5<0.001

Nondiagnostic (B1), Benign (B2), Atypia of undetermined significance or follicular lesion of undetermined significance (B3), follicular neoplasm or suspicious for follicular neoplasm (B4), Suspicious for malignancy (B5), Malignant (B6) p: significance value of the Kruskal-Wallis test

DISCUSSION

In the evaluation of thyroid fine needle aspiration biopsy, The Bethesda System for Reporting Thyroid Cytopathology is the most appropriate cytopathology evaluation system of FNAB in terms of evaluating thyroid malignancies.[5] According to the American Thyroid Academy (ATA), FNAB should be performed on nodules of 1 cm or greater in highly or moderately suspicious lesions, 1.5 cm or more in low suspicions, and 2 cm or more in very low suspicions, while FNAB should not be recommended for nodules smaller than 1 cm.[6] ATA recommends repeating the USG-guided biopsy in the nondiagnostic group and making a follow-up or surgical decision in case a diagnosis cannot be made. In addition, according to the ATA, benign FNAB does not require intervention in terms of diagnosis or treatment, and nodules with benign cytology twice may not be followed up with USG.[6] In this study, the total frequency of B3, B4, and B5 cytology was evaluated at 20–25%, and B5 was the least common cytological diagnosis.

According to the Bethesda cytological reporting system, B1 is between 2-20%, while the ideal is below 10%.[7] In the same study, while the rate of benign cytology was 60-70%, the rate of malignant cytology was 0-3% in all cases.[7] It has been reported that nondiagnostic diagnosis rates decrease by 50% when thyroid FNAB is performed with an accurate technique and accompanied by USG.[6] It is more difficult to obtain biopsy material with a high diagnostic value from mixed and cystic nodules.[8] In their study evaluating the cytopathology results of the USG-guided FNAB for macro-calcified thyroid nodules, Koc et al. encountered B1 cytology at a rate of 19.2% and explained the reason as the inability to aspirate sufficient cells from the calcified area.[9] In other studies, it was emphasized that the rate of B1 cytology varied between 55-74% of the cytology examinations, while the rate of B4-B5 cytology varied between 2-5%. In our study, B1 cytopathology was the most common (41.4%), followed by B2 as the most common diagnostic cytology. We believe the frequency rate of nondiagnostic diagnosis was higher compared to the literature due to the fact that not all samples were obtained with USG in our hospital.

In their study investigating the Bethesda categories of 366 patients who underwent thyroidectomy, Ocak et al. reported category 1 in 6.3% of the patients, category 2 in 21.6% of the patients, category 3 in 35% of the patients, category 4 in 30.9% of the patients, category 5 in 3.8% of the patients, and category 6 in 2.5% of the patients.[12] In this study, the researchers evaluated the Bethesda category of patients who underwent surgery, and the most common diagnoses were Bethesda 3 and 4. In our study, B2 (29.1%) and B3 (16.2%) diagnostic cytology were the most common diagnostic diagnoses. In our study, the frequency of other cytopathological diagnoses was 3.6% for B4 cytology, 6.9% for B5 cytology, and 2.7% for B6 cytology.

Many researchers have stated that thyroid lesions are associated with age and gender.[13,14] Sinna et al. reported in their study that thyroid nodules are more common in women and the frequency of malignant nodules increases with age of over 45 years.[13] In our study, there was a female predominance and the female-to-male ratio was 2.43. Additionally, in our study the suspicion of malignancy or the incidence of malignant cytology increased as the mean age of the patients increased.

CONCLUSION

Thyroid FNAB is an easy-to-apply and cost-effective method with few complications and is a common method used in the follow-up of thyroid nodules and before surgery. The Bethesda System for Reporting Thyroid Cytopathology is a widely accepted and reliable benchmark around the world in the evaluation phase of the cytopathological examination. In this study, we examined the cytopathological evaluation results of the patients who underwent thyroid FNAB at our center according to the Bethesda System for Reporting Thyroid Cytopathology. In terms of diagnostic cytology, we recommend a USG-guided biopsy performed by an experienced clinician.

ETHICAL DECLARATIONS

Ethics Committee Approval: Approval of the ethics committee was obtained from Mardin Artuklu University prior to the study (Date: 08/03/2022 - No:2022-6).

Informed Consent: All participants signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article / Orijinal Araştırma

Investigation of the Relationship Between Chronic Use of Topical Antiglaucomatous Drops and Ocular Demodex Infestation

Topikal Antiglokomatöz Damlaların Kronik Kullanımı İle Oküler Demodeks Enfestasyonu Arasındaki İlişkinin Araştırılması

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Abstract

Aim : To investigate the relationship between chronic use of topical antiglaucomatous drop and ocular Demodex infestation.

Material and Method: This cross-sectional study included a total of 226 eyes, as both eyes of 55 patients with bilateral glaucoma and 58 control subjects. A total of 8 eyelashes, 2 each from the lower and upper eyelids of all the study participants, were taken and the samples were sent to the parasitology laboratory for analysis.

Results: The mean age of the study participants was 66.48±15.19 years in the glaucoma patients, and 64.76±10.89 years in the control group. From analysis of the eyelash samples taken, ocular Demodex was positive in 55% of patients with glaucoma, and negative in 45%. In the control group, Demodex was positive in 34% and negative in 66%. Demodex infestation positivity was determined to be statistically significantly higher in patients with glaucoma (p= 0.042).

Conclusion: The rate of ocular Demodex infestation increases with age, and it is estimated that this rate is further increased by the use of chronic topical antiglaucomatous drops.

Keywords: Antiglaucomatous drops, demodex folliculorum, glaucoma, ocular demodex

Öz

Amaç: Topikal antiglokomatöz damlaların kronik kullanımı ile oküler Demodeks infestasyonu arasındaki ilişkiyi araştırmak.

Gereç ve Yöntem: Bu kesitsel çalışmaya 55 bilateral glokomlu hasta ve 58 kontrol olgusunun her iki gözü olmak üzere toplam 226 göz dahil edildi. Tüm katılımcılardan alt ve üst göz kapaklarından 2'şer adet olmak üzere toplam 8 adet kirpik alındı ve örnekler analiz için parazitoloji laboratuvarına gönderildi.

Bulgular: Çalışmaya katılanların yaş ortalaması glokom hastalarında 66,48±15,19, kontrol grubunda 64,76±10,89 idi. Alınan kirpik örneklerinin analizinden, oküler Demodeks glokomlu hastaların %55'inde pozitif ve %45'inde negatifti. Kontrol grubunda Demodeks %34 pozitif, %66 negatifti. Demodeks enfestasyon pozitifliği glokomlu hastalarda istatistiksel olarak anlamlı derecede yüksek saptandı (p= 0,042).

Sonuç: Oküler Demodeks infestasyon oranı yaşla birlikte artmaktadır ve kronik topikal antiglokomatöz damla kullanımı ile bu oranın daha da arttığı tahmin edilmektedir.

Anahtar Kelimeler: Antiglokomatöz damlalar, demodeks follikülorum, glokom, oküler demodeks

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INTRODUCTION

Glaucoma, a neurodegenerative disease, is the most common cause of irreversible, preventable blindness worldwide, although it usually progresses slowly.[1] The main treatment principle of the disease is to lower the intraocular pressure (IOP).[1,2] Even though lowering IOP is mostly achieved with antiglaucomatous drops (AGDs), laser, or surgical methods[1] the treatment becomes difficult due to the side effects of the AGDs used including redness, itching, burning, stinging, epiphora, and iris discoloration.[3,4] Ocular infections such as blepharitis and conjunctivitis may occur at any time during treatment with topical AGDs, especially those containing preservatives.[5-7]

The most common ectoparasite in humans is Demodex[8], two medically important species of which are Demodex folliculorum and Demodex.brevis, although the Demodex species found in different organisms can be seen incidentally. Demodex brevis is smaller and found deep in the sebaceous glands of the eyelids and the lobules of the meibomian glands. Demodex folliculorum is larger and typically found in clusters in the eyelash follicles.[8] Demodex lives in the anterior structures of the eye, including the eyelashes, eyelids, and ocular surface, causing ocular demodicosis. The mites were thought to be mostly innocuous, forming a normal component of the eyelid flora and causing allergic or immunological reactions only in rare cases. However, a number of ocular surface disorders, such as chronic blepharitis, ocular surface inflammation, and meibomian gland dysfunction, have been connected to Demodex infestation. Undiagnosed or neglected Demodex infestations may be among the causes of chronic blepharitis, conjunctivitis and chalazions.

To our knowledge, no research has been done in the literature examining the relationship between ocular Demodex, and chronic use of topical AGDs. The purpose of this study was to look into the relationship between Demodex and chronic use of topical AGDs.

MATERIAL AND METHOD

This cross-sectional, observational study analyze the prevalence of ocular Demodex infestation in patients with glaucoma was conducted between May 2022 and August 2022. The study included a control group of 58 individuals without glaucoma and 55 bilateral glaucoma patients who were being followed up in the Ophthalmology Department of Bolu Abant Izzet Baysal University Training and Research Hospital. Approval for the study was granted by the Local Ethics Committee (No: 2022/132). Patients signed informed consent forms, and all procedures followed the principles of the Helsinki Declaration.

All patients underwent detailed eye examinations. The control group comprised healthy subjects with the exception of refractive disorders within ±3.0 diopters. Patients using topical AGDs with preservatives for at least 2 years were included in the glaucoma group, and patients were excluded from the study if they were using topical drugs other than topical AGDs, had any known eye disease other than glaucoma, or any known systemic disease.

A total of eight eyelashes per person were taken from each study participant, two from each eyelid. Eye forceps were used to remove the eyelash samples, which were then fixed on slides with cellophane tape, and examined under a light microscope at x5 and x10 magnification in the laboratory of the Department of Medical Parasitology, Bolu Abant Izzet Baysal University Faculty of Medicine. Patients with Demodex infestation in their eyelashes were treated.

Statistical Analysis

Data obtained in the study were analyzed statistically using SPSS version 25.0 (SPSS Inc., Chicago, IL, USA) for Windows software. The Independent Samples t-test was used to evaluate parametric data, and the Chi-square test was used for non-parametric data. Data were calculated as mean ± standard deviation values, number and percentage. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Evaluation was made of a total of 226 eyes of 55 bilateral glaucoma patients and 58 control subjects. The glaucoma group comprised 29 males and 26 females with a mean age of 66.48±15.19 years, and the control group comprised 31 males and 27 females with a mean age of 64.76±10.899 years. No statistically significant difference was observed between the two groups in terms of gender and age ($p= 0.742$, and $p=0.097$ respectively). In the eyelash samples taken, ocular Demodex positivity (Demodex+) was determined in 30 (55%) and ocular Demodex negativity (Demodex-) in 25 (45%) of the glaucoma patients. In the control group, Demodex+ was determined in 20 (34%) patients and Demodex- in 38 (66%) patients. When the glaucoma and control groups were compared, the rate of Demodex+ was found to be statistically significantly higher in the glaucoma group ($p= 0.042$). In the glaucoma patients with Demodex+, involvement was bilateral in 14, in the right eye only in 10, and in the left eye only in 6. On the other hand, in the control group with Demodex+, involvement was bilateral in 5, in the right eye only in 7, and in the left eye only in 8. In the samples taken from the eyelashes of all the patients with Demodex+, D. Folliculorum was present in 38 eyes, D. Breves in 5 eyes, and both parasite species were found in 7 eyes.

DISCUSSION

In this cross-sectional study, the rate of ocular demodicosis was found to be higher in patients with glaucoma than in the control group without glaucoma (55 % and 34 %, respectively).

Antiglaucomatous drops still take the first place in the treatment of glaucoma, which is an insidious disease. In addition to the therapeutic properties of these drops, the frequent occurrence of side-effects, known as ocular surface disease (OSD), is a negative situation for the patient.[3,5] In previous studies, it has been observed that OSD disrupts the normal flora in the cornea and conjunctiva, changes the inflammatory and anti-inflammatory balance, and increases the risk of ocular infection.[6,7,9,10] Schwartz et al. stated that although most of the preservatives used in AGDs are bactericidal and fungicidal, when they are used for a long time bacterial resistance increases and the risk of bacterial and fungal infections increases.[10] Geyer et al. reported that eye infections due to contamination increased after the use of AGDs in patients with glaucoma.[6] Azari et al. also reported that toxic and infectious conjunctivitis is more common in patients using topical drugs.[7] Baudouin et al., however, found that glaucoma itself triggers inflammation in many areas of the eye, from the anterior segment to the posterior segment, and can disrupt the anti-inflammatory response.[11] The results of the current study demonstrated that the rate of ocular Demodex infestation was higher in patients with glaucoma compared to the control group. It was thought that the AGDs used disrupt the ocular flora, disrupt the structure of the ocular surface, damage ocular immunity, and thus, the associated infection rate is higher.

It is known that ocular demodicosis increases with age and may be associated with OSD and especially blepharitis.[8,9,12] Bonnar et al found ocular Demodex+ at a rate of 53 % preoperatively in 100 cataract patients.[13] However, most of those patients were asymptomatic. Many studies have shown that ocular Demodex is closely related to ocular infections (such as blepharitis, conjunctivitis, and keratitis).[8,12,14] The aforementioned studies have revealed that sometimes demodicosis causes OSD and ocular infection, and sometimes in contrast, these primary ocular disorders create a predisposition to demodicosis.[8,12,14] In other words, it is thought that both situations contain complex mechanisms that are intertwined.

From a literature search only one article was found which had examined the relationship between ocular demodicosis and glaucoma.[4] In that study by Polit et al., glaucoma patients using Prostaglandin analogue with preservatives and control subjects were compared. Interestingly, the prevalence of Demodex+ patients was found to be lower in the glaucoma group.[4] In this study, Demodex+ was reported 52% with latanoprost; 42% with travoprost; 7% with bimatoprost.[4] On the contrary, in our study, regardless of the type of AGDs, all patients with glaucoma were included in the study and the rate of demodicosis was found to be statistically significantly higher in the glaucoma group than in the control group. The mean age of the patients with glaucoma was higher than that of the patients in the current study (70.34±9.7 years, and 66.48±15.19 years, respectively).[4] Together with the factors mentioned above, many multifactorial causes, which are as yet unknown, may have affected the results of the study. However, according to the literature information obtained, the use of topical AGDs may increase the risk of many bacterial and fungal infections, including ocular Demodex, by disrupting the structure of the ocular surface and flora.[6,7,9-11]

One of the important drawbacks of this study was the limited number of patients included. More comprehensive results could have been obtained if the patients were classified separately according to the type of AGDs used and according to age groups. However, despite these limitations, this sttudy can be considered of value as statistically significant results were obtained, there were significant differences compared to other studies, and it is one of the very few studies on this subject.

CONCLUSION

It should be known that the rate of ocular Demodex increases with age, and this rate may increase with chronic use of AGDs with preservatives in patients with glaucoma. However, there is a need for more comprehensive studies including more patients and different age groups to clarify the subject.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of the Bolu Abant Izzet Baysal University Clinical Researches Ethics Committee (2022/132).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

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

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Original Article / Orijinal Araştırma

Assessing the Food Disgust Sensitivity and Its Association with Eating Behaviours in Adults

Yetişkinlerde Besin Tiksinme Duyarlılığının İncelenmesi ve Yeme Davranışları ile İlişkisinin Değerlendirilmesi

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Abstract

Aim: The aim of this study was to examine food disgust sensitivity and assess the relationship between food disgust sensitivity and eating behaviours in adults.

Material and Method: In this cross-sectional study, 215 adults were recruited and face-to-face interviews were used to gather data on the demographic information, Food Disgust Scale-short, Adult Picky Eating Questionnaire, and items involving rejection based on texture. Also, anthropometric measurements were taken.

Results: The mean food disgust sensitivity short, adult picky eating questionnaire and texture-based rejection scores of participants were found to be 3.549±0.745, 2.316±0.472, 1.190±0.782, respectively. Income and body mass index were negatively correlated with food disgust sensitivity, although age was positively. People with high food disgust sensitivity were pickier eaters and rejected foods with certain textures more often than those with low scores.

Conclusion: Individuals' food disgust sensitivity should be considered as an important factor influencing picky eating or food rejection.

Keywords: Food disgust; picky eating; texture-based food rejection

Öz

Amaç: Bu çalışmanın amacı yetişkin bireylerde besin tiksinme duyarlılığını incelemek ve besin tiksinme duyarlılığı ile yeme davranışları arasındaki ilişkiyi değerlendirmektir.

Gereç ve Yöntem: Bu kesitsel çalışmaya, 215 yetişkin birey katılmıştır ve demografik bilgiler, Besin Tiksinme Ölçeği-kısa, Yetişkin Seçici Yeme Anketi ve dokuya bağlı besin reddi ile ilgili bilgiler katılımcılardan yüz yüze ve anket teknikleri kullanılarak elde edilmiştir. Ayrıca bireylerin antropometrik ölçümleri alınmıştır.

Bulgular: Katılımcıların besin tiksinme ölçeği-kısa, seçici yeme anketi ve dokuya bağlı ret ortalama puanları sırasıyla 3,549±0,745, 2,316±0,472, 1,190±0,782 olarak bulunmuştur. Besin tiksinme duyarlığı ile gelir ve beden kütle indeksi arasında pozitif yönde ilişki bulunurken, yaş ile negatif yönde ilişki saptanmıştır. Besin tiksinme duyarlılığı fazla olan bireylerin daha fazla seçici yeme davranışı sergiledikleri ve bazı dokulara sahip besinleri daha fazla reddettikleri saptanmıştır.

Sonuç: Besin tiksinme duyarlılığı, bireylerin seçici yeme veya dokuya bağlı besin reddi davranışlarını etkileyen önemli bir faktör olarak değerlendirilmelidir.

Anahtar Kelimeler: Besin tiksinme; seçici yeme; dokuya bağlı besin reddi

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INTRODUCTION

Disgust is an emotion that avoids disease and is characterized by a predisposition to reject and avoid particular stimuli. [1] People are disgusted with a wide range of objects, including food animals and even their own bodies.[2-4] While some disgust-related stimuli differ from culture to culture and from individual to individual, bodily secretions (vomit, sweat, spittle, blood, and pus) are universally assumed to elicit disgust in people.[5] People with disgust sensitivity are predisposed to feel disgusted in response to certain stimuli, known as disgust elicitors.[6,7] The insular cortex of the brain is associated with disgust and its sensitivity may vary considerably between individuals, contributing to differences in sensitivity to disgust.[8]

The term food disgust refers to an emotion that prevents the consumption of substances that might be toxic or pathogenic. [9] Food disgust has functional effects on the eating behaviour of participants by preventing them from consuming potentially toxic foods. On the other hand, high disgust sensitivity may be related to rejection of food sources and a more restrictive eating behaviour.[10,11]

Picky eating is characterised as an aversion to a wide range of familiar or unfamiliar foods, resulting in limited dietary diversity. Picky eaters tend to be disgusted with frequent and multiple stimuli, which underlies their food rejection.[12] Furthermore, even non-spoiled food can cause a disgusting reaction.[13] Although certain foods are safe to eat, their texture characteristics, such as excessive viscosity, can cause disgust. [14] According to this line of reasoning, a significant amount of avoidance and rejection may be justified by the expected textural characteristics of meals when these are evaluated in the visual domain.[15]

To our knowledge, no research has examined the association between food disgust and eating behaviours among Turkish population. This study aimed to investigate food disgust sensitivity and assess the relationship between food disgust sensitivity and eating behaviours in adults.

MATERIAL AND METHOD

Study Design

This cross-sectional study comprised of 215 individuals between the ages of 19 and 65. Face-to-face interviews were used to gather data on sociodemographic characteristics, Food Disgust Scale-short (FDS-short), Adult Picky Eating Questionnaire (APEQ), texture-based rejection behaviours, and anthropometric measures.

Subjects

This participants of the study comprised a random sample of 215 individuals who reside in Gaziantep in Turkey. People with food allergies, chronic illnesses, or those who declined to participate were excluded from the research. Pregnant and breastfeeding women were also omitted from the study due to the fact that their dietary habits may alter during this period.

Participants who agreed to contribute voluntarily to this study were asked to sign a written consent form in accordance with the Declaration of Helsinki. This study was approved by the Ethics Board of Gaziantep Islam Science and Technology University (Date: 27.09.2022, Decision No: 2022/146).

Measurement Tools

Food Disgust Scale

In order to measure food disgust sensitivity, that is people's propensities for disgust toward particular food-related (offensive) stimuli, the food disgust scale's shortened form was used(9). The scale includes eight items that represent various food disgusts: animal flesh, poor hygiene, human contamination, decaying fruits, decaying vegetables, fish, mould, and living contaminants. Items were assessed on a 6-point scale ranging from 1 (not disgusting at all) to 6 (extremely disgusting). All values were averaged to determine the mean. The internal consistency was determined by calculating Cronbach's alpha. Regarding the FDS short scale, Cronbach's Alpha α coefficient was found to be 0.78 in this study. In this scale, a higher score indicates a greater tendency to respond with disgust and to be bothered by the experience of disgust.

Adult Picky Eating Questionnaire

In order to evaluate picky eating behaviours in adults, the Adult Picky Eating Questionnaire (APEQ) was used. [16] The APEQ is comprised of a total of sixteen items and four subscales (meal representation, food variety, meal disengagement, and taste aversion). Items in the scale were scored between 1 (never) and 5 (always). The mean scale score was calculated. Higher APEQ score indicates pickier eating behaviours. Its Turkish validity and reliability study was conducted by Ayyıldız et al.[17]

Texture-Based Rejection

The texture-based rejection items were developed based on those used by Kauer et al.[11] and were previously used by Egolf et al.[18] to assess texture-based rejection in adults. For example, one item asked if the respondent almost always rejects slimy foods. Other textures that were asked in the same fashion included crunchy, gelatinous, or very chewy. These items were scored with a yes (1) or a 'no' (0), and a total score was computed. Cronbach's Alpha coefficient was determined as 0.89 regarding the texture-based rejection.

Anthropometric measurements

The body weight was measured using electronic scale to the nearest 0.1 kilogram while wearing minimal clothing and without shoes. Using a stadiometer, height was measured while standing on the horizontal Frankfort plane. Body mass index (BMI) was computed by dividing weight (kg) by height squared (m^2).

Sociodemographic Characteristics

As sociodemographic characteristics, age, gender, married status, education level (literate, primary school, secondary

school, high school, university), and monthly income (below 3000, 3001–6000, 6001–9000, and over 9001 Turkish Liras) were investigated.

Statistical Analysis

The data was analysed using Statistical Package for the Social Sciences software (version 23.0, Chicago, United States). Visual and analytical methods were used to analyse the normality of data. For continuous and categorical variables, the characteristics of the participants were expressed as mean with standard deviation ($\bar{x}$±SD) or frequency with proportions, respectively. Pearson's correlation coefficient was used to examine bivariate correlations in order to determine whether there was a relationship between general characteristics, FDS-short, APEQ, and texture-based rejection scores. The predictive ability of the general characteristic, food disgust sensitivity, was investigated using a multiple linear regression analysis. Utilizing hierarchical linear regression models, the effects of disgust sensitivity on picky eating and texture-based rejection were analysed. In the first step, control variables (sex, age, and income, BMI) were entered, and in the second step, food disgust sensitivity was entered in all regression models to see how much additional variance there was in food disgust sensitivity. The value of $p<0.05$ was established as statistically significant.

RESULTS

The general characteristics and scale scores of participants are shown in **Table 1**. The study included 109 (50.7%) women participants and 106 (49.3%) men participants. The mean age of participants was 33.897±12.759 years. Almost half of the participants had a university degree (47.0%). The mean BMI of participants was 24.587±4.405 kg/m². The participants' mean FDS short, picky eating, and texture-based rejection scores were 3.149±0.745, 2.316±0.472, 1.190±0.782, respectively.

The results indicated that there was significant correlation between FDS short score and age, gender, income and BMI ($p<0.001$). The FDS short score was also shown to significant positive correlation with APEQ and texture based rejection scores (all $p<0.01$) (**Table 2**).

It was determined through a regression analysis whether food disgust could be predicted using data on age, gender, income, and BMI (**Table 3**). The regression model showed that 14.1 % of food sensitivity could be explained by age, sex, income, and BMI. It was found that there was a positive correlation between age and FDS-short scores which showed that older people had higher FDS-short scores than younger people. One of the predictors was gender and the FDS short scores of women were found to be higher than those men. Additionally, income and BMI were negatively correlated with the FDS short score.

Table 1. General characteristics and scale scores of participants

Variables	Sample (n=215)
Age(years) $\bar{x}$±SD	33.897±12.759
Gender n (%)	
Men	109 (50.7)
Women	106 (49.3)
Education Level n (%)	
Literate	37 (17.2)
Primary school	12 (5.6)
Secondary school	20 (9.3)
High school	45 (20.9)
University	101 (47.0)
Income (TL/monthly) n (%)	
3000 TL or less	27 (12.5)
3000-6000 TL	66 (30.7)
6001-9000 TL	69 (32.1)
9001 TL or more	53 (24.7)
Body weight (kg) $\bar{x}$±SD	68.952±14.165
BMI(kg/m²) $\bar{x}$± SD	24.587±4.405
FDS-short $\bar{x}$±SD	3.549±0.745
APEQ $\bar{x}$± SD	2.316±0.472
Texture based rejection $\bar{x}$± SD	1.190±0.782

$\bar{x}$: mean, SD: standard deviation, TL: Turkish liras, BMI: Body mass index, FDS-short: Food Disgust Scale- Short, APEQ: Adult picky eating questionnaire

Table 3. Multiple linear regression analyses of factors associated with food disgust sensitivity (FDS-short)

Variables	β1 (%95 CI)	SE	β2	t	p
(Constant)	3.991 (3.371 - 4.612)	0.315		12.675	<0.001
Age	0.014 (0.006 - 0.021)	0.004	0.237	3.664	<0.001
Gender	0.229 (0.038 - 0.420)	0.097	0.154	2.366	0.019
Income	-0.157 (-0.248 - -0.066)	0.046	-0.207	-3.403	0.001
BMI (kg/m²)	-0.041 (-0.061 - -0.021)	0.010	-0.242	-4.013	<0.001

BMI: Body mass index, Gender: 0=men, 1=women $F=17.929$; $p<0.001$; Adj. $R^2=0.141$; SE of the estimate=0.649; 1: Unstandardized Coefficients; 2: Unstandardized Coefficients

Table 2. The correlation between FDS-short score and variables investigated

	FDS short	Age	Gender	Education	Income	BMI	APEQ
Age	0.378**						
Gender	0.319**	0.372**					
Education	-0.079	-0.222**	-0.132				
Income	-0.301**	-0.176**	-0.152*	0.073			
BMI	-0.139*	-0.039	-0.122	0.045	0.030		
APEQ	0.468**	0.364**	0.343**	0.005	-0.086	-0.021	
Texture based rejection	0.383**	0.397**	0.301**	-0.158*	-0.048	-0.117	0.383**

FDS-short: Food Disgust Scale- Short, BMI: Body mass index, APEQ: Adult picky eating questionnaire, Gender: 0=men, 1=women, *p< 0.05, **p< 0.01

Table 4 displays the results of hierarchical regression. The model containing age, gender, income and BMI explained R^2=24.5%, 15.7%, respectively, the amount of variation in picky eating and texture-based rejection ($p<0.01$). When FDS short score was added as an independent variable, the model the model R^2 for picky eating (ΔR^2=11.2%, $p<0.001$), texture based rejection (ΔR^2=9.6%, $p<0.001$) increased. Picky eating and texture based food rejection were both predicted by age, gender and food disgust sensitivity. In comparison to those with low FDS-short scores, those with high FDS short scores also had higher APEQ scores. People who report higher FDS short score are more likely to reject foods with a certain texture than people who report lower FDS short score.

DISCUSSION

The purpose of this study was to assess the associations between food disgust sensitivity and eating habits, especially picky eating and texture-based rejection. It was determined that food disgust was connected to picky eating as well as texture based rejection. The findings indicated that age, gender income and BMI are predictors of food disgust sensitivity. The FDS score was a predictor of adult picky eating and texture-based rejection in addition to age and gender. People with high FDS scores were found to have a pickier eating behaviour and more frequently reject foods due to texture than people with low FDS scores.

In a study conducted in ten countries, the FDS short scores ranged from 3.47 to 4.09.[5] In the current research, FDS short score of participants was 3.549±0.745. The mean of the FDS short score is between the means reported in a previous study.[5] Furthermore, the findings indicated that age, gender income, and BMI were predictors of food disgust sensitivity. Women had a higher sensitivity to food disgust than men in this study which is consistent with the findings of previous studies on disgust.[15,18,19] Studies pointing to the existence of an association between food disgust sensitivity and gender have offered varying explanations for the higher food disgust sensitivity among women.[19] First, the reproductive role of women may be one of the factors. In a study conducted by Fessler et al., it was reported that during the first trimester of pregnancy, a woman's sensitivity to disgust is heightened. They thought that since embryos are most vulnerable during the first three months of pregnancy, a higher level of disgust sensitivity might be a way for the foetus to keep safe.[20] Second explanation relates to our ancestry: in the past, women were often more active in food washing, preparation, and cooking than males.[5,21] Consequently, greater disgust sensitivity may have been a characteristic of ancient women (during the period when the disgust system was predominantly created) since it led to more hygienic food-related behaviour.[5]

Contemporary evidence on the connection between age and disgust is contradictory, with indications of negative relationship[13,22] and positive.[18,23] The results of this study corroborate those of other studies in showing that individuals' sensitivity to food disgust increases with age.[18,23] Older people believe themselves to be more susceptible to diseases due to the association between old age and sensitivity to (infectious) diseases.[10,18,23]

Disgust sensitivity is crucial since it can affect eating patterns and, indirectly, body mass index.[7] Higher disgust sensitivity scores are connected with decreased appetite for high-calorie foods, suggesting that a reduced sensitivity to disgust may contribute to obesity by enabling overconsumption of particular foods.[24,25] In line with previous research, we find

Table 4. Hierarchical Linear Models

	Model 1					Model 2				
	β1 (%95 CI)	SE	β2	t	p	β1 (%95 CI)	SE	β2	t	p
Picky Eating										
(Constant)	2.001 (1.592 - 2.41)	0.208		9.633	<0.001	1.424 (0.892 - 1.956)	0.270		5.277	<0.001
Age	0.013 (0.008 - 0.017)	0.002	0.338	5.024	<0.001	0.011 (0.006 - 0.015)	0.003	0.284	4.185	<0.001
Gender	0.191 (0.071 - 0.311)	0.061	0.203	3.138	0.002	0.126 (0.001 - 0.25)	0.063	0.133	1.986	0.018
Income	0.02 (-0.04 - 0.08)	0.030	0.041	0.652	0.515	0.043 (-0.018 - 0.103)	0.031	0.088	1.391	0.166
BMI	-0.01 (-0.023 - 0.003)	0.007	-0.092	-1.466	0.144	-0.004 (-0.017 - 0.01)	0.007	-0.037	-0.578	0.564
FDS		----				0.144 (0.057 - 0.232)	0.045	0.228	3.245	0.001
Adj. R^2	F=18.327; p<0.001; Adj.R^2= 0.245					F=24.630; p<0.001; Adj.R^2= 0.356				
Δ Adj. R^2		----				F=37.204; p<0.001; Adj.R^2= 0.112				
Texture Based Rejection										
(Constant)	0.428 (-0.256 - 1.111)	0.347		1.234	0.219	-1.008 (-1.868 - -0.148)	0.436		-2.310	0.022
Age	0.017 (0.009 - 0.025)	0.004	0.274	4.034	<0.001	0.012 (0.004 - 0.020)	0.004	0.192	2.903	0.004
Gender	0.377 (0.167 - 0.587)	0.107	0.241	3.538	<0.001	0.295 (0.093 - 0.496)	0.102	0.189	2.881	0.004
Income	-0.001 (-0.102 - 0.099)	0.051	-0.002	-0.029	0.977	0.055 (-0.042 - 0.153)	0.049	0.069	1.113	0.267
BMI	0.004 (-0.022 - 0.023)	0.011	0.003	0.044	0.965	0.015 (-0.007 - 0.037)	0.011	0.086	1.379	0.169
FDS		----				0.360 (0.218 - 0.502)	0.072	0.342	4.999	<0.001
Adj. R^2	F=11.697; p<0.001; Adj.R^2= 0.157					F=15.424; p<0.001; Adj. R^2= 0.252				
Δ Adj. R^2		----				F= 24.986; p<0.001; Adj.R^2= 0.096				

Model 1: Age and sex, income, BMI as variables. Model 2: Additionally, added FDS short score. 1: Unstandardized Coefficients; 2: Unstandardized Coefficients Gender: 0=men, 1=women; Bold values p<0.05

that there is an inverse correlation between disgust sensitivity and body mass index.[7,26]

Another demographic component, income level, was found to be associated with food aversion, and a negative correlation was discovered. It is assumed that as one's income increases, so does one's exposure to a wider range of foods. As a result, these individuals are more likely to be exposed to a wider range of food disgust elicitors, which may lead to a decrease in food disgust sensitivity.[27]

We also examined, whether or not a disgust for food is linked to picky eating and the rejection of foods based on their textures in this study. The results indicated that participants with a higher FDS-short score had a higher APEQ score and texture-based rejection score. In the study by Egolf et al.[18] food disgust sensitivity alone explained 11.4% of the variance in picky eating and 14.1% of texture-based rejection. Our results in line with previous study[18] and model (**Table 4**) indicates that food disgust alone accounted for 11.2% of the variance in picky eating and 9.6% of texture-based rejection. It has been hypothesized by Kauer et al. (2015), it's possible that picky eaters are hypersensitive to some aspects of food's sensory qualities (such its taste, appearance, or texture), which causes them to reject food.[11] People who have a high sense of disgust are more prone to reject foods with lumps and foreign objects, which can trigger associations with contamination and decay. Additionally, it's likely that disgust and picky eating can strengthen each other.[18] Food texture may indicate potentially dangerous deterioration conditions.[28] Foods having a chewy, slippery, or creamy texture are more likely to be rejected by people with high food disgust sensitivity.[11] It would appear that slimy surfaces or changes in texture are common causes of disgust since they frequently suggest the presence of microorganisms and foods that might be dangerous.[28] Given the preventative role of disgust in illness avoidance, it is not surprising that sensitivity to certain textural qualities and sensitivity to food distaste are linked.

Finally, some important limitations and strengths must be considered to be evaluated. Firstly, the study's use of a cross-sectional design, which prevented the establishment of causal conclusions. Secondly, only behavioural measurements were utilised in this research, which may restrict the generalizability of the results. In spite of these limitations, the findings of the research are significant for future research since this was the first study to investigate the association between food disgust and picky eating, as well as texture based rejection of foods, in Turkish adults.

CONCLUSION

Finally, the results of this research showed that there was a relationship between food disgust and picky eating and texture-based rejection. This study revealed that those with high FDS scores had pickier eating habits and rejected items with a certain texture more frequently than those with low FDS scores.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was approved by the Ethics Board of Gaziantep Islam Science and Technology University (Date: 27.09.2022, Decision No: 2022/146).

Informed Consent: All participants signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

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

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Original Article / Orijinal Araştırma

Investigation of the Prevalence and Associated Risk Factors of Asthma and Allergic Diseases in Children aged 13-14 in Adıyaman Province

Adıyaman İlindeki 13-14 Yaş Grubundaki Çocuklarda Astım ve Alerjik Hastalıkların Prevalansı ve İlişkili Risk Faktörlerinin İncelenmesi

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Abstract

Aim: While the prevalence of allergic diseases in Turkey cannot be determined clearly due to the regional nature of the studies, it is known that the frequency is increasing. The aim of this study is to evaluate the frequency of allergic diseases and risk factors in Adıyaman province.

Material and Method: The research was conducted as a cross-sectional prospective. The research was carried out in Adıyaman province between November 2021 and January 2022. The universe of the research consists of students aged 13-14 studying in secondary and high schools.

Results: In our study, the prevalence of wheezing in the chest at any time during their lifetime was determined as 10.5%. In the last year, this rate has been determined as 4.9%. 4.9% of the participants stated that their child was diagnosed with asthma by the doctor. The rate of participants diagnosed with hay fever was 3.1%.

Conclusion: Although allergic diseases and asthma are diseases with an increasing frequency, it is striking that they are not yet fully recognized by the society. Studies have shown that family burden is an important risk factor. The necessity of using all communication channels in order to increase the health literacy of the society shows itself in every research.

Keywords: Allergy, eczema, asthma

Öz

Amaç: Alerjik hastalıkların Türkiye'deki prevalansı, çalışmaların bölgesel olması nedeniyle net olarak tespit edilememekle birlikte, sıklığın arttığı bilinmektedir. Bu çalışmanın amacı, Adıyaman ilinde alerjik hastalıkların sıklığını ve risk faktörlerini değerlendirmektir.

Gereç ve Yöntem: Araştırma, kesitsel, prospektif olarak yapılmıştır. Araştırma Adıyaman ilinde Kasım 2021 – Ocak 2022 tarihleri arasında gerçekleştirilmiştir. Araştırmanın evrenini ortaokul ve liselerde öğrenim gören 13-14 yaş arası öğrenciler oluşturmaktadır.

Bulgular: Çalışmamızda yaşamları boyunca herhangi bir zamanda göğüste hışıltı görülme sıklığı %10,5 olarak belirlendi. Son bir yılda bu oran %4,9 olarak belirlenmişti. Katılımcıların %4,9'u çocuğuna doktor tarafından astım teşhisi konduğunu belirtmiştir. Saman nezlesi tanısı konan katılımcıların oranı %3,1'dir.

Sonuç: Alerjik hastalıklar ve astım görülme sıklığı artan hastalıklar olmasına rağmen toplum tarafından henüz tam olarak tanınmamış olmaları dikkat çekicidir. Araştırmalar aile yükünün önemli bir risk faktörü olduğunu göstermiştir. Toplumun sağlık okuryazarlığının artması için tüm iletişim kanallarının kullanılması gerekliliği her araştırmada kendini göstermektedir.

Anahtar Kelimeler: Alerji, egzama, astım

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INTRODUCTION

Allergic diseases are common in children and adolescents. It imposes a serious financial burden on the health system. In addition, school life for children causes lost days for business life for adults. Despite increasing knowledge about its pathophysiology and diversifying treatment protocols, it is considered that the prevalence increases due to the interaction of genetic and environmental factors such as exposure to cigarette smoke, air pollution, and pollen.[1]

Epidemiological studies have been conducted to determine the prevalence of asthma regionally and globally and to understand risk factors. One of them, ISAAC (International Study of Asthma and Allergies in Childhood), was used to measure the frequency and severity of asthma and allergic diseases in children and adolescents, and later the change in frequency, using a standardized questionnaire.[2] While the prevalence of allergic diseases in Turkey cannot be determined clearly due to the regional nature of the studies, it is known that the frequency is increasing.[3]

In this study, we aimed to investigate the prevalence and risk factors of allergic diseases in adolescent children aged 13-14 in Adıyaman province. We questioned the frequency of asthma, rhinitis and eczema, which are among the allergic diseases. Our study should be evaluated within the scope of ISAAC survey studies.

MATERIAL AND METHOD

Study Design

The research is a cross-sectional prospective study. The research was conducted in Adıyaman province. Data collection took place between November 2021 and January 2022. The population of the research consists of students aged 13-14 studying in secondary and high schools in Adıyaman city center. Schools were selected by cluster sampling method. At least 162 people were included in the study with a confidence level of 95% and a margin of error of 0.05. Questionnaire form was used as data collection tool. Consent form was obtained from the students and their parents, and permission was obtained from the Directorate of National Education.

Statistical Analysis

Data were evaluated in SPSS 20 package program. Qualitative data are presented as numbers and percentages (%). Chi-Square and Fisher's exact tests were used in the analysis of categorical data. For statistical significance, a p value of <0.05 was considered significant.

RESULTS

162 parents participated in the study. the mean age of the children is 13.0±0.2 (Min:13 and max:14). The descriptive characteristics of the children are presented in **Table 1**.

Table 1: Descriptive characteristics of children

Characteristics	Number	%
Breastfeeding status		
Breastfed	132	81.5
Not breastfed	10	6.2
Uninformed	20	12.3
Breastfeeding time		
1-5 months	14	8.6
6-12 months	41	25.3
13 and above months	62	38.3
Person who cares for the child		
At home by mother	143	88.3
At home by caregiver	1	0.6
Other	2	1.2
Smoking in the home		
Yes	99	61.1
No	45	27.8

The incidence of asthma in children whose siblings have asthma is significantly higher than those whose siblings do not have asthma (p=0.015). The incidence of eczema in children whose fathers had eczema was significantly higher than those whose fathers did not have eczema (p=0.005). The incidence of hay fever in children whose parents had hay fever was significantly higher than those whose parents did not have hay fever (p=0.022, p=0.45, respectively).

Table 3: Frequency of allergic diseases in mothers, fathers and siblings of children

Disease	Number	%
Asthma		
Mother	17	10,5
Father	8	4,9
Siblings	10	6,2
Hay fever		
Mother	1	0,6
Father	3	1,9
Siblings	4	2,5
Eczema		
Mother	5	3,1
Father	7	4,3
Siblings	10	6,2
Other allergy		
Mother	8	4,9
Father	3	1,9
Siblings	13	8,0

Table 2: Symptoms observed by parents in their children

Symptoms	Number	%
Anyone who hears wheezing / rustling in their child's chest at any time	17	10.5
Those who have heard wheezing / rustling in their child's chest in the last 12 months	8	4.9
Number of wheezing/rustling heard in the child's chest in the past 12 months		
1-3 time	8	4.9
4-12 time	0	0.0
More than 12 times	0	0.0
Number of nights the child was unable to sleep due to wheezing in the last 12 months		
Less than one night a week	6	3.7
Once a week or more	2	1.2
Children with speech difficulties due to wheezing in the last 12 months	2	1.2
Those who say that the child has asthma	8	4.9
Wheezing in the child's chest after exercise in the last 12 months	6	3.7
Nocturnal dry cough attacks without cold or infection in the last 12 months	23	14.2
Children with runny nose, congestion, sneezing without cold and infection	65	40.1
In the last 12 months, the child has runny nose, congestion, sneezing without a cold or infection	59	36.4
In the last 12 months, the child has itchy eyes without a cold or infection	21	13
Month of nose problem		
January	10	6.2
February	2	1.2
March	5	3.1
April	7	4.3
May	5	3.1
June	2	1.2
July	2	1.2
August	1	0.6
September	3	1.9
October	9	5.6
November	19	11.7
December	24	14.8
The degree to which the child's nose problem has affected his/her daily activities in the last 12 months		
None	17	10.5
Slightly	22	13.6
Moderate	15	9.3
Too much	2	1.2
Children diagnosed with hay fever	5	3.1
Child with an itchy rash in the past 12 months	6	3.7
Spill sites		
Front of elbow	2	1.2
Behind the knee	2	1.2
Anterior ankle	3	1.9
Butt bottom	4	2.5
Around the neck	1	0.6
Ear eye area	1	0.6
Age of rash		
Before 12 months	0	0.0
After 12 months	4	2.5
Number of children who had a rash-free period in the last 12 months	4	2.5
Number of nights awakened due to itchy rash in the last 12 months		
None	3	1.9
Less than 1 night per week	1	0.6
1 night per week or more often	1	0.6
Children said to have eczema	10	6.2
Allergy diagnoses made by the doctor from birth		
Asthma	8	4.9
Hay fever	3	1.9
Eczema	7	4.3
Urticaria	4	2.5
Food allergy	11	6.8

Table 4: Frequency of environmental risk factors in children's home environment

	Number	%	Time (month)
Indoor smoking	50	30,9	
Smoking outside the home	47	29	
Carpet in nursery	150	92,6	
Seeing cockroaches/heating beetles at home			
None	108	66,7	
Rarely	44	27,2	
Often	2	1,2	
Pet feeding			
Dog (n=0)			
Cat (n=4)	1	0,6	7
	1	0,6	12
	1	0,6	24
	1	0,6	48
Fish (n=7)	2	1,2	2
	3	1,8	6
	2	1,2	24
	2	1,2	48
Turtle (n=1)	1	0,6	48
Bird (n=12)	2	1,2	1
	2	1,2	3
	3	1,8	6
	3	1,8	24
	2	1,2	72

DISCUSSION

In our study, the prevalence of wheezing in the chest at any time during their life was determined as 10.5%, while this rate was determined as 4.9% in the last year. While 4.9% of the participants stated that their child was diagnosed with asthma by the doctor, the rate of the participants who were diagnosed with hay fever was 3.1%. In the ISAAC survey study conducted with 568 participants in the province of Malatya, the prevalence of wheezing in the chest at any time during their lifetime was determined as 11.4%, while this rate was 6.3% in the last year. In the same study, the rate of those diagnosed with asthma by a physician was 6.5%.[4] In a study conducted within the scope of a master's thesis published in 2019, the rate of life-long wheezing in Konya was 21.8%, while this rate was 53% in the last year. In studies in Malatya and Adıyaman, it was observed that while the rate of symptomatic patients decreased in the last year, this rate increased significantly in Konya.[5] Parallel to this increase, the rate of those who were diagnosed with asthma by a physician in a study conducted in Konya was 8.7%, which is higher than in other provinces.[6] It is considered that the higher prevalence in Konya is due to the rapid industrialization in the city. In a survey conducted with 85 children living in low-income areas in Ankara and their parents, the lifetime wheezing/wheezing frequency was 12.9%, and this rate was 10.5% in the last year.[7] In our study, it was found that children with asthma

Table 5: Asthma, eczema, hay fever status in children according to some variables

	n	Children diagnosed with asthma by the doctor		
		S	%	p
Breastfeeding				
Breastfed	121	7	5,8	0.446
Not breastfed	9	1	11,1	
Smoking at home				
Smoking	93	5	5,4	0,700
No smoking	41	3	7,3	
Mother's history of asthma				
Maternal asthma (+)	16	1	6,3	0,584
Maternal asthma (-)	123	6	4,9	
Father's history of asthma				
Paternal asthma (+)	6	0	0,0	0,553
Paternal asthma (-)	126	7	5,6	
History of asthma in siblings				
Siblings asthma (+)	10	2	20,0	0,015
Siblings asthma (-)	99	3	3,0	
		Children diagnosed with eczema by the doctor		
Breastfeeding				
Breastfed	121	5	4,1	0,356
Not breastfed	9	1	11,1	
Smoking at home				
Yes	93	5	5,4	0,905
No	41	2	4,9	
Mother's history of eczema				
Maternal eczema (+)	5	0	0,0	0,600
Maternal eczema (-)	134	7	5,2	
Father's history of eczema				
Paternal eczema (+)	7	2	28,6	0,005
Paternal eczema (-)	125	5	4,0	
History of eczema in siblings				
Siblings eczema (+)	10	1	10,0	0,513
Siblings eczema (-)	99	5	5,1	
		Children diagnosed with hay fever by the doctor		
Breastfeeding				
Breastfed	121	3	2,5	0,633
Not breastfed	9	0	0,0	
Smoking at home				
Yes	93	3	90	0,533
No	41	0	0,0	
Mother's history of hay fever				
Maternal hay fever (+)	1	1	100,0	0,022
Maternal hay fever (-)	138	2	1,4	
Father's history of hay fever				
Paternal hay fever (+)	3	1	33,3	0,045
Paternal hay fever (-)	129	1	0,9	
History of hay fever in siblings				
Siblings hay fever (+)	4	1	25,0	0,072
Siblings hay fever (-)	105	1	1,00	

in one of their siblings were significantly more likely to have asthma than those without. Exposure to cigarette smoke, presence of pests at home, having siblings at a higher risk when considering family history as risk factors that increase the incidence of the disease support the hypothesis that genetic and environmental factors increase the incidence of the disease.

When life-long rhinitis symptoms were questioned in our study, it was found that these symptoms were observed in 40.1% of the participants, and this rate decreased to 36.4% in the last year. The fact that only 3.1% of the participants was diagnosed with hay fever is considered to be that the severity of the disease does not affect the quality of life or that the families do not take these symptoms seriously when they increase or decrease seasonally. As a matter of fact, in our study, it was observed that although the symptoms of runny nose increased in the spring months, they intensified in the winter months. In addition, a significant number of parents said that their nasal symptoms did not affect their daily activities at all or very little. These situations explain the fact that a small proportion of the disease is diagnosed despite the proportion of those who show symptoms. In the study conducted by Ozbay and Topal in Malatya province, while the lifetime prevalence of rhinitis symptoms was 36%, this rate was 13.9% in the last year, but the rate of those diagnosed with hay fever remained at 3.9%.[4] In the study conducted by Akçay et al. in Denizli, the lifetime prevalence of rhinitis was 34.2%, while it was 9.6% in the last year, and the rate of those diagnosed was 4.3%.[7] In an ISAAC prevalence study conducted in the province of Tokat in 2010, the prevalence of lifetime rhinitis, prevalence of rhinitis in the last year, and those diagnosed with hay fever were 46.7%, 17.7%, and 10.4%, respectively. [8] Children whose parents were diagnosed with hay fever had a significantly higher risk of developing hay fever than those without. This situation coincides with the fact that the probability of having asthma is increased in those with a sibling with asthma, and it shows the necessity of questioning the family burden of allergic diseases. Although it varies regionally, hay fever symptoms are common in the community in general, but the rate of those diagnosed remains low.

While the rate of lifelong eczema symptoms was 4.3% in our study, this rate was 3.7% in the last year, and the rate of children diagnosed with eczema was 6.2%. Again, in this diagnosis, it was determined that children whose fathers were diagnosed with eczema were at a significantly higher risk than those who did not. When the lifetime prevalence of atopic dermatitis, the prevalence in the last year, and the prevalence of diagnosed children in a study conducted in Malatya were examined, these rates were found to be +.9%, 3.9%, and 5.1%, respectively.[4] While the lifetime prevalence of atopic dermatitis was 28.3% in the prevalence study conducted in Sivas province by Arslan et al., this rate was 20.5% in the last year.[9] In the study conducted in Aydın province with 1537 participants aged 12-13 years, the rate of those with lifelong atopic dermatitis symptoms was 12%, while this rate was 7.4% in the last year. The rate of children diagnosed with atopic dermatitis was 2.8%.[10] In the study conducted by Akçay et al. in Denizli, the rates of lifelong symptomatic, symptomatic and diagnosed in the last year were 20.8%, 15.4% and 2.1%, respectively.[7] Although we could not detect any risk other than family burden in our study, it was observed in other studies conducted in the country that the incidence of atopic dermatitis symptoms is higher in regions with dry and cold climate.

CONCLUSION

Although allergic diseases and asthma are diseases with an increasing frequency, it is striking that they are not yet fully recognized by the society. Studies have shown that family burden is an important risk factor. The necessity of using all communication channels in order to increase the health literacy of the society shows itself in every research.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Adıyaman University Non-Interventional Clinical Researches Ethics Committee (Date: 26.10.2021, Decision No: 2021/08-17).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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The Relationship between Attention Deficit Hyperactivity Disorder Symptoms and Bedtime Procrastination

Dikkat Eksikliği Hiperaktivite Bozukluğu Belirtileri ve Uyku Vaktini Erteleme Arasındaki İlişki

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Abstract

Aims: We aimed to examine the relationship between attention deficit and hyperactivity disorder (ADHD) symptoms and bedtime procrastination.

Material and Method: Five hundred fifty-three university students participated in our study. All participants answered the sociodemographic data form, The Adult ADHD Self-Report Scale (ASRS v1.1), Bedtime Procrastination Scale (BPS), Brief Self-Control Scale (BSCS), and Insomnia Severity Index (ISI). All participants were divided into two groups, ADHD and non-ADHD, according to the ASRS score. We compared sociodemographic data, sleep routines, and psychometric scales between these two groups. Finally, we analyzed the factors that could predict bedtime procrastination by hierarchical regression analysis.

Results: The mean age of the participants was 20.55 ± 2.17. Most participants were female and unmarried (69.6% and 98.2%, respectively). BPS, ISI, and ASRS scores were significantly higher in the ADHD group than in the non-ADHD group ($p < 0.01$). BSCS score was significantly lower in the ADHD group than in the non-ADHD group ($p < 0.01$). Sleep duration was significantly lower in the ADHD group than in the non-ADHD group ($p < 0.01$). The correlation coefficients between ASRS and ISI, BPS, and BSCS were 0.461, 0.268, and -0.442, respectively ($p < 0.01$). Self-control and ADHD symptoms separately predicted bedtime procrastination with approximately the same variance (%4 vs. %3.9).

Conclusion: Clinicians should evaluate bedtime procrastination in individuals with adult ADHD by clinical interview or BPS. Individuals with adult ADHD with bedtime procrastination should be assisted with cognitive behavioral therapy-insomnia and sleep hygiene.

Keywords: Attention deficit and hyperactivity disorder, bedtime procrastination, insomnia, self-control.

Öz

Amaç: Dikkat eksikliği ve hiperaktivite bozukluğu (DEHB) semptomları ve uyku vaktini erteleme arasındaki ilişkiyi incelemeyi amaçladık.

Gereç ve Yöntem: Çalışmamıza 553 üniversite öğrencisi katıldı. Tüm katılımcılar sosyodemografik veri formu, Yetişkin DEHB Öz Bildirim Ölçeği (YDEHBÖ), Uyku Vaktini Erteleme Ölçeği (UVEÖ), Kısa Öz Kontrol Ölçeği (KÖKÖ) ve Uykusuzluk Şiddet İndeksi'ni (UŞİ) yanıtladı. Tüm katılımcılar YDEHBÖ puanına göre DEHB'si olan ve olmayan olmak üzere iki gruba ayrıldı. Bu iki grup arasında sosyodemografik verileri, uyku rutinlerini ve psikometrik ölçekleri karşılaştırdık. Son olarak, hiyerarşik regresyon analizi ile uyku vaktini ertelemeyi öngörebilecek faktörleri analiz ettik.

Bulgular: Katılımcıların yaş ortalaması 20.55 ± 2.17 idi. Katılımcıların çoğu kadın ve bekardı (sırasıyla %69,6 ve %98,2). UVEÖ, UŞİ ve YDEHBÖ puanları, DEHB grubunda DEHB olmayan gruba göre anlamlı derecede yüksekti ($p < 0.01$). KÖKÖ puanı, DEHB grubunda DEHB olmayan gruba göre anlamlı olarak daha düşüktü ($p < 0.01$). Uyku süresi, DEHB grubunda DEHB olmayan gruba göre anlamlı olarak daha düşüktü ($p < 0.01$). YDEHBÖ ile UŞİ, UVEÖ ve KÖKÖ arasındaki korelasyon katsayıları sırasıyla 0,461, 0,268 ve -0,442 idi ($p < 0,01$). Öz kontrol ve DEHB semptomları uyku vaktini ertelemeyi yaklaşık olarak aynı varyansla (%4'e karşı %3.9) ayrı ayrı yordamıştır.

Sonuç: Klinisyenler, DEHB'li erişkinlerde uyku vaktini ertelemeyi klinik görüşme veya UVEÖ ile değerlendirmelidir. Uyku vaktini erteleyen yetişkin DEHB'li bireylere bilişsel davranışçı terapi-uykusuzluk ve uyku hijyeni ile müdahale edilmelidir.

Anahtar Kelimeler: Dikkat eksikliği ve hiperaktivite bozukluğu, uyku vaktini erteleme, uykusuzluk, öz-kontrol.

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INTRODUCTION

Attention-deficit hyperactivity disorder (ADHD) is one of the most frequent childhood neurodevelopmental disorders. Persistent symptoms of inattention and hyperactivity-impulsivity are the main symptoms of ADHD (1). It also has been shown to have a high prevalence in adults. The prevalence of adult ADHD is estimated to be between 2.5% and 8.9% (2,3).

Procrastinatination and ADHD

Procrastination is the irrational tendency to postpone any task until after the deadline (4). There are different types of procrastination (i) Academic procrastination is delaying studying for an exam or writing an essay shortly before the deadline. (ii) Daily procrastination is difficulty organizing daily activities and performing to deadlines (for example, inability to pay bills on time). (iii) Decision procrastination describes those with chronic problems making timely decisions (5). All three types of procrastination can be highly associated with ADHD (6). Parents and teachers of children with ADHD provide anecdotal evidence, and a limited number of studies suggest an association between ADHD and procrastination. Procrastination is also involved in identifying problems associated with ADHD. Children with ADHD may likely avoid and procrastinate from complex, unpleasant, demanding, and uninteresting choices, tasks, daily activities, and decisions. They also show organizational problems with school-related activities, such as forgetting homework, difficulty completing long chores, studying for exams, and keeping materials organized. These problems are closely related to procrastination (6). Adults with ADHD present clinically with procrastination, planning difficulties, and missing deadlines (7). In addition, individuals with ADHD are prone to develop negative beliefs about themselves, low self-esteem, and low self-efficacy because they often experience these problems (8). Typical maladaptive beliefs point to feelings of imperfection, failure, and insufficient self-control. When these individuals face negative or stressful situations, they are likely to develop maladaptive coping strategies such as procrastination and avoidance, reinforcing their negative beliefs and creating a vicious circle (9). Therefore, avoidance/procrastination is a compensatory strategy for adolescents and adults with ADHD and it causes them to stop tackling an unpleasant and challenging task beyond their capacity (8).

Bedtime Procrastinatination, Self control, ADHD

In 2014, Kroose et al. described bedtime procrastination. Bedtime procrastination is defined as not being able to go to bed at the desired time even though no external conditions prevent it (10). Bedtime procrastination is now a very common phenomenon after the use of technological devices (11). It impairs sleep quality and predicts insomnia in university students (12). Another important finding is that bedtime procrastination is predictive of depression and anxiety (13). Self-Control can be defined as the ability to change, adapt, or break undesirable behavior patterns to achieve effortful goal-directed action. It has been shown to be particularly associated with health-related behaviors (14). For example, self-control is negatively associated with health-promoting behaviors. Additionally, studies show a negative link between self-control and procrastination. Unsurprisingly, bedtime procrastination reflects a failure of self-control, similar to procrastination in general and health-related behaviors (15).

The present study

Low self-control and procrastination are ordinary among individuals with ADHD (6). Therefore, bedtime procrastination in adults with ADHD may be a significant clinical problem. As far as we know, there is no study examining the relationship between ADHD and bedtime procrastination. We aimed to investigate the relationship between ADHD and bedtime procrastination among university students. Our hypotheses are: First, individuals with ADHD are more likely to have bedtime procrastination and insomnia than those without ADHD. Second, self-control is lower in individuals with ADHD than in those without ADHD. Third, there is a significant correlation between ADHD symptoms, self-control, bedtime procrastination, and insomnia severity. Finally, ADHD symptoms and self-control are independent risk factors for bedtime procrastination.

MATERIALS AND METHODS

Study Population and Procudure

In our study, we included students from the various faculties of Gaziantep University. The researchers explained the definition and aims of the study to all participants in a quiet classroom environment. Afterward, participants who volunteered to participate in the study completed the sociodemographic data form, The Adult ADHD Self-Report Scale (ASRS v1.1), Bedtime Procrastination Scale (BPS), Brief Self-Control Scale (BSCS), and Insomnia Severity Index (ISI), respectively. We removed 163 participants from the study since 75 participants with incomplete responses to ASRS v1.1, BPS, BSCS, and ISI, and 88 gave unreliable answers to the scales as 1111111 or 123123123. Finally, we completed the study with 553 participants. The Clinical Research Ethics Committee of Gaziantep University approved this study (Date: 21.12.2022, decision no:2022/468). We conducted our study between 1 January 2023 and 15 January 2023 in accordance with ethical rules and the principles of the Declaration of Helsinki (16).

Data Collection Tools

Sociodemographic Data Form:

ASRS v1. 1

ASRS is an 18-item self-report scale used to assess ADHD symptoms in adults (17). Each item has responses ranging from 0 (never) to 4 (very often). The total score is obtained by summing up all the items. The first nine items (part A) consist of questions about attention deficit, while the last nine are

about hyperactivity-impulsivity (part B). A score above 24 on Part A or Part B indicates a diagnosis of ADHD (18). ASRS has a high internal consistency coefficient. In our study, ASRS's internal consistency coefficient was excellent (Cronbach's α= 0.90).

BPS

BPS is a 9-item self-report scale that evaluates bedtime procrastination (10). We chose this scale in our study because it is the only scale to assess bedtime procrastination in Turkish (19). Items 2, 3, 7, and 9 are reverse-scored, and a higher score indicates more bedtime procrastination. We found the internal consistency coefficient of the scale 0.76.

BSCS

The BSCS is a 13-item self-report scale that evaluates self-control (20). Each item receives scores ranging from 1 (not at all true of me) to 5 (totally true of me). A high score indicates high self-control (20). We used this scale to analyze the predictive role of self-control in our study. The internal consistency coefficient of the scale was 0.70 in our study.

ISI

We used ISI to determine participants' insomnia severity as it is the most widely used tool for determining insomnia severity (21). It consists of 7 items and evaluates insomnia severity in the last two weeks (22). The total score ranges from 0 to 28, and a high score indicates severe insomnia. We found the internal consistency coefficient of ISI 0.82 in this study.

Statistical Analyses

We used IBM SPSS Statistics Version 23.0 for descriptive statistics, group comparisons, correlation, and regression analyses. We analyzed skewness-kurtosis values to determine whether the data was normal distribution and accepted skewness-kurtosis between -2 and +2 as a normal distribution. We divided all the participants into two groups ADHD with a score of 24 or higher from part A or Part B of ASRS and the non-ADHD group with a lower score. We compared sleep variables and scale scores between these two groups. We used the Student's t-test and chi-square test for these comparisons. Pearson correlation coefficient measured the associations between demographics, BPS, ASRS, ISI, and BSCS. Hierarchical regression analyses investigated the independent effects of self-control and ADHD symptoms on bedtime procrastination. A p-value < 0.05 was considered statistically significant.

RESULTS

Characteristics of all the participants

Table 1 presents the sociodemographic variables and scale mean scores of all participants. The mean age of the participants was 20.55 (SD: 2.17), and the age range was between 16-43. Most participants were female (69.6%), and almost all were unmarried (98.2%). The mean of ASRS, BPS, ISI, and BSCS was 32.77, 27.73,11.89, and 40.71, respectively.

Comparison of sociodemographic data, sleep variables, and scales between ADHD (+) and ADHD (-) groups

Table 1. Sociodemographic and clinical variables of all the participants

Variables	Mean (SD)
Age (years)	20.55 (2.17)
BMI (kg/m2)	22.44 (10.00)
	n (%)
Sex	
Male	168 (30.4)
Female	385 (69.6)
Marital status	
Not married	543 (98.2)
Married	10 (1.8)
Living	
Alone	80 (14.5)
With family	327 (59.1)
With friend	146 (26.4)
Smoking	
Yes	90 (16.3)
No	463 (83.7)
Alcohol use	
Yes	38 (6.9)
No	515 (93.1)
Psychometric Instruments	Mean (SD)
ISI	11.89 (5.18)
BPS	27.73 (5.50)
BSCS	40.71 (6.06)
ASRS	32.77 (11.06)
ASRS inattention	16.62 (6.18)
ASRS hyperactivity-impulsivity	16.14 (5.79)

SD; Standard Deviation, BMI; Body Mass Index, ISI; Insomnia severity Index, BPS; Bedtime Procrastination Scale, BSCS: Brief Self Control Scale, ASRS; The Adult ADHD Self-Report Scale-v1.1.

We divided all participants into two groups, ADHD and non-ADHD, according to the ASRS score. When we compared the two groups, we found no significant difference in terms of age (p = 0.10), gender (p = 0.64), and BMI (p = 0.54). BPS, ISI, and ASRS scores were significantly higher in the ADHD group than in the non-ADHD group (p < 0.01). BSCS score was significantly lower in the ADHD group than in the non-ADHD group (p < 0.01). Sleep duration was significantly lower in the ADHD group than in the non-ADHD group (p < 0.01). Bedtime after 2 am on weekdays was statistically significantly higher in the ADHD group (p = 0.02). On the other hand, bedtime after 2 am on the weekend was more, but not statistically significant (p = 0.17). In addition, this group's wake-up time on weekdays and weekends was also late, statistically significant than the non-ADHD group. **Table 2** shows the detail of the comparison of the two groups.

Correlations Between Psychometric Instruments

There was a statistically significant correlation between all data collection tools. The correlation coefficients between ASRS and ISI, BPS, and BSCS were 0.461, 0.268, and -0.442, respectively (p < 0.01). The correlation coefficient of the ASRS inattention subscale with the BPS was higher than the ASRS hyperactivity-impulsivity subscale correlation coefficient (0.308 vs. 0.264) (p < 0.01). **Table 3** displays details of the correlations between psychometric instruments.

Table 2. Comparison between ADHD group and Non ADHD group

Variables	ADHD group (%15, n= 83)	Non ADHD group (%85, n= 470)	p value
Sex (male)	19 (%22.9)	149 (%31.7)	0.10
Age	20.45 (1.95)	20.57 (2.21)	0.64
BMI	21.82 (4.32)	22.55 (10.69)	0.54
ISI	15.83 (6.02)	11.19 (4.70)	< 0.01
BPS	30.87 (6.70)	27.18 (5.07)	< 0.01
BSCS	37.44 (6.38)	41.29 (5.82)	< 0.01
ASRS	49.77 (9.51)	29.77 (8.24)	< 0.01
ASRS inattention	25.95 (5.52)	14.98 (4.64)	< 0.01
ASRS hyperactivity-impulsivity	23.81 (6.49)	14.78 (4.45)	< 0.01
Sleep duration (< 5 hours)	23 (%27.7)	65 (%13.8)	< 0.01
Bedtime on a weekdays (After 02.00 a.m.)	24 (%28.9)	72 (%15.3)	0.02
Bedtime on weekends (After 02.00 a.m.)	31 (%37.3)	122 (%26)	0.17
Wake-up time on weekdays (After 12.00 p.m.)	11 (%13.3)	18 (%3.8)	< 0.01
Wake-up time on weekends (After 12.00 p.m.)	15 (%18.1)	37 (%7.9)	0.02

ISI; Insomnia severity Index, BPS; Bedtime Procrastination Scale, BSCS; Brief Self Control Scale, ASRS; The Adult ADHD Self-Report Scale-v1.1.

Table 3. Correlations between scales

	1	2	3	4	5	6
ISI	-					
BPS	347**	-				
BSCS	-168**	-207**	-			
ASRS	461**	268**	- 442**	-		
ASRS inattention	447**	308**	- 445**	928**	-	
ASRS hyperactivity-impulsivity	403**	264**	-401**	918**	705**	-

**: p < 0.01. ISI; Insomnia severity Index, BPS; Bedtime Procrastination Scale, BSCS; Brief Self Control Scale, The Adult ADHD Self-Report Scale-v1.1; ASRS

Hierarchical Regression Analysis of Predictors of Bedtime Procrastination

We conducted a hierarchical multiple regression analysis to examine the independent influence of age, sex, self-control, and ADHD symptoms on bedtime procrastination. Most research papers consider a VIF (Variance Inflation Factor) > 10 indicators of multicollinearity, but some choose a more conservative threshold of 5 or even 2.5. As a result of the analysis, the VIF of the final model was 1.26, which did not exceed 2.5, confirming that the multicollinearity problem did not ocur (23).

In the first step, we included age and gender in the model and did not detect any predictive effects of age and gender on bedtime procrastination. In the second step, we added self-control to the model. The predictive effect of self-control on bedtime procrastination (β = -0.20, t = -4.91, p = 0.000). All variables (age, gender, self-control) explain 4% of the variance in bedtime procrastination. Finally, in the third step, We added ADHD symptoms to the model. All variables (age, gender, self-control, ADHD symptoms) explain 8% of the variance in bedtime procrastination. When age, gender, and self-control are controlled, ADHD symptoms significantly present 3.9% of bedtime procrastination (R2= 0.082, R2 change= 0.039, p = 0.000). Self-control and ADHD symptoms separately predicted bedtime procrastination with approximately the same variance (%4 vs. %3.9).

Table 4 displays hierarchical regression analysis of bedtime procrastination.

Table 4. Hierarchical regression analysis of bedtime procrastination (N = 553)

Predictors	Part-cor	t	p	β (standart)	R	R2	R2 change	F
Step 1			0.84		0.025	0.001	0.001	0.175
Age	-0.21	-0.49	0.621	-0.02				
Sex	-0.17	-0.39	0.691	-0.01				
Step 2			0.000		0.207	0.043	0.042	8.182
Age	-0.00	0.60	0.543	0.02				
Sex	0.00	0.69	0.485	0.03				
Self-control	-0.20	-4.91	0.000	-0.20				
Step 3			0.000		0.286	0.082	0.039	12.21
Age	-0.01	-0.30	0.757	-0.01				
Sex	-0.02	-0.48	0.625	-0.02				
Self-control	-0.09	-2.30	0.022	-0.10				
ADHD symptoms	0.20	4.82	0.000	0.22				

ADHD; Attention-deficit hyperactivity disorder.

DISCUSSION

Our study aimed to examine the relationship between ADHD symptoms and bedtime procrastination. To the best of our knowledge, our research is the first study to explore the relationship between ADHD and bedtime procrastination. We found that those with ADHD bedtime procrastinate more, have lower levels of self-control, and experience more insomnia. We also found that ADHD symptoms or self-control independently predicted bedtime procrastination.

While only 20% of adults describe themselves as chronic procrastinators, 33-50% of college students are chronic procrastinators (24). Understanding procrastination is especially important for college students. As in general procrastination, university students are at risk for bedtime procrastination. Therefore, there are many studies on university students about bedtime procrastination (25). That's why we chose our sample group from university students. Previous studies have found negative correlations between bedtime procrastination and self-control and impulsivity (11,15,26). Both self-control and impulsivity are problematic areas in individuals with ADHD (27). Therefore, it is crucial to investigate bedtime procrastination in individuals with ADHD. However, to date, no research has examined the relationship between bedtime procrastination and ADHD.

One of the main findings of our study was that bedtime procrastination was higher in individuals with ADHD than those without ADHD. Adult ADHD patients often exhibit procrastination in their daily lives (6). Descriptive research

on bedtime procrastination was found to be associated with three themes (28). These are deliberate procrastination, mindless procrastination, and strategic delay. Deliberate procrastination, individuals reported that they voluntarily delayed their sleep hours because they thought they deserved some time. On the other hand, mindless procrastination was individuals did not realize how time had passed due to their immersion in evening activities. The strategic delay was defined as those who delay sleep time to fall asleep faster (28). Among these, mindless procrastination may be primarily associated with individuals with ADHD. Adults with ADHD have problems with attention, time planning, organization, and executive functions (29,30). In addition, they may prefer seducers instead of long-range goals and become immersed in evening activities because they have lower self-control (30). Therefore, they may show more bedtime procrastination than individuals without ADHD. Individuals with insomnia can develop some strategies to sleep better, such as going to bed later to fall asleep faster. Insomnia is common in individuals with ADHD, and the prevalence of insomnia has been reported to range from 43% to 80% in adult ADHD. In our study, individuals with ADHD had more severe insomnia and went to bed later than those without ADHD. There may be a role of above mentioned strategic delay in the relationship between ADHD and bedtime procrastination (31).

The other main finding of our study was that ADHD symptoms and self-control predicted bedtime procrastination. In previous studies, academic procrastination, general procrastination, evening chronotype, self-control, negative affect, electronic media use, and smartphone addiction were associated with bedtime procrastination (32-35). One of our study's strongest aspects is examining the relationship between ADHD symptoms and bedtime procrastination. To date, this relationship has never been explored. Inattention may lead to needing to understand how time passes, and impulsivity may lead to engaging in activities, such as using social media, which may lead to preferring not to sleep on time.

Limitations and Future Recommendations

Our study has some limitations. First, we diagnosed ADHD with self-psychometric scales, not clinical interviews. Secondly, the results cannot be generalized to the general population since the sample consists of university students. However, since university students are a risky population in terms of both general and bedtime procrastination, our study is valuable. Third, most of the participants were female, creating gender bias. Despite all these limitations, our study is the first to examine the relationship between ADHD symptoms and bedtime procrastination. Future research should explore bedtime procrastination in individuals diagnosed with ADHD through a clinical interview. What mediates the researchers should investigate the relationship between ADHD and bedtime procrastination. In addition, the relationship between bedtime procrastination and other psychiatric diseases should be examined, especially its effect on insomnia in these patients.

CONCLUSION

People with ADHD symptoms are more likely to delay bedtime. Inattention, impulsivity, and lack of self-control in these individuals may have caused them to delay their bedtime more than others. Psychiatrists should evaluate bedtime procrastination in individuals with ADHD by clinical interview or BPS. Individuals with ADHD with bedtime procrastination should be assisted with cognitive behavioral therapy-insomnia and sleep hygiene.

ETHICAL DECLARATIONS

Ethics Committee Approval: We obtained ethical approval from the Clinical Research Ethics Committee of Gaziantep University (Date: 21.12.2022, decision no:2022/468).

Informed Consent: All participants signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article / Orijinal Araştırma

Comparison of Pre-Operative and Post-Operative Thiol-Disulfide Levels in Acute Abdomen Patients

Akut Karın Hastalarında Pre-operatif ve Post-operatif Tiyol-Disülfid Düzeylerinin Karşılaştırılması

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Abstract

Aim: To evaluate role of thiol and disulfide homeostasis, a novel marker of oxidative stress, in the follow-up acute abdomen.

Material and Method: This prospective study included 107 patients (62 men and 45 women) with a diagnosis of acute abdomen (AA). In all patients, age, gender and cause of acute abdomen were recorded. In addition, native thiol (-SH), total thiol (tSH) and disulfide (-S-S-) levels at baseline, admission and on postoperative day 3 were prospectively recorded and -SS-/-SH, -S-S-/tH and -SH/tSH ratios were calculated.

Results: When the causes of acute abdomen were assessed, it was seen that 72 patients (67.29%) underwent surgery due to appendicitis while 5 patients (4.67%) due to lower GIS perforation, 7 patients (6.54%) due to perforated peptic ulcer, 5 patients (4.67%) due to sigmoid volvulus, 4 patients (3.74%) due to strangulated hernia and 14 patients (13.08%) due to miscellaneous reasons. When thiol and disulfide levels were assessed as a single parameter, mean thiol level was 316.71±78.16 (327.5) at preoperative period and 264.00±72.85 (278.30) at postoperative period. The mean thiol level was significantly decreased at postoperative period (p<0.001). The disulfide//thiol ratio was 5.17±1.56 (5.16) at preoperative period and 5.36±2.45 (5.28) at postoperative period, indicating no significant difference (p=0.563).

Conclusion: In this study, it was found that monitoring these parameters resulting from thiol oxidation are valuable at both preoperative and postoperative period in patients with acute abdomen. Further studies are needed to optimize use of oxidative stress marker together with other established marker.

Keywords: acute abdomen, disulfide, laboratory study, thiol

Öz

Amaç: Akut karın takibinde yeni bir oksidatif stres belirteci olan tiyol ve disülfit homeostazının rolünü araştırmak.

Gereç ve Yöntem: Bu çalışma prospektif olarak planlanmıştır. Bu çalışmaya akut karın(AK) tanılı 107 (62 erkek ve 45 kadın) hasta dahil edildi.107 hastanın yaşı, cinsiyeti, akut karın sebepleri, pre-operatif yatış anındaki ve post-operatif 3. gündeki native thiol (-SH), total thiol (tSH) ve disülfit (-S-S-) seviyeleri prospektif olarak kaydedilmiş ve –SS-/-SH, -S-S -/tSH, -SH/tSH oranları hesaplanmıştır

Bulgular: Akut karına neden olan sebepler incelendiğinde 72 hasta (67,29%) apandisit, 5 hasta (4,67%) alt GIS perforasyonu, 7 hasta (6,54%) peptik ulcus perforasyonu, 5 hasta (4,67%) sigmoid volvulus, 4 hasta (3,74%) strangüle herni, 14 hasta ise (13,08%) diğer nedenlerden opere olmuştu. Hastaların thiol ve disülfit seviyeleri tek parametre olarak incelendiğinde pre-operatif dönemde thiol düzeyi ortalaması 316,71±78,16 (327,5) iken post-operatif dönemde ortalama 264,00±72,85 (278,30) olarak hesaplandı, istatistiki anlamlı farklılık gösterecek şekilde post-operatif dönemde azalma saptandı (p<0,001). Thiol ve disülfit düzeylerinin birbirleri ile oranlarına bakıldığında pre-operatif dönemde disülfit/thiol oranı ortalaması 5,17±1,56 (5,16) iken post-operatif dönemde ortalama 5,36±2,45 (5,28) olarak hesaplandı, istatistiki anlamlı farklılık gözlemlenmedi (p=0,563).

Sonuç: Bu çalışma AK'lı hastalarda tiyol oksidasyonunun bir sonucu olarak ortaya çıkan bu parametrelerin takibinin, gerek preoperatif ve gerekse postoperatif donemde önem arz ettiği bulundu. Bu yeni oksidatif stres belirtecinin diğer yerleşik yaklaşımlarla birlikte kullanımını optimize etmek için daha ileri çalışmalar gereklidir.

Anahtar Kelimeler: akut karın, disülfid, laboratuvar çalışması, tiyol

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INTRODUCTION

Acute abdomen can occur due to an infection, inflammation, vascular occlusion or intestinal obstruction. The patients generally present with sudden onset of abdominal pain with nausea or vomiting.[1]

History and physical examination are important in the approach to the patient with acute abdomen. The localization and character of the pain are key elements in the diagnosis. The presence of free air in the abdomen can be presented with pain in all quadrants. Bowel sounds are assessed during auscultation. Auscultation can reveal lacking or decreased bowel sounds while palpation can show rebound tenderness suggesting peritonitis. The causes of acute abdomen include appendicitis, perforated peptic ulcer, acute pancreatitis, perforated sigmoid diverticulitis, ovarian torsion, volvulus, ruptured aortic aneurysm, splenic or hepatic rupture and intestinal ischemia.[2,3]

Despite advances in the diagnosis and treatment of acute abdomen, laboratory parameters used in the follow-up remain to be routine blood parameters. In recent years, several studies have demonstrated potential role of oxidative stress parameters resulting from acute abdomen in the pathogenesis of the disease.[4-6] In fact, it was proven that some biochemical parameters used to define oxidative stress and inflammation are markers for diagnosis and identification of clinical aspects in acute appendicitis, one of the leading causes of acute abdomen. It is known that plasma thiols are free radical scavengers and have antioxidant function through several mechanisms. Again, it is also known that plasma total thiol measurement and estimation of thiol/disulfide homeostasis are representative for excessive free radical formation in several diseases.[5,6]

In this study, we investigated clinical value of serum thiol and disulfide levels in patients with acute abdomen.

MATERIAL AND METHOD

The study was approved by Ethics Committee on Clinical Research of Health Sciences University, Ankara Keçiören Teaching and Research Hospital (28.12.2016 / 2012-KAEK-15/1245).

In the study, we screened patients who were examined and underwent surgery with a diagnosis of acute abdomen at General Surgery Clinic of Health Sciences University, Ankara Keçiören Teaching and Research Hospital. The patients aged <18 years; those with history of smoking or alcohol consumption; those with presence of any infection foci other than acute abdomen; those with acute or chronic renal disease; those with known hematological-oncologic disease; those with heart disease, chronic obstructive pulmonary disease, diabetes mellitus, hypertension or rheumatic disease; and those using any drug with antioxidant effect were excluded. Overall, 107 patients were included to the study. The plasma samples were obtained from patients and stored at -80°C until assays in central laboratory. Thiol-disulfide homeostasis primarily involves degradation of dynamic disulfide bonds (-S-S-). The degradation into functional thiol groups (-SH) was achieved by sodium borohydride. Total thiol content was analyzed using modified Ellman's reagent. The sodium borohydride residues were removed by formaldehyde during process. The amount of native thiol (-SH) was estimated from total thiol (tSH) content. The half of the difference was defined as amount of -S-S- bound. In all patients, age, gender and cause of acute abdomen were recorded. In addition, native thiol (-SH), total thiol (tSH) and disulfide (-S-S-) levels at baseline, admission and on postoperative day 3 were prospectively recorded and -SS-/-SH, -S-S-/tSH and -SH/tSH ratios were calculated.

All statistical analyses were performed using IBM SPSS for Windows version 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics are presented as count and percent for categorical variables whereas mean ± standard deviation and median for numerical variables. The normality of data distribution was assessed using Shapiro-Wilk test. The relations among variables were analyzed using Pearson's correlation tests and Spearman's correlation coefficient where appropriate. For binary comparisons, Paired samples t test was used to compare -S-S- level while Wilcoxon signed rank test was used to compare SH and tSH levels and -S-S-/-SH, -S-S-/tSH and -SH/tSH ratios. A p value<0.05 was considered as statistically significant.

RESULTS

Mean age was 42.78±18.3 (38) years in the study population. The study included 62 men (57.9+%) and 45 (42.06%) women. When the causes of acute abdomen were assessed, it was seen that 72 patients (67.29%) underwent surgery due to appendicitis while 5 patients (4.67%) due to lower GIS perforation, 7 patients (6.54%) due to perforated peptic ulcer, 5 patients (4.67%) due to sigmoid volvulus, 4 patients (3.74%) due to strangulated hernia and 14 patients (13.08%) due to miscellaneous reasons (**Table 1**).

When thiol and disulfide levels were assessed as a single parameter, mean thiol level was 316.71±78.16 (327.5) at preoperative period and 264.00±72.85 (278.30) at postoperative period. The mean thiol level was significantly decreased at postoperative period (p<0.001). Mean total thiol level was 348.6±83.55 (361.2) at preoperative period and 290.98±77.39 (309.70) at postoperative period. There was a significant decrease in total thiol level at postoperative period (p<0.001). Mean disulfide level was 15.95±5.02 (16.15) at preoperative period and 13.49±5.43 (13.85) at postoperative period. Again, disulfide level was significantly decreased at postoperative period (p<0.001).

Table-1: Comparison of thiol-disulfide levels between preoperative and on postoperative day 3

Variables	Pre-operative	Post-operative	Statistical Significance
Age	42.78±18.3 (38)		
Gender			
Male	62 (57.94%)		
Female	45 (42.06%)		
Thiol (-SH)	316.71±78.16 (327.5)	264.00±72.85 (278.30)	<0.001
Total thiol (tSH)	348.6±83.55 (361.2)	290.98±77.39 (309.70)	<0.001
Disulfide (-S-S-)	15.95±5.02 (16.15)	13.49±5.43 (13.85)	<0.001
-S-S-/-SH	5.17±1.56 (5.16)	5.36±2.45 (5.28)	0.563
-S-S-/tSH	4.65±1.28 (4.68)	4.75±1.94 (4.78)	0.584
-SH/tSH	90.7±2.55 (90.64)	90.49±3.87 (90.45)	0.584
Diagnosis			
Appendicitis	72 (67.29%)		
Peptic ulcer perforation	7 (6.54%)		
Lower GIS Perforation	5 (4.67%)		
Sigmoid volvulus	5 (4.67%)		
Strangulated Hernia	4 (3.74%)		
Other	14 (13.08%)		

When native thiol to disulfide ratio was assessed, it was found that disulfide/native thiol ratio was 5.17±1.56 (5.16) at preoperative period and 5.36±2.45 (5.28) at postoperative period, indicating no significant difference (p=0.563). Mean disulfide/total thiol level ratio was 4.65±1.28 (4.68) at preoperative period and 4.75±1.94 (4.78) at postoperative period, indicating no significant difference (p=0.584). Again, mean native thiol/total thiol ratio was 90.7±2.55 (90.64) at preoperative period and 90.49±3.87 (90.45) at postoperative period, indicating no significant difference (p=0.584).

DISCUSSION

We showed that thiol and disulfide parameters are valuable tests in the diagnosis and differential diagnosis of acute abdomen. These parameters were investigated in the diagnosis of many causes of acute abdomen.[7-9] In addition to inflammatory markers, oxidative stress markers have recently become focus of interest in research efforts. In the literature, there is limited number of experimental and clinical trials addressing relationship between acute abdomen and oxidative stress.[10,11]

Chemically, thiols are formed by carbon atom binding to a sulfur atom. In addition, the carbon also binds a hydrogen atom. This structure also includes a sulfhydryl group.[12] The disulfide bonds can be reduced to thiol group. Thiols comprise majority of total antioxidant capacity, providing protection against reactive oxygen species. In addition, they play role in detoxification and programmed cell death.[13,14]

Recently, it was shown that disruption in thiol/disulfide homeostasis plays role in the pathogenesis of several acute and chronic diseases.[15] The measurement of serum thiol level reveals their roles in antioxidant defense system.[14] Thus, the decreased amounts at postoperative period can be considered as a marker for simultaneous reduction in oxidative stress. Dynamic thiol/disulfide measurement was first introduced by Ere and Neselioglu.[13] In our study, we also investigate dynamic thiol/disulfide homeostasis parameters and their value in the diagnosis in patients with acute abdomen.

In clinical practice, C-reactive protein and white blood cell (WBC) count are used to predict severity of inflammation at preoperative period and during follow-up at postoperative period in patients with acute abdomen. Previous studies have shown that CRP value is increased in case of acute abdomen and reaches to maximum level in case of perforation. Again, it was shown that the serum CRP level increased at preoperative period was decreased at postoperative period.[16-19] Similarly, it was found that serum thiol and disulfide levels were significantly increased at preoperative period, which were, then, decreased at postoperative period. Given the correlation between severity of inflammation and elevation in disulfide/native thiol ratio, it can be suggested that disulfide/native thiol ratio can be used as a marker for disease progression and activity. Although plasma thiol and disulfide measurements aren't routinely used in the diagnosis of acute abdomen, the serum levels with apparent increase at baseline are important in the diagnosis while decreased serum levels at postoperative period can be considered as a marker for regression of disease and recovery.

This study has some limitations, Firstly, it is a pilot study addressing thiol and disulfide homeostasis parameters in the diagnosis and follow-up of acute abdomen for the first time. Secondly, the sample size is relatively limited in this single-center study. Thirdly, we failed to correlate the diagnostic tools such as serum procalcitonin, CRP, WBC count, sonography and/or computed tomography with parameters of thiol and disulfide homeostasis. Finally, thiol/disulfide homeostasis was studied in frozen samples, rather than fresh samples, at a central laboratory. However, the prospective

design is an important strength of the study. In addition, our results showed that these parameters can be used in the diagnosis and follow-up of patients with acute abdomen in the future. Moreover, our study is valuable as it offers novel parameters that can be considered in case of inconclusive clinical and laboratory findings.

CONCLUSION

In this study, it was shown that the serum thiol and disulfide levels were increased at preoperative period while they were decreased at postoperative period in patients with acute abdomen but their ratio to each other showed no significant change. Thus, above-mentioned parameters produced by thiol oxidation are valuable in the diagnosis at preoperative period and as a marker for recovery at postoperative follow-up. Further studies are needed to optimize use this novel oxidative marker together with established parameters.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Kecioren Training and Research Hospital Ethics Committee (Date: 28.12.2016, Decision No: 2012-KAEK-15/1245).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article / Orijinal Araştırma

The Change In Disease Severity and Medication Adherence of Patients Registered in Community Mental Health Center in the COVID-19 Pandemic

COVID-19 Pandemisinde Toplum Ruh Sağlığı Merkezine Kayıtlı Hastaların Hastalık Şiddeti ve İlaç Uyumlarında Ki Değişim

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Abstract

Aim: In this study, we aimed to investigate the changes in disease severity and medication adherence of patients who stayed away from Community Mental Health Center (CMHC) activities during the COVID-19 pandemic period although they participated more regularly in CMHC activities before the COVID-19 pandemic.

Material and Method: 54 patients who regularly attended CMHC were included in the study retrospectively. The first interview in this study was held in January 2020, and the second interview was held in June 2021. Positive and Negative Syndrome Scale (PANSS), Young Mania Rating Scale and Morisky Treatment Adherence Scale (MTAS) were evaluated in the study.

Results: The increase in the PANSS total 2 score compared to the PANSS total 1 score, the increase in the PANSS positive 2 score compared to the PANSS positive 1 score, the increase in the YMRS 2 score compared to the YMRS 1 score, and the decrease in the MMAS 2 score compared to the MMAS 1 score were found to be significant ($p<0.001$, $p<0.001$, $p=0.002$, $p<0.001$, respectively). Those who were registered in CMHC for a longer period of time and who participated in CMHC activities for more active days in a week had higher PANSS total 2 scores, PANSS positive 2 scores, YMRS 2 scores, and lower MMAS 2 scores.

Conclusion: In our study, patients who could not participate actively in CMHC due to social isolation had an increase in the severity of their disease and a decrease in their medication adherence.

Keywords: COVID-19, disease severity, medication adherence, community mental health center

Öz

Amaç: Bu çalışmada COVID-19 pandemisinden önce Toplum Ruh Sağlığı Merkezi (TRSM) faaliyetlerine daha düzenli katılım gösteren hastaların, pandemi sürecinde TRSM'den uzak kalmaları ile hastalık şiddetlerinde ki ve ilaç uyumlarında ki değişimi araştırmayı amaçladık.

Gereç ve Yöntem: TRSM'ye düzenli katılım gösteren 54 hasta retrospektif olarak çalışmaya dahil edildi. Bu çalışmada ki birinci görüşme 2020'nin ocak ayında, ikinci görüşme ise 2021'in haziran ayında yapıldı. Çalışmada Pozitif ve Negatif Sendrom Ölçeği (PANSS), Young Mani Derecelendirme Ölçeği ve Morisky Tedavi Uyum Ölçeği (MTUÖ) değerlendirildi.

Bulgular: PANSS toplam1 puanına göre PANSS toplam 2 puanında ki artış anlamlı bulunmuştur ($p=0,000<0,05$). PANSS pozitif 1 puanına göre PANSS pozitif 2 puanında ki artış anlamlı bulunmuştur ($p=0,000<0,05$). YMDÖ 1 puanına göre YMDÖ 2 puanında ki artış anlamlı bulunmuştur ($p=0,002<0,05$). MTUÖ 1 puanına göre MTUÖ 2 puanında ki düşüş anlamlı bulunmuştur ($p=0,000<0,05$). Daha uzun süredir TRSM'ye kayıtlı olan ve 1 haftada aktif gelinen gün sayısı daha fazla olanlarda PANSS toplam 2, PANSS pozitif 2, YMDÖ 2 puanları daha yüksek; MTUÖ 2 puanları daha düşüktü.

Sonuç: Çalışmamızda sosyal izolasyon nedeniyle TRSM'ye aktif katılım gösteremeyen hastaların hem hastalık şiddetlerinde artış, hem de ilaç tedavisine uyumlarında azalma olmuştur.

Anahtar Kelimeler: COVID-19, hastalık şiddeti, ilaç uyumu, toplum ruh sağlığı merkezi

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INTRODUCTION

The concept of community mental health deals with the psychiatric condition, treatment and care of individuals related to themselves and their environment. The community-based mental health model aims to ensure that patients are treated in the community without leaving the community, in order to continue their follow-up, to enable them to gain the functional skills necessary for vital processes, and to prevent hospitalizations.[1] Community mental health centers (CMHCs) are centers that are run with a community-based mental health model, where patients are supported with psychosocial services and treatments are offered to improve their functionality. In these centers, it is aimed to prevent hospitalizations by performing outpatient follow-up and treatment of patients. In addition, some tasks are offered for the patients to provide them with functionality in their lives. These are the centers where individual and group therapies are provided apart from the drug treatments. In these centers, it is also aimed to increase the quality of life of patients. Individuals with mental illnesses such as bipolar affective disorder, schizophrenia and other psychoses are registered in these centers.[2]

On December 31, 2019, the World Health Organization (WHO) began receiving information about some cases of pneumonia of unknown cause in the city of Wuhan, China. Later research determined that the cause was coronaviruses. This new virus, previously unidentified in humans, was named COVID-19. COVID-19 is a disease that can be fatal and can cause many medical consequences. But apart from all these, it also causes many mental and psychological problems.[3] The stress associated with the pandemic can be manageable for many people. However, the situation may be more challenging in those who are more prone to anxiety or have a previous mental illness.[4] As it is known, schizophrenia has a chronic course and often requires hospitalization. The difficult disease process and ongoing disability, especially in the acute stages of the disease, create a serious financial and moral burden. [5] The episodes of bipolar affective disorder (BAD) can be very intense and challenging, and even manic episodes often require hospitalization. A serious loss of functionality occurs in social, familial and occupational areas.[6] While the course of mental illnesses such as schizophrenia and BAD was already challenging, individuals with serious mental illnesses were affected much more negatively in the COVID-19 pandemic.[7]

Owing to the fact that one of the most important measures to combat the pandemic is social isolation, the living conditions, social aspects, habits, and more importantly, the psychological status of individuals have been significantly affected.[3] In order to control and eliminate COVID-19, many restrictions, especially "lockdowns", were applied and measures were taken in many countries throughout the pandemic.[8] Within the scope of the precautions taken in our country, some measures against the pandemic had to be implemented in CMHCs, like in many other institutions. The patients had to stay away from CMHC activities for a while due to the precautions taken in consideration of the necessity of social isolation and the fact that the patients participating in CMHC services could not follow the important rules such as mask use and social distance.

Since CMHCs have an important place in the follow-up of chronic psychiatric diseases, we wanted to determine the impact of the difficult process experienced during the COVID-19 pandemic on patients. In this study, we aimed to investigate the changes in disease severity and medication adherence of patients who stayed away from CMHC activities during the COVID-19 pandemic period although they participated more regularly in CMHC activities before the COVID-19 pandemic.

MATERIAL AND METHOD

Sample

There are 543 patients registered in Elazığ CMHC. 54 of these patients come to CMHC at least once a week and benefit from CMHC activities. These 54 patients were considered as regular participants in CMHC. Some patients come and participate in CMHC from time to time. Home visits are planned for the remaining patients, and they are invited to the CMHC from time to time and examined. Personal care plan files are available for all patients registered in CMHC. These files are prepared according to the personal needs of that patient and necessary interventions are made. There are many activities such as music, painting, handicrafts and sports for patients who come to CMHC. After the start of the COVID-19 pandemic, the number of patients participating in CMHC began to decrease. The number of days in which patients with active participation came to CMHC decreased. Elazığ CMHC did not provide face-to-face service from January 2021 to May 2021. In this process, patients were followed up with online interviews. After the CMHC was reopened in May 2021, a limited number of patients - approximately 10 patients per day - were admitted into the center.

Implementation

Patients diagnosed with BAD, schizophrenia or other psychosis diagnoses according to DSM-5 diagnostic criteria, who were registered in CMHC and who regularly participated in CMHC activities for at least 6 months before the pandemic, were included in the study. Exclusion criteria from the study were determined as the presence of mental retardation, the presence of a cognitive and a neurological deficit to such an extent that the participants cannot comprehend the requirements of the scales.

54 patients who attended CMHC more regularly were included in this study. Since none of these patients had exclusion criteria, all of them were included in the study. In general, within the care plans of CMHC, patient interviews are made regularly, and their clinical course is recorded in their personal files. All data in the study were obtained from the records retrospectively. The first interview in this study was held in January 2020, before the start of the COVID-19 pandemic in Turkey. The second meeting was held in June 2021, later in the pandemic, after the start of the COVID-19 pandemic and the lockdown and restriction process.

Both interviews were carried out face to face. The Positive and Negative Syndrome Scale was applied to the patients diagnosed with schizophrenia and other psychoses. The Young Mania Rating Scale was applied to the patients with BAD. Morisky Medication Adherence Scale and sociodemographic data form were applied to all patients. The 1st scales were applied in the first interview before the pandemic started, and the 2nd scales were applied in the second interview after the pandemic started.

The study was conducted based on the ethical principles and in accordance with the principles of the Declaration of Helsinki. The study was approved by the Fırat University Clinical Research Ethics Committee (Date: 26.01.2023, No: 2023/02-15).

Data Collection Tools

Sociodemographic Data Form

The sociodemographic data form was prepared by the researchers. There are some data such as age, gender, educational status, background, employment status, and COVID history.

Positive and Negative Syndrome Scale (PANSS)

It was developed by Kay et al. in 1987.[9] The Turkish validity and reliability study of this scale was carried out by Kostakoğlu et al. in 1999. Of the 30 psychiatric parameters evaluated, 7 belong to the positive syndrome subscale, 7 belong to the negative syndrome subscale, and the remaining 16 to the general psychopathology subscale. The scale has a 7-point Likert type. Each item is evaluated between 1 and 7. 4 measurements are made: positive, negative and general psychopathology scores and a total PANSS score. An increase in the scores obtained indicates an increase in the severity of the disease.[10]

Young Mania Rating Scale (YMRS)

Developed by Young et al. (1978), this scale consists of 11 items.[11] It is used to measure the clinical status of the patient in the last week and the severity of the disease. 7 items are in the 5-point Likert type, and the other 4 items are in the 9-point Likert type. A minimum of 0 and a maximum of 60 points can be obtained from the scale. It is thought that the higher the score is, the higher the severity of mania is. The Turkish validity and reliability study of this scale was performed by Karadağ et al. in 2001.[12]

Morisky Medication Adherence Scale-8 Items (MMAS-8)

It is a scale used to evaluate patients' adherence to drug therapy. It was developed by Morisky et al.[13] It consists of 8 questions. In the first 7 questions, one can get 0 point for each "yes" answer and 1 point for each "no" answer. Question 8 is in 5-point Likert type. If the score obtained according to the answers given by the patient to the scale is <6, it is considered as low level of medication adherence, if it is between 6 and <8, it is considered as moderate level of medication adherence, and if it is 8, it is considered as high level of medication adherence. The Turkish validity and reliability study was performed by Aşılar et al. in 2014.[14]

Statistical Analysis

The data obtained in the research were evaluated in the computer environment through the SPSS 22.0 statistical program. Frequency and percentage analyses were used to determine the descriptive characteristics of the patients participating in the study and mean and standard deviation statistics were used to analyze the scales. Kurtosis and Skewness values were examined to determine whether the research variables showed a normal distribution. It was determined that the research variables showed a normal distribution. Parametric methods were used in the analysis of the data. The change in the scores of the first measurement and the second measurement was analyzed with the dependent groups t-test. T-test, one-way analysis of variance (Anova) and post hoc analyses were used to examine the differences in scale levels according to the descriptive characteristics of the patients.

RESULTS

Participants consisted of 44 men and 10 women. The mean age was 41.407. The distribution of sociodemographic characteristics of the patients is shown in **Table 1**.

Table 1. Distribution of Sociodemographic Data

	Frequency (n)	Percentage (%)
Gender		
Male	44	81.5
Female	10	18.5
Marital Status		
Married	10	18.5
Single/Widow(er)	44	81.5
Education Level		
Illiterate	22	40.7
Primary School	22	40.7
Secondary School	10	18.5
Employment Status		
Employed	6	11.1
Unemployed	48	88.9
Diagnoses		
BAD	28	51.9
Schizophrenia and other psychoses	26	48.1
The duration of being registered in CMHC		
<1 Year	9	16.7
1-5 Years	20	37.0
>5 Years	25	46.3
The Number of Days for which Patients Show Active Participation in 1 Week in CMHC		
1 Day	9	16.7
2-3 Days	20	37.0
4-5 Days	25	46.3
History of COVID-19		
Present	14	25.9
None	40	74.1
	Mean	SD
Age	41.407	10.262

BAD: Bipolar Affective Disorder, CMHC: Community Mental Health Center

When the change of the scales in the 2nd interview compared to the 1st interview was examined, the increase in the PANSS total 2 score ($\bar{x}$=66.000) compared to the PANSS total 1 score ($\bar{x}$=55.654) was significant (t=-5.308; p<0,001). The increase in PANSS positive 2 score ($\bar{x}$=31.154) compared to PANSS positive 1 score ($\bar{x}$=21.231) was significant (t=-5.004; p<0.001). The increase in the YMRS 2 score ($\bar{x}$=25.357) compared to the YMRS 1 score ($\bar{x}$=18.214) was found to be significant (t=-3.399; p=0.002<0.05). Compared to the MMAS 1 score ($\bar{x}$=5.741), the decrease in the MMAS 2 score ($\bar{x}$=4.463) was found to be significant (t=4.776; p<0.001) (**Table 2**).

The differentiation status of the scales applied in the 2nd interview according to some sociodemographic data is shown in **Table 3** (**Table 3**).

Table 2. Change of Scales Between the 1st and 2nd Interviews

Scales	N	Mean ± SD	t	p
PANSS total1	26	55.654±9.099	-5,308	<0.001
PANSS total2	26	66.000±14.218		
PANSS positive1	26	21.231±9.446	-5,004	<0.001
PANSS positive2	26	31.154±10.739		
PANSS negative1	26	17.615±7.627	-0,947	0.353
PANSS negative2	26	17.846±7.724		
PANSS general psychopathology 1	26	16.808±6.645	-0,795	0.434
PANSS general psychopathology 2	26	17.000±6.663		
YMRS1	28	18.214±8.774	-3,399	0.002
YMRS2	28	25.357±12.615		
MMAS1	54	5.741±1.277	4,776	<0.001
MMAS2	54	4.463±1.870		

Dependent Groups T-Test, MMAS: Morisky Medication Adherence Scale, PANSS: Positive and Negative Syndrome Scale, YMRS: Young Mania Rating Scale.

Table 3. Differentiation of the Scales Applied in the 2nd Interview According to Some Sociodemographic Data

Sociodemographic Data	n	PANSS total 2	PANSS positive 2	PANSS negative 2	PANSS general psychopathology 2	YMRS 2	MMAS 2
The duration of being registered in CMHC		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
<1year	4	53.50±13.20	17.50±12.61	16.75±4.92	19.25±7.58	11.80±2.16	6.22±0.83
1-5 Years	10	59.20±7.23	26.20±6.40	18.30±6.96	14.70±4.69	19.20±7.36	5.25±1.61
>5 Years	12	75.83±12.83	39.83±3.58	17.83±9.41	18.16±7.68	35.30±10.02	3.20±1.44
F=		9.21	22.05	0.05	1.00	18.88	19.40
p=		0.001	<0.001	0.94	0.38	<0.001	<0.001
PostHoc=		3>1, 3>2 (p<0.05)	2>1, 3>1, 3>2 (p<0.05)			3>1, 3>2 (p<0.05)	1>3, 2>3 (p<0.05)
The Number of Days for which Patients Show Active Participation in 1 Week in CMHC		**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
1 Day	4	58.00±13.31	20.75±11.23	17.00±3.36	20.25±5.50	9.60±2.70	6.33±1.41
2-3 Days	8	60.37±15.48	24.62±10.48	22.00±7.01	13.75±5.87	22.58±8.70	5.05±1.57
4-5 Days	14	71.50±12.12	37.85±5.05	15.71±8.36	17.92±7.01	35.54±9.79	3.32±1.46
F=		2.60	10.90	1.82	1.64	16.99	15.83
p=		0.09	<0.001	0.18	0.21	<0.001	<0.001
PostHoc=			3>1, 3>2 (p<0.05)			2>1, 3>1, 3>2 (p<0.05)	1>2, 1>3, 2>3 (p<0.05)
History of COVID-19 Infection		**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
BAD	Schizophrenia and other psychoses						
Present 6	8	59.62±9.88	27.62±14.97	15.25±6.04	16.75±6.15	23.50±10.40	4.78±2.15
None 22	18	68.83±15.14	32.72±8.29	19.00±8.25	17.11±7.04	25.86±13.32	4.35±1.77
t=		-1.56	-1.12	-1.15	-0.12	-0.40	0.74
p=		0.13	0.39	0.26	0.9	0.69	0.45
Diagnoses		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
BAD	28	-	-	-	-	-	4.92±1.74
Schizophrenia and other psychoses	26	-	-	-	-	-	3.96±1.90
t=							1.94
p=							0.057

F: Anova Test; t: Independent Groups T-Test; PostHoc: Tukey, LSD, BAD: Bipolar Affective Disorder, MMAS: Morisky Mental Adherence Scale, PANSS: Positive and Negative Syndrome Scale, CMHC: Community Mental Health Center, YMRS: Young Mania Rating Scale.

DISCUSSION

According to the results we obtained in our study, PANSS total, PANSS positive and YMRS scores were significantly higher and MMAS scores were significantly lower in the second interview after the start of the COVID-19 pandemic, compared to the first interview we conducted before the COVID-19 pandemic.

The COVID-19 pandemic affected the whole world in a short time and changed the living conditions considerably. In order to prevent the transmission of this viral epidemic all over the world, many measures were taken, such as restricting social and community movements, closing educational institutions, isolating infected cases, quarantining suspected cases and imposing lockdown across the country. These measures were expected to be effective in preventing transmission.[15] On the other hand, while cases with a rate of 96% and deaths with a rate of 76% are expected for communities that do not apply quarantine measures, cases with a rate of 44% and deaths with a rate of 31% are expected in communities where quarantine measures are applied.[16] Taking preventive measures against the pandemic has reduced the incidence and death rates. Although these measures have benefits, it also causes many mental problems such as feeling frustrated, unhappiness, panic and fear.[15] For example, in the general population in a systematic review, depression (14.6% to 48.3%), anxiety (6.33 to 50.9%), post-traumatic stress disorder (7% to 53.8%), psychological distress (34.43% to 38%), and stress (8.1% to 81.9%) were detected.[17] Many measures against the pandemic, such as social isolation, were implemented in our country.

Coronaviruses may be associated with psychotic symptoms through an immune-related mechanism, and therefore COVID-19 infection may exacerbate symptoms in patients with schizophrenia.[18] In addition, corticosteroids can be applied for treatment and these interventions may cause psychosis.[19] As it is known, patients with schizophrenia have lower quality and smaller social networks compared to the general population.[20] Social support has been associated with higher scores on measures of recovery in schizophrenia. And again, living in contact with society and social integration have been associated with recovery in schizophrenia.[21] The social isolation rules that they must follow due to the COVID-19 pandemic will pose a risk for these patients. In our study, we observed an increase in the severity of the disease in patients with schizophrenia and other psychoses during the pandemic period. Compared to the 1st interview before the COVID-19 pandemic, the PANSS total and PANSS positive scores in the 2nd interview after the pandemic started were higher.

Because important life events may trigger mood instability, BAD patients may constitute a vulnerable group against COVID-19 measures.[22] With pandemic measures, there are changes in biological rhythms such as sleep, activity, and social rhythm.[23] Thus, more potential adverse effects occur in patients with bipolar disorder compared to healthy controls.[24] Bipolar disorder patients may experience intense emotions arising from the serious health crisis caused by the COVID-19 pandemic and are vulnerable to stress that may arise from radical changes in life habits such as social isolation.[25] This pandemic and the measures necessary to control it have significant risks for the recurrence of bipolar disorder.[26] In a longitudinal study conducted during the COVID-19 pandemic, it was observed that bipolar disorder patients, compared to the controls, were more affected by "stay at home" calls made within the scope of measures taken against the pandemic.[27] In another study, it was observed that there was an increase in manic symptomatology in the early stages of the pandemic compared to the period prior to COVID-19 era in bipolar disorder patients. And these symptoms decreased after quarantine measures were eased.[28] In our study, there was an increase in the severity of the disease in BAD patients during the pandemic period. Compared to the 1st interview before the COVID-19 pandemic, the YMRS scores in the 2nd interview carried out after the pandemic started were higher.

In the COVID-19 pandemic, recurrences in severe mental disorders may result in failure to implement social distancing or other preventive strategies, failure to seek medical attention, and failure to comply with expected treatment.[29] In a study investigating medication adherence of patients with psychiatric illness during the COVID-19 pandemic, it was observed that 39% of patients did not comply with the treatment, while 26% of them had moderate and 35% of them had poor medication adherence.[30] In a study conducted in a psychiatric hospital, it was found that there was a 49% decrease in the use of long-acting injectable risperidone, and a 70-90% decrease in the use of long-acting injectable olanzapine, aripiprazole, and paliperidone in March 2020 compared to the previous 3-month period.[31] In a study that included patients with schizoaffective disorder, BAD, and schizophrenia during the COVID-19 pandemic, it was observed that the symptoms of 30% of the patients worsened during quarantine and 1 out of 5 patients discontinued their psychiatric medications.[32] In the results of our study, there was a significant decrease in medication adherence of the patients during the pandemic period. Compared to the 1st interview held before the COVID-19 pandemic, the MMAS scores in the 2nd interview carried out after the pandemic started were lower.

It should be emphasized that the perceived distress of those with serious mental illness due to the COVID-19 pandemic and mass quarantine is greater than that perceived by the general population. In a study conducted in Italy one month after the quarantine, those with severe mental illness were compared with the healthy controls. In the results they obtained, it was seen that the risk of perceiving more stress related to the pandemic was four times higher, and they had a twice-three times higher risk of anxiety and depression. Similar results were found in patients with schizophrenia and mood disorders.[33] In our study, according to the results

of the second interview, patients with longer registered time in CMHC and more days for which patients show active participation in 1 week in CMHC had higher disease severity and lower medication adherence. This result may indicate that longer and more participation in CMHC activities will be supportive to maintain disease severity and medication adherence. In a study conducted to show the effects of the services provided in CMHC on the patients, it was found that the patients' adherence to medical treatment, quality of life, general and social functionality and insight increased significantly thanks to the services provided, while the symptoms of their diseases decreased significantly.[34] In another study, it was shown that psychosocial adjustment services in addition to pharmacotherapy received by patients in CMHC contributed positively to patients' functionality, insight, medication adherence, and clinical data.[35] Our study also shows that the pandemic process, in which CMHC services are not sufficient, causes negative consequences for patients in terms of both disease severity and medication adherence.

Our study had some limitations. This study was conducted with a limited sample size and in a single center. New studies to be conducted in centers with more patients participating in CMHC activities are important in terms of supporting the results of this study. In addition, in this study, the drugs used by the patients were not determined while evaluating their medication adherence.

CONCLUSION

Social support is very important in the treatment of mental illnesses. Developed with a community-based mental health model, CMHCs offer important opportunities for patients to adapt to society, for their social lives, their medication adherence, and the follow-up and treatment of their diseases. The COVID-19 pandemic has had negative effects in our country as it has affected the whole world. Social isolation methods applied to prevent the pandemic have also affected individuals with mental illnesses, just like the general population. In our study, it was seen that patients who could not participate actively in CMHC due to social isolation had an increase in the severity of their disease and a decrease in their medication adherence. These results show both the negative effects of the COVID-19 pandemic on patients and the importance of CMHC services for the patients.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was approved by the ethics committee of Fırat University (Date: 26.01.2023, No: 2023/02-15).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Original Article / Orijinal Araştırma

The Comparison of the Comorbidities of Patients with Peritoneal Dialysis and Hemodialysis with the Charlson Comorbidity Index

Periton Diyalizi ve Hemodiyaliz Hastalarının Komorbiditelerinin Charlson Komorbidite İndeksi ile Karşılaştırılması

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Abstract

Aim: Hemodialysis and peritoneal dialysis are renal replacement treatment options in patients with chronic kidney disease. Mortality and morbidity rates are higher in hemodialysis and peritoneal dialysis patients when compared to the healthy population. Comorbidities of the patients play roles in the high mortality and morbidity rates. In the present study, the comorbidities of hemodialysis and peritoneal dialysis patients were evaluated; and the Charlson Comorbidity Index, whose reliability was proven in many studies before, was compared and discussed.

Material and Method: A total of 154 patients (78 hemodialysis and 76 peritoneal dialysis patients), who were followed up for end-stage renal disease, were included in the study. The Charlson Comorbidity Index scores of the patients were calculated. The Charlson Comorbidity Index score and parameters were compared between patient groups on hemodialysis and peritoneal dialysis.

Results: The Charlson Comorbidity Index was found to be significantly higher in peritoneal dialysis patients than in hemodialysis patients (p=0.001). It was also found that the frequency of age,congestive heart failure, cerebrovascular accident and connective tissue which are the parameters of the Charlson Comorbidity Index, were significantly different between the groups (p<0.05).

Conclusion: Charlson comorbidity index parameters, which may cause mortality and morbidity, were found more frequently in peritoneal dialysis patients compared to hemodialysis patients.

Keywords: Hemodialysis, peritoneal dialysis, Charlson Comorbidity Index

Öz

Amaç: Hemodiyaliz ve periton diyalizi kronik böbrek yetmezlikli hastalarda renal replasman tedavisi seçeneklerindendir. Hemodiyaliz ve periton diyalizi hastalarında sağlıklı popülasyona göre mortalite ve morbidite oranları yüksektir. Mortalite ve morbidite oranlarının yüksek olmasında hastaların sahip olduğu komorbiditelerde rol oynamaktadır. Biz bu çalışmada hemodiyaliz ve periton diyalizi hastalarının komorbiditelerini; daha önce güvenilirliği pek çok çalışma ile ispatlanmış olan Charlson Komorbidite İndeksi ile karşılaştırmayı ve tartışmayı amaçladık.

Gereç ve Yöntem: Çalışmamıza son dönem böbrek yetmezliği nedeni ile takip edilen 78 hemodiyaliz, 76 periton diyalizi hastası olmak üzere toplamda 154 hasta dahil edildi. Bu hastaların Charlson komorbidete indeksi puanları hesaplandı. Hemodiyaliz ve periton diyalizindeki hasta grupları arasında Charlson komorbidite indeksi skoru ve parametreleri karşılaştırıldı.

Bulgular: Charlson komorbidite indeksi periton diyalizi hastalarında hemodiyaliz hastalarına göre anlamlı şekilde yüksek bulundu(p=0.001). Gruplar arasında Charlson Komorbidite İndeksi parametrelerinden olan yaş, konjestif kalp yetmezliği, serebrovasküler olay ve konnektif bağ dokusu sıklığının anlamlı şekilde farklı olduğu görüldü(p<0.05).

Sonuç: Periton diyalizi yapan hastalarda hemodiyaliz hastalarına göre mortalite ve morbiditeye neden olabilecek Charlson komorbidite indeksi parametreleri sık bulunmuştur.

Anahtar Kelime: Hemodiyaliz, periton diyalizi, Charlson komorbidite indeksi

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INTRODUCTION

Chronic Kidney Disease (CKD) is a disease characterized by permanent loss of kidney functions because of kidney or systemic diseases and requires cost-effective Renal Replacement Treatments (RRT). Many systems such as the cardiovascular system, musculoskeletal system, and central nervous system can be affected by the disease. When the Glomerular Filtration Rate (GFR) is <15, it is considered as End-Stage Renal Disease and renal replacement treatments come to the forefront(1). Currently, Hemodialysis (HD), Peritoneal Dialysis (PD), and Renal Transplantation are applied as renal replacement treatments.

Mortality and morbidity rates are higher in dialysis patients (HD and PD) when compared to the healthy population. Mortality and morbidity in this patient group are closely related to the comorbidities of patients (Diabetes Mellitus (DM), Hypertension (HT), cardiovascular diseases, malignancy, etc.) (2). Knowing these comorbidities that patients have is important to decide on the appropriate dialysis modality for the patient and prevent complications that may arise.

The Charlson Comorbidity Index (CCI) was developed by Charlson et al. in 1987 as a scoring system used to classify patients' comorbidities, determine their severity, and predict their mortality. Each comorbidity of the patient has a score between 1-6 points and a total score of 0-37 is obtained in this scoring system(3). Many previous studies reported that it is a very reliable scoring system in determining the burden of disease and mortality risk(4,5).

In the present study, the purpose was to calculate and compare the comorbidity status of patients with End-Stage Renal Disease who received HD/PD as RRT using the Charlson Comorbidity Index.

MATERIAL AND METHOD

For the study, the approval of the Afyonkarahisar University of Health Sciences Ethics Committee was received on 04.11.2022 with the number 2022/546. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

A total of 154 patients, who underwent HD and PD with the diagnosis of End-Stage Renal Disease between 2016 and 2022 in Afyonkarahisar Health Sciences University Faculty of Medicine, Department of Internal Medicine, Department of Nephrology, Hemodialysis and Peritoneal Dialysis Unit, were included in the study. The patients were divided into 2 groups as HD and PD groups. The data of the patients were obtained from the hospital data system and the patients were evaluated on 19 parameters in accordance with the Charlson Comorbidity Index. The patients were given points according to the following criteria.

"1" point was given for each of the following parameters: Myocardial Infarction (past, excluding ECG changes only), Congestive Heart Failure, Peripheral Vascular Disease (including ≥6 cm aortic aneurysm), Cerebrovascular Disease (SVO, TIA), Dementia, Chronic lung disease, Connective tissue disease, Peptic ulcer, Mild liver disease (including chronic hepatitis), no target organ damage Diabetes Mellitus organ uncomplicated (except only those controlled by diet).

"2" points for each of the following parameters: Hemiplegia, Moderate or severe kidney disease, Diabetes Mellitus with target organ damage, non-metastatic tumor (not included if 5 years have passed since diagnosis), Leukemia (acute or chronic), Lymphoma.

"3" points were given in case of moderate or severe liver disease.

"6" points were given for each parameter in case of metastatic solid tumor or AIDS (only HIV-positive patients are not scored).

Regardless of these parameters, "1" point was given to the total score for each decade after the age of 40, and the total scores of the patients were then calculated. The age, gender, cause of chronic renal failure, and calculated CCI were compared in HD and PD patients.

Statistical Analysis

The categorical variables were presented as frequency and percentage. The Chi-Square Test was used when comparing the categorical variables between the groups. The conformity of the continuous variables to the normal distribution was checked with the Shapiro-Wilk Test. Continuous variables were expressed as mean and standard deviation if they were normally distributed, and as median and minimum-maximum if they were not normally distributed. The Independent Sample t-Test was used for the continuous variable comparison between groups. Statistical analyzes were performed with the SPSS 26.0 (IBM Corp. 2019 IBM SPSS Statistics for Windows, version 26.0. Armonk, NY: IBM Corp.) program. A $p<0.05$ value was taken as the statistical significance level.

RESULTS

The study was conducted with 154 End-Stage Renal Disease patients. HD was initiated in 78 (50.6%) of the patients who were included in the study and PD was initiated in 76 (49.4%). The mean dialysis time was 5 (1-7) years in HD patients and 4 (1-6) years in PD patients. Although 90 (58.4%) of the patients were female, 64 (41.6%) were male. The mean age was 59.8±12.2 years. It was found that the patients who started PD were significantly older than the patients who started HD ($p=0.036$), and their BMI was significantly higher ($p=0.012$). Hypertension was the most common cause of CKD in the HD group and diabetes was the most common cause of CKD in the PD group. The CKD etiologies of the groups were found to be significantly different from each other ($p=0.012$). The comparison of demographic characteristics of HD and PD patients is given in **Table 1**.

Table 1. The Comparison of the Demographic Characteristics of HD and PD patients

Characteristics	HD (n= 78)	PD (n= 76)	p
Age (Mean±SD)	57.76±12.4	61.87±11.7	0.036
Dialysis time(year)(min-max)	5 (1-7)	4 (1-6)	0.259
Gender Male (%-n) Female (%-n)	 34.6-27 65.4-51	 48.7-37 51.3-39	0.102
Smoking (%-n)	32.1-25	39.5-30	0.401
Alcohol (%-n)	9-7	14.5-11	0.324
BMI (kg/m2)	24.18±3.4	26.1±5.7	0.012
CKD Etiology DM (%-n) HT (%-n) Cr. Glomerulonephritis (%-n) Polycystic Kidney (%-n) Obstructive (%-n) Unknown	 34.6-27 41-32 16.7-13 6.4-5 1.3-1 0	 47.4-36 21.1-16 10.5-8 9.2-7 7.9-6 3.9-3	0.012
DM (%-n)	43.6-34	52.6-40	0.333
HT (%-n)	85.9-67	75-57	0.105
HL (%-n)	17.9-14	28.9-22	0.129
Myocardial infarct (%-n)	25.6-20	40.8-31	0.060
Congestive heart failure (%-n)	20.5-16	39.5-30	0.013
Peripheric vascular disease (%-n)	11.5-9	15.8-12	0.488
Cerebrovascular disease (%-n)	6.4-5	19.7-15	0.017
Dementia (%-n)	6.4-5	14.5-11	0.119
COPD (%-n)	9-7	19.7-15	0.067
Connective tissue disease (%-n)	0	7.9-6	0.013
Peptic ulcer (%-n)	10.3-8	13.2-10	0.623
Chronic liver disease (%-n)	1.3-1	2.6-2	0.618
Hemiplegia (%-n)	1.3-1	5.3-4	0.207
Solid organ malignity (%-n)	7.7-6	7.9-6	1
Leukemia (%-n)	0	1.3-1	0.494
Lymphoma (%-n)	0	3.9-3	0.118
AIDS (%-n)	0	0	NS

BMI: body mass index, CKD: Chronic kidney disease, DM: diabetes mellitus, HT: hypertension, HL: hyperlipidemia, COPD: chronic obstructive pulmonary disease, AIDS: Acquired Immune Deficiency Syndrome KC

When the groups were compared in terms of the Charlson Comorbidity Index parameters, it was found that congestive age, heart failure, cerebrovascular disease, and connective tissue disease were statistically and significantly higher in the PD group (**Table 1**).

The mean Charlson Comorbidity Index score of the HD group was 5.9±2.1 and the mean Charlson Comorbidity Index score of the PD group was 7.17±2.6. When the groups were compared in terms of the mean Charlson Comorbidity Indices, it was found that the PD group had a statistically and significantly higher Charlson Comorbidity Index score (p=0.001). **Figure 1** shows the comparison of the mean Charlson Comorbidity Indices of the HD and PD groups.

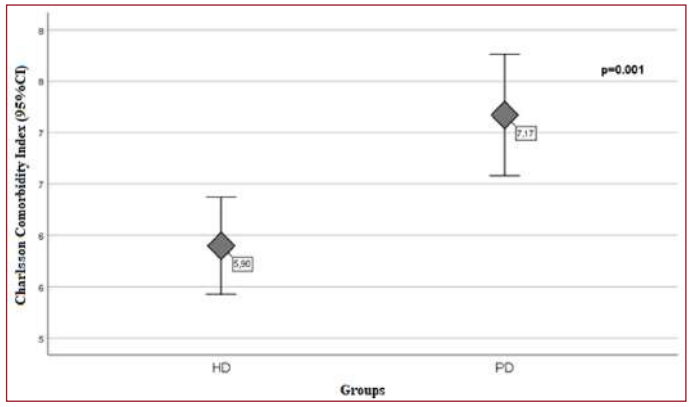

Figure 1. The Comparison of the Mean Charlson Comorbidity Indices of HD and PD Patients. HD: Hemodialysis PD: Peritoneal dialysis

DISCUSSION

Chronic kidney disease is an important public healthcare issue that can lead to systemic complications with increasing frequency. Renal replacement treatments come to the forefront when the GFR rate falls below 15. Diseases such as DM and HT are the leading causes in the etiology of CRF in patients receiving HD and PD treatment. According to the report published by the Turkish Society of Nephrology in 2020, 36.46% DM and 26.5% HT are responsible for the etiology of CRF in patients who have HD in Turkey. In patients with PD, 27.45% HT plays the first role in etiology, and it comes second with 24.28% DM (6). According to the findings of the present study, HT and DM are responsible for the etiology of CRF in HD patients, respectively, and DM and HT are the first two in etiology in PD patients, respectively. No statistical differences were detected between the patient groups in terms of DM and HT.

The Charlson Comorbidity Index is a scoring system that determines the comorbidities of patients and scores them according to the severity of their comorbidities. Previous studies showed that it can be used reliably as an indicator of morbidity and mortality(7,8). In a study that evaluated patients followed in the Intensive Care Units because of Coronavirus-19, it was shown that a 1-point increase in CCI increased mortality by approximately 32% (7). In the current study, CCI was compared in HD and PD patients. Although the CCI score was 7.17 in PD patients, it was 5.90 in HD patients. The CCI score in PD patients was statistically and significantly higher than in HD patients (p=0.001). The reason for the difference in CCI in the current study was that the parameters of age, congestive heart failure, connective tissue disease and cerebrovascular disease showed a statistically significant difference in PD patients when compared to HD patients. No statistical differences were detected between HD and PD groups in terms of myocardial infarction, peripheral vascular disease, dementia, Chronic Obstructive Pulmonary Disease, peptic ulcer, chronic liver disease, hemiplegia, solid organ malignancy, leukemia, lymphoma, and AIDS in other parameters of CCI.

According to the data of the Turkish Society of Nephrology, in Turkey at the end of 2020; There are a total of 60,558 patients receiving HD treatment and a total of 3,387 patients receiving PD treatment. Approximately 66.62% of HD patients and approximately 57.31% of PD patients are between the ages of 45-74(6). In our study, the mean age was; 57.76 in HD patients, 61.87 in PD patients. In our study, PD patients were older than HD patients; It may be related to the fact that it is an option for RRT that can be applied at home in immobile patients and in elderly patients where transport may be difficult.

Congestive Heart Failure (CHF) is very common in patients who receive hemodialysis and peritoneal dialysis as renal replacement treatment and is one of the leading causes of mortality in these patient groups. Approximately 30% of hemodialysis patients were found to have heart failure in a study that involved multicenter dialysis centers in the United States(9). Approximately 29.8% of patients who received HD and PD had CHF in the current study. There are different results in the literature regarding the frequency of heart failure in HD and PD patients. In a study conducted by Chien-Yao Sun et al. with 4754 HD and PD patients, the cumulative incidence of CHF was found to be significantly higher in HD patients than in PD patients (10). Patients who received HD and PD as Renal Replacement Treatment (RRT) were divided into 4 groups in another study in terms of treatment duration after their ages were equalized, and Left Ventricular Hypertrophy (LVH) and Ejection Fractions (EF) of the patients were compared. Long-term HD (U-HD: 165.1±52.7 months), short-term HD (F-HD: 71.3±28.9 months), long-term PD (U-PD: 76.5±13.2 months), short-term PD (F -SAPD:28.41±1.9 months) LVH ratio and EF were found to be lower in long-term PD patients when compared to other groups (11). In the current study, when compared to HD patients, HF was significantly higher in PD patients. This can be associated with dialysis time the volume load. In our study, the mean dialysis time of HD patients was 60 months, while the mean dialysis time of PD patients was 48 months, and there was no statistical difference between the two groups. Although there is a survival advantage over HD in the first months of PD in previous studies, this situation decreases especially after 2 years(12).Although a more effective volume control is expected compared to HD as the residual renal functions are preserved in PD patients between both treatment options, it becomes difficult to control hypervolemia in PD patients with the decreased residual kidney functions and urine output over time for PD patients. In a study conducted by Menon et al., it was shown that there is a deterioration in the volume status and an increase in blood pressure with the decrease of RRF in PD patients (13). Ultrafiltration is expected to be followed up regularly by the physician in each session and to occur effectively in HD patients.

Cerebrovascular events are the most common disease group among neurological diseases and are the third most common cause of mortality on a global scale (14). Regardless of the type of renal replacement, it was reported that the frequency of CVO in patients with the end-stage renal disease increases 4 to 10 times when compared to the general population (15). In a study conducted by Kebapçıoğlu et al. with 30 HD, 40 PD, and 50 control group patients, it was shown that ischemic stroke is significantly higher in HD-PD patients when compared to the control group. In the same study, no differences were detected between the HD and PD groups in terms of the frequency of ischemic stroke (14). In a study conducted by Wang et al. with HD and PD patients, it was determined that patients with hemorrhagic and ischemic stroke have similar risk factors (16). In the current study, cerebrovascular diseases were found to be higher in PD patients when compared to HD patients (p=0.017). Conditions such as DM, HT, HL, and gender, which may pose a risk for SVO, were found to be similar between the groups. This difference may be associated with the increased excretion of albumin in dialysate and the increased synthesis of some coagulation factors in PD patients, which may predispose to a thrombophilic state. Martins et al. reported that the risk of thrombosis increased in the group that underwent PD as pre-transplant RRT compared to the group that underwent HD(17).

Connective tissue diseases are a group of autoimmune diseases with involvement of other organs rich in skin, joint, and connective tissue such as systemic lupus erythematosus, Sjögren's Syndrome, polymyositis, and dermatomyositis. There is no study in the literature on the frequency of these patients in HD and PD patients. In the current study, these diseases were found to be statistically higher in the PD group than in the HD group (p=0.017).

CONCLUSION

In conclusion, there is no statistically significant difference between the dialysis times of PD and HD patients in our study. CCI scores were found to be significantly higher in PD patients compared to HD patients. However, there is a need for multicenter prospective studies in which the number of patients is higher and mortality data are included.

ETHICAL DECLARATIONS

Ethics Committee Approval: For the study, the approval of the Afyonkarahisar University of Health Sciences Ethics Committee was received on 04.11.2022 with the number 2022/546.

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Original Article / Orijinal Araştırma

QT and P-Wave Dispersion of Patients with Antisocial Personality Disorder

Antisosyal Kişilik Bozukluğu Olan Hastalarda QT ve P Dalgası Dispersiyonu

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Abstract

Aim: The purpose was to examine the electrocardiographic arrhythmia risk determinants of the QT and P-wave dispersions of the patients who have Antisocial Personality Disorder (ASPD) by comparing them with the healthy control group.

Material and Method: A total of 52 patients who were diagnosed with ASPD according to DSM-5 Criteria and a healthy control group that consisted of 54 people were included in the study. Twelve lead Electrocardiograms (ECGs) were obtained from all participants in the supine position and at rest, and P-wave dispersion and QT dispersion were also calculated. The participants were administered the Beck Anxiety Inventory (BAI), Beck Depression Inventory (BDI), Buss-Durke Aggression Scale (BDAS), Barratt Impulsivity Scale (BIS-11) and sociodemographic clinical data form. The SPSS version 22 package program was used for statistical analyses.

Results: The mean age of the ASPD group and control group were no statistically significant differences between them (p=0.092). QT max (p=0.016), QTd (p<0.001), P max (p<0.001), and Pd (p<0.001) values of the ASPD group were higher than the values of the control group at significant levels; and QT min (p<0.001) and P min (p<0.001) values were found to be significantly lower. The BAI, BDI, BDAS scores and the motor impulsiveness score of the BIS-11 subscales of the ASPD group were significantly higher than those of the control group (p<0.001).

Conclusion: The findings of study showed that ASPD patients were at risk for electrical problems of the heart, especially cardiac arrhythmia, and this must be considered in the general psychiatric follow-up of these patients.

Keywords: Antisocial personality disorder, QT dispersion, P dispersion

Öz

Amaç: Antisosyal kişilik bozukluğu (ASKB) olan hastalarda elektrokardiyografik aritmi risk belirleyicileri olan QT ve P dalgası dispersiyonlarının sağlıklı kontrol grubuyla karşılaştırılarak incelenmesi amaçlanmıştır.

Gereç ve Yöntem: Çalışmamıza DSM-5 kriterlerine göre ASKB tanısı alan 52 hasta ve 54 kişiden oluşan sağlıklı kontrol grubu dahil edildi. Tüm katılımcıların boy, kiloları, tansiyonları ölçüldü ve vücud kitle indeksleri(VKİ) hesaplandı. Vücut kitle indeksi > 24,9 kg/m2 olan katılımcılar çalışma dışı bırakıldı. Tüm katılımcılardan on iki derivasyon elektrokardiyogramı (EKG) yatar pozisyonda ve istirahatte alındı. P dalga dispersiyonu ve QT dispersiyonu hesaplandı. Ayrıca katılımcılara Beck Anksiyete Ölçeği (BAÖ), Beck Depresyon Ölçeği (BDÖ), Buss-Durke Saldırganlık Ölçeği (BDSÖ), Barratt Dürtüsellik Ölçeği (BrDÖ) ve sosyodemografik klinik veri formu uygulandı. İstatiksel analizler için SPSS versiyon 22 paket programı kullanıldı.

Bulgular: ASKB grubunda bulunanların yaş ortalaması 29,6±6,5, kontrol grubunun yaş ortalaması ise 31,8±7,1 olarak bulunmuş olup aralarında istatistiksel olarak anlamlı farklılık görülmemiştir (p=0,092). ASKB grubunun QT max (p=0,016), QT disp (p<0,001), P max (p<0,001), P disp (p<0,001) değeri kontrol grubunun değerlerinden anlamlı şekilde yüksek; QT min (p<0,001) ve P min (p<0,001) değeri ise anlamlı şekilde düşük bulunmuştur. ASKB grubunun BAÖ ve BDÖ puanı kontrol grubunun puanından anlamlı şekilde yüksek bulunmuştur (p<0,001). BDSÖ tüm alt ölçek puanları kontrol grubuna kıyasla daha yüksekti (p<0,001), BrDÖ alt ölçeklerinden ise motor dürtüsellik puanı ASKB grubunda daha yüksekti (p<0,001).

Sonuç: Çalışma bulgularımız ASKB hastalarının kardiyak aritmi başta olmak üzere kalbin elektriksel temelli sorunları açısından risk taşıdıkları ve bu durumun bu hastaların genel psikiyatrik takibinde göz önünde bulundurulması gerektiğini göstermektedir.

Anahtar Kelimeler: Antisosyal kişilik bozukluğu, QT dispersiyonu, P dispersiyonu

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INTRODUCTION

The mental disorder known as antisocial personality disorder (ASPD) is chronic and treatment-resistant. It does not significantly impair a person's basic affection, thinking, or cognitive abilities, but it does cause problems in their relationships with others, their ability to function in family and work settings, and it disturbs society.[1] The tendency to infringe another person's rights without feeling regret is described as ASPD in the Diagnostic and Statistical Manual of Mental Disorders (DSM) by the American Psychiatric Association (APA).[2] Such people are given a behavior disorder diagnosis when they are young, and an ASPD diagnosis when they are over the age of 18. The most accurate diagnostic for personality disorders is ASPD.[3]

Due to the complexity of ASPD, many variables, including genetic, environmental, biological, psychodynamic, cognitive, and psychosocial factors, are highlighted in connection to its etiology.[4] The etiopathogenesis of ASPD has not yet been fully elucidated, despite the fact that many of these factors are thought to interact to cause the illness.

It is argued that the Hypothalamic-Pituitary-Adrenal (HPA) axis, which is involved in the regulation of the emotion of fear, creating sensitivity to punishment, and triggering the withdrawal signs, has a lower activity in ASPD. In a study that was conducted with adolescents who had antisocial behavior, it was shown that both basal and stress-induced blood cortisol levels were significantly lower than in those without antisocial behavior.[5]

An extensively used non-invasive heart imaging technique is electrocardiography (ECG). QT dispersion (QTd) on the Electrocardiogram demonstrates ventricular repolarization and depolarization. Due to the fact that an elevated QTd is a sign of ventricular instability, it can result in severe ventricular arrhythmia and sudden cardiac mortality.[6] P wave dispersion (Pd) is the difference between the lengthiest and shortest P wave lengths on an ECG.[7] Longer Pd intervals are thought to be an independent risk factor for atrial fibrillation that is linked to abnormal electrical signaling.[8] Since QTd and Pd have a clear correlation with cardiac autonomic function, they both have the potential to predict abnormalities of the autonomic nervous system.

The purpose of the present this study was to investigate the relations between cardiac autonomic markers and clinical variables such as QT and P-wave dispersion to better explain the etiopathogenesis of ASPD.

MATERIAL AND METHOD

Participants

A total of 60 patients, who had antisocial personality disorder according to the DSM 5 diagnostic criteria, who were admitted to the psychiatry clinic, were included in the study. Five of the patients were excluded from the study because they could not fill in the given scales, and 3 of them had a BMI of >24.9 kg/m². For this reason, 52 patients were included in the study. The control group included 60 healthy individuals who did not have any psychiatric, neurological, or cardiological diagnoses. Three were excluded from the study because they did not meet the study criteria, and three were excluded because they did not agree to participate in the study.

Both groups were excluded if they had any cardiological (cardiac arrhythmias, unstable coronary artery disease, atrioventricular blocks or bundle branch block, or heart failure), neurological, endocrinological, pulmonary, neoplastic, autoimmune, metabolic, or psychiatric disorders, any electrolyte imbalance, systolic blood pressure less than 140 mm Hg or diastolic blood pressure less than 90 mm Hg. All participants included in the research were normotensive. In addition, the participants who were included in the study have not been using an active drug for the last 3 months.

The research was given the go-ahead by Firat University's Ethics Committee (24.01.2019-02.04). The protocols adhered to the Declaration of Helsinki and the Institutional and National Human Experiments Committee's ethical guidelines.[9]

Procedure

All participants signed the Informed Consent Forms. The detailed history of the participants who were included in the study was taken, and their height, weight, and blood pressure values were measured. All participants were normotensive. Participants were formed from people who have not used alcohol or drugs for at least 3 months. The Sociodemographic and Clinical Data Form, Beck Depression Inventory (BDI), Beck Anxiety Inventory (BAI), Barratt Impulsivity Inventory (BIS-11), and Buss-Durke Aggression Scale (BDAS) were administered to all participants by the same psychiatrist. After the scale application, Twelve lead electrocardiograms (ECGs) were obtained from all participants in the supine position and at rest in the ECG recording room in the same hospital .

1) Sociodemographic and Clinical Data Form

The study made use of the sociodemographic and clinical data form that the researchers had created in accordance with their clinical experience, data from their sources, and their understanding of the study's objectives. This semi-structured form collects sociodemographic information, including age, gender, marital status, level of education, profession, place of residence, family structure, and place of employment.

2) Beck Depression Scale (BDI)

Beck created the scale in 1961 to assess adult depressive symptom severity, chance of developing depression, and level of depressive symptoms.[10] Hisli performed the validity and reliability study in Turkey in 1989.[11] The scale's cutoff was found to be 17 points. It is commonly used as a 21-item Likert-type self-assessment scale in studies on depression. Each item pertains to a personality characteristic associated with depression. The things are graded from 0 to 3 depending on how severe the depression is. The total number can be

anything from 0 to 63. If the number is 0 to 9, there are no depressive symptoms; 10 to 16 points, mild symptoms; 17 to 24 points, moderate symptoms; and 25 or more, severe symptoms.

3) Beck Anxiety Scale (BAI)

It was created by Beck and associates.[12] It is a self-assessment tool with 21 items on a Likert-type measure with a score range of 0 to 3. It is used to gauge how frequently people experience anxiety symptoms. Ulusoy et al. conducted research on the validity and dependability of it in Turkey.[13]

4) Barratt Impulsivity Scale (BIS-11)

One of the key clinical characteristics of many distinct psychiatric disorders is impulsivity. The most widely used tool to assess impulsivity is the 11th edition of the Barratt Impulsivity Scale (BIS-11). H Güleç et al.[14] evaluated the validity and reliability of the measure created by Barrat ES[15] in Turkey.

5) Buss-Durke Aggression Scale (BDAS)

It was created to gauge a person's propensity for violence. It is a 34-item self-report measure of the Likert type, with a 1–5 scale for each item. Sub-dimensions include physical aggression, verbal aggression, hostility, rage, and indirect aggression. Additionally, general aggression is calculated along with the overall score, and high scores suggest an aggressive tendency.[16] Can performed the validity and reliability tests on the Turkish version of the scale.[17]

ECG Procedure

After 10 minutes of relaxation, the participants' blood pressures were discreetly assessed using an automatic sphygmomanometer (Omron HEM-7113, Omron Healthcare, Lake Forest, Illinois). Participants whose systolic and diastolic blood pressures were less than 140 and 90 mm Hg, respectively, were excluded from the categories. After 10 minutes of relaxation, a 12-derivation ECG (Cardiofax S, Nihon Kohden, Japan) was used. It had three standard (I-III), three unipolar (aVR, aVL, aVF), and six precordial (V1-V6) leads. The cardiologist (M. Y.), who was unaware of the groups, manually assessed the findings. The QT interval, which is measured in milliseconds, is the distance from the Q-start wave's to the place where the T-wave transitions to the isoelectric line. If there was a U-wave, the lowest point of the combined T-wave and U-wave section was recognized as the T wave's endpoint.[18] The final number was determined to be the mean over three successive QT intervals. The highest and minimum values in each of the 12 derivatives of each range were measured, and the minimum and maximum were then subtracted to determine the QTd values. According to Bazett's Algorithm, QTc times were computed: QTc equals QT/(RR).[19] In accordance with the rules, QTc was deemed long (long QT) at 450 and 460 milliseconds in males and girls, respectively. The distance between the P-beginning wave's point and the points where the isoelectric line, the P-end wave's point, and those lines meet was measured to determine how long the P-wave lasted. The difference between the maximum and minimum P-wave durations was used to determine Pd.[20]

Body Mass Index (BMI, kg/m²)

Calculation was made with the equation of body weight/height2 (kg/m²). Using the World Health Organization (WHO) BMI classification, patients were divided into four groups: normal weight (18.5–24.9 kg/m²), overweight (25–29.9 kg/m²), obese (30–39.9 kg/m²), and morbidly obese (>40 kg/m²).[21]

Statistical analysis

The SPSS 22 package application was used to conduct the analyses (Statistical Package for Social Sciences; SPSS Inc., Chicago, IL). The study's descriptive data were presented as n and % values for categorical data and as mean, SD, median, and interquartile range (numbers between 25 and 75 percentiles) for continuous data. The categorical factors were compared between the groups using the Chi-Square Analysis (Pearson Chi-Square). The Kolmogorov-Smirnov Test was used to assess how closely the continuous factors adhered to a normal distribution. The Mann-Whitney U-Test was employed to compare non-normally distributed variables between groups, and the Independent Samples T-Test was used to compare normally distributed variables between groups. The relationships between the continuous factors were investigated using the Spearman Correlation Test. In the analyses, p0.05 was accepted as the statistically significant threshold.

RESULTS

A total of 106 people, which included 52 patients and 54 healthy controls, were included in the study. The mean age of the patients was 29.6±6.5 (min=20-max=48) in the patient group, and the mean age was 31.8±7.1 (min=20-max=44) of the control group, which was not statistically significant (p=0.092). Only one person in the patient and control group was female, and the rest were male. No significant differences were detected between the groups in terms of gender (p=1.000). It was found that the rate of being single (59.6%) was higher in the patient group than the rate of being single (40.7%) in the control group (p<0.001). It was found that the education level of the control group was significantly higher than the education level of the patient group (p<0.001). The working rate was found to be significantly lower in the patient group (57.7%) than that of the control group (100%) (**Figure 1**). A total of 59.6% of the patient group was in prison. Although in the past, alcohol and substance use was higher in the patient group than in the control group (p<0.001), smoking was higher in the control group (p<0.001). Tattoo, incision scar, and prison history rates of the patient group were significantly higher than those of the control group (p<0.001). When the patient group was evaluated in terms of crime types, there were 40.6% injury, 15.6% theft, 12.5% using drugs, 12.5% murder, 9.4% extortion, 6.3% terrorism, and 3.1% abuse (**Table 1**).

Table 1. The comparison of some variables of the patient and control group

		Patient		Control		p*
		Numbers	%	Numbers	%	
Age. Mean±SD		29.6±6.5		31.8±7.1		0.092
Income level	<1000 ₺	39	75.0	1	1.9	<0.001
	1000-2000 ₺	9	17.3	0	.0	
	2000-3000 ₺	3	5.8	19	35.2	
	3000-4000 ₺	1	1.9	0	.0	
	>4000 ₺	0	.0	34	63.0	
Patient came from	Home	13	25.0	51	94.4	<0.001
	Other hospital	5	9.6	1	1.9	
	Emergency	3	5.8	0	.0	
	Other (Prison)	31	59.6	2	3.7	
Arrival form	Willingly	30	57.7	53	98.1	<0.001
	Not willingly	2	3.8	0	.0	
	With family	5	9.6	0	.0	
	Alone	1	1.9	1	1.9	
	Police	1	1.9	0	.0	
	Other	13	25.0	0	.0	
Smoking	Yes	2	3.8	15	27.8	0.001
	No	49	94.2	36	66.7	
	Quit	1	1.9	3	5.6	
Tattoo/ incision scar	Yes	46	88.5	0	.0	<0.001
	No	6	11.5	54	100.0	
History of prison	Yes	32	61.5	0	.0	<0.001
	No	20	38.5	54	100.0	
Type of offence	Injury	13	40.6	0	.0	-
	Theft	5	15.6	0	.0	
	Drug abuse	4	12.5	0	.0	
	Murder	4	12.5	0	.0	
	Extorsion	3	9.4	0	.0	
	Teror	2	6.3	0	.0	
	Abuse	1	3.1	0	.0	
Alcohol use	No	18	34.6	49	90.7	<0.001
	Quit (3 monts ago)	34	65.3	5	9.3	
Substance use	No	19	36.5	54	100.0	<0.001
	Quit (3 monts ago)	33	53.5	0	.0	

Chi-Square analysis was applied.

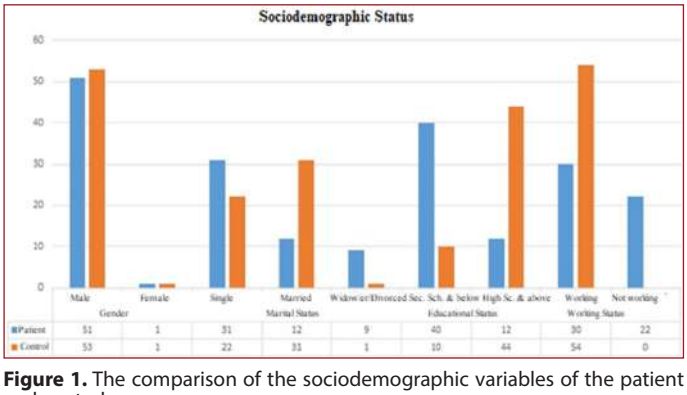

Figure 1. The comparison of the sociodemographic variables of the patient and control group
Sec.Sch & below: Secondary school and below
High Sc & above: High school and above

It was discovered that the case group's BAI and BDI scores were noticeably greater than those of the control group (p 0.001). The case group's Tp-e gap, Tp-e/QT ratio, and Tp-e/QTc ratio were all noticeably greater than those of the control group (p<0.001). It was discovered that the case group's incidence of fragmented QRS (60%) was significantly greater than the control group's incidence (36%) (p=0.016) (**Table 2**).

It was found that the height (p=0.022) and weight (p=0.041) of the patient group were significantly lower than those of the control group, but BMI was similar in both groups (**Table 2**).

Table 2. The comparison of the anthropometric values of the patient and control group

	Patient	Control	
	Mean (IQR)	Mean (IQR)	
Height	172.0 (170.0-180.0)	176.0 (173.0-180.0)	0.022
Weight	70.5 (65.0-75.5)	75.0 (68.0-80.0)	0.041
BMI	23.2 (21.4-24.9)	23.5 (22.6-24.9)	0.309

*Mann Whitney U-test was used.

QT max (p=0.016), QTd (p<0.001), P max (p<0.001), Pd (p<0.001), BDI (p<0.001), BAI (p<0.001), motor impulsiveness (p<0.001), physical aggression (p<0.001), indirect aggression (p=0.007), irritability (p<0.001), negativizm (p<0.001), verbal aggression (p<0.001) and general aggression (p<0.001) values of the patient group were significantly higher than those of the control group; and QT min (p<0.001) and P min (p<0.001) values were significantly lower (**Table 3**).

In the correlation analysis, a negative correlation was detected between QTd and age, and there was a significant and positive correlation between motor impulsiveness with QTd. (**Table 4**).

Table 3. The comparison of the ECG and scale scores of the patient and control group

	Patient	Control	p*
	Median (IQR)	Median (IQR)	
QT Max	362.5 (350.0-390.0)	352.0 (333.0-375.0)	0.016
QT Min	310.0 (285.0-330.0)	340.0 (320.0-352.0)	<0.001
QTd	60.0 (40.0-70.0)	31.5 (23.0-55.0)	<0.001
P Max	100.0 (90.0-110.0)	80.0 (75.0-100.0)	<0.001
P Min	40.0 (40.0-50.0)	60.0 (40.0-93.0)	<0.001
Pd	60.0 (50.0-70.0)	30.0 (19.0-50.0)	<0.001
BDI	28.0 (9.0-40.0)	1.0 (.0-5.0)	<0.001
BAI	24.0 (9.5-36.0)	2.5 (.0-8.0)	<0.001
BIS-11			
Attention Impulsiveness	31.5 (28.0-34.0)	31.0 (27.0-34.0)	0.254
Motor Impulsiveness	15.0 (12.0-18.0)	11.0 (10.0-13.0)	<0.001
Non-planning impulsiveness	19.0 (17.0-21.0)	18.5 (16.0-20.0)	0.136
BDAS			
Physical aggression	5.0 (3.5-7.0)	3.0 (2.0-4.0)	<0.001
Indirect aggression	4.0 (3.0-5.0)	3.0 (2.0-4.0)	0.007
Irritability	6.0 (4.0-7.0)	3.0 (2.0-5.0)	<0.001
Negativism	4.0 (3.0-4.0)	2.0 (1.0-4.0)	<0.001
Verbal aggression	11.0 (9.0-12.0)	7.0 (5.0-10.0)	<0.001
General Aggression Score	25.5 (20.5-29.5)	17.0 (12.0-20.0)	<0.001

*Mann Whitney U test was used. BDI: Beck Depression Scala, BAI: Beck Anxiety Scala, BIS-11: Barratt Impulsivity Scala, BDAS: Buss-Durke Aggression Scale

Table 4. The correlation of the ECG values with age and scale score in the patient group

		QT Max	QT Min	QT Disp	P Max	P Min	P Disp
Age	r	-.062	.114	-.286	.138	.232	-.003
	p	.664	.423	.040	.333	.102	.986
BDI	r	.008	-.184	.264	-.146	-.084	-.147
	p	.954	.191	.059	.306	.556	.303
BAI	r	.029	-.139	.163	-.114	.035	-.202
	p	.840	.327	.249	.424	.810	.155
BDAS-General	r	-.070	-.169	.132	-.034	.097	-.169
	p	.621	.230	.349	.812	.499	.237
BIS-11-Attention Impulsiveness	r	-.136	-.176	.060	-.064	-.214	.123
	p	.338	.212	.670	.656	.132	.391
BIS-11-Motor Impulsiveness	r	-.039	-.198	.294	-.010	-.179	.136
	p	.783	.160	.035	.947	.209	.341
BIS-11-Non-planning impulsiveness	r	-.169	-.265	.157	.102	-.131	.257
	p	.230	.057	.267	.476	.360	.069

BDI: Beck Depression Scala, BAI: Beck Anxiety Scala, BIS-11: Barratt Impulsivity Scala, BDAS: Buss-Durke Aggression Scale

DISCUSSION

In this study, QT and P dispersion in ASPD cases and healthy controls were examined, as well as their associations with clinical variables. The patient group was found to have considerably higher QT max, QTd, P max, and Pd, while having significantly lower QT min and P min. It was discovered that the patient group had higher BDI and BAI ratings. All BDAS subscale scores were higher than the control group ($p<0.001$), and the motor impulsivity score of the BIS-11 subscales was higher in the sick group than the control group. The rate of smoking was found to be higher in the control group, despite the fact that alcohol and drug abuse were considerably higher in the patient group than in the control group. BMI scores were similar in both groups. In the correlation analysis, a negative correlation was detected between QTd and age, and there was a significant and positive correlation between motor impulsiveness with QTd.

ECG is used widely in the clinical settings for the diagnosis of heart diseases, rhythm and conduction disorders as well as in the analysis of the side effects of some drugs. The repolarization time between the electrodes is different on the ECG. Since the QT dispersion employed to measure this difference varies according to heart rate, it is calculated by correcting with the Bazett Formula. This correction prevents the changes occurring because of heart rate and allows for a more accurate analysis. Recently, ECG has been used not only in the context of heart diseases, but also in many risky psychiatric disorders, especially in studies conducted to identify ventricular arrhythmias.[22,23]

In the literature, there is no such study that was conducted in ASPD cases. Previous studies showed that QTd is significantly higher in panic disorder such as anxiety disorder and social phobia than in controls. This correlation was evaluated as a result of prolonged anxiety.[6] The group of psychiatric patients with eating problems who scored highly on depression and anxiety had a longer QTd. In a meta-analysis of five studies, people with elevated levels of anxiety had longer QTds.[24] Similarly, when compared to controls, patient anxiety and QTd levels were considerably higher.

Studies in Major depressive disorder (MDD) patients have found QTd prolongation findings supporting ventricular arrhythmia. It has been thought that both depression and antidepressant drugs used in the treatment of the treatment of the autonomic nervous system and cause some ECG findings.[25-27] In our research, the patient group's BDI scores were significantly higher than those of the controls. The participants had no treatment for the last three months, including antidepressant and antipsychotic drugs. The results of our study may also be considered to affect depressive disorder due to the height of BDI scores.

In a research with bipolar disorder patients, it was discovered that fundamental electrocardiographic indicators, such as QTcmax, QTcd, and Pmin, increased at significant levels in the patient group when compared to the control group.[28] Additionally, it was claimed that a longer QTd is a sign of ventricular tachycardia. Atrial fibrillation is already known to be linked to sudden cardiac death and a lengthy Pd interval.[6,29] Bipolar illness has been shown to interact with Cluster B personality disorders like borderline and antisocial personality disorders. Bipolar disorder and Cluster B personality disorders were linked to traits like impulsivity, self-destructive behavior, and criminal activity.[30] QTcd was found to be increased in a study conducted with borderline personality disorder, one of the cluster B personality disorders.[31] Our findings were similar to those found in bipolar and borderline personality disorders.

Pd is a sensitive and specific precursor of atrial fibrillation (AF) in a variety of clinical conditions because it displays the difference between the lowest and maximum P-wave durations. According to reports, it was used to forecast paroxysmal AF.[32] Significant variations in cardiac atrial conduction are associated with systemic autonomic symptoms of anxiety attacks.[33] In a research that looked at the connection between arrhythmia and Pd in anxiety disorders, it was discovered that state anxiety had a greater impact on Pd than trait anxiety.[34] Pd times in the patient group of a study with 30 hypochondriasis sufferers were discovered to be considerably higher.[35] Another study examined 40 patients and 40 controls and found that Pd was prolonged in patients with panic disorder who also had significant somatic symptoms.[36] The BAI scores and Pd in the current research were significantly higher.

A total of 65.3% of the patient group had used alcohol and 53.5% had used the substance in the past (had not used it for at least three months). Chronic alcohol use can cause the immune system damage, lead to heart failure, myocardial infarction, and sudden death by causing arrhythmia and contractile dysfunction, as well as autonomic nervous system dysfunction, QT prolongation,

myocarditis, and myocyte degeneration.[37-39] In a study that was performed with patients with alcohol use disorder, a significant prolongation of QTd and Pd values was found in the patient group.[40] The results of the present study show that ASPD patients are at risk for electrical-based problems of the heart, especially cardiac arrhythmia, and this must be considered in the general psychiatric follow-up of such patients.

It is suggested that HPA axis has lower efficacy in ASPD. It was shown in a study that was conducted with adolescents who had antisocial behaviors that both basal and stress-induced blood cortisol levels were significantly lower than those without antisocial behaviors.[5] Abnormalities in the functioning of the HPA axis were associated with the characteristics of psychopathy.[41,42] It has long been believed that low cortisol may contribute to the reduction of fear in the psychopathic personality. However, cortisol levels can be affected by environmental factors such as chronic stress conditions. Loomans et al. (2016) measured CAR (Cortisol Awake Response) and afternoon and evening cortisol levels in a group of inpatient male ASPD patients and two healthy comparison groups (male employees in the same hospital and general population sampling) grouping the ASPD patients as those with high and low psychopathy scores (26 points and above, 25 points and below).[43] Although no differences were detected in cortisol secretion between the groups that had personality disorder, both ASPD and psychopathic groups (and hospital staff) had more tendency to show high cortisol levels, which was interpreted as evidence that patients live in more stressful conditions than the general population, which, also suggests that cortisol daily rhythms may be affected by environmental events.[43] The fact that 59.6% of the patient group was in prison and the elevated BAI scores predict that this group led a stressful life. Assuming that the normally low expected cortisol levels might have been elevated because of these reasons, it may have affected the ECG findings.

A cohort study published in 2022 revealed that QRS duration was associated with age, gender, HDL, TG, LDL, and BMI.[44] In another study, obesity was found to prolong Pd and QTcd.[45] In our study, the height and weight of the patient group were significantly lower than the control group, but BMI values were similar. While the median BMI value of the patient group was 23.2, it was 23.5 in the control group. It is in the normal weight class according to the WHO classification.[21]

In our correlation analysis results, a negative correlation was found between age and QTd. However, literature information was that there was no significant relationship between age and QTd.[46,47] There are studies showing an increase in QT dispersion in patients with predominant impulsivity such as manic episode and borderline personality disorder.[28,31] In our study, the results supported the literature and found a positive correlation between motor impulsivity and QTd.

The present study had some limitations. The first was that the majority of the patient and control group were men. The second was that more samplings are needed in terms of numbers. Also, it was considered that uncontrollable factors e.g. high alcohol and substance use in the patient group, high rate of being in prison, and differences in daily living activities e.g. nutrition and exercise affected the results. On the other hand, the fact that the levels of the related hormones, especially cortisol, which may be associated with ASPD and autonomic dysfunction, were not checked can be considered as another limitation. For this reason, another study can be planned with a larger sampling by minimizing the variables which may affect the parameters such as alcohol and substance use.

CONCLUSION

The findings of the present study showed that ASPD patients are at risk for electrical problems of the heart, especially cardiac arrhythmia, and this must be considered in the general psychiatric follow-up of these patients. Treatment of depression and anxiety disorders, providing treatment in alcohol and substance rehabilitation centers, changing nutritional habits and adding exercise to daily living activities in ASPD patients are important both in terms of cardiac and psychiatric.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Fırat University Ethics Committee (Date: 24.01.2019, Decision No: 02/04).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

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Original Article / Orijinal Araştırma

Gastrointestinal System Involvement of Multisystem Inflammatory Syndrome in Children (MIS-C): A Single Center Experience of 47 cases

Pediatrik Multisistem İnflamatuvar Hastalıkta (MIS-C) Gastrointestinal Sistem Tutulumu: 47 Olgunun Tek Merkez Deneyimi

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Abstract

Aim: Multisystem inflammatory syndrome in children (MIS-C) is a hyperinflammatory syndrome which was newly described during the coronavirus disease 2019 (COVID-19) pandemic in children and characterized by fever, inflammation, multiorgan dysfunction. One of the major clinical presentation is gastrointestinal system involvement. The aim of the study is to evaluate the clinical course and outcome according to the severity of gastrointestinal presentation, focusing on MIS-C cases with gastrointestinal system involvement.

Material and Method: We performed a retrospective study of 47 MIS-C patients with gastrointestinal involvement in our clinic between October 2020 and March 2022. The patients were divided into two groups according to the severity of gastrointestinal involvement. The groups were compared in terms of demographic characteristics, gastrointestinal symptoms, laboratory parameters, other system involvement, length of hospital stay, treatment modalities, and clinical outcomes.

Results: According to the severity of gastrointestinal system involvement, 44.7% (n=21) of the cases were mild to moderate, 55.3% (n=26) were severe. The most common gastrointestinal symptoms at presentation were abdominal pain (78.7%), vomiting (59.6%), and nausea (55.3%). Transaminase elevation was present in 29.8% of the cases. The most common radiological findings were ascites (36.2%) and pancreatic edema (27.7%). In cases presenting with acute pancreatitis (n=9), intensive care unit admission rates (n=6) were statistically significantly higher. Brain natriuretic peptide (p=0.020) and d-dimer (p=0.032) were statistically significantly higher in the severe group than in the mild to moderate group.

Conclusion: In a significant part of the MIS-C cases with gastrointestinal involvement, severe findings is observed. Especially in cases presenting with pancreatitis, a more severe clinical course may be observed. Therefore, when managing patients presenting with gastrointestinal symptoms, the evaluation for pancreatitis is essential.

Keywors: Multisystem inflammatory syndrome in children, gastrointestinal involvement, pancreatitis

Öz

Amaç: Pediatrik multisistem inflamatuvar hastalık (MIS-C), koronavirüs 2019 (COVID-19) pandemisi sırasında yeni tanımlanan, ateş, inflamasyon ve çoklu organ yetmezliği ile karakterize hiperinflamatuvar bir sendromdur. Başlıca klinik prezentasyonlardan birisi gastrointestinal sistem tutulumudur. Çalışmanın amacı, gastrointestinal sistem tutulumu olan MIS-C olgularına odaklanarak, gastrointestinal tablonun şiddetine göre klinik seyir ve sonucu değerlendirmektir.

Gereç ve Yöntem: Ekim 2020-Mart 2022 tarihleri arasında kliniğimizde gastrointestinal tutulumu olan 47 MIS-C hastasının retrospektif bir çalışmasını gerçekleştirdik. Hastalar gastrointestinal tutulumun şiddetine göre iki gruba ayrıldı. Gruplar demografik özellikler, gastrointestinal semptomlar, laboratuvar parametreleri, diğer sistem tutulumları, hastanede kalış süreleri, tedavi yöntemleri ve klinik sonuçlar açısından karşılaştırıldı.

Bulgular: Gastrointestinal sistem tutulumunun ciddiyetine göre olguların %44.7'si (n=21) hafif-orta, %55.3'ü (n=26) şiddetli idi. Başvuru anında en sık görülen gastrointestinal semptomlar karın ağrısı (%78.7), kusma (%59.6) ve bulantı (%55.3) idi. Olguların %29.8'inde transaminaz yüksekliği mevcuttu. En sık radyolojik bulgu asit (%36.2) ve pankreas ödemi (%27.7) idi. Akut pankreatit ile başvuran olgularda (n=9), yoğun bakıma yatış oranları (n=6) istatistiksel olarak anlamlı derecede yüksekti. Brain natriüretik peptid (p=0.020) ve d-dimer (p=0.032), şiddetli grupta, hafif-orta gruba göre istatistiksel olarak anlamlı derecede yüksekti.

Sonuç: Gastrointestinal tutulumu olan MIS-C olgularının önemli bir kısmında ciddi bulgular görülmektedir. Özellikle pankreatit ile başvuran olgularda daha ağır bir klinik seyir gözlenebilir. Bu nedenle gastrointestinal semptomlarla prezente olan hastalarda pankreatit açısından değerlendirme gereklidir.

Anahtar Kelimeler: Pediatrik multisistem inflamatuvar hastalık, gastrointestinal tutulum, pankreatit

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INTRODUCTION

Multisystem inflammatory syndrome in children (MIS-C) is a newly recognized severe clinical condition which affects multiple systems and may result in shock and death. The most common organ involvements are cardiovascular, respiratory, renal, neurologic, hematologic and gastrointestinal systems.[1] Some patients with MIS-C with gastrointestinal system involvement may present with a clinic resembling viral gastroenteritis such as abdominal pain, nausea and vomiting. But, a considerable number of serious gastrointestinal manifestations such as diffuse mesenteric lymphadenitis, appendicitis, pancreatitis and terminal ileitis have also been reported in the literature.[2] In the literature, the presence of gastrointestinal symptoms at presentation was associated with a more severe clinical course for MIS-C.[3] The aim of this study is to describe the MIS-C patients presenting with gastrointestinal system involvement (clinical/radiological findings) and to evaluate patients according the severity of gastrointestinal involvement and its relationship with clinical course and outcome.

MATERIAL AND METHOD

In this study, the files of 53 patients followed with a diagnosis of MIS-C in the pediatric infectious diseases service of Necmettin Erbakan University Meram Medical Faculty between October 2020 and March 2022 were evaluated retrospectively. The diagnosis of MIS-C was made according to the CDC criteria:

1. Age < 21 years.
2. Clinical presentation in accordance with MIS-C, including all of the following:

Fever: Documented fever > 38.0°C (100.4 °F) for> 24 hours or report of subjective fever lasting> 24 hours.

Laboratory evidence of inflammation, including any of the following:

- Elevated C-reactive protein, erythrocyte sedimentation rate, fibrinogen, procalcitonin, D-dimer, ferritin, lactate dehydrogenase, IL-6 level.
- Neutrophilia, lymphocytopenia, hypoalbuminemia.

Multisystem (≥2) organ involvement:

- Cardiovascular (e.g., shock, elevated troponin, elevated brain natriuretic peptide (BNP), abnormal echocardiography, arrhythmia).
- Respiratory (e.g., pneumonia, ARDS, pulmonary embolism).
- Renal (renal failure).
- Neurologic (e.g., seizure, stroke, and aseptic meningitis).
- Hematologic (e.g., coagulopathy).
- Gastrointestinal (e.g., abdominal pain, vomiting, diarrhea, elevated liver enzymes, and ileus).

Severe illness requiring hospitalization.

3. No alternative potential diagnosis.
4. Evidence of infection with SARS-CoV-2 including positive SARS-CoV-2 reverse-transcriptase polymerase chain reaction (RT-PCR) or positive serology.[4]

Demographic data, clinical features, laboratory parameters, radiological findings, treatment options and clinical outcome of the patients were retrospectively scanned from hospital records. MIS-C divided into three groups according to clinical severity as mild, moderate and severe:

- **Mild MIS-C:** No vasoactive requirement or respiratory support and minimal organ injury.
- **Moderate MIS-C:** Mild or isolated organ injury.
- **Severe MIS-C:** Moderate or severe organ injury, including moderate-to-severe ventricular dysfunction and requirement of inotropic support.[5]

Gastrointestinal involvement was determined by evaluating symptoms, laboratory and imaging findings at admission. The symptoms were nausea, vomiting, abdominal pain, diarrhea, hematemesis, and hematochezia. A transaminase level elevated more than twice the upper limit of normal was accepted as increased. The diagnosis of acute pancreatitis was made by evaluating clinical, laboratory and radiological findings in accordance with the recommendations of the International Study Group Of Pediatric Pancreatitis: In Search For A Cure consortium.[6] The liver size, findings consistent with pancreatitis (heterogeneity, edema), gallbladder wall thickening, wall thickening in the terminal ileum and/or cecum, findings compatible with appendicitis, presence of ascites and diffuse mesenteric lymphadenitis were examined by abdominal ultrasonography and computed tomography.

Patients were divided into 3 groups according to the severity of gastrointestinal involvement:

a. Severe gastrointestinal involvement: cases with clinical and radiological findings suggesting appendicitis, diffuse adenomesenteritis, ascites, terminal ileitis and acute pancreatitis, which require surgical consultation
b. Mild to moderate gastrointestinal involvement: cases with symptoms such as nausea, vomiting, abdominal pain, and diarrhea, without severe gastrointestinal findings and/or mild findings such as liver enzyme elevation, and gallbladder wall thickening
c. Cases without gastrointestinal symptoms and signs.[7]

According to this classification scheme, 6 patients without gastrointestinal involvement were excluded from the study. The remaining 47 patients were divided into two groups as mild to moderate group and severe group. The groups were compared in terms of demographic characteristics, gastrointestinal symptoms, laboratory parameters, other system involvement, length of hospital stay, treatment modalities, and clinical outcomes.

The study protocol was approved by the Ethics Committee of Necmettin Erbakan University Meram Medical Faculty (approval no:2022/4060) and conformed to the principles outlined in the Declaration of Helsinki. Informed consent was obtained from all individual participants included in the study.

Statistical Method

The data were analyzed by using IBM-Statistical Package for Social Sciences (IBM-SPSS Inc., Chicago, IL, USA) 22.0 package program. The conformity to the normal distribution was examined by using the 'Shapiro-Wilk test'. Continuous variables were expressed as mean ± standard deviation or median (25-75 percentile) and categorical variables were expressed as frequency (percentage ratio). In the analysis of continuous variables, when parametric test assumptions were met the 'Independent Sample T-test' was used and when parametric test assumptions were not met the 'Mann-Whitney U test' was used. In the analysis of categorical variables Chi-square Test or Fischer's Exact Test were used. Logistic regression analysis was used to identify possible independent risk factors for severe gastrointestinal involvement. A value of $p<0.05$ was accepted as statistically significant level.

RESULTS

The study was conducted on 47 patients with MIS-C with gastrointestinal system involvement. The mean age was 126.53±44.88 months and, 27 (57.4%) patients were male and 20 (42.6%) were female. All cases were previously healthy. According to the severity of the MIS-C clinic, 27.7% of the cases were considered as mild, 46.8% moderate and 25.5% severe. According to the severity of gastrointestinal involvement, 44.7% (n=21) of the cases were mild to moderate and 55.3% (n=26) were severe.

The most common gastrointestinal symptoms at admission were abdominal pain (78.7%), vomiting (59.6%), and nausea (55.3%). Seventeen (36.2%) children presented with diarrhea. Gastrointestinal bleeding was observed in 2 patients. Transaminase elevation was present in 29.8% of the patients. Cholestatic hepatitis was not observed in any of the cases. Additionally, acute liver failure was not observed in any of these cases. Abdominal USG was performed in all cases. Abdominal computed tomography was performed in 7 cases. The most common radiological findings were ascites (36.2%) and pancreatic edema (27.7%). Other radiological findings were mesenteric lymphadenitis (12.8%), hepatomegaly (10.6%), gallbladder wall thickness (8.5%), terminal ileitis (6.4%), and appendicitis (4.3%). MIS-C symptoms developed after appendectomy in one of 2 patients who had an clinical findings of acute abdomen and had radiological findings consistent with appendicitis. The other patient had clinical and laboratory findings of MIS-C at the first presentation and appendectomy was not performed. 3 patients had radiographic findings similar to those seen in inflammatory bowel disease, such as bowel wall thickening. These findings improved in 2 of patients, but persisted in a patient and were diagnosed with inflammatory bowel disease 3 months later.

Median duration of hospitalization was 9 days (25-75 percentiles, 7–11). All patients received intravenous immunoglobulin and methylprednisolone. 45 (95.7%) patients received acetylsalicylic acid and 35 (74.5%) patients received enoxaparin. Interleukin-1 receptor antagonist was administered to 22 patients (46.8%) and interleukin-6 receptor inhibitor was administered to a patient (2.1%). 9 children (19.1%) required intensive care unit admission and 2 of those who need hospitalization in the intensive care unit died. 95.7% of MIS-C cases with gastrointestinal presentation were discharged. Gastrointestinal symptoms and findings, treatment methods and clinical outcome of the cases are shown in **Table 1**.

Table 1. Gastrointestinal symptoms and findings, treatment, clinical outcomes in MIS-C patients with gastrointestinal involvement.

Symptoms N (%)	Abdominal Pain	37 (78.7%)
	Vomiting	28 (59.6%)
	Nausea	26 (55.3%)
	Diarrhea	17 (36.2%)
	Hematemesis	1 (2.1%)
	Hematochezia	1 (2.1%)
Findings N (%)	Ascites	17 (36.2%)
	Pancreatic Edema	13 (27.7%)
	Mesenteric lymphadenitis	6 (12.8%)
	Hepatomegaly	5 (10.6%)
	Gallbladder wall thickness	4 (8.5%)
	Terminal Ileitis	3 (6.4%)
	Appendicitis	2 (4.3%)
Clinical Course and Outcomes N (%) or median (25-75 persentile)	Hospitalization Time (days)	9 (7-11)
	Hospitalization in the ICU	9 (19.1%)
	Inotrope Support	12 (25.5%)
	Invasive or Noninvasive MV support	10 (21.3%)
	Acetylsalicylic acid	45 (95.7%)
	IVIG	47 (100%)
	Methylprednisolone	47 (100%)
	Enoxaparin	35 (74.5%)
	IL-1 receptor antagonist	22 (46,8%)
	Il-6 receptor inhibitor	1 (2.1%)
	Mortality	2 (4.3%)
	Discharge	45 (95.7%)

The severe gastrointestinal presentations such as pancreatitis, adenomesenteritis, ascites, appendicitis were compared according to the clinical courses and outcomes (intensive care unit hospitalization requirement, inotropic support, invasive or noninvasive mechanical ventilation requirement, mortality). It was found that the rate of intensive care unit admission was statistically significantly higher in cases presenting with acute pancreatitis (p=0.008). 2 patients died and these patients had acute pancreatitis and ascites. There was no significant difference between the other severe clinical presentations (ascites, diffuse mesenteric lymphadenitis, terminal ileit, appendicitis) in terms of clinical course and outcome (**Table 2**).

Table 2. The severe gastrointestinal presentations according to the clinical courses and outcomes

		Ascites			Pancreatitis			Mesenteric lymphadenitis			Terminal Ileit			Appendicitis		
		Yes	No	p	Yes	No	p	Yes	No	P	Yes	No	p	Yes	No	p
ICU admission	Yes	5 (55.4)	4 (45.4)	0.168	6[a] (66.7)	3[b] (33.3)	0.008*	1 (11.1)	8 (88.9)	0.678	0 (0)	9 (100)	0.520	0 (0)	9 (100)	0.650
	No	12 (31.6)	26 (68.4)		7[a] (18.4)	31[b] (81.6)		5 (4.9)	33 (86.8)		3 (7.9)	35 (92.1)		2 (5.3)	36 (94.7)	
Inotrop support	Yes	6 (50)	6 (50)	0.208	6 (50)	6 (50)	0.055	0 (0)	12 (100)	0.151	0 (0)	12 (100)	0.404	0 (0)	12 (100)	0.550
	No	11 (31.4)	24 (68.6)		7 (20)	28 (80)		6 (12.8)	29 (82.9)		3 (8.6)	32 (91.4)		2 (5.7)	33 (94.3)	
MV	Yes	4 (40)	6 (60)	0.526	5 (50)	5 (50)	0.087	1 (10)	9 (90)	0.622	0 (0)	10 (100)	0.479	0 (0)	10 (100)	0.616
	No	13 (35.1)	24 (64.9)		8 (21.6)	29 (78.4)		5 (13.5)	32 (86.5)		3 (8.1)	34 (91.9)		2 (5.4)	35 (94.6)	
Mortality	Yes	2 (100)	0 (0)	0.126	2 (100)	0 (0)	0.072	0 (0)	2 (100)	0.759	0 (0)	2 (100)	0.875	0 (0)	2 (100)	0.916
	No	15 (33.3)	30 (66.7)		11 (24.4)	34 (75.6)		6 (13.3)	41 (87.2)		3 (6.7)	42 (93.3)		2 (4.4)	45 (95.7)	

*: significant at 0.05 level according to Fischer's Exact test. a,b: same superscript letters in each row denote the significant pairwise comparison of columns. ICU: Intensive Care Unit, MV: Mechanical Ventilation (invasive or/and noninvasive)

When the patients were evaluated according to the severity of gastrointestinal involvement, it was found that there was no statistically significant difference between the groups in terms of age (p=0.427) and gender (p=0.579). Body mass index was lower in the severe group, but the difference was not statistically significant (p=0.627). The most common gastrointestinal complaint was abdominal pain in both groups. There was no statistically significant difference between the groups in terms of the distribution of symptoms (p>0.05 for all) (**Table 2**). It was found that respiratory (p=0.284) and cardiovascular involvement (p=0.181) were more common in the severe group, however the difference was not statistically significant. Neurological involvement was significantly higher in the severe group (p=0.037) (**Table 3**).

When the groups were compared according to the severity of MIS-C, it was found that the rate of mild to moderate gastrointestinal involvement was significantly higher in cases with mild MIS-C. Similarly, severe gastrointestinal involvement was significantly higher in cases with moderate and severe MIS-C (p=0.002).

When the groups were evaluated in terms of clinical course and outcome, it was found that there was no statistically significant difference between the groups. However, intensive care unit requirement (26.9%, p=0.160), inotropic support (30.8%, p=0.360), invasive or noninvasive mechanical ventilation requirement (26.9%, p=0.475) and mortality rates (7.7%, p=0.475) were higher in the group with severe gastrointestinal involvement compared to the group with mild to moderate gastrointestinal involvement (9.5%, 19%, 14.3%, 0%, respectively) (**Table 3**).

When the groups were compared according to laboratory parameters, it was found that BNP (p=0.020) and d-dimer (p=0.032) levels were statistically significantly higher in the severe group compared to the mild to moderate group. The median BNP level was 8,632 ng/mL (2.252-16,414 ng/mL) in the severe group, it was 2,227 ng/mL (909-5,444 ng/mL) in the mild to moderate group. D-dimer level was 3,771 ng/mL (2.005-4.800 ng/mL) [median (25-75 percentiles)] in the severe group and it was 2,022 ng/mL (725-3,100 ng/mL) [median (25-75 percentiles)] in the mild to moderate group. In addition, lymphocyte count (p=0.195) and albumin levels (p=0.334) were lower in the severe group, but the difference was not statistically significant. Additionally, there was no statistically significant difference between severe and mild to moderate groups in terms of other laboratory parameters such as hemoglobin, white blood cell count, platelet count, erythrocyte sedimentation rate, C-reactive protein, interleukin-6, aspartate transaminase, alanine transaminase, fibrinogen and ferritin. (**Table 3**).

DISCUSSION

Gastrointestinal symptoms, which were thought to be less common in the early stages of the COVID-19 pandemic, have been reported more frequently after the identification of MIS-C.[3] It is increasingly recognized that gastrointestinal symptoms and signs are the most common clinical presentation of MIS-C.[8] Cases ranging from mild gastrointestinal symptoms such as isolated nausea and vomiting to severe manifestations such as terminal ileitis, pancreatitis, and acute abdomen have been reported.[9-11] Our study focused on MIS-C cases with gastrointestinal system involvement and evaluated the severity of gastrointestinal involvement, clinical features, laboratory findings, and clinical outcome.

In a meta-analysis in which 8 studies including a total of 440 MIS-C cases were evaluated, it was reported that the age of the cases ranged from 7 to 10 years, with a dominance of male gender 59%.[12] Sayed et al., in their study, which evaluated gastrointestinal involvement in patients with SARS-CoV-2 infection and MIS-C, reported that the mean age of MIS-C cases with gastrointestinal involvement was approximately 8 years higher compared to those without gastrointestinal involvement and there was no significant difference in terms of gender.[13] Vecchio et al evaluated the severity of gastrointestinal findings in children with SARS-CoV-2 infection. They reported that the mean age of the patients with severe gastrointestinal findings was higher in their cohort, which also included cases with MIS-C and age was a risk factor for severe gastrointestinal involvement.[7]

Table 3. Demographic characteristics, clinical and laboratory parameters according to the severity of gastrointestinal involvement

Parameters		Mild to moderate (n:21)	Severe (n:26)	p value
Demographic characteristics	*Age (months)	120.67±48.65	131.27±41.96	0.427
	‡2-5 years of age	4(19%)	1(3.8%)	0.158
	‡>5 years of age	17(81%)	25(96.2%)	
	§Male	13(61.9%)	14(53.8%)	0.579
	§Female	8(38.1%)	12(46.2%)	
	*BMI SDS	0.26±1.31	0.07±1.26	0.627
Symptoms	Nausea	12 (46.2%)	14 (53.8%)	0.528
	Vomiting	13 (46.4%)	15 (53.6%)	0.503
	Abdominal pain	17 (45.9%)	20 (54.1%)	0.512
	Diarrhea	7 (41.2%)	10 (58.8%)	0.478
	Hematemesis	0 (0.0%)	1 (100%)	0.553
	Hematochezia	0 (0.0%)	1 (100%)	0.553
MIS-C severity	Mild	11(84.6%)a	2(15.4%)a	0.002
	Moderate	8(36.4%)b	14(63.6%)b	
	Severe	2(16.7%)b	10(83.3%)b	
Other system involvement	Neurological	5(23.8%)	14(53.8%)	0.037
	Respiratory	5(23.8%)	10(38.5%)	0.284
	Cardiac	8(38.1%)	15(57.7%)	0.181
	Renal	3(14.3%)	3(11.5%)	1
Clinical course	†Hospitalization duration	9(7-10)	11(8-13)	0.093
	‡Hospitalization in the ICU	2(9.5%)	7(26.9%)	0.160
	§Inotrop	4(19%)	8(30.8%)	0.360
	‡Invasive/Noninvasive MV	3(14.3%)	7(26.9%)	0.475
Clinical outcomes	Mortality	0(0%)	2(7.7%)	0.495
	Discharge	21(100%)	24(92.3%)	
Laboratory parameters	*Hemoglobin (g/dL)	12.16±2.18	12.25±1.31	0.859
	*White blood cell (µg/L)	8,409±3,807	10,744±5,175	0.171
	†Platelet count (109/L)	140 (122-195)	161 (121-206)	0.669
	†Lymphocyte count (µg/L)	0.850 (0.640-1.220)	0.730 (0.600-0.970)	0.195
	*Neutrophil count (µg/L)	6,748±3,582	9,482±5,077	0.069
	†Erythrocyte sedimentation rate (>20mm/h)	55 (40-73)	40 (26-78)	0.624
	†AST (U/L)	27.3 (13.0-48.0)	27.5 (16.3-87.0)	0.556
	†ALT (U/L)	30.0 (17.4-43.0)	31.5 (16-72)	0.889
	†Albumin (g/dL)	3.5(3.0-3.8)	3.1 (2.8-3.6)	0.334
	†BNP (>25 ng/mL)	2,227 (909-5,444)	8,632 (2,252-16,414)	0.020
	†IL-6 (>6.4 pg/mL)	51.35 (8.30-230)	96.85 (56.55-1,253.5)	0.187
	*C- reactive protein (mg/L)	177.49±96.31	201.74±84.24	0.362
	†D-dimer (>500 ng/mL)	2,022 (725-3,100)	3,771 (2,005-4,800)	0.032
	†Fibrinogen (>400 mg/dL)	457 (334-547)	481.5 (437-732)	0.149
	†Ferritin >336 ng/mL)	601(410-903)	854 (551-1080)	0.239

* Independent Sample T-test, † Mann-Whitney U test, ‡ Fischer's Exact test, §Chi-Square test. Continuous variables were expressed as mean±SD or median (25-75 percentiles), while categorical variables were presented as N (%). BMI SDS: Body mass index standard deviation score; ICU: Intensive care unit; MV: Mechanical ventilation

In our study, the mean age and male dominance (57.4%) were similar to the previously reported MIS-C cases. However, the mean age of mild to moderate involvement and severe involvement groups were very close to each other. In addition, there was no difference between the severity groups in terms of gender.

The most common gastrointestinal symptoms of MIS-C include abdominal pain vomiting, and diarrhea. Radia et al., in their review analyzed the results of 35 studies conducted on a total of 783 MIS-C cases. They reported that 36% of cases with MIS-C had abdominal pain, 27% had diarrhea, and 25% had vomiting.[14] In our study, the most common symptoms of the cases with gastrointestinal involvement were abdominal pain (78.7%) and vomiting (59.6%). When the groups according to the severity of gastrointestinal involvement were compared no statistically significant difference was found in terms of presenting symptoms. The results of our study findings show that patients may present with different gastrointestinal symptoms regardless of the severity of gastrointestinal involvement.

It has been reported that elevated liver enzymes are frequent in MIS-C and associated with more severe clinical manifestations.[15] Giannattasio et al., in their study evaluating acute liver injury in MIS-C cases, reported that

16 (29%) of 55 MIS-C cases had increased transaminases and cholestasis findings were accompanied in 2 cases at admission and no acute liver failure developed in any of the patient.[16] In our study, consistent with the literature, increased transaminases were present in 29.8% of our cases. No cholestasis or acute liver failure was detected in any of the patients included in our study. There was no difference between the mild to moderate and severe gastrointestinal involvement groups in terms of transaminase levels. The results of our study suggest that there is no relationship between increased transaminase levels and severity of gastrointestinal involvement.

In patients with MIS-C abdominal imaging findings are rarely normal. Frequently reported imaging findings include mesenteric lymphadenitis, ascites, intestinal wall thickening including terminal ileum and/or cecum, hepatomegaly, and gallbladder wall thickening.[17,18] The some clinical and radiological features in gastrointestinal involvement of MIS-C can lead to an unneccesary surgery or late-diagnosis of some disease such as inflammatory bowel disease, as in our cohort.[9] Ilieva et al evaluated abdominal imaging findings in a cohort of 51 patients with MIS-C. They reported ascites in 65% of cases, mesenteric lymphadenitis in 37%, and ileitis and/or colitis in 35%.[19] The most common findings in our study were ascites (36.2%) and pancreatic edema (27.7%). The patients with severe findings including ascites, pancreatitis, diffuse mesenteric lymphadenitis and terminal ileitis were included in the severe group. When the relationships between these findings and parameters related to poor clinical outcome, such as intensive care admission, inotropic support, invasive/noninvasive mechanical ventilation requirement, and mortality were examined, it was found that the rate of intensive care hospitalization was significantly higher, especially in the presence of pancreatitis.

Liu et al., investigated the expression of angiotensin-converting enzyme 2 (ACE2) receptors of SARS-CoV-2 in the pancreas and they showed that ACE2 receptors are more abundant in the pancreas than in the lung.[20] Acharyya et al., reported that 53% of 17 patients with MIS-C had acute pancreatitis at admission. In addition, they reported that 55% of the cases with acute pancreatitis required hospitalization in the ICU and 22% resulted in mortality. Acharya et al. suggested that pancreatitis should be included in the MIS-C diagnostic criteria, due to its frequency and poor clinical course.[10] In our study, similar to the literature, we found that the rate of hospitalization in the intensive care unit is higher in the presence of acute pancreatitis.

The results of our study showed that neurological involvement was significantly higher in MIS-C cases with severe gastrointestinal involvement. It has been suggested that neuroinvasion of the central nervous system by SARS-CoV-2 may worsen the clinical course.[21] In addition, it has been shown that two proteinases required for neuroinvasion (ACE2 and TMPRSS2) are expressed in the enteric nervous system and the blood brain barrier and have been implicated in neurological findings.[22] The fact that neurological involvement is more frequent in MIS-C cases with severe gastrointestinal involvement may be the clinical reflection of this expression.

Vecchio et al. found that leukocyte, C-reactive protein, and ferritin levels were higher in MIS-C cases with severe gastrointestinal involvement.[7] In our study, there was no difference between the severe and mild to moderate groups, in terms of these inflammatory biomarkers. We found that BNP and d-dimer levels were higher in MIS-C with severe gastrointestinal involvement group compared to the mild to moderate group. In the literature, it has been reported that BNP and d-dimer levels are associated with the severity of MIS-C.[23] In our study, we observed that severe MIS-C clinic was significantly more common in patients with severe gastrointestinal involvement and we suggest that these findings may be related to the severity of the MIS-C. In a study evaluating the severity of gastrointestinal involvement in children with SARS-CoV-2 infection, it was reported that severe gastrointestinal findings are associated with MIS-C, prolonged hospitalization, and increased need for hospitalization in the intensive care unit.[7] Sayed et al. reported that the rate of critical illness was higher in patients with MIS-C, in the presence of gastrointestinal involvement. However, the severity of gastrointestinal involvement was not evaluated in this study.[13] Our study focused only on MIS-C cases with gastrointestinal involvement and showed that severe gastrointestinal involvement was more common in severe MIS-C cases. The rates of need for hospitalization in the intensive care unit, inotropic support, need for invasive/noninvasive mechanical ventilation, and mortality were higher in patients with MIS-Cs with severe gastrointestinal involvement; however, the differences were not statistically significant. We think that the lack of statistically significant results is due to the small number of patients in the groups, which we formed according to severity.

The limitations of the study are its retrospective design and single-center study. Although the number of patients in the groups seems to be small, this is the study with the largest patient cohort from a single center on a specific aspect of MIS-C in the current literature. We focused only on cases with MIS-C with gastrointestinal involvement and evaluated the severity of gastrointestinal system involvement along with clinical course and outcome.

CONCLUSION

Gastrointestinal involvement is one of the major findings of MIS-C. In a significant part of the cases severe gastrointestinal system involvement is observed. Patients with pancreatitis may have more severe course. However,

pancreatitis is not one of the organ involvements in the MIS-C diagnostic criteria. We think that, clinic and laboratory evaluation for pancreatitis should be part of the management of MIS-C with gastrointestinal presentations.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of the Ethics Committee of Necmettin Erbakan University Medical Faculty (protocol code:2022/4060 and date of approval: 02.12.2022).

Informed Consent: All participants signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article / Orijinal Araştırma

Prognostic Value of Systemic Immune-Inflammation Index in Head and Neck Carcinoma Patients Undergoing Definitive Radio(Chemo)Therapy

Definitif Radyo(kemo)terapi ile Tedavi Edilen Baş Boyun Kanserli Hastalarda Sistemik İmmun-İnflamasyon İndeksinin Prognostik Etkisi

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Abstract

Aim: The aim of this study is to investigate the prognostic effect of the systemic immune-inflammation index (SII) in non-surgically managed head and neck carcinoma patients who underwent definitive radio(chemo)therapy.

Material and Method: Twenty four patients who were all treated with radio(chemo)therapy with curative intent for head and neck cancer (HNC) were included in the study. All patients were analyzed in terms of age at diagnosis, gender, body mass index, stage, radiotherapy dose/ fraction, chemotherapy (CT), pre-treatment complete blood count parameters, the pre-treatment systemic immune-inflammation index, local relapse, distant failure, overall survival (OS), and disease-free survival (DFS).

Results: SII index was observed to be higher in locally advanced patients than in stage I/II patients (p=0.004). In addition, as a result of the evaluation made with ROC (receiver operating characteristic) analysis, it was observed that the SII index had a diagnostic value in predicting locally advanced disease (AUC: 0.867, 95% CI: 0.721-1.00, p=0.002). DFS and OS rates were 79% and 90% at a median follow-up of 9 months.

Conclusion: The systemic immune-inflammation index predicts more advanced disease in non-surgically managed head and neck cancer patients. It can be considered as a biomarker that can contribute to the management of definitive radio(chemo)therapy.

Keywords: Head and neck carcinoma, radiotherapy, systemic immune-inflammation index

Öz

Amaç: Cerrahi uygulanmayıp definitif radyo(kemo)terapi ile tedavi edilen baş boyun kanserli hastalarda sistemik immun –inflamasyon indeksinin prognostik etkisinin araştırılmasıdır.

Gereç ve Yöntem: Küratif yaklaşımla radyo(kemo)terapi uygulanan baş boyun kanseri tanılı yirmi dört hasta çalışmaya dahil edilmiştir. Hastalar tanı yaşı, cinsiyet, vücut kitle indeksi, evre, radyoterapi doz/ fraksiyon verisi, uygulanan kemoterapiler, tedavi öncesi tam kan sayım parametreleri, tedavi öncesi sistemik immun –inflamasyon indeksi, lokal relaps, uzak hastalık, genel sağkalım (OS), and hastalıksız sağkalım (DFS) açısından retrospektif olarak incelenmiştir.

Bulgular: Sistemik immun –inflamasyon indeksinin lokal ileri evre hastalarda, evre I/II olan hastalara göre daha yüksek olduğu gözlenmiştir (p=0,004). Bununla birlikte yapılan ROC analiz sonucuna göre sistemik immun –inflamasyon indeksinin lokal ileri evre hastalığı öngörmede tanısal değeri olduğu belirlenmiştir (AUC: 0,867, %95 CI: 0,721-1,00, p=0,002). Medyan 9 aylık takip sonunda DFS ve OS oranı sırasıyla %79 ve %90 olarak bulunmuştur.

Sonuç: Sistemik immun –inflamasyon indeksi cerrahi uygulanmadan tedavi edilen baş boyun kanserli hastalarda lokal ileri evre hastalığı öngördürmektedir. Definitif radyo(kemo)terapi yönetimine katkı sağlayabilecek bir biyobelirteç olarak gözönünde bulundurulabilir.

Anahtar Kelimeler: Baş boyun kanseri, radyoterapi, sistemik immun–inflamasyon indeksi

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INTRODUCTION

The head and neck region includes the upper aerodigestive tract (oral cavity, paranasal sinuses, pharynx, larynx, cervical esophagus), thyroid, associated lymph nodes, bone and soft tissues.[1] As with other cancers, there is increasing evidence that inflammation is associated with prognosis in head and neck cancers. Tumor cells also secrete various chemotactic substances and invoke macrophages, secrete damage-related molecular structures , activate neutrophils, and acidify the tumor microenvironment and prepare the environment for inflammatory responses.[2]

The cancer-induced inflammatory response leads to changes in peripheral blood neutrophils, lymphocytes, monocytes, and platelets, and this can be used to predict survival rates of patients with cancer. There have been many studies investigating the effects of systemic inflammatory responses such as neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR) and monocyte/lymphocyte ratio (MLR) on prognosis.[3] Systemic immune-inflammation index (SII) is an inflammatory response marker calculated based on absolute platelet, neutrophil, and lymphocyte counts. It shows the patient's systemic immune stimulation level and immune response level. SII has been shown to have a prognostic effect in different malignancies.[4,5] It has also been observed to be associated with decreased DFS and OS in different cancer types.[6] In this study, the importance of SII in head and neck cancers (HNC) treated with definitive radio(chemo)therapy was examined.

MATERIAL AND METHOD

Study Population

Between February 2021 and August 2022, twenty four patients who were all treated with radio(chemo)therapy with curative intent for head and neck cancer were included in the study. All patients were analyzed in terms of age at diagnosis, gender, body mass index, stage, radiotherapy (RT) dose/fraction, chemotherapy (CT), pre-treatment complete blood count parameters, the pre-treatment systemic immune-inflammation index, local relapse, distant failure, overall survival, and progression-free survival. The tumors were staged according to the American Joint Committee on Cancer (AJCC, 8th ed.,2017) TNM staging system.[7] The patients were followed up 1-3 times a week during the RT treatment, and enteral and/or parenteral nutrition support was provided if necessary. The systemic immune-inflammation index (SII), was calculated as : platelet count * neutrophil count/lymphocyte count. The study was carried out with the permission of Istanbul Prof. Dr. Cemil Tascioglu City Hospital. Ethics Committee (Date: 2023, Decision No: E-48670771-514.99-210779036).

Statistical Analysis

The descriptive statistics of the numerical variables obtained in the study are given as the median (range) value. The descriptive statistics of the categorical variables are given as numerical values and percentages. Data distribution was assessed by the Kolmogorov–Smirnov test. In consideration of the sample size, the non-normal distribution of variables was assumed, and nonparametric tests were used for between-group comparisons. So the categorical and numerical variables were compared using the chi-square test and Mann–Whitney U-test, respectively. Receiver operating characteristic (ROC) curves were also used to analyze the SII for predicting the advanced stage (T3-T4) disease . Kaplan–Meier curves were generated for overall survival (OS) and disease-free survival (DFS) and significance was assessed using the log-rank test . Statistical analyses were performed using SPSS 25 software (SPSS Inc., Chicago, IL, USA). A probability value of $p<0.05$ was considered significant.

RESULTS

Patient Characteristics

The median age of the patients was 65,5. Median follow-up was 9 (range, 3-22) months. All of the patients had squamous cell carcinoma histology and none of them had undergone surgical treatment. 67% of the patients were node-positive patients, and the incidence of N2-N3 disease was higher in patients aged 65 years and younger than those over 65 years of age (83% vs 25% ,p=0,004). The rate of locally advanced patients was 71% and SII index was observed to be higher in locally advanced patients than in Stage I/II patients (p=0.004). In addition, as a result of the evaluation made with ROC analysis, it was observed that the SII index had a diagnostic value in predicting locally advanced disease (AUC: 0.867, 95% CI: 0.721-1.00, p=0.002) (**Figure 1**). Curative RT was applied to all patients with intensity-modulated radiotherapy (IMRT) and 79% of the patients received concomitant chemotherapy. The baseline characteristics of the patients are presented in **Table 1**.

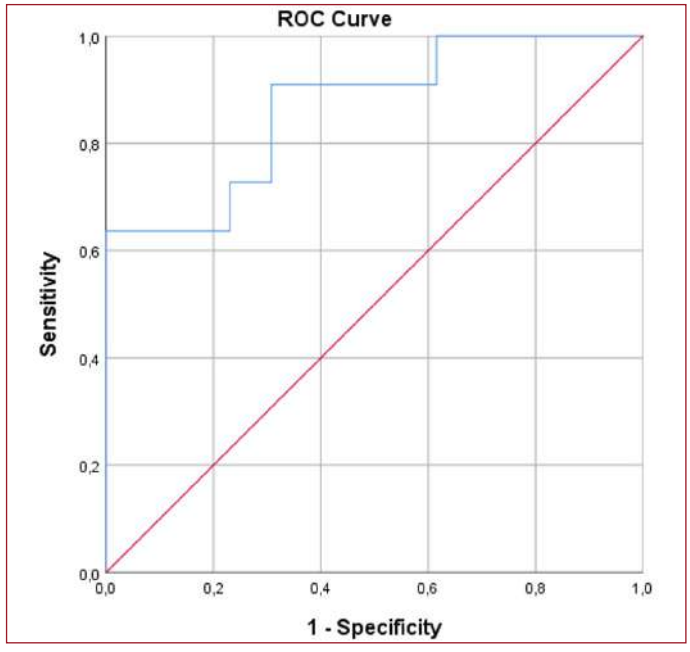

Figure 1. ROC analysis for the SII index predicting locally advanced disease

Table 1: Patient characteristics and basic statistical findings

	Patients (n:24 , %) / median (range)
Age	65.5 (32-82)
Gender	
Female	4 (16.7%)
Male	20 (83.3%)
BMI (kg/m²)	24.2 (18.7-36.1)
Location	2 (8.4%)
Paranasal sinus	8 (33.2%)
Nasopharynx Oropharynx	1 (4.2%)
Oral cavity	1 (4.2%)
Hypopharynx	1 (4.2%)
Larynx	10 (41.6%)
Unknown primary	1 (4.2%)
T stage	
X	1 (4.2%)
I	5 (20.8%)
II	8 (33.3%)
III	6 (25%)
IV	4 (16.7%)
N stage	
0	8 (33.3%)
I	3 (12.5%)
II	7 (29.2%)
III	6 (25%)
Stage	
I	5 (20.8%)
II	2 (8.3%)
III	6 (25%)
IVA	9 (37.5%)
IVB	2 (8.3%)
Radiotherapy (Gy)	70 (63-70)
Chemotherapy	
Yes	19 (79.2%)
No	5 (20.8%)
Lymphocyte ($\times 10^3/\mu L$)	1.62 (0.3-3.9)
Neutrophil ($\times 10^3/\mu L$)	4.6 (1.8-9.1)
Platelet ($10^3/L$)	248 (133-374)

Oncological Results

After a median follow-up of 9 months, local recurrence was observed in 12.5% of patients. Distant metastasis was encountered three of the patients. Two patients died due to disease-related reasons. DFS and OS rates were 79% and 90% at a median follow-up of 9 months (**Figure 2**-**3**).

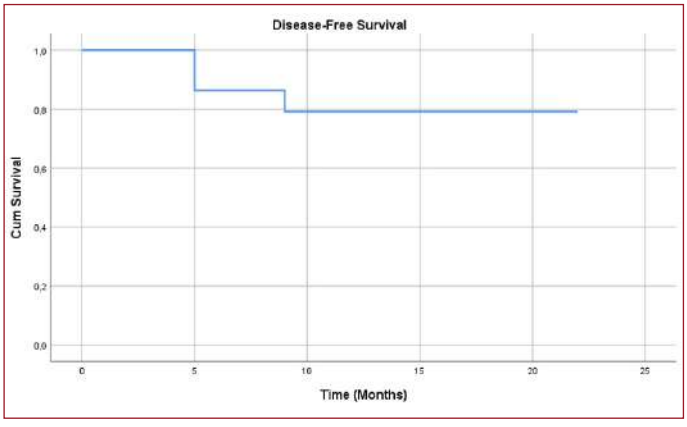

Figure 2. Kaplan-Meier plots of disease free survival.

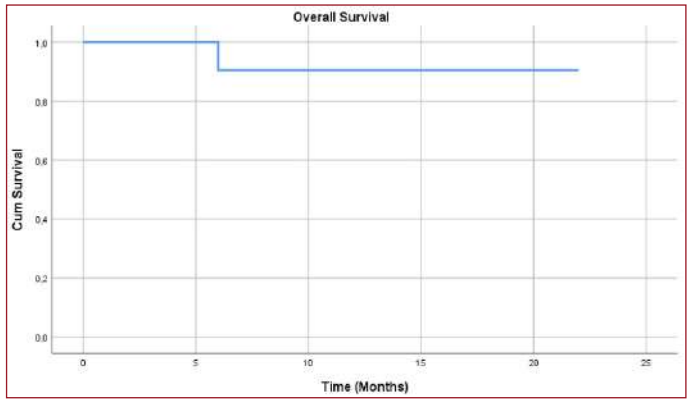

Figure 3. Kaplan-Meier plots of overall survival.

DISCUSSION

In different studies, it has been observed that SII is a better prognostic marker than other inflammation indices in many cancer types.[8-11] Also , most of the studies on head and neck cancers are on patients who underwent surgical treatment.

Zhilin Li et al. retrospectively evaluated 147 patients who underwent surgery for laryngeal cancer. They observed that high SII was associated with advanced T stage ($p<0.005$), locoregional recurrence ($p<0.005$), lower PFS ($p<0.001$) and OS ($p<0.001$).[12]

Kubota et al. retrospectively analyzed 183 patients with oral cavity cancer diagnosis. Most of the patients in the study were surgical patients and were evaluated in terms of SII and inflammation-based prognostic scores. Worse DFS results ($p=0.003$) were observed in patients with higher SII, and higher SII was also found to be an independent predictive factor on OS ($p=0.016$).[13]

Ruiz-Ranz et al. retrospectively analyzed 348 patients who underwent surgical treatment for oral cavity cancer. They reported that patients with high SII had a larger tumor volume ($p<0.001$) and these patients were more advanced stage ($p=0.003$).[14]

Cho et al. analyzed 269 patients who underwent surgical treatment for oral cavity tumor.

74% of these patients underwent surgery for tongue cancer, and 52% of them were stage 3-4. It was observed that patients with high SII had lower disease-specific survival and PFS rates ($p<0.05$).[15]

In a multicenter retrospective analysis covering the years 2004-2018, Rizzo et al. examined 925 patients with a diagnosis of HNC. All of the patients were surgical patients and patients who underwent curative RT were excluded from the study. Besides different systemic inflammatory response parameters, SII was also investigated in the study. Patients were analyzed by dividing them into 3 groups according to SII value, and it was observed that OS and DFS decreased significantly in the group with high SII value ($p=0.013$ and $p=0.003$,respectively).[16]

Lu et al. aimed to develop a nomogram on the prognostic effect of different inflammation indices such as neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), lymphocyte- to- monocyte ratio (LMR), and SII in predicting survival. For this purpose, 120 patients who underwent primary surgery for tongue cancer were analyzed. At the end of the study, it was observed that high SII was associated with tumor differentiation (P=0.011), tumor size (P=0.002), tumor invasion depth (P=0.011) and lymph node density (P=0.003). In the multivariate analysis, it was observed that SII had an independent prognostic effect on DFS and OS.[17]

Wang et al. published a meta-analysis of 12 studies involving 4369 patients to investigate the effect of SII on survival outcomes in patients with head and neck cancer treated with different treatment modalities. Worse OS results were observed in patients with high SII ($p<0.001$). In addition, when they looked at nasopharynx, larynx, and oral cavity tumors separately, they again found that SII elevation was significantly associated with poor OS results ($p=0.004$, $p<0.001$,and $p<0.002$, respectively) . It was also observed that high SII was associated with worse DFS and PFS results (both $p<0.001$). In this meta-analysis, no relationship was observed with tumor differentiation or gender, but it was observed that SII elevation was associated with more advanced T stage ($p<0.001$) and lymphatic involvement ($p=0.002$).[18]

All of the patients in our study were patients treated with definitive radio(chemo)therapy . 71% of the patients are stage 3-4 patients and 67% of them have lymph node positivity. Similar to these studies, also, we observed that high SII predicted more advanced stage disease.

Wu-Chia Lo et al. studied 147 patients with oropharyngeal cancer. 87% of this patient group is stage IV and all of them are treated with chemoradiotherapy. It was observed that SII was an independent risk factor for death ($p=0.011$) and patients with high SII had lower OS ($p<0.001$).[19]

Zeng et al. analyzed the data of 2169 patients from 6 studies conducted only on patients with nasopharyngeal carcinoma between 2017-2021. It was observed that patients with higher SII values exhibited worse OS and PFS results (HR=1.69, 95% CI=1.36–2.09, $P<0.001$ and HR=1.60, 95% CI=1.29–1.98, $P<0.001$, respectively).[20]

CONCLUSION

SII is a noninvasive, accessible and prognostically effective marker in patients with head and neck cancer treated with curative radio(chemo)therapy without surgery. A pre-treatment SII elevation indicates more advanced disease and may contribute to treatment management.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Istanbul Prof. Dr. Cemil Tascioglu City Hospital. Ethics Committee (Date: 2023, Decision No: E-48670771-514.99-210779036).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article / Orijinal Araştırma

Brucellosis; Difficulty of Diagnosis in Endemic Areas

Bruselloz; Endemik Bölgelerde Tanı Zorluğu

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Abstract

Introduction: Brucellosis is a zoonotic disease distributed worldwide and very important public health problem especially in the developing countries. In this study, we aimed to evaluate the clinical/laboratory findings of brucellosis patients and contribute of coombs testing to diagnosis at Iğdır State Hospital's Infection Diseases and Clinical Microbiology department.

Material and Method: One hundred and forty-five brucellosis patients followed up in our clinic between September 2012 and February 2013 were evaluated retrospectively. Demographic characteristics, laboratory findings, diagnostic methods of the patients were presented.

Results: The mean age of the patients were 39±15 (18-80) and 59% (n=86) of the patients were female, 41% (n=59) were male. Most frequent risk factors were animal breeding (n=115, 79%) and using underdone milk and milk products (n=98, 69%). Most reported complaints were weakness (92%), arthralgia (89%), sweating (74%), lack of appetite (70%) and fever (68%). Fifty-seven of the brucellosis patients could not diagnosed with standard tube agglutination. Therefore, Coombs test was used for these undiagnosed patients (39%, n=57). Eighty patients were evaluated as acute (55%), 53 as subacute (37%) and 12 as chronic (8%) brucellosis.

Conclusion: Brucellosis can affect all organ systems and cause different clinical manifestations. Therefore, difficulties are encountered in the diagnosis of the disease. Brucellosis should be kept in mind in the differential diagnosis especially in the endemic regions. When the clinical suspicion exists detailed laboratory evaluation must be performed.

Keywords: Brucellosis, diagnosis, coombs test.

Öz

Giriş: Bruselloz tüm dünyada yaygın olarak görülen zoonotik bir hastalıktır ve özellikle gelişmekte olan ülkelerde çok önemli bir halk sağlığı sorunudur. Bu çalışmada, Iğdır Devlet Hastanesi Enfeksiyon hastalıkları ve klinik mikrobiyoloji bölümünde takipli bruselloz hastalarının klinik/laboratuvar bulgularının ve coombs testinin tanıya katkısının değerlendirilmesi amaçlandı.

Gereç ve Yöntem: Eylül 2012-Şubat 2013 tarihleri arasında kliniğimizde izlenen 145 bruselloz hastası geriye dönük olarak değerlendirildi. Hastaların demografik özellikleri, laboratuvar bulguları, tanı yöntemleri sunuldu.

Bulgular: Hastaların yaş ortalaması 39±15 (18-80) olup, hastaların %59'u (n=86) kadın, %41'i (n=59) erkekti. En sık görülen risk faktörleri hayvancılık (n=115, %79) ve az pişmiş süt ve süt ürünleri kullanımıydı(n=98, %69). Bildirilen şikayetlerin çoğu halsizlik (%92), artralji (%89), terleme (%74), iştahsızlık (%70) ve ateş (%68) idi. Hastaların %60,6'sında (n=88) standart tüp aglutinasyonu pozitifti (≥ 1/160 titrasyonda), 57'sine standart tüp aglütinasyonu tanısı ile konulamadığından bu hastalarda Coombs testi kullanıldı (%39, n=57). Seksen hasta akut (%55), 53 hasta subakut (%37) ve 12 hasta kronik (%8) bruselloz olarak değerlendirildi.

Sonuç: Bruselloz tüm organ sistemlerini etkileyebilir ve farklı klinik bulgulara neden olabilir. Bu nedenle hastalığın tanısında güçlüklerle karşılaşılmaktadır. Özellikle endemik bölgelerde ayırıcı tanıda bruselloz akılda tutulmalıdır. Klinik şüphe mevcut olduğunda ayrıntılı laboratuvar değerlendirmesi yapılmalıdır.

Anahtar Kelimeler: Bruselloz, tanı, coombs testi

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INTRODUCTION

Brucellosis is a zoonosis that seen commonly all over the world, and continues to be an important health problem for developing countries.[1] It is endemic in Turkey, particularly concentrated in Central Anatolia, Eastern and Southern Anatolian cities.[2]

Brucellosis can involve all organs and systems, cause different clinical pictures, so diagnosis may be difficult.[3]

The gold standard method in the diagnosis of brucellosis is culture;however, its sensitivity decreases depending on several factors (e.g.disease duration and history of antibiotic use). For this reason, serological methods such as Rose-Bengal Test -as a screening test-, Standard Tube Agglutination (STA), and Coombs'Antiglobulin Test are often used for diagnosis.[4]

In this study, we aimed to evaluate brucellosis cases that followed-up at Iğdır State Hospital, which is a small city in northeastern Turkey. and importance of Coombs test for diagnosis of brucella cases that cannot be detected by screening test in hospitals where blood culture is not possible.

MATERIAL AND METHODS

One hundred and forty-five adult patients that followed-up at infectious diseases department with a diagnosis of brucellosis between September 2012 and February 2013 were retrospectively evaluated. The age, sex, risk factors, complaints, duration of complaints, physical examination and laboratory findings were recorded.

The inclusion criteria were as follows:[5]

- Serum agglutination titer over 1/160 with clinical sign and symptoms, or
- Coombs test over 1/160 with clinical sign and symptoms, or
- Brucella spp positivity in blood culture.

Patients under the age of 18, and patients treated for brucellosis in the past year were excluded.

For the serologic diagnosis, slide antigen that was produced by Turkish Public Health Institution (TPHI) was used for Rose Bengal test, and B. abortus antigen (that was also produced by TPHI) was used for STA. The STA was prolonged until a negative tube was seen in each sample. The agglutination test with the Coombs serum was used to prevent blocking antibodies and eliminating false negativity. The Coombs Test was used in every patient who could not be diagnosed with STA but was clinically considered to have brucellosis. Blood cultures were incubated for seven days in BACTEC9120 (Becton-Dickinson) system and conventional methods were used to identify microorganisms.

Consuming rare-cooked milk and dairy products, working in livestock, being a butcher, veterinarian or laboratory worker were accepted as the risk factors for the disease. Patients were classified as acute (less than 8 weeks), subacute (8-52 weeks), and chronic (more than 52 weeks) according to duration of clinical symptoms.

Statistical Analysis

Statistical analysis was performed using the SPSS v15.0 package program. In descriptive statistics, the continuous variables were expressed as mean and standard deviation and the categorical variables were expressed as frequencies and percentages. The Kolmogorov-Smirnov test was used to assess the normality of the data. ESR values were distributed normally, and this variable was compared with one-way ANOVA test between disease duration groups (acute, subacute, chronic). Since CRP and ferritin values were not normally distributed, these variables were compared with Kruskal-Wallis test between groups. A p value of <0.05 was considered statistically significant.

The study was carried out with the permission of Diskapi Yildirim Beyazit Training and Research Hospital Ethics Committee (Date: 27.06.2016, Decision No: 31/04). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

RESULTS

When the demographic characteristics of 145 cases of the study were evaluated, mean age was 39±15 (18-80) years, 59% (n=86) were females, and 41% (n=59) were males. Risk factors were livestock (n=115, 79%), consuming raw milk and dairy products (n=98, 69%) and being butcher (n=5, 3%).

Eighty (55%) patients had acute, 53 (37%) had subacute, and 12 (8%) had chronic brucellosis, 26% (n=38) had a history of brucellosis longer than one year ago, and 36% (n=53) had family history. When the complaints at first admission were evaluated, the most frequent symptoms were found to be weakness, arthralgia and sweating (**Table 1**). Physical examinations revealed fever over 38.3°C in 42 (29%) patients, hepatomegaly in 20 (14%), splenomegaly in 10 (7%), hepatosplenomegaly in six (4%), peripheral arthritis in six (4%), and erythema nodosum in two (1.4%) patients. Correlation between organomegaly and disease duration was summarized in **Figure 1**. Splenomegaly was more frequent in acute brucellosis cases. Hepatomegaly was detected in all periods of the disease.

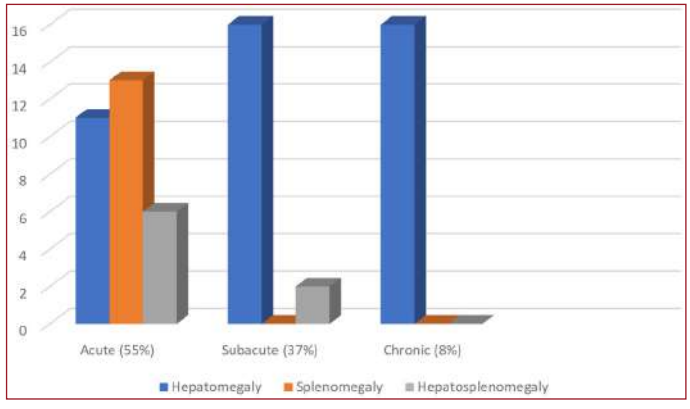

Figure 1: Disease durations of patients with organomegaly

Table 1: Patients' symptoms	
Symptoms	**n (%)**
Weakness	134 (92)
Arthralgia	129 (89)
Sweating	107 (74)
Loss of appetite	101 (70)
Fever	99 (68)
Headache	93 (64)
Lumbalgia	92 (63)
Myalgia	62 (43)
Stomachache	53 (37)
Weight loss	32 (22)
Joint swelling	25 (17)
Scrotal pain	6 (10)

The serologic tests revealed that Rose Bengal test was negative in 16 patients, and STA test was lower than 1/160 in 57 (39%) patients.They applied to the polyclinic after an average of one month after the onset of the compliant. STA was not repeated, as there was admission more than two weeks after the onset of symptoms. Both Rose Bengal and STA tests were found negative in 14 (9.7%) cases. STA test was 1/160 in 32 (22%), 1/320 in 44 (30%), 1/640 in seven (5%), 1/1280 in four (3%), and 1/5120 in one (0.7%) cases. Fifty-seven cases that could not be diagnosed with STA test were diagnosed by the Coombs test (**Table 2**). Thirty-six percent (n=29) of acute cases, 40% (n=21) of subacute cases, and 58% (n=7) of chronic cases were diagnosed with the Coombs test. In four acute cases with positive blood cultures, STA test was below 1/160 and the diagnosis was confirmed with the Coombs test. Blood cultures were obtained from only 28 cases due to the unavailability of the hospital resources, and 17 (60.7%) had positive results (**Table 2**). Of importance, we have noticed that 65% of positive blood cultures were obtained from patients without fever. Of the cases with positive blood culture, 14 (82%) were acute, and 3 (18%) were subacute brucellosis cases. Laboratory findings were summarized in **Table 3**. The assessment of ferritin levels revealed that 24 of the 112 cases (21%) had elevated ferritin levels.The elevations detected in the acute phase reactants decreased to the normal limits following the treatment - in all patients. The effect of disease duration on factors such as age, blood culture, and serological test positivity, and the elevation in acute phase reactants are discussed in **Table 4**. Comparisons of disease durations of cases with increased ESR, CRP, and ferritin were shown in **Figure 2**. The increases in these values were found to be significantly higher in acute brucellosis when compared to subacute and chronic brucellosis ($p<0.05$).

Table 2: The results of diagnostic tests performed			
Test performed	**Number of tests performed**	**Negative result (%)**	**Positive result (%)**
Rose Bengal	145	16 (11%)	129 (89%)
STA*	145	57** (39.3%)	88*** (60.7%)
Coombs Agglutination	57	-	57***
Blood culture	28	11 (39.3%)	17 (60.7%)

*STA: Standard Tube Agglutination, **< 1/160 recorded as negative, ***1/160 and above recorded as positive

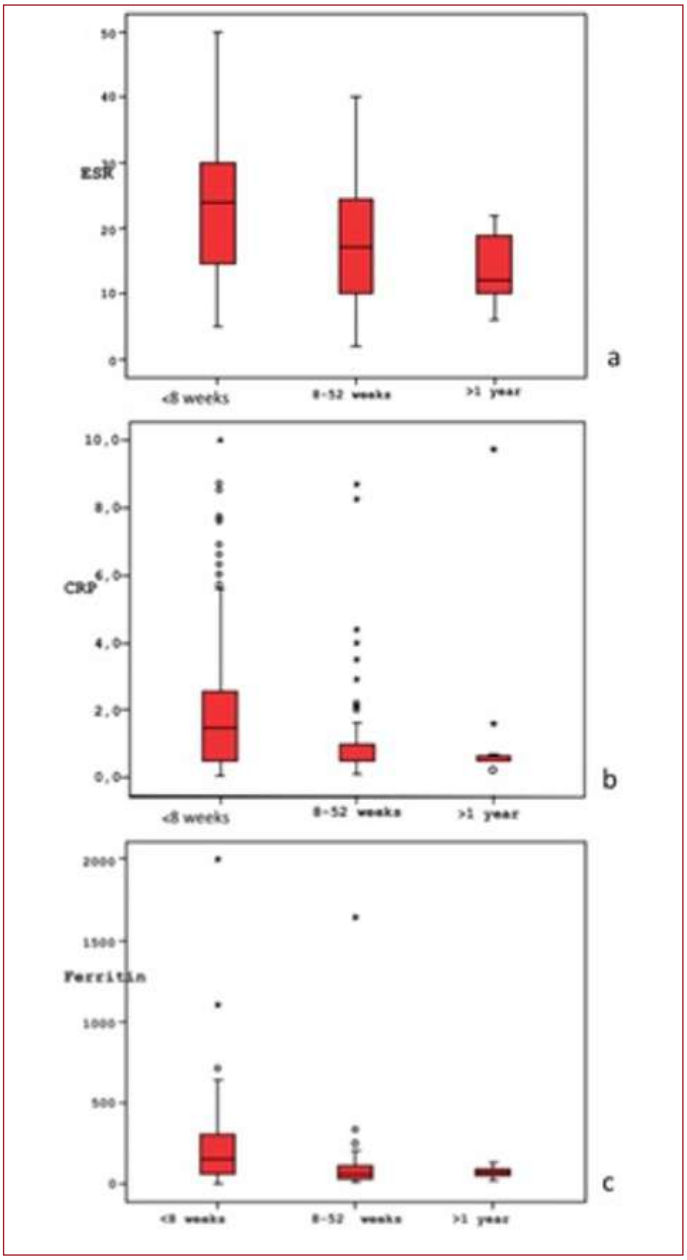

Figure 2. Comparison of disease durations of patients with (a) ESR, (b) CRP, and (c) fernitin

Table 3: Patients' laboratory findings	
Laboratory finding	**n (%)**
Anemia (women: <12 mg/dl; men: <14 mg/dl)	27 (19)
Leucopenia (<4500/mm³)	18 (12)
Lymphomonocytosis	67 (46)
Leukocytosis (>10500/mm³)	9 (6)
Thrombocytopenia (<150000/mm³)	16 (11)
ESR (>20 mm/h)	75 (52)
CRP (>1 mg/dl)	65 (45)
Ferritin (women >150ng/ml; men > 400 ng/ml)	24 (17)
ALT (>40 IU/L)	51 (35)
AST (>40 IU/L)	34 (23)

ESR: Erythrocyte sedimentation rate, CRP: C reactive protein, ALT: Alanine transaminase, AST: Aspartate transaminase

Table 4: Comparison of age and laboratory findings of patients with Brucellosis according to disease duration. Data presented as mean±standard deviation, median (min-max) or percent

	Acute	Subacute	Chronic	p
Age (n=145)	39.22±14.89	39.38±15.74	43.42±12.27	0.659*
Positivity of blood culture % (n=17, 100%)	14, 82%	3, 18%	-	0.104**
ESR (mm/h)	23.20±10.38	13.38±10.01	20.68±10.34	<0.05*
CRP (mg/dl)	2.24±2.35	1.23±1.75	1.36±2.65	<0,05**
Leukocyte (/mm³)	6945.8±2250.1	7062.2±1903.5	7823.3±2602.3	0,425*
Hb (mg/dl)	13.81±1.78	14.16±1.01	14.16±1.01	0.753*
Rose-bengal, % (n=145)	91.3	84.9	91.7	0.498**
STA	1/160 (0-1/5120)	1/160 (0-1/1280)	1/40 (0-1/1280)	0.657**
Coombs testi	1/320 (1/160-1/1280)	1/320 (1/160-1/2560)	1/320 (1/160-1/320)	0,998**
Ferritin	230.48±297.81	127.93±272.07	70.82±31.07	<0.05**

ESR: Erythrocyte sedimentation rate, CRP: C reactive protein, Hb: hemoglobin, STA: Standart tube agglutination. Acute: Disease duration shorter than 8 weeks, Subacute: Disease duration 8 weeks 1 years, Chronic: Disease duration more than 1 year, *:ANOVA test, **:Kruskall-Wallis test

DISCUSSION

Brucellosis is a zoonotic disease seen worldwide. Each year, it is estimated that more than 500000 new cases are diagnosed and its prevalence is more than 1/100000 in endemic countries. It is hyper-endemic in Mediterranean countries, Arabian Peninsula, India, Mexico, and Central and South America. It is also endemic in our country, but its prevalence is unknown due to lack of reporting.[6] Çetin et al. evaluated 70000 individuals for brucellosis seropositivity, and found that 1.8% of the entire population was seropositive, and this was 6% in high-risk groups.[7] In a seroprevalence study conducted in blood donors, brucella was found at a rate of 2.7%.[8]

Brucellosis is recognized at every age and sex in Turkey, but it is more frequent especially between 15-35 years of age and in males. It is a prevalent infectious disease in animals, so it is frequently seen in people working with livestock, and consuming raw milk and dairy products.[9] In this study, which evaluated 145 cases that followed-up in our department, mean age was 39, 59% of the patients were females, and 79% were working with livestock. Sixty-eight percent of patients had a history of consuming undercooked milk and dairy products. These findings were in accordance with previous reports from Turkey and suggested that brucellosis is a highly prevalent disease in northeastern Turkey mainly due to consumption of raw milk products and not following personal protective measures when dealing with animals.[10]

Most frequent complaints of brucellosis are weakness, arthralgia, fever, sweating, and loss of appetite.[6,11-13] These complaints were found in our cases with similar frequencies. Fever was the major complaint of admission in 68% of the cases, but it was found as a physical examination finding in only 28% of cases. Similarly, Buzgan et al. found that 72.2% of cases had a complaint of fever, but only 28.8% of them had fever in physical examination.[6] This may be explained by the undulant nature of fever in brucellosis.

Every system and organ can be involved in brucellosis. In our study, hepatomegaly was present in 14%, splenomegaly was present in 7%, and hepatosplenomegaly was present in 4% of the patients. These rates were lower than previous reports.[6,12,13] When the association between organomegaly and disease duration was evaluated, splenomegaly was found to be more frequent in acute periods. Hepatomegaly was detected in all periods. Similar results were also reported in Buzgan et al. study.[6] Because hepatosplenomegaly is a result of replication of Brucella in the reticuloendothelial system, it was thought to be of splenomegaly is more frequent in acute and subacute periods.

Hematological abnormalities are frequent in brucellosis, but mostly they are not diagnostic.[2,14] Anemia, leukopenia, and lymphomonocytosis are frequent, and thrombocytopenia, pancytopenia, and disseminated intravascular coagulation are rarely seen.[11] In our study anemia was present in 19%, leukopenia in 12%, lymphomonocytosis in 46%, leukocytosis in 6%, and thrombocytopenia in 11% of the patients. These findings are compatible with other reports.[2,14]

ESR, CRP, and ferritin are known as acute phase reactants and they are used as inflammation indicators, not used for diagnosis.[15] Elevations of acute phase reactants in brucellosis were reported previously. ESR and CRP values were reported to be higher in acute and subacute cases.[3,5] Similarly, ESR and CRP elevations were more frequently found in acute and subacute cases in our study. Elevation of serum ferritin in brucellosis cases may be due to nonspecific tissue damage, and abnormalities in iron metabolism and/or hematopoiesis. Ferritin levels as high as 250 ng/L in patients with brucellosis was reported previously.[17] Serum ferritin level elevations were present in 17% (n=20) of our patients, and 88% (n=17) of these patients were in the acute period. Extremely high levels of serum ferritin as high as 2000 ng/L were detected in our patients. The correlation of disease duration and ferritin elevation has not been reported previously (**Figure 2**). These elevations may be related with the inflammatory over-response against bacterial load. The high levels were normalized after successful treatment in all cases.

Definitive diagnosis of brucellosis is based on the isolation of bacteria from clinical samples.[10] The positivity rates in blood cultures were reported between 11.4%-68.8% in previous studies.[6,11,12,18] In our study, blood cultures were taken from 28 patients, and 60.7% (n=17) of them had positive results. It was possible to obtain blood cultures from a small number of

patients because of the lack of materials in our hospital, but high positivity rates were found. It was remarkable that blood cultures were taken in the fever-free period of the patients who were positive.This shows us the importance of obtaining blood cultures when clinical findings are present, even in the absence of fever.

Of the serologic tests, Rose Bengal as screening test and STA are frequently used in the diagnosis. The sensitivity of commercially available Rose Bengal tests is between 96% and 100%. With STA, infection can be determined serologically in more than 97% of cases after three weeks of a disease period.[19] IgA and IgG antibodies, which are called as blocking antibodies, may cause false negative results.Also, false negativity can be detected in the early stages of the disease and in the Prezon Phenomenon in which agglutination may be masked at low dilution of the serum, especially when the serum has high titers of antibodies. It is frequently seen at 1/20 dilution and is rare at 1/80 and above dilutions. False positive results occur because of cross-reactivity with antibodies against Francisella tularensis, Escherichia coli O116 and O157, Salmonella urbana, Yersinia enterocolitica O: 9, Vibrio cholerae, Xanthomonas maltophilia and Afipia chevelandensis. False positive and negative results can be avoided by making dilutions of 1/320 or higher.[20] For these reasons, the STA dilutions were prolonged until there was a negative tube in the present study. The Coombs Test is used to prevent blocking anchors.[5] The cases diagnosed by Coombs Test were reported as 1%-6% in different studies.[7,21] In the study of Uysal et al., the diagnostic value of STA was reported to be low, especially in chronic patients, and it was recommended to repeat the tests with Brucellacapt and/or ELISA.[22] Coombs Agglutination Test was used in all our patients who were found to be negative for STA. The sensitivity of Rose Bengal test was found to be 89%, and STA was found positive in only 60,7% of brucellosis cases. All patients with negative STA results (n=57) were found to be positive with the Coombs test. In accordance with the literature, the contribution of the Coombs test to diagnosis was found to be more in chronic cases rather than acute and subacute cases. Diagnosis was based on Coombs test in 36% of acute cases, 40% of subacute cases, and 58% of chronic cases. Interestingly, in four acute cases with positive blood cultures, STA test was below 1/160 and the diagnosis was confirmed with the Coombs test.

The rate of cases diagnosed with the Coombs test was higher than literature, and this was thought to be related with that study was conducted in an endemic region and the Coombs test was performed more frequently by a careful evaluation of risk factors and clinical findings of patients.

Previous studies reported that diagnostic sensitivity of ELISA IgG and IgM was higher than STA.[3,18] But, Memish et al. reported that STA was more valuable in the diagnosis of brucellosis in cases with bacteremia, and should be evaluated together with ELISA IgM.[21] In our study, ELISA IgM and IgG could not be analyzed due to the limited resources.

The limitations of our study were that blood culture have not been studied for all patients (because of lack of equipment in our hospital) and ELISA IgG and IgM have not been studied for anyone (because there were no ELISA kits for brucellosis).

CONCLUSION

In conclusion, brucellosis is still an endemic disease in our country, and detailed physical examination and further analyses should be performed in the presence of a clinical suspicion since the sensitivity of screening tests are low in the acute period. ESR, CRP, elevated ferritin, and hepatosplenomegaly were found to be more frequent in acute and subacute periods of the disease. Especially in endemic regions, it should be kept in mind that contribution of screening tests and STA to the diagnosis is limited. In the presence of a clinical suspicion, Coombs test should be performed.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Diskapi Yildirim Beyazit Training and Research Hospital Ethics Committee (Date: 27.06.2016, Decision No: 31/04).

Informed Consent: All participants signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article / Orijinal Araştırma

Is there a Relation between Sleep Apnea, Tinnitus, and Hearing Loss?

Uyku Apnesi, Tinnitus ve İşitme Kaybı Arasında Bir İlişki Var Mı?

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Abstract

Aim: Chronic hypoxia may lead to auditory dysfunction in patients with obstructive sleep apnea (OSA), and this dysfunction may worsen OSA, creating a vicious circle. The aim of this study was to investigate tinnitus and hearing loss in OSA patients.

Material and Method: A total of 147 patients were included in this prospective study. After polysomnography (PSG), the patients with an apnea-hypopnea index (AHI) ≥ 5 were included in OSA group, and the ones with an AHI<5 were included in the simple snoring group. The OSA patients were divided into three OSA severity subgroups as mild, moderate and severe OSA subgroups. Standard pure-tone audiometry (PTA) (0.5, 1, 2, 3 kHz) and high-frequency audiometry (8, 10, 12 kHz) were performed, and Tinnitus Handicap Inventory (THI) was applied to all participants. Audiological results and THI scores were compared among the study groups.

Results: The OSA group consisted of 46 (36.5%) female, 80 (63.5%) male, and a total of 126 patients. The simple snoring group included 13 (61.9%) female, 8 (38.1%) male, and a total of 21 patients. The mean body mass index, hearing thresholds in all tested frequencies and THI scores were significantly higher in OSA group (p=0.024, p=0.001, p=0.015, p=0.017, p=0.039, p=0.002 and p=0.001, respectively). THI score showed a statistically significant and positive correlation with the AHI (p=0.004; r=0.252). The mean THI score was significantly lower in mild OSAS subgroup compared to moderate and severe OSA subgroups (p=0.019).

Conclusion: OSA, hearing impairment and tinnitus are either comorbidities or related etiologically. Higher prevalence of hearing impairment in OSA patients compared to simple snorers suggests that intermittent nocturnal hypoxia, rather than sound of snoring plays a role in hearing impairment.

Keywords: Hearing loss, obstructive sleep apnea, polysomnography, tinnitus

Öz

Amaç: Kronik hipoksi, tıkayıcı uyku apnesi (TUA) olan hastalarda işitme sisteminde işlev bozukluğuna yol açabileceği gibi, işitsel işlev bozukluğu da TUA'yı kötüleştirerek bir kısır döngü oluşturabilir. Bu çalışmanın amacı TUA'da tinnitus ve işitme kaybını araştırmaktı.

Gereç ve Yöntem: Bu prospektif çalışmaya toplam 147 hasta dahil edildi. Polisomnografi (PSG) sonrası apne-hipopne indeksi (AHİ) ≥5 olan hastalar TUA grubuna, AHI<5 olanlar ise basit horlama grubuna dahil edildi. TUA hastaları hafif, orta ve şiddetli TUA alt grubu olarak üç alt gruba ayrıldı. Tüm katılımcılara standart saf ses odyometri (SSO) (0,5, 1, 2, 3 kHz), yüksek frekans odyometri (8, 10, 12 kHz) ve Tinnitus Handikap Envanteri (THE) anketi uygulandı. Çalışma grupları arasında odyolojik sonuçlar ve THE skorları karşılaştırıldı.

Bulgular: TUA grubu 46 (%36,5) kadın, 80 (%63,5) erkek olmak üzere toplam 126 hastadan oluşmaktaydı. Basit horlama grubuna 13 (%61,9) kadın, 8 (%38,1) erkek olmak üzere toplam 21 hasta dahil edildi. Ortalama vücut kitle indeksi, test edilen tüm frekanslarda işitme eşikleri ve THE skorları TUA grubunda anlamlı olarak yüksekti (sırasıyla, p=0,024, p=0,001, p=0,015, p=0,017, p=0,039, p=0,002 ve p=0,001). THE skoru, AHİ ile istatistiksel olarak anlamlı ve pozitif korelasyon gösterdi (p=0,004; r=0,252). Ortalama THE skoru hafif TUA alt grubunda orta ve şiddetli TUA alt gruplarına göre anlamlı olarak düşüktü (p=0,019).

Sonuçlar: TUA, işitme kaybı ve kulak çınlaması ya komorbiditelerdir ya da etiyolojik olarak ilişkilidir. Basit horlayanlara kıyasla TUA hastalarında işitme bozukluğu prevalansının daha yüksek olması, horlama sesinden ziyade aralıklı gece hipoksisinin işitme bozukluğunda daha fazla rol oynadığını düşündürmektedir.

Anahtar Kelimeler: İşitme kaybı, obstrüktif uyku apnesi, polisomnografi, tinnitus

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INTRODUCTION

Obstructive sleep apnea (OSA) is an important health problem affecting 15-30% of men and 10-15% of women in North America. Hypoxia due to repetitive upper airway obstructions during sleep cause systemic inflammation and vascular endothelial damage through several proinflammatory cytokines and mediators, leading to various comorbidities.[1-4]

Tinnitus impairs quality of life and has been defined as perception of sound in absence of an external acoustic stimulus.[5] Tinnitus and OSA may interact in a negative way. Most of the patients with tinnitus have insomnia, however tinnitus is more frequent in OSA patients.[6] OSA may also be associated with a higher risk of hearing impairment.[7] Several studies that investigated the relation of OSA with auditory dysfunction reported that the patients with sleep apnea had a higher risk of developing both tinnitus and hearing loss.[7-9] Tinnitus Handicap Inventory (THI) is the most widely used measure to determine perceived tinnitus handicap severity.[10]

The aim of this study was to investigate the tinnitus and hearing impairment in OSA patients through THI, standard pure tone audiometry (PTA) and high frequency audiometry.

MATERIAL AND METHOD

A total of 291 consecutive patients who admitted to the sleep laboratory of a tertiary referral center with complaints of snoring and/or daytime sleepiness and witnessed sleep apnea between the July 2017 August 2018 were included in this prospective study. The patients with any mental or neurological disorders (18 patients), diabetes mellitus (15 patients), hypertension (18 patients), middle/inner ear disorders/significant air-bone gap in standard PTA or results other than a Type A tympanogram (8 patients), age ≥65 years (10 patients), history of otological surgery (1 patient), hyperlipidemia (9 patients) and the ones who did not accept to participate in the study and/or left without completing the study (65 patients) were excluded. A total of 147 patients were included in the study.

All patients had full-night standard polysomnography (PSG) during spontaneous sleep under the supervision of a sleep technician. PSG was recorded with Alice 5 PSG device (Philips Respironics, The Netherlands). Recorded data were electrooculogram (EOG), electroencephalogram (EEG), nasal airflow, thoracic and abdominal respiratory efforts, blood oxygen saturation, body position and submental and bilateral tibialis anterior electromyograms (EMG). A PSG and sleep disorders-certified ENT physician scored the PSG data manually according to the standard criteria of American Academy of Sleep Medicine. Complete interruption of airflow for at least 10 sec was regarded as apnea. At least 30% decrease in airflow amplitude accompanied by 3% oxygen desaturation and a reduction in chest/abdominal respiratory effort amplitude and/or related arousal was considered as hypopnea. The number of apneas and hypopneas per hour of sleep was used to calculate the apnea-hypopnea index (AHI).[11]

In relation with their AHI, the patients were divided into following groups: The patients with an AHI ≥ 5 were included in OSA group, and the ones with an AHI < 5 were included in the simple snoring group. Moreover, the patients with OSA were divided into three subgroups in relation with the severity of OSA as "Mild OSA" (5 ≤ AHI < 15), "moderate OSA" (15 ≤ AHI < 30) and "severe OSA" (AHI ≥ 30) subgroups.

The standard PTA (0.5, 1, 2, and 4 kHz) and high-frequency audiometry (8, 10 and 12 kHz) were performed using Interacoustic AC-40 (Assens, Denmark) clinical audiometer. The air and bone conduction thresholds were measured at four frequencies (0.5, 1, 2, and 3 kHz).The hearing threshold at 3 kHz was calculated by obtaining the mean of the hearing thresholds at 2 and 4 kHz.

Subjective tinnitus severity was assessed using a validated Turkish version of a standardized outcome measure, the THI.[12] THI consists of 25 items answered as yes (4 points), sometimes (2 points), or no (0 point). The total score may range between 0 and 100.[13] The THI and audiometric tests were completed within two days after PSG. The THI scores and audiology test results were evaluated and compared among the study groups.

All participants provided their informed consents, and the study protocol was approved by the local ethics committee (Date: 05.10.2017, Decree no: E17-1411).

IBM-SPSS for Windows v.21.0 software (IBM Corporation, Armonk, NY, USA) was used for statistical analysis of the data. The normality of the distribution of data was analyzed with Kolmogorov-Smirnov test. The descriptive data were presented as mean±standard deviation, median, minimum and maximum. Categorical variables were expressed in numbers and percentages. One-way ANOVA test was used to compare three or more groups with normal distributions, while Student t test was employed for the two-group comparisons of data with normal distributions. The comparison of quantitative data among three or more groups without normal distributions was done with Kruskal Wallis test, and Mann Whitney U test was used to detect the group that caused the difference. Spearman's Correlation Analysis was used for analysis of the correlations among the study parameters. The significance level was set at $p<0.05$.

RESULTS

A total of 147 patients were included in the study. The OSA group consisted of 46 (36.5%) female, 80 (63.5%) male and a total of 126 patients. The simple snoring group consisted of 13 (61.9%) female, 8 (38.1%) male and a total of 21 patients. The mean ages of OSA and control groups were 47.26±6.93 and 43.47±6.40 years, respectively. There was a statistically significant difference between the groups in terms of age ($p=0.019$). On PSG, it was evident that both simple snoring and OSA patients snored during the study in the sleep

laboratory.

Comparisons of THI scores, audiometry and polysomnography (PSG) findings between the OSA and simple snoring groups

Table 1 shows THI scores and audiometry and polysomnography (PSG) results of the OSA and the simple snoring groups. The mean body mass index (BMI), AHI, mean hearing thresholds at standard PTA (0.5, 1, 2 and 3 kHz), 8, 10 and 12 kHz as well as THI scores were significantly higher in OSA group compared to the simple snoring group (p=0.024, p=0.001, p=0.015, p=0.017, p=0.039, p=0.002 and p=0.001, respectively) (**Table 1**).

Table 1. The demographic characteristics and the audiometric, THI, polysomnography (PSG) findings among the groups.

Variables	AHI < 5 (n=21) Mean±SD	AHI ≥ 5 (n=126) Mean±SD	p
Age (years)	43.47±6.40	47.26±6.93	[a]p=0.019
Female Male	13 (61.9%) 8 (38.1%)	46 (36.5%) 80 (63.5%)	[b]p=0.028
BMI (kg/m²)	28.42±1.99	30.38±3.68	[a]p=0.024
AHI (ev/hr)	2.98±1.13	22.58±14.87	[a]p=0.001
PTA	10.57±2.78	12.83±4.30	[a]p=0.015
8 kHz	24.76±8.13	30.55±10.22	[a]p=0.017
10 kHz	27.85±7.51	33.37±10.95	[a]p=0.039
12 kHz	31.66±8.26	40.75±12.44	[a]p=0.002
THI	32.38±9.02	40.07±7.68	[c]p=0.001

aMann Whitney U Test bPearson's chi-squared Test cStudent T Test
AHI: Apnea-hypopnea index, PTA: Pure-tone audiometry, THI: Tinnitus Handicap Inventory, BMI: Body mass index, kHz: Kilohertz.

Comparisons of THI scores, audiometry and polysomnography (PSG) findings among the subgroups determined by OSA severity

Table 2 shows THI scores, audiometry and polysomnography (PSG) findings of the subgroups determined by the severity of OSA. In relation with the AHI scores of the OSA group, 47 patients had mild OSA, 46 patients had moderate OSA, and 33 patients had severe OSA (**Table 2**).

The mean standard PTA, 8 kHz and 12 kHz thresholds did not significantly differ among the OSA subgroups (p=0.179, p=0.600 and p=0.155, respectively). The mean 10 kHz value differed significantly among the subgroups (p=0.024). The paired comparisons performed to determine the subgroup that caused the difference indicated a significantly higher mean 10 kHz hearing threshold in the patients with severe OSA (p=0.007). The paired comparisons of other subgroups did not yield any significant differences (**Table 2**).

The mean THI scores differed significantly among the subgroups (p=0.019). Mild OSA subgroup had significantly lower THI scores compared to moderate and severe OSA subgroups (p=0.014 and p=0.027, respectively) (**Figure 1, Table 2**).

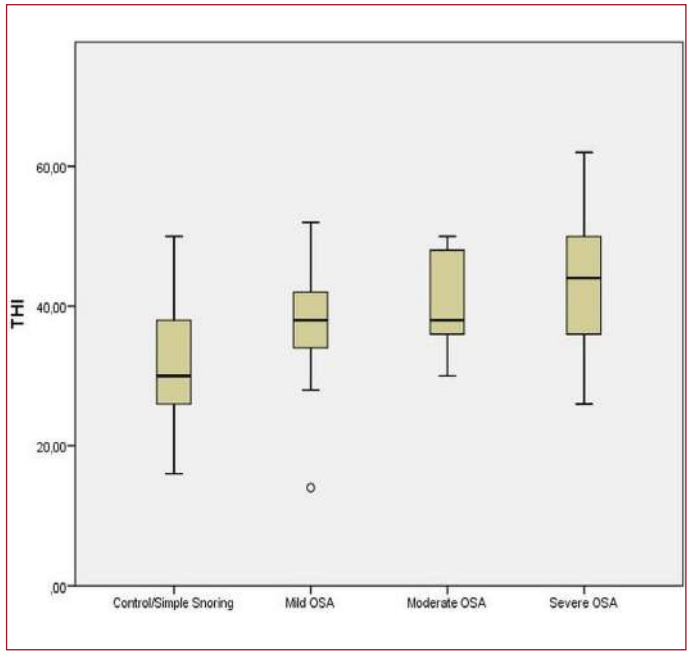

Figure 1. The mean tinnitus handicap inventory scores in control and OSA subgroups

Table 2. Comparisons of the audiometric and THI scores according to degree of OSA.

Variables	15 ≤ AHI < 15 (n=47) Mean±SD	215 ≤ AHI < 30 (n=46) Mean±SD	3AHI ≥ 30 (n=33) Mean±SD	p	Binary Comparisons dp
Age (years)	43.29±5.95	48.36±6.01	51.36±6.64	[c]p=0.001*	
Female Male	14 (29.8%) 33 (70.2%)	18 (39.2%) 28 (60.8%)	14 (42.5%) 19 (57.5%)	[b]p=0.017*	
BMI (kg/m²)	28.43±2.15	31.03±3.75	32.25±4.09	[a]p=0.001*	
AHI (ev/hr)	10.48±2.31	19.94±2.83	43.49±13.10	[a]p=0.001*	
PTA (dB)	12.19±3.76	12.30±3.30	14.48±5.72	[a]p=0.179	
8 kHz (dB)	31.06±11.88	28.91±7.21	32.12±11.18	[a]p=0.600	
10 kHz (dB)	32.55±10.26	31.30±11.51	37.42±10.31	[a]p=0.024*	[1-2]p=0.368 [1-3]p=0.062 [2-3]p=0.007*
12 kHz (dB)	38.82±12.47	40.65±13.60	43.63±10.32	[a]p=0.155	
THI	37.70±6.35	40.91±6.06	42.30±10.32	[c]p=0.019*	[1-2d]p=0.014* [1-3d]p=0.027* [2-3d]p=0.492

aKruskal Wallis Test, bPearson's chi-squared Test, c One Way ANOVA Test, dStudent T Test, *p<0,05
AHI: Apnea-hypopnea index, PTA: Pure-tone audiometry, THI: Tinnitus Handicap Inventory, BMI: Body mass index, kHz: Kilohertz

The age was significantly and positively correlated with all mean standard PTA hearing thresholds, 8 kHz, 10 kHz, 12 kHz hearing thresholds and THI scores (p=0.001; r=0.291, p=0.011; r=0.226, p=0.001; r=0.482, p=0.001; r=0.488 and p=0.001; r=0.395, respectively). In addition, the mean THI score showed a statistically significant and positive correlation with the AHI and BMI (p=0.004; r=0.252 and p=0.001; r=0.290, respectively) (**Table 3**).

Table 3. The correlation coefficients and p values among demographic characteristics and the audiometric, THI, polysomnography (PSG) findings in patients with OSA.

N=126	AHI (ev/hr)	BMI (kg/m²)	Age
THI Correlation Coefficient	0.252	0.290	0.395
P value	0.004*	0.001*	0.001*
PTA Correlation Coefficient	0.162	0.152	0.291
P value	0.069	0.090	0.001*
8 kHz Correlation Coefficient	0.051	0.030	0.226
P value	0.567	0.738	0.011*
10 kHz Correlation Coefficient	0.105	0.161	0.482
P value	0.242	0.071	0.001*
12 kHz Correlation Coefficient	0.106	0.172	0.488
P value	0.238	0.054	0.001*

Spearman's Correlation test, p < 0.05 AHI: Apnea-hypopnea index, BMI: Body mass index, PTA: Pure-tone audiometry, THI: Tinnitus Handicap Inventory, kHz: Kilohertz.

The audiometry findings (standard PTA, 8 kHz, 10 kHz and 12 kHz hearing thresholds) did not show any statistically significant correlations either with AHI or BMI (p>0.05) (**Table 3**).

DISCUSSION

In our study, we found that both THI scores and hearing thresholds were higher in OSA patients compared to simple snorers. Tinnitus scores were positively correlated with AHI.

OSA is a syndrome that affects almost every cell of human body, and it may cause various comorbidities such as cardiovascular and neurological disorders.[14] Hypoxia caused by recurrent upper airway obstruction results in systemic inflammation and activation of sympathetic nervous system, and vascular endothelial damage follows oxidative stress and systemic inflammation.[15,16]

The exact pathophysiology of hearing loss in OSA is not yet clearly put forward, however several possible mechanisms have been proposed. Small cerebral blood vessels maintain blood to the arterioles of cochlea.[17] The arterioles of cochlea are terminal blood vessels, and cochlea does not have any collateral blood supply. Cerebral blood flow velocity decreases and blood viscosity increases in patients with OSA leading to hypercoagulability.[18] Moreover, cochlear and brainstem microcirculations may be impaired due to autonomic nervous system dysfunction, hypoxia and inflammation, resulting in hearing impairment.[8] Hearing loss may also lead to tinnitus.[19]

Vorlova et al. investigated the effect OSA on hearing, and reported that OSA resulted in hearing impairment possibly due to hypoxia, heart rate variations, decreased brain perfusion and changes in intracranial pressure. The authors also reported that high-frequency auditory thresholds were higher in patients with severe OSA, and this was correlated with the severity of OSA.[20]

Most of OSA patients complain of snoring. Persistent and/or loud snoring sound may damage cochlea through acoustic trauma. Hearing impairment in OSA is a multifactorial mechanism that may also involve cochlear ischemia and hypoxia that causes degeneration of hair cells and cochlear spiral organ. Lu et al. investigated the relationship between OSA and auditory dysfunction, and studied the role of snoring sound contributing to the auditory dysfunction, and reported that rather than hearing loss, OSA could result in tinnitus. They also reported that high-frequency snoring sounds transmitted to the ear canal might have contributed development of tinnitus.[21] A recent meta-analysis reported that 4 and 8 kHz hearing thresholds of the OSA patients were significantly higher than those of the control group, and snoring might have caused hearing impairment in those patients.[8] Similarly, Chopra et al. reported that presence of OSA was significantly correlated with hearing impairment, and both presence of OSA and increased OSA severity were correlated with increased likelihood of hearing impairment in both high and low frequencies.[7] Seo et al. indicated that lowest oxygen saturation was the only variable correlated with hearing thresholds, and severe OSA might be a trigger for hearing loss.[22] In another study, it was reported chronic nocturnal intermittent hypoxia, particularly due to severe OSA, played the major role in development of high-frequency hearing impairment and early cochlear damage. The authors revealed that there was no difference in the standard PTA thresholds between OSA and control groups, however the patients with severe OSA had higher thresholds at high (6 –16k kHz) frequencies.[23]

In our study, we found higher hearing thresholds on standard PTA and high frequencies including 8, 10 and 12 kHz in OSA group compared to the simple snoring group. Our results imply that intermittent nocturnal hypoxia related to sleep apnea causes hearing loss rather than snoring sounds. However, the patients in our OSA group were older compared the simple snoring group, and it may be supposed that older age of might have contributed to this result. OSA subgroup comparisons showed that there was no statistically significant difference among the OSA severity subgroups for standard PTA or 8 and 12 kHz hearing thresholds, however severe OSA subgroup had significantly higher hearing thresholds at 10 kHz. Although not statistically significant, hearing thresholds increased as the AHI scores increased. Accordingly, this result may suggest that the level of hearing impairment increases as the AHI score increases.

Hearing loss (particularly at high frequencies) and tinnitus are closely interrelated. Both conditions may be associated

with various factors and disorders including exposure to noise, otologic infections, obesity, hypertension, Meniere's disease, medications and aging.[24] Several studies reported prevalence of tinnitus in a wide range (5.1% to 42.7%) in relation with different demographic criteria.[25,26] OSA has been reported as a risk factor for tinnitus.[21,23] Lu et al. reported the prevalence of tinnitus in OSA patients as 66%, which was higher compared to the general middle-aged population.[21] Similarly, in a large population-based study it was reported that the risk of tinnitus increased 1.36 times in middle-aged patients with OSA.[27] Another study reported a higher prevalence of tinnitus in OSA patients compared to the individuals without OSA.[23] In our study, we found higher THI scores in patients with OSA compared to simple snoring group indicating that OSA patients suffer from tinnitus more frequently compared to the simple snorers. We also found that moderate and severe OSA patients had higher THI scores compared to mild OSA patients. THI sores showed a significant and positive correlation with AHI. We may say that tinnitus is an early sign of auditory impairment in patients with OSA.

Our study has several limitations. First, it is a single-center study. The mean age and BMI were higher in the OSA group and those might have affected hearing thresholds and THI scores. The changes in study parameters after positive airway pressure treatment were not investigated. The duration of OSA and/or snoring was not included as a study parameter into our study. This may be an important factor for hearing loss and tinnitus. Inclusion of the duration of OSA into the study would enable us to obtain more valuable results.

CONCLUSION

The prevalence of both tinnitus and hearing loss is higher in OSA and particularly in severe OSA compared to simple snorers. OSA, hearing impairment and tinnitus are either comorbidities or related etiologically. Higher prevalence of hearing impairment in OSA patients compared to simple snorers suggests that intermittent nocturnal hypoxia, rather than sound of snoring plays role in hearing impairment. THI scores are significantly and positively correlated with AHI, and tinnitus may be an early sign of auditory impairment of patients with OSA. Further multi-center studies including a larger and more homogeneous study population are required to further clarify the correlation of OSA with hearing impairment and tinnitus.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was approved by the ethics committee of Ankara Numune Education and Research Hospital, and conducted in accordance with the ethical principles of Declaration of Helsinki. (Date: 05.10.2017, Decree no: E17-1411).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

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Original Article / Orijinal Araştırma

Posture Analysis and Presence of Sacroiliac Joint Dysfunction in Patients with Chronic Lower Extremity Edema

Kronik Alt Ekstremite Ödemi Olan Hastalarda Sakroiliyak Eklem Disfonksiyonu Varlığı ve Postür Analizi

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Abstract

Aim: Chronic lower extremity edema has been associated with postural impairment, sacroiliac joint dysfunction (SIJD), and abnormal gait. Lymphedema and lipedema are important causes of chronic lower extremity edema. This study aimed to detect the presence of SIJD and postural disorders in patients with lower extremity edema and the relationship between them.

Material and Method: This study is a comparative prospective cross-sectional study. Fifty-three patients with lower extremity edema and 53 healthy subjects were included in the study. Pain provocation tests were used to determine SIJD. Postural analysis was conducted with PostureScreen® Mobile 11.2 (PostureCo, Inc., Trinity, FL) software. The life quality of participants was determined by the Lymphedema Quality of Life (LYMQOL) scale. The functional status of the patients was determined by the Oswestry Disability Index and Lower Extremity Functional Scale.

Results: SIJD (18.9%) was more common in the edema group. There was a positive correlation between volume differences, percentages, and the presence of SIJD. We found deviations in the head, shoulder, and hip angulations in the edema group. Q angle and lateral shoulder angulation were significantly higher in patients with SIJD in the edema group. In the edema group, LYMQOL-leg total score was higher in patients with SIJD.

Conclusion: Chronic lower extremity edema was found to be associated with postural deviations and SIJD. Besides edema control, postural disorders and SIJD should also be considered in these patients.

Keywords: Lower extremity edema, sacroiliac joint dysfunction, quality of life, posture

Öz

Amaç: Kronik alt ekstremite ödemi, postür bozukluğu, sakroiliak eklem disfonksiyonu ve anormal yürüyüş paterni ile ilişkilendirilmiştir. Lenfödem ve lipödem, kronik alt ekstremite ödeminin önemli nedenleridir. Bu çalışmada alt ekstremite ödemi olan hastalarda sakroiliyak eklem disfonksiyonu ve postürel bozuklukların varlığı ve aralarındaki ilişkiyi araştırmayı amaçladık.

Gereç ve Yöntem: Çalışmamız prospektif, karşılaştırmalı kesitsel bir çalışmadır. Çalışmaya alt ekstremite ödemi olan 53 hasta ve 53 sağlıklı kişi dahil edildi. Sakroiliak eklem disfonksiyonu tanısı içi ağrı provokasyon testleri kullanıldı. Postür analizi, PostureScreen® Mobile 11.2 (PostureCo, Inc., Trinity, FL) yazılımı ile gerçekleştirildi. Katılımcıların yaşam kaliteleri Lenfödem Yaşam Kalitesi Ölçeği (LYMQOL) ile, fonksiyonel durumları ise Oswestry Dizabilite İndeksi ve Alt Ekstremite Fonksiyonel Skalası ile belirlendi.

Bulgular: Alt ekstremite ödemi olan hastalarda sakroiliyak eklem disfonksiyonu daha sıktı (%18.9). Alt ekstremite hacim farkları ve yüzdeleri ile sakroiliyak eklem arasında pozitif korelasyon saptandı. Ödem grubunda baş, omuz ve kalça açılarında deviasyonlar saptandı. Ödem grubunda sakroiliyak eklem disfonksiyonu olan hastalarda Q açısı, lateral omuz angulasyonu ve LYMQOL-leg ölçeği total skoru daha yüksekti.

Sonuç: Çalışmamızda kronik alt ekstremite ödemi ile postural sapma ve sakroiliyak eklem disfonksiyonu arasında ilişki bulundu. Kronik alt ekstremite ödemi olan hastalarda ödem kontrolünün yanı sıra, postür analizi ve sakroiliak eklem değerlendirmesi uygun bir yaklaşım gibi gözükmektedir.

Anahtar Kelimeler: Alt ekstremite ödemi, sakroiliyak eklem disfonksiyonu, yaşam kalitesi, postür

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INTRODUCTION

Chronic lower extremity edema is a multifactorial condition mainly caused by venous and lymphatic insufficiency. The prevalence varies between 7-20%. Lymphedema and lipedema have an important place among the factors that cause chronic lower extremity edema.[1] Lymphedema causes a progressive decrease in joint movements and muscle strength, musculoskeletal pathologies, gait abnormalities, and postural instability due to immobility.[2] The presence of lower extremity lymphedema has a negative effect on balance.[3] Lipedema causes gait disorders by affecting the hip and knee joints due to abnormal fat accumulation in the lower extremities.[3,4]

The sacroiliac joint (SIJ) connects the spine to the pelvis and transfers body weight to the lower extremities.[5] SIJ pain is one of the most common causes of chronic low back pain and accounts for 15-30% of patients.[6] SIJ dysfunction (SIJD) results from sliding and torsional forces during activities such as walking, running, and squatting. Extremity volume load and differences cause asymmetrical stress to the pelvis during walking. It is suggested that this situation will increase the shear force in SIJ and cause damage to the joint.[7]

To the best of our knowledge, there is no study yet on which of the postural alignment and stability parameters are affected by lower extremity edema. This study aimed to detect the presence of SIJD and postural disorders in patients with lower extremity edema and the relationship between them.

MATERIAL AND METHOD

Data collection/recruitment procedure

This study is a comparative prospective cross-sectional study. The study was conducted between September 2020 and October 2021 and approved by the Clinical Research Ethics Committee of Süleyman Demirel University Faculty of Medicine (dated 25.09.2020, numbered 281). The universe of the study consisted of patients diagnosed with lymphedema and lipedema and admitted to our outpatient clinic. The control group was selected from volunteer relatives of the hospital staff in a similar age range to patients. All cases were informed in detail about the study's content, purpose, and application, and their written informed consents were obtained.

Fifty-three volunteered patients with lower extremity edema (lipedema and lymphedema) for more than six months and healthy volunteers of similar age were included in the study. Exclusion criteria were as follows: being younger than 18 years of age, illiteracy, being pregnant, a cognitive impairment that prevented answering the questionnaire questions for the study, extremity pathology due to rheumatological disease, history of major musculoskeletal trauma and malformations, presence of neurological disease and active malignancy.

The demographic characteristics of the patients, duration, stage, and localization of edema, presence of accompanied disease and disease duration, surgery history, lymph node dissection history, chemotherapy and radiotherapy history, and pain status of all patients included in the study were recorded.

The staging and volumetric circumference measurements of patients diagnosed with lymphedema and lipedema and physical examination consisting of goniometric joint range of motion (ROM) measurement, sacroiliac joint dysfunction tests, and postural analysis were performed and recorded. The same physiatrist performed all measurements and examinations.

Lymphedema quality of life for lower extremity (LYMQOL-Leg) questionnaire, lower extremity functional scale (LEFS), and Oswestry disability index (ODI) were filled out by the patients.

Assessment tools and scales

Volumetric Circumference Measurement

The extremity volumes of the patients were evaluated by the circumferential measurement method. In both lower extremities, the circumference of the metatarsophalangeal joint, the ankle (2 cm proximal to the medial malleolus midpoint), and the entire lower extremity towards the proximal 4 cm intervals were measured symmetrically with a tape measure (**Figure 1**).[8]

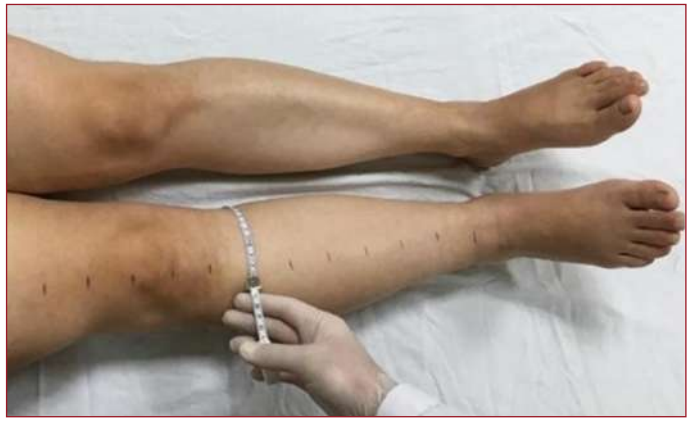

Figure 1. Lower extremity circumferential measurement method

The obtained data were transferred to the Excel Cone program, and the edema volume and percentage were calculated.

Staging

Lymphedema and lipedema grading is done in 4 stages. Lymphedema staging was done according to the 2016 consensus report of the International Society of Lymphology:

- Stage 0: This stage describes a subclinical state where swelling is not evident despite impairments in lymph transport.
- Stage 1: Pitting edema occurs without secondary tissue changes. In this stage elevation reduces swelling.
- Stage 2: Non-pitting irreversible edema with positive Stemmer's sign (The skin on the second toe of the foot cannot be lifted when it is grasped, squeezed, and tried to be lifted).

- Stage 3: This is the stage of lymphocytic elephantiasis with acanthosis, fat deposits, fibrosis, hyperpigmentation, and trophic skin changes.

The Meier-Vollrath and Schmeller classification system was used for lipedema staging.[9]

- Stage 1: The skin is smooth but the tissue under the skin has a pebble-like feel, which suggests fibrosis in the tissue
- Stage 2: There is more lipedema tissue, the skin has dimpling due to fibrotic changes in the skin and underlying loose connective tissue, and the nodules are larger.
- Stage 3: Large fat lobules are seen medial to the knee and thigh.

Sacroiliac Joint Examination and Dysfunctional Evaluation

Pain provocation tests were used in the evaluation of SIJD. These tests are FABER (Patrick) test, compression test, distraction test, Gaenslen test, sacral thrust test, and thigh thrust test. Positive tests are interpreted as an indicator of increased SIJ sensitivity.[10]

Three or more test positivity has a sensitivity of 85% and a specificity of 76-79% and is considered significant in terms of SIJD.[11-13] In this study, at least three positive tests were accepted as diagnostic for SIJD.

Postural Evaluation

Postural assessment was performed with PostureScreen® Mobile 11.2 (PostureCo, Inc., Trinity, FL) application in all cases included in the study.

PostureScreen® Mobile (PSM) is a specially designed app to objectively evaluate patients' posture, movement, and body composition with a photographic method.[14] Its validity and reliability have been demonstrated.[15] The patient should be minimally dressed during the analysis.[16]

Gender, date of birth, height, and body weight of all cases were recorded in the application. The subjects were positioned with their feet parallel to each other, medial malleolus at the same level, and arms free to the sides. The camera of the mobile phone was fixed at a distance of 3 m and a height of 1.30 m. A total of 4 photographs were taken in the anterior, posterior, right, and left lateral planes. A green target-like screen appears when the device was level to ensure standardization in photography.

Postural evaluation in PSM application is based on the principle of marking the anatomical reference points on the photographs taken.[16] The reference points determined after each photo shoot were marked. These reference points are;

- Anterior plane; right and left pupil, nasal filter, acromioclavicular joint, upper end of the sternum, right and left lateral ribs (T8), right and left SIAS, patella, tibial tuberosity, and ankle joint midline,
- Lateral plane; lateral edge of the eye, external meatus, C7 vertebra, acromioclavicular joint, thoracic kyphosis apex, lower thoracic vertebra (T12), SIPS, SIAS, greater trochanter, knee articulation line, and lateral malleolus,
- Posterior plane; earlobe, C7 spinous process, acromioclavicular joint, T4 spinous process, right and left rib (T8), T12 spinous process, L3 spinous process, right and left SIPS, and bilateral Achilles tendon.

After the reference points were marked manually, measurements were made with the PSM application, and analysis results were obtained in pdf format (**Figure 2**).

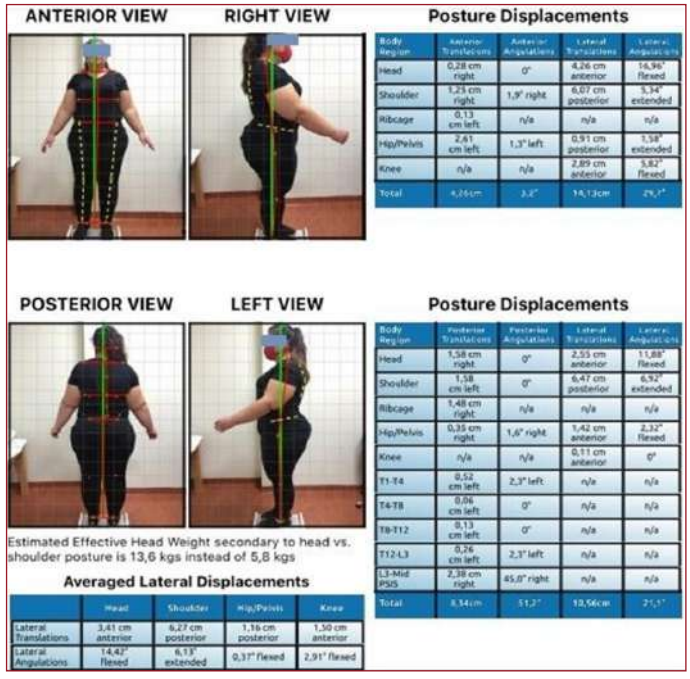

Figure 2: Postural analysis pdf document achieved with PostureScreen® Mobile application

In the results obtained, various postural variables were analyzed in the coronal and sagittal planes. The postural variables used in the PSM application were as follows: (1) anterior head angulation, shoulder angulation, hip angulation, right and left Q angle; (2) sagittal: head angulation (right and left), shoulder angulation (right and left), hip angulation (right and left), knee angulation (right and left), thoracic kyphosis angle, pelvic tilt; (3) posterior: head angulation, shoulder angulation, hip angulation.

Lymphedema Quality of Life for Lower Extremity Questionnaire (LYMQOL-Leg)

The LYMQOL-Leg is a 27-item, a 4-part scale developed to evaluate the impact of lower extremity lymphedema on quality of life.[17] It consists of 26 multiple-choice questions assessing symptoms, appearance, daily physical activities, emotional state, and a visual analog scale that questions the general quality of life. Each item is scored between 1 and 4 on a Likert-type scale (1: not at all, 2: a little, 3: quite a lot, 4: a lot). If any item is left blank or more than 50% of the questions per section are not answered, a score of "0" is given. Individual

scores are added in each section, and the calculation is made by dividing the total by the number of questions answered. A high score indicates poor quality of life of the patient. The Turkish validity and reliability study of the scale was conducted.[18]

Lower Extremity Functional Scale (LEFS)

LEFS was developed to assess the functional status of patients with musculoskeletal dysfunction affecting the lower extremities.[19] The scale consists of 20 items. Each question has five options from 0 to 4 (0: extremely difficult or unable to do the activity, 1: quite difficult, 2: moderately difficult, 3: somewhat difficult, 4: not difficult at all). The total score ranges from 0 to 80, with higher scores indicating better functional status. The Turkish validity and reliability study of the scale was conducted.[20]

Oswestry Disability Index (ODI)

ODI was developed to evaluate functional disability in patients with low back pain.[21] In this scale, ten questions evaluate daily life activities such as pain intensity, self-care, lifting, walking, standing, sleep status, travel, and social life. Each question is scored between 0-5. The total score in scoring is a minimum of 0 and a maximum of 50. The higher the total score, the higher the disability level. The Turkish validity and reliability study of the scale was conducted.[22]

Statistical Analysis

SPSS 25.0 (IBM Inc., Chicago, IL, USA) program was used in the statistical analysis of the study. Descriptive measures are presented as mean±standard for normally distributed data, median (min-max) for non-normally distributed data, and frequency (percentage ratio) for categorical variables. The conformity of continuous numerical data to normal distribution was analyzed by the Kolmogorov-Smirnov test. Independent Sample t-Test was used to analyze the difference between groups of normally distributed numerical variables, and the Mann-Whitney U test was used for non-normally distributed variables. The difference in nominal variables between groups was analyzed with the Chi-square test. Correlation analyzes were performed using the Spearman correlation test. Type 1 error value was taken as 5%, and the $p<0.05$ value was considered statistically significant.

Before starting the study, the minimum number of participants was determined as 51 by applying Power Analysis with 80% power and type 1 error level (5%) to ensure the study's reliability. The G power 3.1.9.2 Software (Universität Düsseldorf) program was used for power analysis.

RESULTS

Fifty-three lymphedema and lipedema patients and fifty-three healthy controls were included in the study. Twelve patients were not included in the study because they did not want to be photographed. Four patients were excluded because of the presence of chronic rheumatic disease, and seven patients were due to active malignancy. There were forty-eight female and five male patients in the edema group and forty-nine females and four males in the control group (P=0.870). There was no statistical difference in the mean age of the patient group and control group (58.92±9.84, 58.19±8.12, respectively; P=0.457). The mean BMI of the patient group was statistically higher than the control group (33.7±5.9, 30±3.8, respectively; P<0.001). Lipedema was detected in 42.5% and lymphedema in 54.8% of the patients. Edema was bilateral in 60.4% of the patients, on the right side in 11.3%, and on the left in 28.3%. Stage 2-3 edema was detected in 88.7% of patients, stage 1 in 9.4%, and stage 4 edema in 1.9%. Edema was located in the entire lower extremity in 83% of the patients, distal in 15.1%, and proximal in 1%.

SIJD was detected in 10 (18.9%) cases in the edema group and 3 (5.6%) cases in the control group. A statistically significant difference was found between the two groups in terms of SIJD (P=0.038). In the edema group, left-sided SIJD was detected in all patients with bilateral edema, and contralateral SIJD was detected in all patients with unilateral edema. The edema duration, volume difference, and volume percentage of the patients in the edema group with and without SIJD are compared in **Table 1.**

Table 1. Comparison of the demographic and clinical characteristics of the cases with and without SIJD in the edema group

Clinical characteristics	Patients with SIJD (n=10, 18.9%)	Patients without SIJD (n=43, 81.9%)	P
Age (year)	60.50 (47-74)	61 (31-74)	0.486*
BMI (kg/m2)	30.70 (25-61)	33.55±4.57	0.643*
Disease duration (month)	98.10±67.83	84 (7-480)	0.706*
Duration of edema (month)	67.30±44.89	72 (7-480)	0.298*
Volume difference (ml)	983.5 (385-7190)	326 (36-2621)	0.002*
volume percentage (%)	15 (6-150)	5.8 (1-71)	0.001*

SIJD: sacroiliac joint dysfunction; SD: standard deviation; BMI: body mass index, *Mann-Whitney U test **Independent Sample t-Test, Mean±SD for normally distributed data and median (min-max) for non-normally distributed data are used.

The correlation between the presence of SIJD and edema localization, duration, stage, and the correlation between the presence of SIJD and the volume difference and percentage are shown in **Table 2.**

Table 2. Correlation analysis between the presence of SIJD and edema localization, stage, duration, and volume in the edema group

	Cases with SIJD (n=10)	
	r	P
Localization	-0.034	0.810
Stage	0.153	0.276
Duration of edema (month)	-0.063	0.653
Volume difference (ml)	0.433	0.001
Volume percentage (%)	0.397	0.003

SIJD: sacroiliac joint dysfunction *Spearmen Correlation Analysis

The comparison of the postural analysis results of edema and control groups is shown in **Table 3**.

Table 3. Posture analysis of edema and control groups

Variables		Edeme group (n=53)	Control group (n=53)	P
	Head angulation	2.10 (0-6.4)	1.36±11.59	0.008*
	Shoulder angulation	1.90 (0-7.9)	1.12±0.94	<0.001*
Anterior	Hip angulation	2.52±2.02	1.20 (0-7.9)	0.002*
	Q angle (right)	7.27±4.86	6.79±3.10	0.053**
	Q angle (left)	7.74±4.86	6.12±2.78	0.031**
	Head angulation	14.94±7.18	8.05±5.05	<0.001**
	Shoulder angulation	3.12±1.98	2.30 (0-7.72)	0.098*
Lateral	Hip angulation	2.47 (0.02-14.97)	2.17 (0-8.25)	0.102*
	Knee angulation	6.93 (0.89-23.59)	4.82±2.36	0.001*
	Pelvic tilt	20.61±5.62	19.66±4.78	0.350**
	Thoracic kyphosis	30.8 (14.5-41.2)	30.05±5.49	0.832*
	Head angulation	1.60 (0-6.6)	1.30(0-5.9)	0.014*
Posterior	Shoulder angulation	1.40 (0-4)	1.2 (0-3)	0.004*
	Hip angulation	1.60 (0-12.2)	2.14±1.44	0.601*

* Mann-Whitney U test **Independent Sample t-Test Mean±SD for normally distributed data and median (min-max) for non-normally distributed data are used.

In the edema group, the left knee Q-angle in the anterior plane and the shoulder angle in the lateral plane were higher in patients diagnosed with SIJD compared to those not diagnosed with SIJD (P=0.025, P=0.036, respectively). No statistically significant difference was found between the groups in the posture analysis of the cases with unilateral and bilateral edema (P>0,05).

The comparison of the LYMQOL-leg total score, ODI, and LEFS scores of the edema subgroups is shown in **Table 4**. Functional status and general quality of life, which are sub-parameters of the LYMQOL-leg scale, were statistically higher in patients with bilateral edema, and emotional status was statistically higher in the presence of SIJD (P=0.036, P=0.037, P=0.003, respectively).

DISCUSSION

This study found that SIJD developed more frequently in patients with lymphedema/lipedema, and contralateral dysfunction developed more in patients with unilateral edema. The increase in the volume of edema in the lower extremities was correlated with the development of dysfunction. The patients with edema had deviations in the angulations of the head, shoulders, and hips. Patients with edema and SIJD had poor quality of life. Functional status and general quality of life of patients with bilateral edema were more adversely affected.

Leg length difference, scoliosis, abnormal gait pattern, and abnormal or asymmetric loading are risk factors for SIJD.[23,24] It has been suggested that unilateral volume increase due to unilateral lower extremity lymphedema causes asymmetrical loading during walking, leading to tension in SIJ due to increased shear force.[24] There is no study on the mechanism of the SIJD caused by chronic lower extremity edema, except for only one case report dealing with this relationship. Crane reported.[7] 50 years old female patient with SIJD secondary to unilateral lower extremity lymphedema. The volume difference and percentage between the lower extremities of our patients with SIJD were high, and a correlation was found between the presence of SIJD and the volume difference.

SIJ is a component of the lumbopelvic system and effectively transmits compressive loads between the lumbar spine and the lower extremities.[25] Vleeming et al.[26] defined that the posterior thoracolumbar fascia provides load transfer between the ipsilateral latissimus dorsi and the contralateral gluteus maximus. This myofascial connection, called the posterior oblique sling, provides lumbopelvic stability and the functional connection between the lumbar spine and the lower extremity during walking. The load on one side affects the contralateral side with this myofascial sling mechanism.[27] We found that SIJD developed on the contralateral side in patients with unilateral edema. We interpreted that the contralateral side may be affected by the increased stress on the contralateral joint with the myofascial sling or by the effect of the pelvic torsion resulting from abnormal loading.

The ODI is a reliable and validated scale for assessing disability caused by the lumbar region and SIJ pain.[21] There was no difference in ODI scores between our patients with and without SIJD in the edema group. Patients without SIJD may have high ODI scores due to other possible causes of low back pain.

Lower extremity edema significantly affects the quality of life due to physical and psychosocial problems.[28,29] However, a limited number of studies evaluated the quality of life in patients with lipedema and lymphedema in the lower extremities.[17] Greene and Meskell[30] determined that edema has physical, psychological, and social effects. It was found that all subgroup measurement scores of the LYMQOL scale improved by decongestive lymphatic therapy.[31] In accordance with the literature, we observed that the functional status and general quality of life were affected in patients with bilateral edema. Telli et al.[32] suggested that the presence of SIJD in patients with lumbar disc herniation caused an increase in depression. Similarly, we found that the emotional state was more affected in patients with dysfunction.

Table 4. Comparison of the quality of life and functional scores of the edema subgroups

Groups, n(%)	LYMQOL-leg	P	ODI	P	LEFS	P
Unilateral edema, 21 (39.7)	6 (1-9)	0.212*	30 (0-46)	0,270*	44 (28-77)	0.315*
Bilateral edema, 32 (60.3)	4 (1-9)		32 (8-71)		42.5 (17-70)	
SIJD (+), 10 (18.9)	4.5 (1-7)	0.017*	34.5 (22-71)	0.098*	40 (17-64)	0.084*
SIJD (-), 43 (81.9)	5 (1-9)		32 (0-64)		44 (22-77)	

LYMQOL-leg: Lymphedema Quality of Life for Lower Extremity Questionnaire; ODI: Oswestry Disability Index; LEFS: Lower Extremity Functional Scale, SIJD: Sacroiliac joint dysfunction, * Mann-Whitney U test, Median (min-max) for non-normally distributed data are used.

Lymphedema causes a progressive decrease in joint movements, muscle strength, gait abnormalities, and postural instability due to inactivity.[2] Posture analysis evaluates the deviations resulting from asymmetry on the right and left sides of the body and segmental rotations in the frontal, sagittal and transverse planes.[33] The use of postural assessment tools with mobile applications has increased in the past decade.[34] The PSM was the most used application tool in posture analysis between 2012 and 2020.[35] We used the PSM application for postural assessment and found that postural disorders occur in cases with edema.

In the postural analysis of our study, we found an increase in head, shoulder, and hip angulations in edematous cases. We did not detect significant changes among those with unilateral and bilateral involvement in the edema group. To our knowledge, this is the first study to evaluate posture in chronic lower extremity edema. Therefore, we could not find any data to compare these results in the literature.

An increase in the Q angle is associated with patellofemoral pain syndrome, chondromalacia patella, patellar subluxation, and patellar hypermobility.[36] We found a difference in left-sided Q angle in patients diagnosed with SIJD in the edema group. This result might be because patients with dysfunction were predominantly affected on the left side. Since the possible patellofemoral pathologies of the patients were not evaluated in our study, it is not possible to mention this with certainty.

Study limitations: 1) lower extremity volumes of the cases with bilateral edema were not given separately, 2) the 3D analysis could not be performed, 3) not all of the cases were minimally dressed, and 4) the inability to use anatomical markers.

CONCLUSION

Postural changes and SIJD may occur in patients with chronic lower extremity edema. Both edema and SIJD reduce the quality of life. Patients with chronic lower extremity edema should be evaluated regarding postural disorders and SIJD.

ETHICAL DECLARATIONS

Ethics Committee Approval: Approved by the Clinical Research Ethics Committee of Süleyman Demirel University Faculty of Medicine (dated 25.09.2020, numbered 281).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

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

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Original Article / Orijinal Araştırma

Changing Trends in Cesarean Section Deliveries in a Tertiary Hospital Using the Robson Ten Group Classification

Robson On Grup Sınıflandırması Kullanılarak Üçüncü Basamak Bir Hastanede Sezaryenle Doğumlarda Değişen Eğilimler

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Abstract

Aim: This study aimed to identify and highlight the changing trends in cesarean deliveries in a tertiary hospital using the Robson Ten Group Classification.

Material and Method: A retrospective cohort study included 103745 patients admitted to Istanbul Kanuni Sultan Suleyman Training and Research Hospital's Obstetrics and Gynecology Department between January 1, 2012, and December 31, 2021. Ten groups were established based on five basic obstetric factors: parity, labor initiation, gestational age, number of fetuses, and fetal presentation. All live or dead births over 500 grams or 20 gestational weeks were included in the study. The total number of cesarean sections in the group, the total number of women in each group, group size (%), cesarean rate (%), absolute group contribution to general cesarean section rate (%), and relative group contribution to general cesarean section rate (%) was calculated. Cesarean section indications were evaluated in 10 categories. their group sizes and cesarean section rates were recorded. Statistical analyzes were performed using SPSS Statistics for Windows, Version 24.0.

Results: Our study's average CS rate from 2012 to 2021 was 45.77%. The largest contributions to the total cesarean section rate were in group 5 (20.69%), group 3 (5.99%) and group 1 (5.75%).

Conclusion: Reducing cesarean rates, which have been high for years, is only possible with multidisciplinary studies. For this purpose, clinical practices should be combined with evidence-based practices.

Keywords: Robson ten group classification, cesarean section, pregnancy

Öz

Amaç: Bu çalışmanın amacı, üçüncü basamak bir hastanede sezaryen doğumlardaki değişen eğilimleri Robson on grup sınıflandırmasını kullanarak belirlemek ve vurgulamaktır.

Gereç ve Yöntem: Bu retrospektif çalışmaya 01.01.2012 - 31.12.2021 tarihleri arasında SBÜ İstanbul Kanuni Sultan Suleyman Eğitim ve Araştırma Hastanesi Kadın Hastalıkları ve Doğum polikliniğine başvuran 103745 hasta dahil edildi. Robson sınıflamasına göre doğumlar fetüs sayısı parite, doğum başlangıcı, gebelik yaşı, fetal presentasyon gibi beş temel karakteristik özelliklerine göre on gruba ayrıldı. 500 gramın üzerindeki veya 20 haftanın üzerindeki ölü ya da canlı tüm doğumlar çalışmaya dahil edildi. Gruptaki toplam sezaryen sayısı, her gruptaki toplam kadın sayısı, grup büyüklüğü (%), grup sezaryen oranı (%), genel sezaryen oranına mutlak grup katkısı (%) genel sezaryen oranına göreli grup katkısı (%) hesaplandı. Sezeryan endikasyonları 10 kategoride değerlendirilerek sayıları ve oranları kaydedildi. İstatistiksel analizler SPSS programının Windows için 24.0 versiyonu kullanılarak yapıldı.

Bulgular: Araştırmamızda 2012–2021 yılları arası ortalama sezaryen oranı %45,77 olarak saptandı. Toplam sezaryen oranına en büyük katkısı olan gruplar, grup (20.69%), grup 3(%5.99) ve grup 1(5.75%) olarak saptandı.

Sonuç: Yıllardır yüksek seyreden sezaryen oranlarının düşürülmesi ancak multidisipliner çalışmalarla mümkündür. Bu amaçla klinik uygulamalar kanıta dayalı uygulamalar ile birleştirilmelidir.

Anahtar Kelimeler: Robson on grup sınıflaması, sezaryen, gebelik

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INTRODUCTION

The fetus is delivered through an abdominal incision during a cesarean section, when a vaginal birth is not indicated. It is a routine surgical operation that is carried out all over the world.[1] Cesarean sections (CS) deal with several immediate and long-term risks, such as increased feto-maternal morbidity and mortality, stillbirths due to uterine rupture and postpartum hemorrhage. CS should not be a routine surgical procedure.[2,3]

In recent years, cesarean rate has gradually increased in many countries, becoming a public problem.[4,5] It is very difficult to identify and compare the risk factors of the cesarean section without using global classification. A reliable and consistent classification system should identify and highlight the factors affecting an increasing trend in cesarean delivery rates.[6] World Health Organization proposed and established the Robson Ten Group Classification (RTGC) as the international benchmark for tracking, contrasting, and assessing cesarean section rates.[7] The International Federation of Gynecology and Obstetrics (FIGO) also proposed this classification method.[8] RTGC divides women into ten groups based on parity, plurality, presentation, the start of labor, and gestational age.[9] This classification's advantages were that it was straightforward, similar, trustworthy, and adaptable.[10] This study aimed to identify and highlight the changing trends in cesarean deliveries in a tertiary hospital using the RTGC.

MATERIAL AND METHOD

This retrospective cohort study included 103745 patients who were admitted to Istanbul Kanuni Sultan Suleyman Training and Research Hospital's Obstetrics and Gynecology Department between January 1, 2012, and December 31, 2021. The characteristic features of patients, such as average age, the number of pregnancies, the history of prior cesarean sections, the number of birth, body mass index, and the indication for cesarean section, were assessed retrospectively. The information about the cases was obtained from the patient's files in the hospital archive and computer records.

Table 1 shows RTGC. Ten groups were established based on basic obstetric factors: parity (nulliparous, multiparous), previous cesarean section, labor initiation (natural, induced, or cesarean before labor starts), gestational age (less than 37 weeks, "preterm," more than 37 weeks, "term," the number of fetuses (single, multiple), and fetal presentation (head, breech, transverse). After classifying the deliveries into ten groups based on the year, the cesarean rates were estimated for all births per year for each of the ten groups.

The contribution of each Robson Group to the total cesarean rate for each year was calculated. In addition, each Robson Group's contribution to the shift in other total cesarean rates between the starting period of 2012 and the ending period of 2021 was compared. Patients with more than 20 weeks of gestational week or more than 500 grams of living or dead births were included in the study. Patients with less than 20 weeks of the gestational week or fewer than 500 grams of living or dead births were excluded from the study.

Statistical Analysis

Fisher's exact test, chi-square, and descriptive statistics such as mean and standard deviation were used to examine the data statistically. $p<0.05$ was the cutoff for statistical significance. The statistics were carried out using the SPSS Statistics for Windows, Version 24.0.

This study was approved by the ethics committee of Istanbul Kanuni Sultan Suleyman Training and Research Hospital. (KAEK/2022.10.217 Request Number).

RESULTS

The study enrolled a total of 103745 participants. The CS rate was 45.77% between 2012 and 2021. The CS rate increased slightly from 43,57% in 2012 to 47,47% in 2021, as shown in **Table 1** ($p>0.05$). Group 5 was the most significant contributor to the total CS rate. (multiparous, single, head presentation before uterine scar, greater than 37 weeks), which accounted for 20.61% of all CS. The second-highest contribution in the total CS rate was Robson group 3 (5.99%). The third highest contribution to the CS rate was Robson group 1 (5.75%) with nulliparous, single-head presentation, gestational age greater than 37 weeks, and spontaneous labor.

Table 1: Robson 10 group classification system

Group 1	Nulliparous, single, head presentation, greater than 37 weeks, spontaneous in labor.
Group 2	Nulliparous, single, head presentation, greater than 37 weeks, birth induction or cesarean section before the labor.
Group 3	Multiparous, no prior uterine scar, single, head presentation, greater than 37 weeks, spontaneous labor.
Group 4	Multiparous, no prior uterine scar, single, head presentation, induction before labor, or cesarean section.
Group 5	Multiparous, single, head presentation, prior uterine scar, greater than 37 weeks.
Group 6	Nulliparous, singular, breech presentation.
Group 7	Multiparous, single, with or without a prior uterine scar, breech presentation
Group 8	All multiple pregnancies, with or without a prior uterine scar.
Group 9	All pregnancies, single, transverse, or oblique presentation, with or without a prior uterine scar.
Group 10	All preterm births single, head presentation with or without a prior uterine scar.

All preterm births, single, head presentation, and either a previous uterine scar or not (group 10) constituted 5.60% of all CS. All women who presented breech, transversely, or obliquely (groups 6, 7, and 9) provided 2.41% of the total CS.

According to **Table 2**, the prior cesarean was the most frequent reason for a cesarean section (24.01%), followed by fetal distress (3.72%) and an abnormal presentation (3.59%).

Table 2: Evaluation of the CS rate between 2012 and 2021 using the RTGC

Groups 2012-2021	CS in the group	Number of women delivered	Group size* (%)	Group CS rate † (%)	Absolute group contribution to total CS rate ‡ (%)	Relative group contribution to all CS rate § (%)
1	5968	22116	21.31	26.98	5.75	12.57
2	2268	4228	4.07	53.65	2.18	4.78
3	6214	37899	36.53	16.40	5.99	13.08
4	1080	2972	2.86	36.34	1.04	2.27
5	21392	21467	20.69	99.65	20.62	45.04
6	1335	1368	1.32	97.59	1.29	2.81
7	1045	1121	1.08	93.22	1.00	2.20
8	2246	2515	2.42	89.30	2.16	4.73
9	125	127	0.12	98.42	0.12	0.26
10	5814	9932	9.57	58.54	5.60	12.25
Total*	47487	103745	100%		45.77 %	100%

*Group size (%) = number of women in the group / total number of women delivered in hospitals multiplied by 100
†Group CS rate (%) = number of CS in the group / total number of women multiplied by 100.
‡, Absolute contribution (%) = number of CS performed in the group / total women delivered in hospitals multiplied by 100.
§ Relative contribution (%) = number of CS performed in the group/ overall CS rate in the hospital, multiplied by 100

Table 3: Indications of cesarean sections

Indications	Number of Women (n)	Percent in Group (%)
Previous CS	24916	24.01
Fetal distress	3867	3.73
Abnormal presentation	3863	3.72
Cephalo pelvic distortion	3799	3.58
Prolonged labor	3149	3.02
Twin pregnancy	2496	2.38
Pregnancy-induced hypertension	2176	2.08
Macrosomia	1474	1.40
Placental abnormalities	1092	1.04
Other reasons	955	0.92
Total	47487	45.77

DISCUSSION

Our research shows that the average CS rate from 2012 to 2021 was 45.77%. Most cesarean sections were performed on members of Group 5, who were multiparous, single, head presenters, previously scarred uteri, and who were more than 37 weeks pregnant. Most cesarean deliveries (24.01%) were due to prior cesarean surgery. The largest size was in group 1, spontaneous labor, nulliparous, single, head presentation, more than 37 weeks.

To help analyze cesarean delivery rates, all healthcare providers can utilize the RTGC tool. It also acts as a reference for efforts in response to changes in the CS rate.[11] The average CS rate for this study was 45.77%, which was higher than the rates reported by Jain R,[12] (42.13%), RC Prameela et al.[13] (29.33%), and Sidara Gilani et al. (33.3%).[14] Due to its criteria, Group 5 contributes the most to the total CS rate. Researchers worldwide validated the most prevalent contribution, with findings ranging from 15.4% to 67.7%. Group 5 represented most of the total CS in our study (20.62%).[15-17]

In our study group, 3 was the largest group of in terms of all types of deliveries (group size: 36.34%) and the lowest CS rate (16.40%). Group 3 (multiparous women with a single fetus in a cephalic presentation who spontaneously went into labor at term) was the second highest contributor (5.99%) to the total CS rate. The multiparous women in group 3 are a low-risk obstetric population and therefore, more likely to give birth vaginally. It is reasonable to assume that this group has a low CS rate. The CS rate in (group 3) was found to be 9.7% by Arpita Y et al.[18] and 2.6% by Tahira Kazmi et al.[19]

In this study, the Robson group 1 was the second-largest group (21.31%) and the third-highest contributor (5.75%) to the total cesarean section rate. Before spontaneous or artificially induced labor started, ultrasound was utilized to evaluate pregnant women. A cesarean section was typically performed on the patient when fetal macrosomia was suspected or predicted to exceed 4000 grams. Additionally, an elevated cesarean rate in Robson was linked to non-reactive stress test (NST) results. Khan MA et al.[20] reported that groups 5, 2, and 10 contributed most to the total CS rates. Parveen et al.[21] reported that groups 10 and 5 were the groups that contributed the most overall CS rate. Bolognani, C. Vet al.[22] reported that groups 5, 1, and 2 contributed the most to the overall CS rate. In groups 6 and 7 (breech presentation), the cesarean section rates exceeded those noted in the literature.[23,24] The lack of an external cephalic version in our clinic during the preterm period and potential medico-legal issues that could arise in breech birth may be the causes of these rates. In our study, major CS indication was a previous cesarean section, similar to other studies.[25,26]

Strengths and Limitations of the Study: The data were meticulously collected, and the sample size was sufficient compared to the literature studies. This study determined the importance of experience which could be beneficial to each group. This study was conducted at a single center, limiting generalization.

CONCLUSION

Reducing cesarean rates, which have been high for years, is only possible with multidisciplinary studies. For this purpose, clinical practices should be combined with evidence-based

practices. Therefore, indications such as fetal distress, non-progressive labor, and cephalopelvic discordance, which constitute the majority of cesarean section indications, should be based on more objective criteria.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Istanbul Kanuni Sultan Süleyman Training and Research Hospital Ethics Committee (Date: 28.10.2022, Decision No: KAEK/2022.10.217).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Original Article / Orijinal Araştırma

Therapeutic Efficacy of Malachite Green-Based Photodynamic Therapy in Acute Myeloid Leukemia

Akut Miyeloid Lösemide Malahit Yeşili-Bazlı Fotodinamik Tedavinin Terapötik Etkinliği

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Abstract

Aim: Acute myeloid leukemia (AML) is a disease characterized by relapse and treatment resistance in most patients. Therefore, there is a need for targeted therapies in AML. Photodynamic therapy (PDT) is a promising alternative for the treatment of malignant tumors. Also, PDT has the potential to be used individually or complementally in the treatment of leukemia. In this study, it was aimed to investigate possible the effect of malachite green (MG)-based PDT on acute myeloid leukemia cells.

Material and Method: Cells were incubated with 0.19, 0.39, 0.78,1.56, 3.125, and 6.25 µM MG for one hour and irradiated with 46.4 J/cm² of light. The trypan blue test was used to assess the viability of cells, and the change in mitochondrial activity was determined by MTT. Morphological features were determined by Giemsa staining and scanning electron microscopy. Cell cycle and Annexin V/PI assays (measuring fluorescence emitted by staining reagents) were measured by flow cytometry.

Results: With the combination of MG and light, HL60 cell viability was found to be significantly reduced compared to the control group. Giemsa staining and SEM results showed that 3.125 µM MG-based PDT induced various morphological changes in cells typical for apoptosis. Late apoptosis was observed in cells treated with 3.125 µM MG combined PDT according to Annexin/PI staining, further showing that it caused an arrest in the subG1 phase of the cell cycle.

Conclusion: MG-based PDT has the potential to inactivate HL60 cells. Thus, MG-based PDT may ensure a promising approach for treating acute myeloid leukemia cells.

Keywords: HL60 cells, Malachite green, Photodynamic therapy, apoptosis

Öz

Amaç: Akut miyeloid lösemi (AML), çoğu hastada nüks ve tedavi direnci ile karakterize bir hastalıktır. Bu yüzden AML de hedefleme tedavilerine ihtiyaç vardır. Fotodinamik tedavi (FDT), malign tümörlerin tedavisi için umut verici bir alternatiftir. Aynı zamanda FDT, lösemi tedavisinde tek başına veya tamamlayıcı olarak kullanılma potansiyeline sahiptir. Bu çalışmada malahit yeşili (MG) aracılı FDT'nin akut miyeloid lösemi hücreleri üzerindeki olası etkisinin araştırılması amaçlanmıştır.

Gereç ve Yöntem: Hücreler 0,19, 0,39, 0,78, 1,56, 3,125 ve 6,25 µM MG ile bir saat süreyle inkübe edildi ve 46,4 J/cm² ışık ışınına tabi tutuldu. Hücrelerin canlılığını değerlendirmek için tripan mavisi testi kullanıldı ve mitokondriyal aktivite değişikliği MTT ile belirlendi. Morfolojik özellikler Giemsa boyama ve taramalı elektron mikroskobu ile belirlenmiştir. Hücre döngüsü ve Annexin V/PI testleri (boyama reaktifleri tarafından yayılan floresan ölçümü) akım sitometrisi ile ölçüldü.

Bulgular: MG ve ışık kombinasyonu ile HL60 hücre canlılığının kontrol grubuna göre anlamlı derecede azaldığı bulunmuştur. Giemsa boyama ve SEM sonuçları, 3,125 µM MG aracılı FDT'nin,hücrelerde apoptoz için tipik olan çeşitli morfolojik değişikliği indüklediğini göstermiştir. Annexin/PI boyamasına göre 3,125 µM MG kombine FDT ile tedavi edilen hücrelerde geç apoptoz gözlenmiş, ayrıca hücre döngüsünde subG1 fazında bir durmaya neden olduğunu göstermiştir.

Sonuç: MG aracılı FDT, HL60 hücrelerini inaktive etme potansiyeline sahiptir. Bu nedenle, MG bazlı FDT, akut miyeloid lösemi hücreleri için umut verici bir yaklaşım sağlayabilir.

Anahtar Kelimeler: HL60 hücreleri, Malahit yeşili, Fotodinamik tedavi, apoptoz

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INTRODUCTION

Photodynamic therapy (PDT) is a non-invasive method based on the simultaneous combination of a photosensitizer and light and the generation of reactive oxygen species in cells in the presence of molecular oxygen.[1] PDT consists of the combination of three different components: photosensitizer, light, and molecular oxygen. These components, which are non-toxic individually, form cytotoxic reactive oxygen species when combined. Once activated by visible light, the photosensitizer can react in two photooxidative pathways classified as type I and II.[2] Type I reactions involve the formation of free radicals via a photosensitizer. These radicals react with oxygen to form reactive oxygen species, including hydroxyl radicals, hydrogen peroxide, and superoxide anions. In a type II reaction, on the other hand, the photosensitizer transfers energy directly to oxygen to produce $^1O_2$, which induces apoptosis. Both reactions can lead to cell and oxidative damage, including necrotic, autophagic, and apoptotic cell death.[3,4]

PDT is an approved method for use in many countries for various types of cancer, Barrett's esophagus, age-related macular degeneration, actinic keratosis,atherosclerotic vascular disease, etc.[5] PDT has several advantages over traditional therapeutic methods, including repetitive treatment potential, minimal side effects, and potential for combination with other forms of treatment, including radiotherapy and chemotherapy.[6]

According to the course of the disease, leukemia can be classified as acute or chronic leukemia. Acute leukemia is usually characterized by overgrowth and rapid accumulation of immature malignant blood cells, whereas chronic leukemia is usually characterized by slower overgrowth of mature blood cells, and its progression may take months or even years.[7] Although current treatments for leukemia are mainly chemotherapy, radiation therapy, and allogeneic stem cell transplantation, these treatments can lead to serious late effects such as a drug resistance, high risk of infection, graft-versus-host disease,and cytotoxicity to normal cells.[8] Therefore, seeking alternative approaches has become the focus of research. PDT is a promising alternative to chemotherapy or radiation therapy for the treatment of malignant tumors.[9] One of the advantages of PDT is that the maximum cumulative dose in both radiation therapy and chemotherapy does not cause cumulative toxicity in the patient.[10] It accumulates in photosensitizing cancer cells at higher concentrations compared to normal cells.[11] With photosensitizer-based PDT, it can be applied locally to a certain area by selectively illuminating the lesion without damaging normal tissues.[12]

Malachite green (MG) is a triphenylmethane cationic dye obtained from dimethylaniline and benzaldehyde with an absorption band of approximately 617 nm and used for staining.[13] Triarylmethane dyes have received attention as anticancer and antimicrobial agents due to their structural properties and selective localization.[14] To the best of our knowledge, no studies have been conducted in HL60 cells to determine morphological imaging (Giemsa staining and SEM), cell viability, mitochondrial activity, and apoptotic effects (Annexin V-PI staining and SubG1 peak analysis) of MG-based PDT. This study aimed to determine the effects of MG-based PDT on acute myeloid leukemia cells in terms of cell viability, mitochondrial activity, morphology, and apoptosis.

MATERIAL AND METHODS

Cell Culture

HL60 myeloid leukemia cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 1 mmol/L L-glutamine, and 1% penicillin-streptomycin, and incubated at 37°C in a 5% humidified CO_2 incubator.

Photosensitizer

In this study, the MG, which is in the cationic structure, was used as a photosensitizer. It was dissolved in PBS. Concentration experiments were performed for MG at doses of 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.39, and 0.19 µM. Since no alive cells were observed above 6.25 µM; 0.19, 0.39 ,0.78,1.56, 3.125, and 6.25 µM MG concentrations were used throughout this study. Cells ($1x10^5$) were exposed to MG for 1 hour at 37°C in the dark.

Experimental Design

Four different groups were formed in the PDT study using different MG concentrations. 1-Control group: No MG or No light; 2-Light Control: HL60 cells were exposed to light for 30 minutes; 3-MG group: HL60 cells were exposed to MG in all concentrations for one hour with no light; 4-MG-based PDT group: HL60 cells were exposed to MG for one hour and then exposed to light for 30 minutes

Photodynamic Therapy

After the cells were exposed to MG for one hour, they were centrifuged at 1000 rpm for five minutes, and free MG was removed from the medium. After the cells were washed three times with PBS, fresh PBS was added. A LED50 device with spectral ranges at λ=420 nm and 780 nm was used for irradiation, and a 550 nm longpass filter was used for the wavelength of MG. The light output was measured with a power meter (Newport, USA), and the cells were exposed to light at a distance of 10 cm for 30 minutes with an irradiance of 25.8 mW/cm² and a fluence of 46.4 J/cm².

Cell Viability and Mitochondrial Activity Assay

Cell viability was determined using the trypan blue exclusion method. At the end of treatment, 0.4% trypan blue dye was added to the 50 µL cell suspension. The number of viable cells was calculated by using the following formula: percentage of viable cells=[1.00-(number of blue cells÷total number of cells)]*100. The 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay was used to assess the mitochondrial activity of HL60 cells. MTT reagent was added to all groups 24 hours after the treatment and

incubated for 4 hours. At the end of the four-hour period, a solubilization buffer was added, and the cells were incubated overnight at 37°C. HL60 cells were evaluated with a microplate reader at 550 to 600 nm. Percentage of mitochondrial activity was calculated according to the following formula: %=[(Sample OD value- Blank OD value) / (Control OD value-Blank OD value)] × 100.

Giemsa Staining

Three samples were prepared from each group. The samples were dried on slides and fixed in methanol for 10 minutes. Then, the slides were stained with May-Grunwald for 1 minute, washed with distilled water, and kept at room temperature for 5 minutes with Giemsa staining (1:1). All samples were then backwashed with tap water and visualized with a light microscope.

Scanning electron microscope (SEM)

Cells in the control, light control, MG, and MG-based-PDT groups were fixed in 2.5% glutaraldehyde. The cells were then allowed to dry after a series of ethanol dilutions. After the samples were coated with palladium-gold, they were observed under a JSM 5600 model scanning electron microscope.

Annexin/PI staining

The apoptosis induced by MG-based PDT was analyzed by flow cytometry (BD Accuri C6 Plus, USA). Apoptotic cells represent green fluorescence. Late apoptotic cells represent both green and red fluorescence. Living cells show low fluorescence. After the treatments, 5 µl of PI and 1 µl of Annexin V-FITC were added to the cells and incubated for 15 minutes in the dark.Percentages of late and early apoptotic cells were determined for apoptosis.

SubG1 Peak Analysis

SubG1 peak analysis is a method used to detect cells that have lost some of their DNA in the late phase of the apoptosis. SubG1 analysis of treatment groups was performed using the BD Cycletest Plus DNA kit (BD Biosciences, USA). Post-treatment cells were centrifuged at 1000 rpm for 5 minutes. Trypsin buffer containing 250 µl solution was added to each sample. Then, 200 µl of solution containing Trypsin inhibitor and RNase buffer was added to the cells and incubated for 10 minutes. After 10 minutes of incubation, 200µl of buffer containing PI staining solution was added to the cells and incubated in the dark (10 minutes). The percentage of cells in SubG1 (apoptotic peak) was determined with the BD Accuri C6 Plus software.

Statistical Analysis

The results were calculated as mean±standard deviations (SDs). All data analyses were performed using SPSS 25 (San Diego, CA, USA). One-way ANOVA analysis of variance was used for data analysis, followed by Tukey post hoc test. A p value of <0.05 was considered a significant difference in all tests

RESULTS

Damage to the outer cell membrane was assessed by using the trypan blue exclusion test. Metabolically active cells with intact plasma membranes that could not divide were evaluated by the MTT test. It was determined that the cells in the group with MG-based PDT were significantly lower at 0.78, 1.56, 3.125 and 6.25 µM concentrations compared to the control group. The observed IC50 of MG-based PDT was 3.125 µM for HL60 cells. It was determined that the cells in the group with MG-based PDT were significantly lower at 0.78, 1.56, 3.125 and 6.25 µM concentrations compared to the control group. According to the trypan blue exclusion test, the light group did not show cytotoxic effects on HL60 cells.No effect was found on HL60 cells treated with light and MG groups. These results results indicated that MG-based PDT decreased the viability of HL60 cells with both the trypan blue exclusion test and the MTT (**Figure 1A** and **B**).

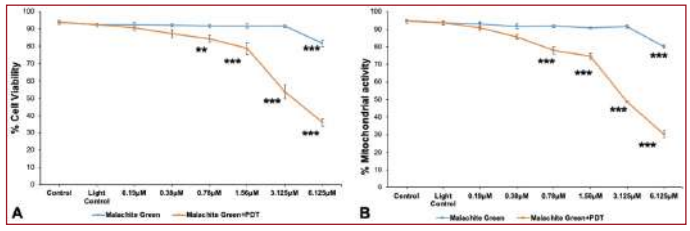

Figure 1. A. Evaluation of cytotoxicity after treatment with control, light control, MG and MG-based PDT. B. Evaluation of mitochondrial activity after treatment with control, light control, MG and MG-based PDT. The data represent the means±standard deviations (SDs) of 3 independent experiments. ** indicates statistically significance compared to control group ($p<0.001$);*** indicates statistically significance compared to control group ($p<0.0001$)

Giemsa staining and SEM were employed to assess morphological alterations in the HL60 cells exposured by MG-based PDT. In the 3.25 µM group, leukemia cells treated with MG-based PDT exhibited features not found in control cells such as chromatin condensation, irregular cytoplasmic contours (**Figure 2**).

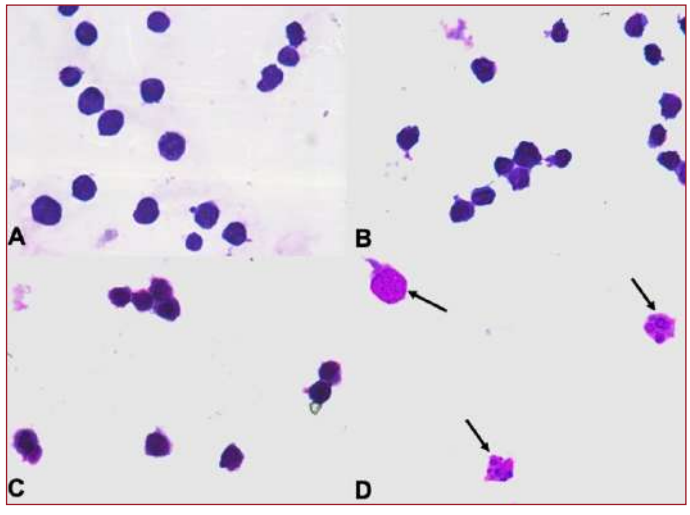

Figure 2. Morphology of HL60 cells with Giemsa staining for all experimental groups (x100). A. Control group; B. Light Control; C. 3.125 µM MG; D. 3.125 µM MG + PDT. Black arrows indicate apoptotic bodies and damaged cells

SEM analysis of the control, MG and light groups cells, revealed normal cell structure without cell damage. However, in the treatment group, some cells had shrunk in volume and showed typical apoptotic properties such as apoptotic bodies, holes, ırregular cytoplasmic contours, and broken cell membranes (**Figure 3**).

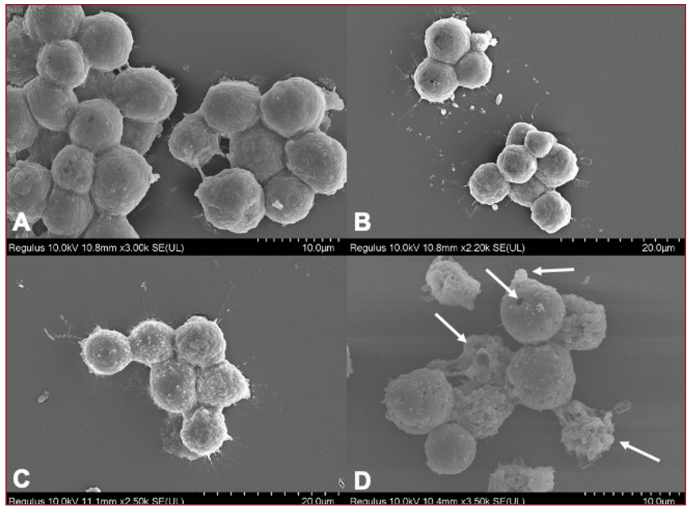

Figure 3. SEM images of HL60 cells. A. Control; B. Light control; C. treatment with 3.125 µM MG in the dark; D. treatment with 3.125 µM MG + light. White solid arrows indicate apoptotic cell with holes, shrunking cell with apoptotic bodies and broken cell membranes

To determine the mechanism of cell death induced by MG-based PDT, HL60 cells were evaluated using Annexin V-FITC and PI staining. Q1 in the histogram only represents dead cells stained with PI (Annexin V–/PI+); Q2 represents late apoptotic and necrotic cells stained with both Annexin V and PI (Annexin V+/P+); Q3 represents viable cells that were not stained with both Annexin V and PI (Annexin V–/PI–); and Q4 represents early apoptotic cells stained with Annexin V (Annexin V+/PI–) only. 93.23% of the control group cells were detected in Q3. A similar cell population was observed in Q3 only in the MG group compared to the control group (91.23%). In the MG (3.125 µM)-based PDT group, the rate of early apoptotic cells (Q4) was determined to be approximately 15.4%. Treatment of HL60 cells with MG-based PDT caused an increase in the percentage of late apoptotic cells (Q2) compared to the control group (0.7-30.36%). In addition, the percentage of late apoptotic cells in the MG-based PDT group was approximately 19 times higher than in the light-control group. When the percentage cell values obtained after Annexin V/PI staining were compared in terms of late apoptotic, early apoptotic, and viable cells, it was found that MG-based PDT showed a significant difference compared to the control group (***p< 0.0001). These results demonstrate that MG-based PDT induces late apoptosis in HL60 cells (**Figure 4**).

The control, light-control, MG only, and MG-based PDT groups were stained with PI 24 hours after treatment and analyzed by flow cytometry. DNA content in cells has a direct relationship with the amount of fluorescence intensity, and DNA degradation in apoptotic cells translates to a lower PI intensity than that of cells in the G1 phase (subG1 peak). 24 hours after the application, it was determined that 37.6% of the cells treated with 3.125 µM MG-based PDT were in the subG1 peak region, but only 2.1% of the cells in the control group were in the subG1 peak region, while 2.2% and 3.5% of the light control and MG-only groups were in the subG1 peak region, respectively. The results of this analysis showed that apoptosis was induced in cells treated with 3.125 µM MG-based PDT (**Figure 5**).

DISCUSSION

There are several issues with the use of the treatments that are commonly used to treat leukemia. The side effects of high-dose radiotherapy on normal cells are evident.[15] Chemotherapy has similar side effects and drug resistance possibilities.[16] Hematopoietic stem cell transplantation is one of the most used methods in the treatment of leukemia. There are significant risks in terms of recipient and donor for this treatment.[17] Not all therapeutic approaches are effective in destroying leukemia cells completely. PDT is a minimally invasive or non-invasive therapeutic technique for diseases and especially cancer.[18] Clinical studies reveal that PDT can prolong survival and significantly improve quality of life in patients with inoperable cancer.[19] Several previous studies have shown that PDT can be applied to leukemia cells with different photosensitizers.[20-22] This application has a cytotoxic effect on leukemia cells and allows them to differentiate into phagocytic cells.[23] The effectiveness of PDT varies depending on the photosensitizer applied, the light source, and the type of treatment.[24] The severity of the phototoxic effect is related to the dose of the drug and irradiation. The ideal agent to use in clinical applications was reported to be one whose applied dose is reduced as much as possible and whose skin toxicity is low to avoid side effects.[25]

Recently, ex vivo applications such as immunotherapy and replacement therapy have been used in leukemia patients. PDT application is a non-invasive method with fewer side effects. Recent studies have shown that this treatment is also effective in various leukemia cell lines.[26] It can be used to remove leukemic cells from the bone marrow intended for autologous transplant.

The HL60 cell line and other leukemia lines are widely used in chronic myeloid leukemia studies. Salmeron et al. reported that phenelonone-based PDT showed strong antitumor cell activity in HL-60 promyelocytic leukemia cells, and that the formed free oxygen radicals induced apoptosis.[27] In another study Sun et al. reported that ALA-based PDT induced DNA damage and apoptosis in K562 leukemia cells.[28] Cisariková et al. showed that PDT mediated by acridin-3,6-dialkyldithiourea hydrochlorides increased cytotoxicity and arrested cell cycle in the subG0 phase in the mouse leukemia line L1210.[29] In another study was reported that nanoparticle-ZnPc-based PDT caused apoptosis in leukemia cells and that PDT could be an excellent alternative for leukemia treatment.[30] Xu et

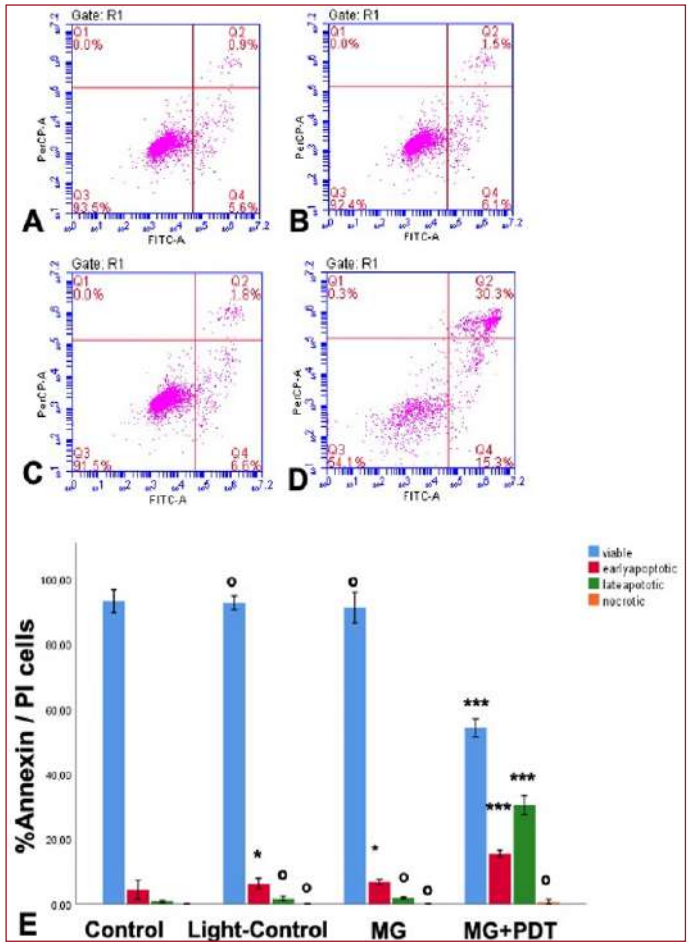

Figure 4. A. Control, untreated HL60 cells; B. Control, treated with only light; C. HL60 cells treated with 3.125 µM MG; D. HL60 cells treated with light combined with 3.125 µM MG; E. Graphical presentation of the percentage of the Annexin V positive apoptotic cell. The data represent the means±standard deviations (SDs) of 3 independent experiments. *indicates statistically significance compared to control group ($p<0.05$);***indicates statistically significance compared to control group ($p<0.0001$); °indicates not statistically significant when compared with the control group ($p>0.05$).

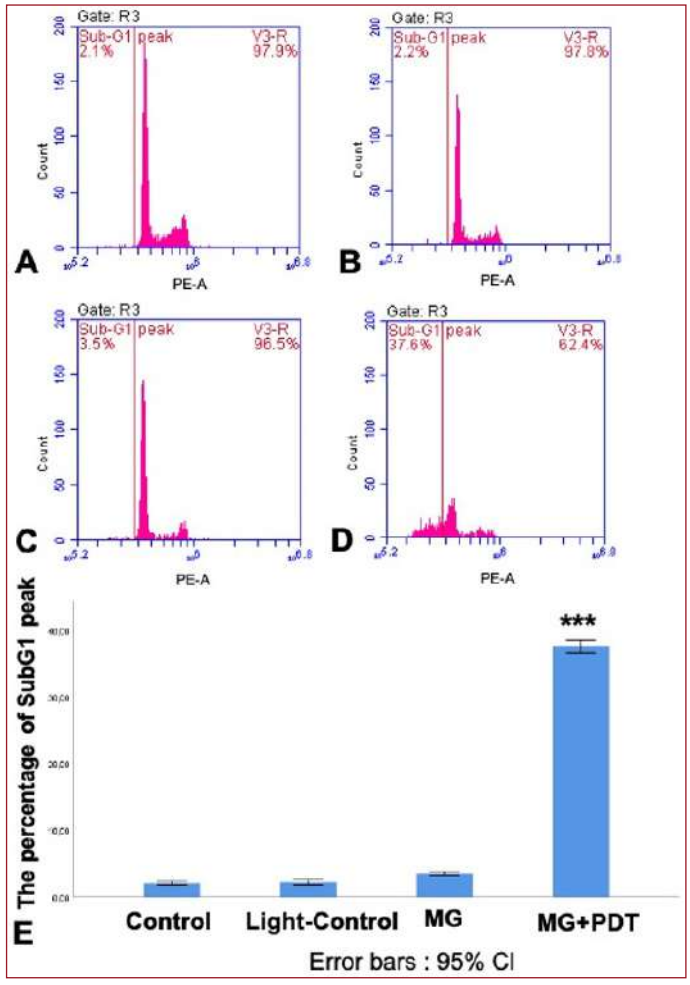

Figure 5. SubG1 apoptotic peak was determined by flow cytometry. Flow cytometry histograms A. Control, untreated HL60 cells; B.Control, treated with only light; C. HL60 cells treated with 3.125 µM MG; D. HL60 cells treated with light combined with 3.125 µM MG; E. Graphical presentation of the percentage of the SubG1 peak apoptotic cell. ***indicates statisticaly significance compared to control group ($p<0.0001$)

al. showed that hypericin-based PDT reduces cell viability in leukemia cells and can be developed as an effective treatment for leukemia.[21] Philchenkov et al. demonstrated that ALA- and fotolon®-based PDT resulted in dose-dependent cell death in human T cell lines of acute lymphoblastic leukemia.[31] Zhang et al. reported that ALA-based PDT inhibited cell proliferation in K562 leukemia cells.[32] Ettorre et al. showed that LycoC-based PDT is a good photosensitive agent that can induce apoptosis in HL60 cells.[33] Čunderlíková et al. reported that hexaminolevulinate-based PDT for tumor treatment was effective in photodynamically clearing leukemia cells from bone marrow grafts.[34] Unlike these studies, Chen et al. demonstrated that platinum-based PDT causes cell cycle arrest in different human leukemia cell lines at the G0/G1 stage, induces autophagy-induced cell death, and inhibits cell growth in leukemia cells in a mitochondria-based and caspase-independent manner.[35] Zhang et al. proposed the idea that ALA-based PDT on HL60 cells could be used to inactivate leukemia cells.[36] Di Stefano et al. demonstrated that purpurin-18-based PDT causes rapid apoptotic cell death in human leukemia cell lines at low doses and necrosis at higher concentrations.[37] Grebenova et al. showed that PDT inhibited proliferation and viability and caused an interphase arrest of the cycle of human promyelocytic leukemia HL60 cells and human erythroleukemia cells.[38]

To demonstrate the effects of MG-based PDT on HL60 cells and to develop an ideal PDT protocol, MG-based PDT experiments were performed in this study with different MG concentrations. HL60 cells viability decreased to 53.6% in the concentration of 3.125 µM MG after 30 minutes of irradiation with a light intensity of 25.8 mW/cm². However, the effects were minimal at low MG concentration. The morphological structure was examined by Giemsa staining and SEM analysis. It was observed that MG-based PDT had typical apoptotic cell features such as the formation of apoptotic bodies,and bubble-like protrusions on the HL60 cell surface compared to the MG alone, light-control, and control groups. Annexin-V staining was performed to examine the role of MG-based

PDT in cell apoptosis. Annexin V staining, which binds to phosphatidylserine passing from the plasma membrane's cytoplasmic surface to the plasma membrane's outer surface and is one of the indicators of apoptosis, is a major technique for detecting early-stage apoptosis. Late apoptotic and necrotic cells can be detected by adding propidium iodide to Annexin V-stained cells. In this study, 15.4% of apoptotic cells observed in HL60 cells were determined to be early apoptotic, while 30.36% were late apoptotic. This suggests that MG-based PDT treatment is effective in the action of membrane phospholipid phosphatidylserine in these cells, and that some of them are in the final stage of apoptosis. The results of the SubG1 analysis show that MG-based PDT induces a subG1 phase arrest in the cell cycle of the HL60 cells. These results suggest that MG-based PDT application may have important potential as a therapeutic option for leukemia.

CONCLUSION

PDT has been used clinically for the last 25 years as an alternative therapy in cancer treatment. Given the studies conducted, this subject is the current focus of research, and new studies emerge every day. Therefore, this study, with its multidisciplinary subject, is original research that will lead to promising new research topics. Although autologous bone marrow transplantation is one of the methods used in the treatment of leukemia, tumor cell contamination in autografts affects transplantation success. The effect of photosensitizers on penetrating malignant cells using PDT could be used as an auxiliary method in bone marrow purification. This is a study that includes an in vitro experiment. There is no study in the literature focusing on the mechanisms by which MG-based PDT affects leukemia cells. The results shown in this study provided insights about the effects of PDT on HL60 cells and created a new perspective for an alternative therapy against cells. This suggests that apoptosis may influence cell death after MG-based PDT administration in HL60 cells. This study is significant in that it will provide the basis for future ex vivo studies.

ETHICAL DECLARATIONS

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

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article / Orijinal Araştırma

Investigation of the Effect of Initial Cardiac Rhythm on Survival in Patients Admitted with Cardiopulmonary Arrest to the Emergency Department

Acil Servise Arrest Nedeniyle Getirilen Hastalarda İlk Tespit Edilen Kardiyak Ritmin Sağkalım Üzerine Olan Etkisinin Araştırılması

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Abstract

Aim: Cardiopulmonary arrest is the condition of insufficient oxygen delivery to tissues as a result of sudden cessation of circulatory and respiratory functions. This study aimed to investigate the causes of arrest in patients admitted with cardiopulmonary arrest and the effect of initial cardiac rhythm on patient survival.

Material and Method: Out of 1126 patients who had an in-hospital and out-of-hospital cardiac arrest and were admitted to our emergency department, 1009 patients were included in this retrospective study following the exclusion criteria. In addition to the demographic characteristics of patients, their initial rhythms and mortality states were assessed.

Results: There was a significant relationship between patients' clinical outcomes and initial cardiac rhythms ($p<0.001$). The mortality rate (77.1%) was higher in patients whose initial cardiac rhythm was asystole. While the rate of acidosis was higher in inpatients the rate of trauma was higher in mortal patients ($p<0.001$). ST elevation was higher in patients whose cardiac rhythm returned and right bundle branch block was higher in those who died, however, these were not statistically significant.

Conclusion: The initial rhythm analysis in arrest patients is crucial specifically in the detection of ventricular fibrillation and pulseless ventricular tachycardia which are shockable rhythms and patient survival increases with early diagnosis and intervention.

Keywords: Cardiopulmonary arrest, cardiac rhythm, mortality

Öz

Amaç: Kardiyopulmoner arrest, dolaşım ve solunum fonksiyonlarının aniden durması sonucu dokulara yetersiz oksijen taşınması durumudur. Bu çalışmada kardiyopulmoner arrest ile başvuran hastalarda arrest nedenlerini ve başlangıç kardiyak ritminin hasta sağkalımına etkisini araştırmak amaçlandı.

Gereç ve Yöntem: Geriye dönük olarak yapılan bu çalışmaya, hastane içi ve hastane dışı kardiyak arrest geçiren ve acil servisimize başvuran 1126 hastadan dışlama kriterleri sonrasında 1009'u dahil edildi. Hastaların demografik özelliklerinin yanı sıra başlangıç ritimleri ve mortalite durumları değerlendirildi.

Bulgular: Hastaların klinik sonuçları ile başlangıç kardiyak ritimleri arasında anlamlı bir ilişki vardı ($p<0.001$). Başlangıçta kalp ritmi asistoli olan hastalarda mortalite oranı (%77,1) daha yüksekti. Yatan hastalarda asidoz oranı daha yüksek iken, ölümlü hastalarda travma oranı daha yüksekti ($p<0.001$). Kalp ritmi dönen hastalarda ST elevasyonu, ölenlerde sağ dal bloğu daha fazlaydı ancak bunlar istatistiksel olarak anlamlı değildi.

Sonuç: Arrest hastalarında ilk ritim analizi, özellikle şoklanabilir ritimler olan ventriküler fibrilasyon ve nabızsız ventriküler taşikardinin saptanmasında çok önemlidir ve erken tanı ve müdahale ile hasta sağkalımı artar.

Anahtar Kelimeler: Kardiyopulmoner arrest, kardiyak ritim, mortalite

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INTRODUCTION

Cardiopulmonary arrest (CPA) is the condition of insufficient oxygen delivery to tissues as a result of sudden cessation of circulatory and respiratory functions. The emergency response in which the required oxygen is provided and circulatory functions are restored by chest compression for the survival of a patient diagnosed with CPA is called cardiopulmonary resuscitation (CPR). In case CPR is not performed effectively or early death or permanent brain damage may happen.[1] An immediately and effectively performed CPR positively affects the chance of survival. About 15-20% of deaths worldwide are caused by cardiac arrest. Only 5-10% of out-of-hospital cardiac arrests (OHCA) can be returned and about 6% of these patients are discharged from the hospital with neurological well-being.[2] Cardiac arrest is a condition that often results in the death of patients. However, most cardiac causes are treatable and thereby reversible.[3] In this sense, the latest international guidelines released by the International Liaison Committee on Resuscitation (ILCOR)[4], the European Resuscitation Council (ERC)[5], and the American Heart Association (AHA)[6] recommend the treatment of potentially reversible causes of cardiac arrest. CPR Guidelines of both ERC and AHA divide these causes into two groups of 5 by their first letters: 5H (Hypovolemia, Hypoxia, Hydrogen ion [acidosis], Hypo-/Hyperkalemia, and other electrolyte disorders; Hypothermia) and 5T (Tension pneumothorax, Tamponade [cardiac], Toxins, Thrombosis coronary, and Thrombosis pulmonary).[7,8]

Initial rhythms in cardiac arrest are divided into two non-shockable rhythms (asystole and pulseless electrical activity [PAE]) and shockable rhythms (ventricular fibrillation [VF] or pulseless ventricular tachycardia [PVT]). The initial presence of a shockable rhythm in out-of-hospital cardiac arrest is a sign of a good prognosis.[9] Several studies in the literature have revealed that the detection of a shockable rhythm as the initial rhythm is a sign of good prognosis compared to the detection of a non-shockable rhythm.[10,11] Therefore, the initial rhythm gives an opinion about the patient's possibility of survival.

This study aimed to detect the causes of arrest in patients admitted with CPA to our hospital and investigate the effect of initial cardiac rhythm on patient survival.

MATERIAL AND METHOD

Patients who were diagnosed with OHCA and in-hospital cardiac arrest (IHCA) in the Tertiary Emergency Clinic of Kayseri City Hospital between 01.06.2018 and 15.04.2022 were included in this study which was designed as a retrospective cohort study. Out of 1126 patients included in the study, 54 patients diagnosed with non-traumatic pediatric arrest and 63 with missing data in their files were excluded. As a result, a total of 1009 patients were included in the study. The principles in the Declaration of Helsinki were considered during the study and the ethical approval required for the study was obtained before the study. The study was approved by the Kayseri City Education and Research Hospital Clinical Ethics Committee with date:16.06.2022, and number:653.

Age, gender, location of arrest, detected cause of arrest (5H/5T), initial cardiac rhythm, initial return rhythm in case of return of spontaneous circulation (ROSC), and clinical outcomes of patients admitted with CPA were recorded after the patient files were scanned on the health management information system (HBYS) and emergency department (ED) patient file archive.

Statistical Methods

SPSS 26.0 (IBM Corporation, Armonk, New York, United States) software program was used in the analysis of variables. Normality in the distribution of data was assessed with the Shapiro-Wilk/Francia Test. Mann-Whitney U test with Monte Carlo results was used in the comparison of patients' clinical outcomes and ROSCs and in-hospital mortality states of patients if they were hospitalized according to their ages. Pearson Chi-Square and Fisher-Freeman-Halton tests tested with the Monte Carlo Simulation technique were used in the comparison of categorical variables such as gender, location of arrest, cause of arrest, initial cardiac rhythm, and stable rhythm detected following ROSC and clinical outcome and ROSCs by the state of mortality inwards the patients were hospitalized. Comparison of the rates in columns with each other was expressed with Benjamini-Hochberg adjusted p values. The sensitivity and specificity rates for the relationship between the classification separated by the cutoff value calculated according to the state of mortality of ROSC patients in their wards by age and real classification were assessed and expressed with ROC (Receiver Operating Curve) analysis. Quantitative variables were expressed in mean (standard deviation) and median (minimum-maximum) values while categorical variables were expressed with n (%) in the tables. Variables were assessed in 95% confidence interval and p-values lower than 0.05 were accepted significantly.

RESULTS

There was no significant difference between clinical outcomes and age of patients and clinical outcomes and gender of patients ($p=0.054$ and $p=0.088$). When the relationship between patients' location of arrest and survival was assessed it was observed that 85.1% of OHCA patients died while 20.3% of IHCA patients survived following intervention. A statistically significant difference was detected in both parameters ($p=0.033$). There was a significant relationship between the clinical outcomes of patients and detected causes of arrest ($p<0.001$). While the rate of acidosis was higher in inpatients the rate of trauma was higher in mortal patients ($p<0.001$).

There was a significant relationship between the clinical outcomes of patients and their initial cardiac rhythms ($p<0.001$). The mortality rate (77.1%) was higher in patients whose initial cardiac rhythm was asystole. The most commonly detected initial cardiac rhythm among patients who were hospitalized following ROSC was asystole (63.7%). A statistically significant difference was found between patients whose initial cardiac rhythm was VF and the mortal group among patients who were hospitalized following ROSC ($p<0.001$). In the mortal group, there was a statistically significant difference between

patients in whom atrioventricular (AV) block and left bundle branch block (LBBB) were detected following ROSC (p=0.003 and p=0.008). ST elevation was higher in patients who were hospitalized following ROSC and the right bundle branch block (RBBB) was higher in those who died, however, they were not statistically significant (**Table 1**).

The median age of mortal patients in the ward where individuals with ROSC were hospitalized was 72 (minimum-maximum= 6-100) years and the median age was significantly higher compared to the age of surviving patients (non-mortal individuals) (<0.001). The rate of mortality was higher among women with ROSC (0.047). There was a significant relationship between the detected causes of arrest and mortality states in the wards where they were hospitalized (p=0.001). While hypoxia (24.7%) and acidosis (23.6%) were higher in mortal patients, cardiac thrombosis (MI) (75.0%) was higher in the surviving group in the ward. There was a significant relationship between initial cardiac rhythm and survival (p<0.001). There was a significant difference between asystole (69.1%) in mortal patients and VF (50%) and PVT (8.9%) in surviving patients. There was a significant difference among mortality states of patients according to their stable electrocardiogram (ECG) rhythms detected following ROSC (p<0.001). Accordingly, while atrial fibrillation (AF) (18.8%) and RBBB (18.4%) were higher in mortal patients ST-segment elevation (STE) (50%) was higher in surviving patients (**Table 2**).

Patients' ages and mortality states in the wards where they were hospitalized were compared with ROC analysis. According to that ROC analysis, sensitivity was 63.2% and specificity was 62.5% for 66 years of age as the obtained cutoff value of age by mortality. The AUC value was 0.655 (0.039) and this cutoff value was statistically significant in the differentiation of mortality (p<0.01) (**Figure 1**).

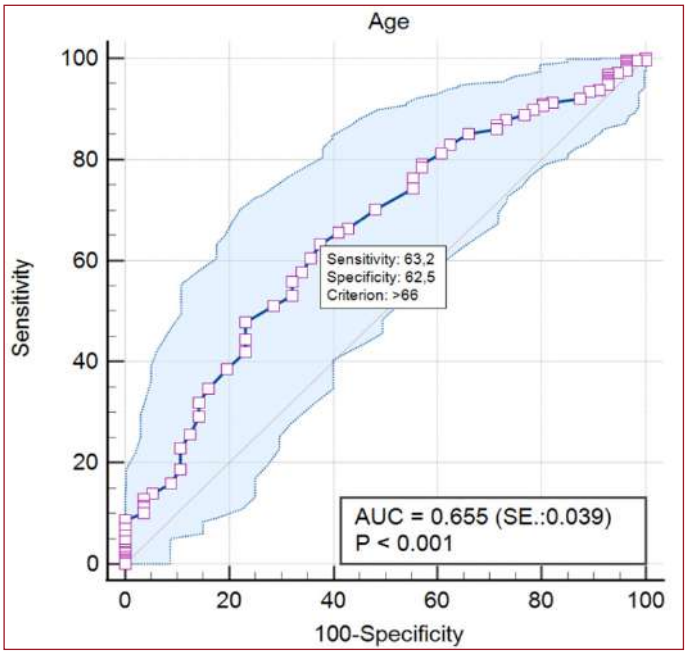

Figure 1. Receiver Operating Curve (ROC) analysis of patients' ages and mortality states in the ward they were hospitalized

Table 1. Patient's demographic data, causes of arrest, initial cardiac rhythms, rhythms following the return of spontaneous circulation

	Total	Clinical Outcome		P
	(n=1009)	Mortal (n=665)	Hospitalization (n=344)	
	Median (min-max)	Median (min-max)	Median (min-max)	
Age	72 (2-105)	72 (2-105)	70 (6-100)	0.054[u]
	n (%)	n (%)	n (%)	
Gender				0.088[c]
Female	389 (38.6)	269 (40.5)	120 (34.9)	
Male	620 (61.4)	396 (59.5)	224 (65.1)	
Location of Arrest				0.033[c]
In-hospital	169 (16.7)	99 (14.9)	70 (20.3)	
Out-of-hospital	840 (83.3)	566 (85.1)	274 (79.7)	
Detected Cause of Arrest				<0.001[f]
Hypoxia	229 (22.7)	152 (22.9)	77 (22.4)	
Hypovolemia	32 (3.2)	21 (3.2)	11 (3.2)	
Hyperkalemia	85 (8.4)	52 (7.8)	33 (9.6)	
Acidosis	140 (13.9)	69 (10.4)	71 (20.6)	<0.001
Hypoglycemia	11 (1.1)	8 (1.2)	3 (0.9)	
Tension pneumothorax	5 (0.5)	5 (0.8)	0 (0.0)	
Cardiac tamponade	3 (0.3)	3 (0.5)	0 (0.0)	
Pulmonary thrombosis (emboli)	38 (3.8)	23 (3.5)	15 (4.4)	
Cardiac thrombosis (MI)	397 (39.3)	271 (40.8)	126 (36.6)	
Intoxication	9 (0.9)	5 (0.8)	4 (1.2)	
Trauma	60 (5.9)	56 (8.4)	4 (1.2)	<0.001
Initial Cardiac Rhythm				<0.001[c]
Asystole	732 (72.5)	513 (77.1)	219 (63.7)	<0.001
PEA	75 (7.4)	46 (6.9)	29 (8.4)	
VF	170 (16.8)	88 (13.2)	82 (23.8)	<0.001
PVT	32 (3.2)	18 (2.7)	14 (4.1)	
A stable rhythm was detected following the return of spontaneous circulation				0.013[f]
NSR	54 (12.6)	7 (8.3)	47 (13.7)	
Bradyarrhythmias (AV block)	20 (4.7)	9 (10.7)	11 (3.2)	0.003
Tachycardia (Sinus Tachycardia)	44 (10.3)	8 (9.5)	36 (10.5)	
AF	70 (16.4)	12 (14.3)	58 (16.9)	
SVT	19 (4.4)	2 (2.4)	17 (4.9)	
ST elevation (MI)	86 (20.1)	12 (14.3)	74 (21.5)	
ST depression	17 (4.0)	1 (1.2)	16 (4.7)	
RBBB	74 (17.3)	18 (21.4)	56 (16.3)	
LBBB	32 (7.5)	12 (14.3)	20 (5.8)	0.008
Extrasystoles	12 (2.8)	3 (3.6)	9 (2.6)	

[c] Pearson Chi-Square Test(Monte Carlo), [f] Fisher freeman Halton Test(Monte Carlo); posthoc test: Benjamini-Hochberg correction, [u] Mann Whitney u Test(Monte Carlo)
PEA: Pulseless Electrical Activity, VF: Ventricular Fibrillation, PVT: Pulseless Ventricular Tachycardia, ECG: Electrocardiography, NSR: Normal sinusoidal rhythm, AV: Atrioventricular, AF: Atrial Fibrillation, MI: Myocardial Infarction, RBBB: Right bundle branch block, LBBB: Left bundle branch block

Table 2. Relationship of re-arrest and mortality state following the return of spontaneous circulation with the location of arrest, causes of arrest, and cardiac rhythm

	Did those who returned die in their wards?		P
	Yes (n=288)	No (n=56)	
	median (min-max)	median (min-max)	
Age	72 (6-100)	63 (18-88)	<0.001[u]
	n (%)	n (%)	
Gender			0.047[c]
Female	107 (37.2)	13 (23.2)	
Male	181 (62.8)	43 (76.8)	
Location of arrest			0.718[c]
In-hospital	60 (20.8)	10 (17.9)	
Out of hospital	228 (79.2)	46 (82.1)	
Cause of Arrest			<0.001[f]
Hypoxia	71 (24.7)	6 (10.7)	0,022
Hypovolemia	10 (3.5)	1 (1.8)	
Hyperkalemia	31 (10.8)	2 (3.6)	
Acidosis	68 (23.6)	3 (5.4)	0,002
Hypoglycemia	3 (1.0)	0 (0.0)	
Tension pneumothorax	0 (0.0)	0 (0.0)	
Cardiac tamponade	0 (0.0)	0 (0.0)	
Pulmonary thrombosis (emboli)	14 (4.9)	1 (1.8)	
Cardiac thrombosis (MI)	84 (29.2)	42 (75.0)	<0.001
Intoxication	3 (1.0)	1 (1.8)	
Trauma	4 (1.4)	0 (0.0)	
Initial Cardiac Rhythm			<0.001[f]
Asystole	199 (69.1)	20 (35.7)	<0.001
PEA	26 (9.0)	3 (5.4)	
VF	54 (18.8)	28 (50.0)	<0.001
PVT	9 (3.1)	5 (8.9)	0,044
A stable ECG Rhythm was detected after the return of spontaneous circulation			<0.001[f]
NSR	35 (12.2)	12 (21.4)	
Bradyarrhytmia (AV block)	11 (3.8)	0 (0.0)	
Tachyarrhythmia (Sinus Tachycardia)	33 (11.5)	3 (5.4)	
AF	54 (18.8)	4 (7.1)	0,034
SVT	16 (5.6)	1 (1.8)	
ST elevation (MI)	46 (16.0)	28 (50.0)	<0.001
ST depression	14 (4.9)	2 (3.6)	
RBBB	53 (18.4)	3 (5.4)	0.016
LBBB	18 (6.3)	2 (3.6)	
Extrasystoles	8 (2.8)	1 (1.8)	

[c] Pearson Chi-Square Test(Monte Carlo), [f] Fisher freeman Halton Test(Monte Carlo); psthoc test: Benjamini-Hochberg correction, [u] Mann Whitney u Test(Monte Carlo)
PEA: Pulseless Electrical Activity, VF: Ventricular Fibrillation, PVT: Pulseless Ventricular Tachycardia, ECG: Electrocardiography, NSR: Normal sinusoidal rhythm, AV: Atrioventricular, AF: Atrial Fibrillation, MI: Myocardial Infarction, RBBB: Right bundle branch block, LBBB: Left bundle branch block

DISCUSSION

Modern CPR methods have started to be tried to keep CPA patients alive since the 1960s and still continue to be developed today. On the other hand, both ERC and AHA CPR guidelines recommend early CPR in case of asystole and PEA rhythms and early defibrillation in the detection of VF and PVT rhythms which are called shockable rhythms. The importance of early CPR and early defibrillation is emphasized in both guidelines.[4,6] In this study, there was a significant relationship between clinical outcomes and initial cardiac rhythms of CPA patients. Roberts D et al. reported in their study that initial rhythm in CPA patients was strongly associated with in-hospital mortality,[12] which is similar to the findings in our study. The mortality rate was higher (77.1%) in patients whose initial cardiac rhythm was asystole in our study. In addition, the survival rate after CPR was the highest in patients whose initial cardiac rhythm was VF. It was stated in a study in Australia that the prevalence of shockable rhythm was 82% in surviving patients who were OHCA.[13] In another study in America, 69.33% of 3952 patients initially had non-shockable rhythm.[14] Thompson L. E. et al. found that initial rhythm was VF in 12.5% of CPA patients, PEA in 42.2%, and asystole in 37.6%.[15] In another study, 295 (4%) of OHCA patients initially had PEA and then a shockable rhythm, and 155 (2%) initially had asystole and then a shockable rhythm.[9] In another study performed in 2021, the prevalence of ROSC was higher in IHCA patients whose initial rhythm was PEA compared to those whose initial rhythm was asystole.[16] In our study, the most commonly detected initial cardiac rhythm was asystole (63.7%) among patients who were hospitalized following ROSC. The rate of shockable rhythm was 58.9% in surviving patients following CPA. The rates of patients' initial rhythms were stated as 72.5% for asystole, 7.4% for PEA, 16.8% for VF, and 3.2% for PVT. In other words, a shockable rhythm was detected in 20% of patients. Survival rates of patients whose initial rhythms were VF and PVT were higher (48.2% for VF, 43.7% for PVT, 38.6% for PEA, and 29.9% for asystole). Survival rate was reported higher in patients whose initial rhythm was VF in a study performed in Sweden.[17] Similarly, the rate of hospitalization following ROSC was higher in patients whose initial rhythm was VF in our study. Findings in our study support the result that early defibrillation has a positive effect on patient survival in presence of a shockable rhythm in CPA patients as emphasized in ERC and AHA CPR guidelines.

Acute coronary syndromes (ACS) are a common cause of arrest in patients with cardiac arrest. A 12-lead ECG should be performed as soon as possible in order to exclude ACS following ROSC.[18] The European Association of Percutaneous Cardiovascular Interventions and ERC recommend emergency coronary angiography (CAG) in patients in whom STE is detected on ECG.[19] In our study, the survival rate was higher (50%) in patients in whom STE was observed on ECG performed following ROSC. ECG should be assessed in detail following ROSC.[19] Non-specific changes and specific changes mimicking myocardial infarction (i.e. STE, ST segment depression, and abnormal T wave morphology) are observed in 3-16% of patients who had just been resuscitated.[20] It was reported in a study that the presence of combined/extended ECG criteria including the presence of ST elevation and/or depression and/or LBBB and/or non-specific QRS widening and/or RBBB on ECG could help detection of patients who could benefit from CAG following ROSC.[21] In our study, 86 (20.1%) patients in whom STE was detected on ECG following ROSC underwent CAG. On the other hand, AV-block was higher in 9 patients (10.7%) and LBBB was higher in 12 patients (14.3%) in the groups dying

of arrest following ROSC. It was confirmed in our study that undergoing CAG for patients who had STE on ECG performed following ROSC had a positive effect on patient survival. Therefore, we think patients should definitely receive an ECG examination following ROSC. On the other hand, the presence of AV-block, LBBB, and RBBB on ECG can be a sign of poor prognosis in terms of patients' clinical outcomes.

Whether patient age is indicated alone to start CPR is controversial in the literature. Although survival rates differ survival decreases by age in studies assessing age and survival in literature. An increase was observed in mortality by age in our study. The mortality rate was interestingly calculated higher as a result of re-arrest in the female gender following ROSC. According to a study performed on the elderly population, in-hospital survival rates following IHCA and OHCA got better in the past ten years but did not become more than 28.5% and 11.1% respectively.[22] When locations, where patients had arrested, were assessed it was observed that most of the patients (83.3%) were OHCA in our study. Additionally, the mortality of patients was compared according to their states of IHCA and OHCA and it was observed that 20.3% of IHCA patients survived after intervention and that 85.1% of OHCA patients died. The reason for that result was commented as fast access to IHCA patients in the hospital and the opportunity to initiate early CPR and early defibrillation; however, there may be delays in access to OHCA patients and initiating early CPR. According to a study performed on a total of 136,328 IHCA and OHCA patients in Sweden, the survival rate increased by years accompanied by progresses in CPR, the highest survival rate was among patients between the ages of 0 and 39, the survival rate increased to 17.5% in 2020 from 9.1% in 1990, and the highest increase in survival rate was in the age group of 40-49 years.[23] On the other hand, the mean survival rate ranged from 0.5% to 0.8% in a study on 234,767 IHCA patients and the survival rate decreased with age according to that study. In addition, the arrest rate of the male gender was reported higher in all age groups.[24] Kazaure H. S. et al. reported in their study assessing 813,493 arrest patients at and above the age of 18 that 54.3-55% of the patients were male, 80.7% were ≥65 years old, and the mean survival rate was 23%.[25] In a study in which 45,567 arrest patients at and above the age of 65 were assessed by Thompson L. E. et al., 55.5% of patients were male, the mean age was 72, and the survival rate was calculated as 16.6%.[15] In our study, the rate of male patients was higher (61.4%), and the mean age of patients was 72, which is similar to findings in the literature. In addition, the rate of survival was initially calculated as 34.9%. Compared to the available studies, the high rate of survival in our study can be explained by conditions that patients can access to hospital by ambulance in a short time as transportation to our hospital is easy and that CPA patients receive CPR according to the current ERC and AHA guidelines as our ED is a clinic providing training on the specialty of emergency medicine.

Detection of underlying causes in CPA patients also affects patient survival. AHA and ERC CPR guidelines abbreviated the causes of reversible arrest by their first letters as 5H/5T.[7,8] According to a study, while cardiac causes of arrest increased with age traumatic arrests decreased with age.[24] In another study, the rate of cardiac causes of arrest was 65.1%, the rate of pulmonary causes of arrest was 4.6% and the rate of traumatic causes of arrest was 2.3%.[23] In our study, while the survival rate of arrest resulting from cardiac causes (ACS) was higher the patient's survival rate of patients with hypoxia, acidosis, and traumatic arrests was lower. We believe that CPR performed effectively, on time, and in good quality can prevent patients from hypoxia. In addition, arterial blood gas analysis performed as soon as possible during CPA can help detect acidosis. Trauma is still a social problem today and the number of cars, industrialization, building industry, and domestic accidents are gradually increasing. Traumatic injury is the leading cause of death among individuals between the ages of 1 and 44 in industrialized countries and may result in more than 30,000 preventable deaths only in the USA.[26] In our study, traumatic cardiac arrests were associated with mortality. Therefore, we recommend taking preventive measures for traumas and the transport of traumatic patients to the hospital as fast as possible.

Limitations

Our study was performed prospectively and in a single center and pediatric arrest patients and pregnant arrest patients excluding pediatric traumatic arrest patients were not included in the study, which can be accepted as the limitations of our study.

CONCLUSION

CPA is a condition reported in all age groups and survival of patients can increase through early diagnosis, early CPR, early defibrillation, and early transport to the hospital. Analysis of initial rhythm is crucial specifically in the detection of VF and PVT which are shockable rhythms and increases the survival of patients. Increasing age and male gender can be accepted as risk factors the for increase in mortality in CPA patients. Undergoing CAG in an early period for patients who have STE on ECG performed following ROSC has a positive effect on patient survival. We believe that the detection of the reversible causes of arrest in CPA patients and initiating a cause-specific treatment can increase patient survival.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was approved by the Kayseri City Education and Research Hospital Clinical Ethics Committee with date:16.06.2022, and number:653.

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article / Orijinal Araştırma

Evaluation of the Correlation of Immunohistochemical Findings with Flow Cytometric Findings in Newly Diagnosed Pediatric Acute Lymphoblastic Leukemia Patients

Yeni Tanı Pediatrik Akut Lenfoblastik Lösemide, Kemik İliği Biyopsisinde İmmünohistokimyasal Bulguların Akım Sitometrik Bulgular ile İlişkisinin Değerlendirilmesi

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Abstract

Aim: The development of new therapeutic options to treat leukemia (therapies targeting chimeric antigen receptor (CAR) T cells) down-regulates markers expressed on the cell surface. Therefore, conventional immunophenotyping panels no longer make these antigens unreliable for identifying a B cell immunophenotype. In our study, we methodically compared multiparametric flow cytometry (FC) in bone marrow aspiration and immunohistochemical (IHC) analysis in bone marrow biopsy in childhood acute lymphoblastic leukemia (ALL). We sought to answer whether these two methods could be alternatives to each other in the diagnosis of leukemia.

Material and Method: Twenty-eight patients diagnosed with ALL were included in the study. A Kappa test was performed between the expression rates of the antibodies studied in simultaneous FC and IHC studies in bone marrow aspiration and biopsy samples performed at the initial diagnosis.

Results: Twenty-three of the patients were precursor B-ALL (BCP-ALL) and 5 were T-ALL. In the immunophenotyping of patients with BCP-ALL using FC and IHC, MPO, CD79A, CD14, CD3 expressions were the same, while CD19, CD7, CD117, CD33, CD 56, CD34 expressions were very good, good concordance for CD20 expressions and moderate for CD10 expressions. In immunophenotyping of patients diagnosed with T-ALL using FC and IHC, CD20, CD19, CD14, CD79a, MPO, CD22 expressions were the same and excellent agreement was found in terms of CD2, CD10, CD34 expressions.

Conclusion: In cases where there are treatments that affect immunophenotyping, costly methods such as FC are not available, or bone marrow aspiration cannot be taken adequately, immunophenotyping with IHC can be safely performed in the diagnosis of pediatric ALL in bone marrow biopsy.

Keywords: Acute Lymphoblastic leukemia, immunohistochemical, flow cytometry, immunophenotyping

Öz

Amaç: Lösemiyi tedavi etmek için yeni terapötik seçeneklerin geliştirilmesi (kimerik antijen reseptörü (CAR) T hücrelerini hedefleyen tedaviler), hücre yüzeyinde eksprese edilen belirteçlerin down regülasyonuna neden olmaktadır. Bu nedenle, geleneksel immünofenotipleme panelleri artık bu antijenleri bir B hücresi immünofenotipinin tanımlanması için güvenilmez hale getirmektedir. Çalışmamızda, çocukluk çağı akut lenfoblastik lösemisinde (ALL) kemik iliği aspirasyonunda multiparametrik akım sitometrisi (AS) ile kemik iliği biyopsisinde immünohistokimyasal (İHK) analizi metodolojik olarak karşılaştırdık. Lösemi tanısında bu iki yöntemin birbirine alternatif olup olamayacağını yanıtlamaya çalıştık.

Gereç ve Yöntem: ALL tanısı alan 28 hasta çalışmaya dahil edildi. İlk tanı sırasında yapılan kemik iliği aspirasyonu ve biyopsi örneklerinde eş zamanlı AS ve İHK çalışmalarında kullanılan antikorların ekspresyonları arasındaki uyum için Kappa testi yapıldı.

Bulgular: Hastaların 23'ü pre-B ALL (BCP-ALL) ve 5'i T-ALL idi. BCP-ALL'li hastaların AS ve İHK'da kullanılan MPO, CD79A, CD14, CD3 antikorlarında ekspresyonlar aynı iken, CD19, CD7, CD117, CD33, CD 56, CD34 antikor ekspresyonları arasında çok iyi, CD20 antikor ekspresyonunda iyi ve CD10 ekspresyonunda ise orta düzeyde uyum mevcuttu. T-ALL tanılı hastaların AS ve İHK'da kullanılan CD20, CD19, CD14, CD79a, MPO, CD22 antikorlarının ekspresyonları aynıydı, CD2, CD10, CD34 antikorlarının ekspresyonları açısından çok iyi uyum mevcuttu.

Sonuç: İmmünfenotiplemeyi etkileyen tedavilerin olduğu, AS gibi maliyetli yöntemlerin bulunmadığı veya kemik iliği aspirasyonunun yeterince alınamadığı durumlarda, kemik iliği biyopsisinde pediatrik ALL tanısında İHK ile immünfenotipleme güvenle yapılabilir.

Anahtar Kelimeler: Akut lenfoblastik lösemi, immünohistokimya, akım sitometri, immünofenotiplendirme

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INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most common childhood cancer and constitutes approximately 25% of cancer diagnoses among children under the age of 15.[1-3] ALL is the most successful treatment paradigm in pediatric cancer medicine as illustrated by the significant survival rate. İmprovement from ~10% in the 1960s to >90% today.[4] Despite high treatment rates, it is an important cause of mortality and morbidity.[5] ALL originates from B-cell precursors (BCP-ALL) in the bone marrow (BM) and T-cell precursors (T-ALL) in the bone marrow and thymus.[1] BCP-ALL constitutes the majority of childhood leukemias, while T-ALL constitutes less. T-ALL incidence increased in adolescents.[6] The incidence of ALL in children varies by country and ranges from 2.5 to 4.1/100.000.[7] Classification of leukemias has changed over time,[8] but the latest classification includes morphological, immunophenotypic, and identification of genetic aberrations evaluations.[6] After acute leukemias can quickly spread to other parts of the body, particularly the spleen, lymph nodes, liver, and brain.[9] For the diagnosis of acute leukemia, the blast rate in the BM should be above 20%.[6] BM biopsy and aspiration are essential for the diagnosis and treatment of leukemia. Classification of leukemia should be done for treatment. Immunophenotyping is one of the most important parameters in ALL classification.[6] For this, multiparametric flow cytometry (FC)[10] and immunohistochemical study (IHC) in BM biopsy are used as methods.[11] Multiparametric FC has important advantages in terms of rapid results and its use in the detection of minimal residual disease,[12] but it also has challenges in the evaluation such as low cell volume, low cell viability, and increased data.[13] In the presence of fibrosis in the BM, the amount of cells taken for FC is considerably reduced, and sometimes it prevents the diagnosis with FC, and BM biopsy becomes the most important tool in the diagnosis.[14]

Even if they seem rare, in biphenotypic leukemias with diagnostic difficulties, especially if MPO positivity cannot be detected in FC, an IHC study in biopsy may be required for diagnosis.[6] Current immunotherapy models (CD 19 or CD22 targeting chimeric antigen receptor (CAR) T cells, blinatumomab, inotuzumab) downregulate cell surface-expressed markers.[15] Therefore, conventional immunophenotyping panels can no longer rely on these antigens to define a B cell immunophenotype. Therefore, there is a need for alternative immunophenotyping besides creating new panels.

Our aim in this study is to compare immunophenotyping with FC in pediatric ALL cases and with IHC in bone marrow biopsy. To find alternative methods in immunophenotyping.

MATERIAL AND METHOD

Twenty-eight patients diagnosed with ALL between 2017-2021 were included. The diagnosis of ALL was made by peripheral smear, BM aspirate, BM biopsy, FC analysis, and IHC studies performed on biopsies. ALL cases were divided into two groups as BCP-ALL and T ALL. BM biopsies were subjected to routine tissue procedures after 10% formaldehyde fixation, decalcified using 14% EDTA solution for 24 hours were embedded in paraffin. Four-micron deparaffinized tissue sections were routinely stained with hematoxylin eosin (H&E). For each antibody in the study, four micron-thick sections from the tissues in the paraffin blocks were included in the research, and we took the control group on poly -L-lysine coated slides. In the IHC studies, the antigen retrieval technique was used, and the avidin-biotin-peroxidase complex method was applied. The antibodies were examined in a Leica band max automatic immunohistochemical device. The Bond Polymer Refine Detection kit (Leica, DS9800) was used for each antibody. The necessary staining procedure on the data sheet was applied, and appropriate positive and negative controls were used for each antibody. For most antibodies, normal cells from bone marrow were used as positive and negative controls, endothelial cells for CD34 (**Figure 1**-**2**), thymus tissue for tdt. The characteristics of the primary anticipation used in the immunohistochemical study are listed (**Table 1**). The prepared samples were examined under an Olympus BX51 model microscope after the coverslip was covered with the ultra-mount.

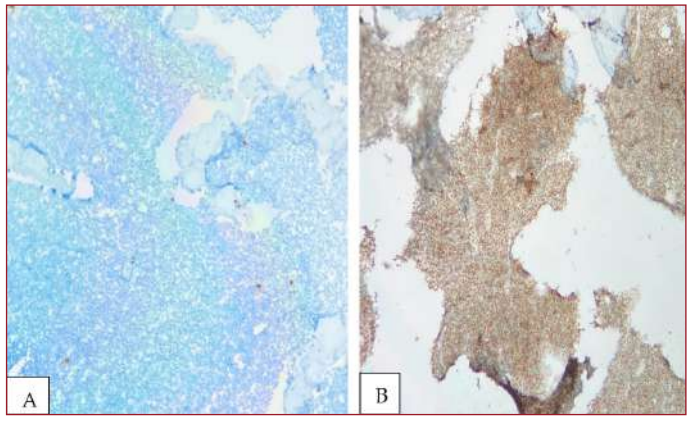

Figure 1. While there is a negative reaction with MPO antibody in leukemia cells, cytoplasmic positive reaction is observed in cells belonging to myeloid series. Positive myeloid cells were considered as internal control (A, x100). While there is nuclear reaction with PAX-5 antibody in leukemia cells, more severe nuclear reaction is observed in normal B lymphocytes other than leukemia cells (B, x100).

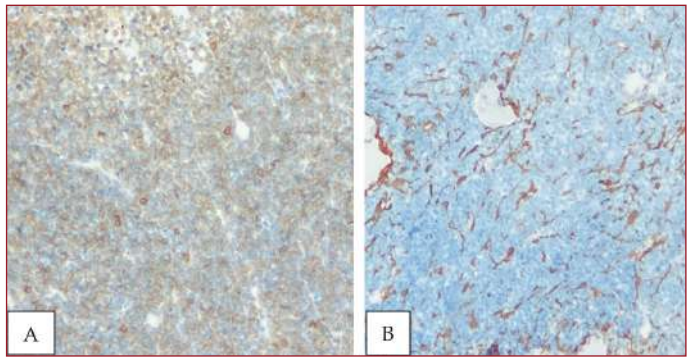

Figure 2. While cytoplasmic reaction is present with CD8 antibody in leukemia cells, more severe cytoplasmic reaction is observed in normal T lymphocytes other than leukemia cells (A,x100). While there is a positive cytoplasmic reaction in vascular endothelium with CD34 antibody, a negative reaction is observed in leukemia cells (B,x100).

Table 1. Antibodies used in immunohistochemical study and their properties.

Antibody	Clone	Dilution	Incubation Time	Antigen retrieval	Company
MPO	Polyclonal	1:200	5minute	ER1	Thermo
CD3	LN10	1:300	40minute	ER2	Leica
CD19	ZR212	1:100	30minute	ER2	Zeta
CD14	7	1:100	30minute	ER1	Bias
TdT	SEN28	1:50	30minute	ER2	Leica
CD34	ABEND/10	1:200	20minute	ER1	Leica
CD117	EP10	1:200	20minute	ER2	Leica
CD22	SP104	1:50	20minute	ER1	Zeta
CD10	56C6	1:100	40 minute	ER2	Leica
CD20	L26	1:200	40 minute	ER2	Leica
CD79A	HM47/A9	1:150	40 minute	ER2	Thermo
PAX5	Polyclonal	1:80	40 minute	ER2	Thermo
CD56	CD564	1:100	30minute	ER1	Leica
CD33	PWS44	1:100	30minute	ER2	Leica
CD4	EP204	1:100	20minute	ER2	Epitomics
CD8	4B11	1:50	30minute	ER2	Leica
CD1A	O10	1:70	30minute	ER2	Thermo
CD99	EPR3097Y	1:50	40 minute	ER2	Biocare
CD5	4CY	1:100	20minute	ER2	Leica
CD13	304	1:80	30minute	ER2	Novocastra
CD123	BR4MS	1:25	30minute	ER2	Leica

ER1: Citrate buffer ; ER2: EDTA buffer.

Staining in 10% and more of leukemia cells was considered positive for each antibody in the immunohistochemical evaluation.[16-18]

In the FC study, the analyses on the BM samples taken into tubes containing Ethylenediaminetetraacetic acid (EDTA) were completed within 24 hours. CD45, CD19, CD20, CD79a, CD22, CD3, CD5, CD123, CD38, CD10, CD7, CD79a, CD34, CD117, CD14, CD33, MPO, CD13, CD33, HLA-DR antibodies were used in the BCP-ALL immunophenotyping panel and CD45, surface CD3, cytoplasmic CD3, CD2, CD7, CD5, CD4, CD8, CD10, CD38, CD19, CD123, CD34, CD117, CD99, CD1A, TDT, CD33, CD14, CD20, CD22, CD56 antibodies were used in the T-ALL immunophenotyping panel. The reading results of the samples obtained were evaluated after the application of antibodies and the lysing procedure. Readings were performed on the Navios Ex model device of Beckman Coulter, (Miami, USA) using antibodies from the same company (**Table 2**). First, gating was performed on the CD45-Side Scatter (SSC) graph. In MPO FC evaluation, 10% was accepted for cut-off expression and 20% cut-off was accepted for other antibodies (**Figure 3**-**4**).[19-21] For each antibody, positive and negative expressions in normal cells were used as controls whenever possible. During our applications, the cut-off values created for our laboratory were taken into consideration.

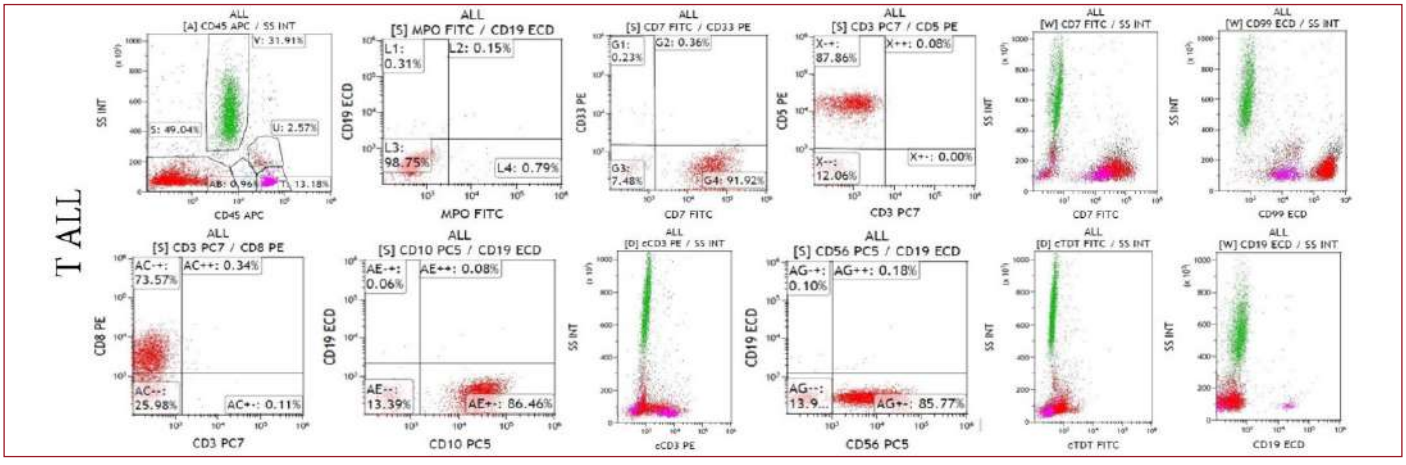

Figure 4. Leukemia cells are negative with CD45, Tdt, CD3, MPO, CD19 antibodies and positive with CD5, CD7, CD8, CD10, CD56, cCD3 antibodies. Myelocyte cells are used as internal control for MPO, and lymphocytes as internal control for both T cell markers and B cell markers.

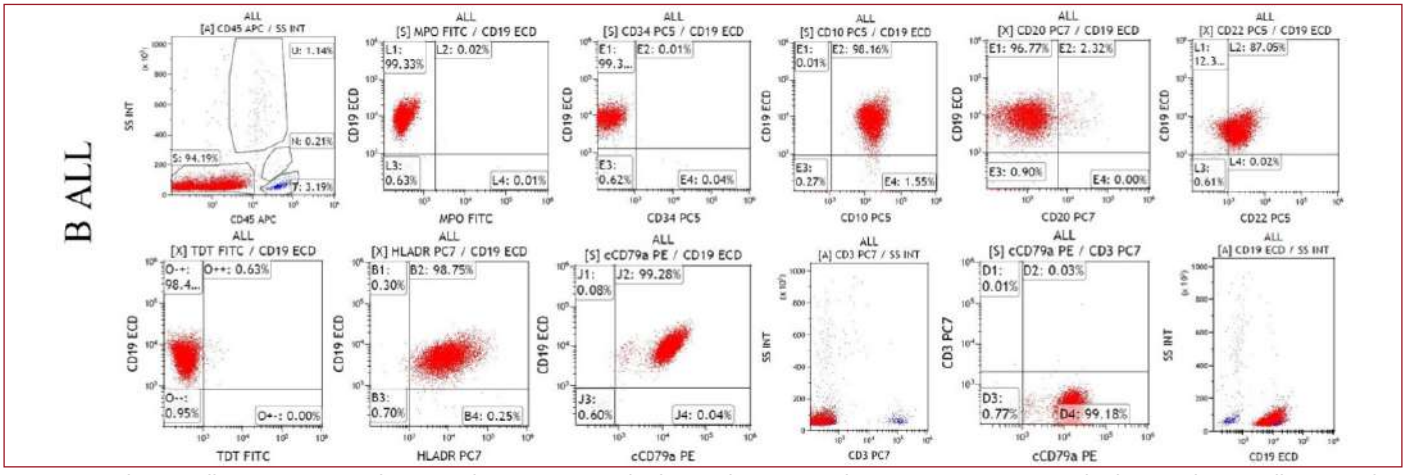

Figure 3. Leukemia cells are negative with CD45, Tdt, CD3, MPO antibodies, and positive with CD19, CD10, CD79A antibodies. Myelocyte cells are used as internal control for MPO antibody and lymphocytes are used as internal control for both T cell markers and B cell markers.

Table 2. Antibodies used in flow cytometry and their characteristics.

BPC-ALL, T-ALL	Antibody	Color	Clone
1	CD1a	PC5	BL6 (IgG1 mouse)
2	CD2	FITC	39C1.5 (IgG2a Rat)
3	CD3	PC7	UCHT1 (IgG1 mouse)
4	CyCD3	PE	UCHT1 (IgG1 mouse)
5	CD4	ECD	SFCL12T4D11(IgG1 mouse)
6	CD5	PE	BL1a (IgG2a mouse)
7	CD7	FITC	8H8.1 (IgG2a mouse)
8	CD8	PE	B9.11 (IgG1 mouse)
9	CD10	PC5	ALB1 (IgG1 mouse)
10	CD11a	PE	25.3 (IgG1 mouse)
11	CD13	PE	SJ1D1 (IgG1 mouse)
12	CD14	PC7	RMO52 (IgG2a mouse)
13	CD19	ECD	J3-119 (IgG1 mouse)
14	CD20	PC7	B9E9 (IgG2a mouse)
15	CD22	PC5	SJ10.1H11 (IgG1 mouse)
16	cyCD22	PC7	SJ10.1H11 (IgG1 mouse)
17	CD33	PE	D3HL60.251 (IgG1 mouse)
18	CD34	PE	581 (IgG1 mouse)
19	CD34	PC5	581 (IgG1 mouse)
20	CD38	PC7	LS198-4-3 (IgG1 mouse)
21	CD45	APC	J33 (IgG1 mouse)
22	CD56	PC5	N901 (IgG1 mouse)
23	CD58	FITC	ALCD58 (IgG2a mouse)
24	CD71	PE	YDJ1.2.2 (IgG1 mouse)
25	CyCD79a	PE	HM47 (IgG1 mouse)
26	CD99	ECD	HCD99 (IgG2a mouse)
27	CD117	ECD	104D2D1 (IgG1 mouse)
28	CD123	PC5,5	SSDCLY107D2 (IgG1 mouse)
29	HLA-DR	PC7	Immu-357 (IgG1 mouse)
30	IgM	FITC	SA-DA4 (IgG1 mouse)
31	CyIgM	PE	SA-DA4 (IgG1 mouse)
32	MPO	FITC	CLB-MPO-1 (IgG2a mouse)
33	TCRalb	PE	1P26A (IgG1 mouse)
34	TCRgld	FITC	IMMU510 (IgG1 mouse)
35	TDT	FITC	HT1+HT4+HT8+HT9 (IgG mouse)

BPC-ALL: B-cell precursors acute lymphoblastic leukemia, T-ALL: T-cell acute lymphoblastic leukemia.

Statistical Method

Continuous variables were presented as median, maximum and minimum, categorical variables were presented as percentage (%) and frequency (n). Kappa statistics were performed for antibodies studied in both BM biopsy and FC. In order for this analysis to be performed, it is necessary to have a 2*2 table layout. Kappa coefficient (kappa value) was not calculated for cases where only one of the negative or positive results was observed. Kappa coefficient varies between -1 and 1; classifies as , perfect agreement (0.81-1), good agreement (0.61-0.80), moderate agreement (0.41-0.60), low agreement (0.21-0.40), weak agreement (0.00-0.20), and very weak agreement (<0.00). If the p value was < 0.05, it was considered significant.[22]

Ethical approval

Ethics The study was approved by the Afyonkarahisar Health Sciences University Clinical Research Ethics Committee (2021/382).

RESULTS

A total of 28 patients, 23 (82.1%) of whom were diagnosed with BCP-ALL and 5 (17.9%) with T-ALL, were included in the study. Of the children diagnosed with BCP-ALL, 16 (69.6%) were male. Of the children diagnosed with T-ALL, 4 (80.0%) were male. The median age of children with BCP-ALL was 7 years (2-17 years) and T-ALL was 8 years (3-14 years).

In BCP-ALL, according to the FC result, CD19 and CD79A were positive in all the cases, while CD22 and CD10 were positive over 90% of the cases. All MPO, CD19 and CD3 and 80% of CD56 and CD117 were negative, while according to the results of IHC in BCP-ALL, positivity was observed in all CD79 A and TST, and over 80% of CD19 and CD10. Negativity was observed in all MPO, CD14, and CD3 and in more than 80% of CD56 and CD117 (**Table 3**).

Table 3. Results of antibodies in BCP-ALL that were studied in all patients with both FC and IHC.

Antibody	Flow-cytometry		Immunohistochemistry	
	+	-	+	-
	n(%)	n(%)	n(%)	n(%)
CD19	23 (100%)	0 (0%)	22 (95.7%)	1 (4.3%)
CD79A	23 (100%)	0 (0%)	23 (100%)	0 (0%)
CD22	21 (91.3%)	2 (8.7%)	18 (78.3%)	5 (21.7%)
CD10	22 (95.7%)	1 (4.3%)	20 (87.0%)	3 (13.0%)
CD20	8 (34.8%)	15 (65.2%)	6 (26.1%)	17 (73.9%)
CD34	19 (82.6%)	4 (17.4%)	18 (78.3%)	5 (21.7%)
CD33	7 (30.4%)	16 (69.6%)	6 (26.1%)	17 (73.9%)
CD117	3 (13.0%)	20 (87.0%)	4 (17.4%)	19 (82.6%)
MPO	0 (0%)	23 (100%)	0 (0%)	23 (100%)
CD14	0 (0%)	23 (100%)	0 (0%)	23 (100%)
CD56	2 (8.7%)	21 (91.3%)	2 (8.7%)	21 (91.3%)
TDT	18 (78.3%)	5 (21.7%)	23 (100%)	0 (0%)
CD3	0 (0%)	23 (100%)	0 (0%)	23 (100%)
PAX5	-	-	23 (100%)	0 (0%)

BPC-ALL: B-cell precursors acute lymphoblastic leukemia. IHC: Immunohistochemistry. FC: Flow Cytometry.

CD123, CD7, CD13 could be applied in different numbers. PAX 5 was performed as IHC only on biopsy and all cases were positive. CD38 and HLA-DR were studied in FC and all cases were positive (**Table 4**).

Table 4. Results of antibodies in BCP-ALL that were studied with both FC and IHC in some patients.

Antibody	Flow-cytometry		Immunohistochemistry	
	+	-	+	-
	n (%)	n (%)	n (%)	n (%)
CD123	2 (22.2)	7 (87.8)	9 (69.2)	4 (30.8)
CD7	1 (4.3%)	22 (95.7%)	1 (10%)	9 (90.0%)
CD13	6 (26.1%)	17 (73.9%)	0 (0%)	8 (100%)

BPC-ALL: B-cell precursors acute lymphoblastic leukemia. IHC: Immunohistochemistry. FC: Flow Cytometry.

In BCP-ALL, as a result of both FC and IHC studies, all MP0, CD3, CD14 results were negative and CD79a CD13 results were all positive. There was substantial agreement between FC and IHC studies for CD19, CD7, CD117, CD33, CD56 (k>0,80)(**Table 5**).

Table 5. Level of agreement of antibodies studied by FC and IHC in BCP-ALL.

Antibody	k	Agreement level	p
CD19	1.000	PERFECT	<0.001
CD123	0.125	WEAK	0.495
CD22	-0.142	VERY WEAK	0.435
CD10	0.465	MODERATE	0.008
CD20	0.796	GOOD	<0.001
CD34	0.862	GOOD	<0.001
CD7	1.000	PERFECT	0.002
CD117	0.832	PERFECT	<0.001
CD33	0.893	PERFECT	<0.001
CD56	1.000	PERFECT	<0.001

BPC-ALL: B-cell precursors acute lymphoblastic leukemia. IHC: Immunohistochemistry. FC: Flow Cytometry.

In T-ALL cases, according to the FC result, CD1A, CD5 and CD7 were found to be positive in all cases. CD117, MPO, CD14, CD22, CD20, CD33, CD79A and CD19 were found to be negative in all cases, and according to the results of IHC in T-ALL, CD3 and CD7 were all positive. CD117, MPO, CD14, CD22, CD20, CD33, CD79A and CD19 were all negative (**Table 6**).

Table 6. Results of antibodies in T-ALL that can be studied in all patients with both FC and IHC.

Antibody	Flow-cytometry		Immunohistochemistry	
	+	-	+	-
	n(%)	n(%)	n(%)	n(%)
CD3	3 (60%)	2 (60%)	5 (100%)	0 (0%)
CD2	4 (80%)	1 (20%)	4 (80%)	1 (20%)
CD4	4 (80%)	1 (20%)	2 (40%)	3 (60%)
CD8	5 (100%)	0 (0%)	4 (80%)	1 (20%)
CD99	4(80%)	1(20%)	3 (60%)	2 (40%)
CD1A	5 (100%)	0 (0%)	4(80%)	1(20%)
CD5	5 (100%)	0 (0%)	4 (80%)	1(20%)
CD34	2 (40%)	3 (60%)	2 (40%)	3 (60%)
CD117	0 (0%)	5(100%)	0 (0%)	5 (100%)
MPO	0 (0%)	5 (100%)	0 (0%)	5 (100%)
CD14	0 (0%)	5 (100%)	0 (0%)	5 (100%)
TDT	2 (40%)	3 (60%)	3 (60%)	2 (40%)
CD22	0 (0%)	5 (100%)	0 (0%)	5 (100%)
CD20	0 (0%)	5 (100%)	0 (0%)	5 (100%)
CD10	2 (40%)	3 (60%)	2 (40%)	3 (60%)
CD33	0 (0%)	5(100%)	0 (0%)	5 (100%)
CD7	5 (100%)	0 (0%)	5 (100%)	0 (0%)
CD79A	0 (0%)	5 (100%)	0 (0%)	5 (100%)
CD19	0 (0%)	5 (100%)	0 (0%)	5 (100%)

T-ALL: T-cell acute lymphoblastic leukemia. IHC: Immunohistochemistry. FC: Flow Cytometry.

CD56 was studied in FC and BM biopsy in 2 patients, and CD123 was studied in FC and BM biopsy in three cases . CD123 was positive in both FC and IHC (**Table 7**). PAX-5 was only studied in BM biopsy in all cases and was found to be negative.

Table 7. Results of antibodies in T-ALL that were studied in both FC and IHC in some patients.

Antibody	Flow-cytometry		Immunohistochemistry	
	+	-	+	-
	n(%)	n(%)	n(%)	n(%)
CD123	0 (0%)	3 (100%)	0 (0%)	3 (100%)
CD56	1(50%)	1 (50%)	0 (0%)	2 (100%)

T-ALL: T-cell acute lymphoblastic leukemia. IHC: Immunohistochemistry. FC: Flow Cytometry.

As a result of both FC and IHC studies in T-ALL in CD117, MPO, CD22, CD14, CD33, CD19, CD20 expressions were found to be negative in all, and CD7 results were positive in all. Among the Kappa Statistic antibodies, CD2, CD10 and CD34 were found to have perfect agreement (k>0.80) (p<0.05) (**Table 8**).

Table 8. Level of agreement of antibodies studied by Flow Cytometry and Immunohistochemical in T-ALL.

Antibody	k	Agreement level	p
CD3	-.154	WEAK	0.361
CD2	1.000	PERFECT	0.025
CD4	0.286	WEAK	0.361
CD99	0.545	LOW LEVEL	0.171
CD10	1.000	PERFECT	0.025
CD34	1.000	PERFECT	0.025
Tdt	0.615	GOOD	0.136

T-ALL: T-cell acute lymphoblastic leukemia. IHC: Immunohistochemistry. FC: Flow Cytometry.

DISCUSSION

In a study conducted with data from 62 countries in 2018, leukemia has been the most common malignancy seen in children aged 0-14 with 284,649 cases out of 140.6 million cases.[2] The immunophenotypic types of childhood acute leukemia have been observed in the literature as 85-86% BCP-ALL.[23,24] In our study, 82.14% of the cases were BCP-ALL. T-ALL rates in the literature ranged between 7.3-27%[25,26] and it was 17.86% in our study. In terms of gender distribution in ALL cases in the literature, it was observed as 55.6% male, 44.4% female and 61.9% male, 38.1% female,[27] and it is more common in male.[28]

In our study, 71.42% of the cases were male and 28.58% were female, and our cases in the male gender were higher compared to the literature. In the study of Noronha et al.[25] the mean age of BCP-ALL cases was four years and the mean age of T-ALL cases was 8 years and in our study, the mean age of BCP-ALL cases (between 2 and 17 years) was 7.22, the age of T-ALL cases was between 3 and 14, with a mean age of 8.80.

The most important method in the classification of acute leukemia is immunophenotyping. Immunophenotyping can be done with both FC in BM aspiration and[21] IHC study in BM biopsy.[6] In our study, the agreement was 100% in terms of biopsy in both BCP-ALL and T-ALL, and leukemia diagnosis in FC. When the literature is examined, studies on this subject are limited, and the diagnostic agreement between the two methods varies between 100%[29] and 95.8%.[30] The most sensitive markers in the diagnosis of BCP-ALL are CD19 and CD79a.[6] In BCP-ALL cases, the presence of 100% and near

100% expression in FC is detected.[21] In our study, CD19 and CD79a in FC were expressed in all our BCP-ALL cases, but CD19 expression was not detected in IHC in only one case.

CD10 is positive in common B-ALL and is approximately 93%.[6,32] In our study, it was found to be 95.7%. In addition, in our study, CD10 was positive in FC in 2 (8.7%) cases, but negative in IHC. In a study in the literature, such a result was found in 1 of 25 ALL cases.[33] CD10 negativity in B-ALL is largely associated with MLL gene rearrangement.

Most BCP-ALL cases have positive expression of CD34 and TdT. In our study, TdT was expressed at a rate of 78.3% and CD34 82.6% in FC in our BCP-ALL cases.[34] In the BM biopsy IHC study, Tdt had positive expression in all BCP-ALL cases. However, in a study in the literature, 18 of the 25 BCP-ALL cases were found to be TdT positive in IHC.[35] TdT expression is absent in approximately 2% of B-ALL cases, and little is known about the clinicopathological and genetic features of this unusual and potentially diagnostically challenging immunophenotypic subtype.

Isolated MPO positivity[36] or false high-density MPO positivity have been reported in BCP-ALL cases while other myeloid markers have been negative.[37]

In all of our cases, MPO was negative in both IHC and FC. Although it is negative in FC, positivity in IHC has been detected in various studies, and these rates go up to 26.7%.[38]

Aberrant expression of CD117 in BCP-ALL is seen as a myeloid marker. Its expression in the literature varies between 0.5-36%.[36,39,40] In this study, it was found to be 13.0% and 17.4% in FC and IHC. CD7 aberrant expression was 2.9% and it was 4.3% in our study.[41] While the expression of CD13 and CD33, myeloid markers, in BCP-ALL was 4.5% and 10.5%, respectively, in our study they were significantly higher than the study and determined to be 26.1% and 30.4%, respectively.[41]

In CD3 and CD14 BCP-ALL, all of our cases were negative in both FC and IHC; they are negative in many studies in the literature, as in our study.[33-35]

CD56 expression in BCP-ALL has generally been associated with poor prognosis. While the[42] expression rate was 11.6% in the study of Aref S et al., it was quite close to another study with 8.7% in both FC and IHC[41] in our study.

CD22 expression in FC and IHC was over 75% in our cases, which was considerably higher than previous studies.[35]

In our study, when the p value was significant (<0.05) in the kappa agreement analysis, good agreement was found in terms of CD20 and CD34 expressions, moderate agreement was found in terms of CD10 expressions, weak agreement was found in terms of CD123 expressions, and very weak agreement was found in terms of CD22 expressions in BCP-ALL.

In this study, myeloid markers MPO, CD14, CD33, CD117, and B lineage markers CD19, CD20, CD22, CD79A were negative in both FC and IHC in T-ALL cases. These results are consistent with previous studies.[7,34,41]

Similar to previous studies, CD5, CD7, CD1a FC were positive in all cases.[41] In IHC, CD3 and CD7 were positive in all cases. In our study, CD10 positivity was 40% in both FC and IHC in T-ALL, and it was found 27.3% in pediatric cases under 14 years old in the previous study, which is lower than our study.[41]

In our study, CD8 was 100% positive and CD4 was 80% positive in FC in T-ALL cases. In the previous study, it was 90% and 72%, respectively.[41] We found CD34 to have 40% positivity in both FC and IHC in T-ALL, which is 9.1%[41] higher compared to the previous study. In our T-ALL cases, we found Tdt positivity as 40% in FC and 60% in IHC, which was relatively low when compared to a study in the literature.[31]

CD99 has been used to detect minimal residual disease (MRD) in various studies. In our T-ALL cases, 80% positivity was found in FC and 60% in IHC. This was determined to be 96% in the previous study, which is higher compared to our study.[43]

In our study, when the p value was significant (< 0.05) in the kappa agreement analysis, perfect agreement was found in terms of CD2, CD10, CD34 expressions, good agreement was found in terms of Tdt expressions, poor agreement was found in terms of CD3, CD4 expressions and low level agreement was found in terms of CD99 expressions in T-ALL.

CONCLUSION

FC is a reliable and rapid method to detect minimal/measurable residual disease (MRD) in diagnosis and post-treatment follow-up in acute leukemia. However, panel selection, high cost and training of the evaluator are the main problems. It is very difficult or impossible to diagnose FC in myelofibrosis, especially when the degree of necrosis is high and bone marrow biopsy cannot be taken. Today, treatments that specifically target surface antigens reduce the expression of these antigens and cause diagnostic difficulties. Therefore, alternative immunophenotyping methods are required.

According to the results of our study, immunophenotyping with IHC in bone marrow biopsy in the new diagnosis of pediatric ALL may be an alternative to immunophenotyping with FC.

In addition, antibodies such as Pax-5, which can only be studied in bone marrow biopsy and are positive in all B-ALL, will be the most important diagnostic tool in the diagnosis of leukemia and bone marrow biopsy in the treatment effects.

Some of the genetic studies on leukemia have been associated with negative cell antigens. For example, in the Tdt negative leukemia group, this negativity was associated with various genes and poor prognosis. In this case, is Tdt negativity immunohistochemical? floow cytometric? should be.

We should look for the answer to this in future studies.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was approved by the Afyonkarahisar Health Sciences University Clinical Research Ethics Committee (2021/382).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article / Orijinal Araştırma

New Proteins in the Differentiation of Papillary Renal Cell Carcinoma From Clear Cell Renal Cell Carcinomas; Importance of DARS2, Reelin and Enkurin

Papiller Renal Hücreli Karsinomun Berrak Hücreli Renal Hücreli Karsinomlardan Ayrımında Yeni Proteinler; DARS2, Reelin ve Enkurin'in Önemi

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Abstract

Aim: The purpose of the present study was to examine the roles of new proteins DARS2, Reelin, and Encurin in the differentiation of Clear-Cell Renal Cell Carcinoma (RCC) and Papillary Renal Cell Carcinoma (RCC). Clear-cell RCC is the most common malignancy of the kidney, and papillary RCC is the second most common malignant malignancy in this respect. They are neoplasms and show similarity to each other, both histologically and morphologically, in some cases. Differential diagnosis is important because treatment approaches and prognoses are different. Although careful histopathological examination and specific immunohistochemical markers are important for diagnosis, there are no specific antibodies that can be used reliably and the search for biomarkers continues in this regard.

Material and Method: A total of 30 Clear-Cell RCC and 30 Papillary RCC cases were included in the present study. Patients were identified retrospectively by reviewing the hospital database and pathological reports. Pathological data were obtained from hospital medical archives and pathology reports.

Results: It was found that DARS2, Reelin, and Encurin proteins were significantly higher in papillary RCC when compared to clear-cell RCC.

Conclusion: It was concluded that DARS2, Reelin, and Encurin proteins may be potential biomarkers for the differentiation of Papillary RCC and Clear-Cell RCC.

Keywords: DARS, Reelin, Encurin, papillary renal cell carcinoma, clear-cell renal cell carcinoma, biomarker, immunohistochemistry

Öz

Amaç: Bu çalışma ile berrak hücreli böbrek hücreli karsinomlardan (BHK), papiller böbrek hücreli karsinom (BHK)'un ayırmında yeni proteinler olan DARS2, reelin ve enkurinin rollerinin incelenmesi amaçlandı. Berrak hücreli BHK, böbreğin en yaygın, papiller BHK ise ikinci sıklıkta görülen malign neoplazmları olup bazı durumlarda histolojik ve morfolojik olarak birbirleri ile benzerlik göstermektedir. Tedavi yaklaşımları ve prognozları farklı olduğu için ayırıcı tanıları önemlidir. Dikkatli histopatolojik inceleme ve spesifik immünohistokimyasal belirteçler tanı için önemli olmasına rağmen güvenilir bir şekilde kullanılabilecek spesifik antikorlar yoktur ve biyobelirteç arayışları devam etmektedir.

Gereç ve Yöntem: Bu çalışmaya 30 adet berrak hücreli BHK ve 30 adet de papiller BHK olgusu dahil edildi. Hastalar, patolojik bir veri tabanının gözden geçirilmesiyle geriye dönük olarak tanımlandı. Patolojik veriler hastane tıbbi arşivlerinden ve patoloji raporlarından elde edildi.

Bulgular: DARS2, reelin ve enkurin proteinleri papiller BHK da berrak hücreli BHK ya kıyasla anlamlı derecede yüksek bulundu.

Sonuç: DARS2, reelin ve enkurin proteinlerinin papiller BHK ve berrak hücreli BHK ayrımı için potansiyel birer biyobelirleyiciler olabileceği sonucuna varılmıştır.

Anahtar Kelimeler: DARS, Reelin, Enkurin, papiller böbrek hücreli karsinom, berrak hücreli böbrek hücreli karsinom, biyobelirteç, immunohistokimya

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INTRODUCTION

Kidney cancer consists of adenocarcinomas that emerge in the kidney parenchyma, often called Renal Cell Carcinomas (RCCs). RCCs consist of various subtypes that have different tumor histologies, chromosomal changes, and different molecular pathways and 60-70% of RCC cases are clear-cell carcinomas.[1,2] Its microscopy consists of prominent cytoplasmic structures surrounded by thin-walled vessels, often arranged in layers, tumor cells with membranes, transparent cytoplasm, nuclei of varying sizes, and the nucleolus of which varies according to the grade of the tumor.[3]

Papillary RCC is the second most common type of RCC in the kidney and is more common in men. In microscopy, single-row or pseudostratified tumor cells lining the papillary structures containing fibrovascular cores are detected. The tumors in Type 1 Papillary Tumor cells in RCC have an ovoid nucleus, indistinct nucleoli, pale cytoplasm, and edematous cores containing histiocytes. Tumor cells are larger and their cytoplasm is abundant with eosinophilic appearance and prominent nucleoli in Type 2 papillary RCC.[4]

The differential diagnosis of a kidney tumor that shows the papillary structure and consists of cells with clear cytoplasm can be quite difficult for pathologists.[3]

Correct diagnosis is crucial for the management of patients because biological behaviors differ depending on the histological subtype. The diagnosis of the RCC subtypes can usually be achieved by careful histological and immunohistochemical (IHC) examination. However, the search for novel markers for diagnosis continues.

DARS2 is a newly identified protein contributing to high mitochondrial efficiency, and its association with tumors has been the subject of many studies conducted to date.[5,6]

Reelin, on the other hand, is a glycoprotein that is critical for neuronal positioning, migration, and synaptic activity in the brain as a novel molecule that has been suggested to suppress the invasion in tumors.[7]

Encurin is a protein that has been shown to have significant anti-cancer effects, especially in lung and colorectal cancers, nasopharyngeal cancers nasopharyngeal carcinoma, and is localized in the Ca2+ ion channel.[8]

The purpose of the study was to determine the roles of novel molecules DARS2, Reelin, and Encurin in the differential diagnosis of Papillary RCC and Clear-Cell RCC.

MATERIAL AND METHOD

The study was approved by the Fırat University Non-Interventional Health Research Ethics Committee (Date: 15.12.2022 Decision No: 2022/14-14). A total of 30 Clear-Cell RCC and 30 Papillary RCC cases were included in the study. Patients were identified retrospectively by reviewing a pathological database and the pathological data were obtained from hospital medical archives and pathology reports.

Immunohistochemistry

Immunohistochemical procedures were used as previously described by Kocaman and Artas.[9] Immunohistochemistry (IHC) was performed by using 3 µm-thick histological tissue microarray slides. The following antibodies were also used; Anti AspRS antibody (Sc-166535, Santa Cruz Biotechnology, Oregon, USA) and anti-Reelin antibody (Sc, MyBioSource, Santa Cruz Bioyechnology, Oregon, USA), and Polyclonal Antibody ENKUR (PA5-58028, ThermoFisher Waltham, Massachusetts, USA). A histoscore was calculated for the measurement of tissue levels of DARS2, Reelin, and Encurin by using indirect immunohistochemical staining.

Microscopic evaluation of staining intensity

The staining distribution was scored as 0.1, < 25%; 0.4%, 26-50%; 0.6%, 51-75%; 0.9, 76-100%, and staining intensity 0, no staining; 0.5, very little staining; 1, little staining; 2, moderate staining; 3, very strong staining. A histoscore was calculated as histoscore = distribution × intensity.[9]

Statistical Method

The data were evaluated with the Statistical Package for social sciences for Windows version 22.0 program (SPSS, Chicago, IL). The descriptive data were expressed as mean±standard error and numbers. The distribution property of the data was evaluated with the Shapiro-Wilk Test. An Independent t-test was used to compare the data that showed normal distribution. The significance level was evaluated as $p<.05$.

RESULTS

Histopathological Results

In the histopathological examination of Clear-cell RCC, a tumor with congested capillary vascular network, prominent cytoplasmic borders, clear cytoplasm, hyperchromatic nucleus, and solid cell nests was observed (**Table 1a**).

On the other hand, papillary RCC showed a tumor characterized by papillary structures with clear-pale eosinophilic cytoplasm, prominent cytoplasmic borders, lined with cells with hyperchromatic nuclei, and anastomosing fibrovascular cores (**Table 1b**).

Table 1. Histoscores of DARS2, Reelin, and Enkurin

	*Papillary RCC	**Clear Cell RCC	P*
DARS2	2.40±0.43	0.94±1.55a	.000
Reelin	1.09±0.15	0.79±0.20a	.000
Enkurin	0.15±0.06	.000a	.000

Values are given as median, min-max. a Compared to the papillary RCC group (p< .05).* Independent t test, *Papillary cell RCC: Papillary cell renal cell carcinoma, **Clear cell RCC: Clear cell renal cell carcinoma

Immunohistochemical Results

DARS2, reelin and encurin immunoreactivity: The following results were obtained as a result of the examination of the immunohistochemical staining used for immunoreactivity under light microscopy for DARS2, Reelin, and Encurin.

As seen in **Table 1**, DARS2 expression was detected in Papillary RCC and clear cell RCC groups. DARS2 expression was mostly observed in papillary RCC. When DARS2 expression was compared between groups, it was observed that it differentiated papillary RCC from clear cell RCC and the difference between them was statistically significant (**Figure 1A-1D, 2a-2d**) (**Table 1**) (p< .05).

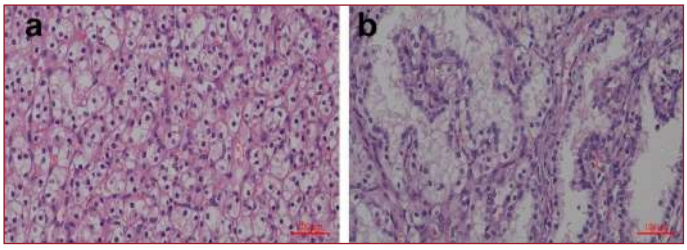

Figure 1. Hematoxylin-eosin image of *papillary cell RCC and **clear cell RCC lesion areas.

*Papillary cell RCC: Papillary cell renal cell carcinoma
**Clear cell RCC: Clear cell renal cell carcinoma

The expression of reelin and encurin proteins was highest in papillary RCC. Reelin expression was found to be significantly lower in clear cell RCC compared to papillary RCC (**Figure 1B-1E, 2b-2e**) (p< .05), Encurin expression was not observed in clear cell RCC (**Figure 1C-1F, 2c-2f**).) (p< .05). The difference in expression between the groups was found to be statistically significant (**Table 1**) (p< .05).

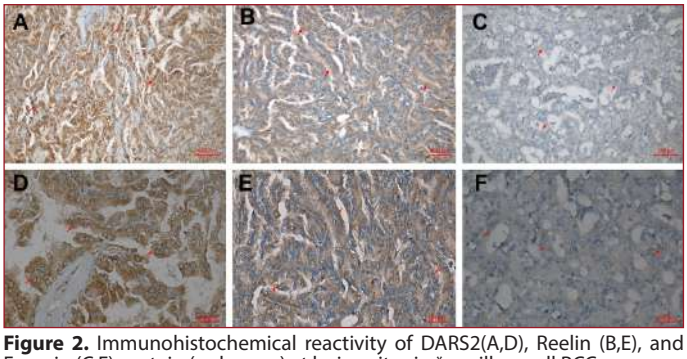

Figure 2. Immunohistochemical reactivity of DARS2(A,D), Reelin (B,E), and Encurin (C,F) protein (red arrow) at lesion sites in *papillary cell RCC.

*Papillary cell RCC: Papillary cell renal cell carcinoma

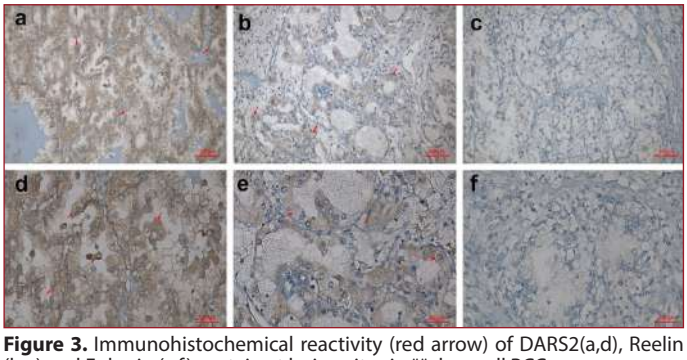

Figure 3. Immunohistochemical reactivity (red arrow) of DARS2(a,d), Reelin (b,e) and Enkurin (c,f) protein at lesion sites in **clear cell RCC.

**Clear cell RCC : Clear cell renal cell carcinoma

DISCUSSION

The typing of a kidney tumor that consists of cells with papillary structure and clear cytoplasm can be challenging for pathologists. As clear-cell RCC sometimes shows the papillary structure, papillary RCC may also be clear-cell in some cases. Accurate identification of these different entities is important in prognostic and therapeutic terms. Although histomorphological characteristics in routine Hematoxylin-Eosin Staining are the gold standard method in reaching the correct diagnosis in many cases, immunohistochemical studies are life-saving as an auxiliary diagnostic method for cases with difficult differential diagnosis.[3] Although many IHC markers have been investigated for this purpose to date, unfortunately, a specific biomarker that can be used for problematic cases has not yet been found.

DARS2, Reelin, and Encurin proteins were investigated as IHC in papillary and clear-cell RCC in the present study. Although DARS2 was reactive for both tumors, it was found to be significantly increased in papillary RCC.

Normally, mitochondrial dysfunction causes an increase in the production of reactive oxygen derivatives, which often leads to the accumulation of oxidized proteins in the cell and the regulation of antioxidant responses. DARS2 is a mitochondrial protein, and it is already known that pathologies in the DARS2 gene cause direct disruption of mitochondrial protein synthesis and tissue-specific activation of cellular stress responses.[10] In a previous study, it was determined that mitochondrial dysfunction causes reactive oxygen release that mediates epithelial-mesenchymal transition through intracellular signal transduction and cell invasion in lung cancer and attention was drawn to the relationship of DARS2 with tumorigenesis.[11]

In our previous study, it was shown that there is a relationship between DARS2 and adenocarcinoma of the lung and malignant mesothelioma, and it was suggested that it can be used as a biomarker for the differential diagnosis of these 2 tumors.[12]

The findings of this study were also very important in terms of demonstrating that DARS2 is a marker that can be used in the differential diagnosis of papillary and clear cell RCCs because mitochondrial mechanisms that affect tumorigenesis were studied recently, and some specific nuclear mitochondrial genes are considered to be potential targets for the development of next-generation cancer therapeutics.[6]

Reelin protein is associated with various brain disorders such as Alzheimer's Disease, schizophrenia, and depression, and its expression was also described in non-neuronal tissues such as the liver, breast, and kidney in more recent studies.[13] Reelin gene expression is regulated by various genetic and epigenetic mechanisms. In a recent study, it was stated that an increase in reelin prevents the development of colon cancer, and its decrease might trigger the formation of colon carcinoma, and it was suggested as a biomarker in the prediction of prognosis.[14]

In the present study, it was found that reelin was more expressed in papillary RCC, but the expression decreased in clear cell carcinoma, which is a more aggressive tumor, compared to papillary RCC, and the difference between them was statistically significant. Decreased expression of reelin in clear cell RCC, which is quite aggressive, is a finding that was expected, and it was in line with the literature data. In a different study, the antitumor effect of reelin was emphasized by showing that blockade of reelin expression increases tumor aggressiveness in breast cancer, in line with the findings of the present study.[15]

Encurin, on the other hand, is a Ca2 channel protein and was also found to induce an anti-tumor effect in the proliferation and metastasis of cancer cells by binding to β-catenin and suppressing the nucleocytoplasmic transport of β-catenin.[16] It was also shown that encurin, which is also effective as a tumor suppressor protein, inhibits proliferation, migration, and invasion of non-small cell lung cancer cells, and the absence of encurin accelerates tumor progression.[17] It was reported in previous studies that encurin plays tumor suppressor roles in lung adenocarcinoma cells through PI3K/Akt signaling pathways, and it was argued that encurin-targeted therapies would be promising for patients.[18] It is already known that the members of the Protein Kinase C (PKC) family contribute to intracellular signaling in cancer, and one of its subforms, Protein Kinase Cε (PKCε), was accepted as an oncogene. The overexpression of PKCε plays critical roles in different processes leading to cancer development, including RCC. It was shown that increased PKCε expression correlates with tumor grade in RCC and PKCε regulates cell proliferation. Its effects on the invasion ability, migration, and chemo-resistance of tumor cells in Clear-cell RCC were investigated and it was emphasized that PKCε is important for the survival of tumor cells in Clear-cell RCC.[19] In the present study, it was found that encurin was secreted very little in papillary RCC, but not at all in Clear-cell RCC, which is a very aggressive tumor. As the aggression increases, the decreased secretion of encurin may be a finding that can be used in the differential diagnosis of papillary and Clear-cell RCCs, as well as a guide for encurin-targeted treatment procedures in RCCs.

Of course, the study also had limitations, as in many other studies. The most important limitation was that it had a retrospective design, and therefore, Western Blot Analyzes of the proteins examined were not performed. Also, not including grades of tumoral tissues in the study was another limitation. For this purpose, studies to be conducted in larger series will reveal the relationship of DARS2, reelin, and encurin proteins with papillary and clear cell BCCs.

CONCLUSION

It was determined in the present study that DARS2, reelin, and encurin proteins may be biomarkers in the differential diagnosis of papillary and Clear-cell RCCs. Decreased levels of reelin and encurin in tumoral tissues in RCC were determined to increase aggressiveness, and they were shown to be candidate molecules for the development of new cancer therapeutics specific to these tumors.

ETHICAL DECLARATIONS

Ethics Committee Approval: The permission was received with the date 01.12.2022 and number 2022/14-14 from Fırat University Non-Interventional Health Research Ethics Committee.

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article / Orijinal Araştırma

Patterns of Dental Anxiety in Primary Schoolers Attending Oral Health Education Program

Ağız Sağlığı Eğitim Programına Katılan İlköğretim Okulu Öğrencilerinde Dental Kaygı Paterni

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Abstract

Aim: Dental anxiety is a common problem which develops mostly in childhood. This study aimed to determine the level and the patterns of dental anxiety perceived by the attendants of oral and dental health education in their classroom environment using Facial Image Scale (FIS).

Material and Method: FIS were applied to 163 third-grade primary schoolers while they were having oral health education course in an interactive way. Gender, white dental attire, and past dental experience(s) were pre-determined variables evaluating the pattern of dental anxiety in the third-grade primary schoolers in this study.

Results: The overall anxiety level in children was 46.01%, with no significant difference observed between genders (p=0.4593). Students who were educated by instructors wearing white dental attire were more likely to have a 1&2 FIS score (OR: 3.9 (1.3-11.7); p=0.0156). However, students who had past dental experience expressed significantly more 4&5 FIS scores (OR: 4.38 (2.17-8.85); p < 0.001).

Conclusions: Regardless of gender white dental attire created a positive perception in 9-year-old students, but the presence of past dental experience and a experience of a tooth extraction especially under local anesthesia caused negative perception in the study.

Keywords: Dental anxiety, child, dental attire, tooth extraction, facial image scale

Öz

Amaç: Dental anksiyete çoğunlukla çocukluk çağında gelişen yaygın bir sorundur. Bu çalışma, sınıf ortamında gerçekleştirilen ağız ve diş sağlığı eğitim programına katılan öğrencilerinin dental anksiyete düzeyinin ve paterninin Görsel Yüz Skalası (GYS) ile belirlemeyi amaçlamaktadır.

Gereç ve Yöntem: Programa katılan 163 üçüncü sınıfa giden ilkokul öğrencisine ağız ve diş sağlığı eğitimi dersi verilirken interaktif bir şekilde GYS uygulandı. Dental anksiyete paterninin değerlendirilmesinde: cinsiyet, beyaz diş hekimi önlüğü ve geçmiş diş tedavisi deneyimleri değişkenleri ele alınmıştır.

Bulgular: Yaş ortalaması 9.05±0.54 bulunan katılımcı popülasyonunun genel dental anksiyete düzeyi %46.01 olup, cinsiyetler arasında anlamlı fark gözlenmemiştir (p=0.4593). Beyaz diş hekimi önlüğü giyen eğitmenlerle etkileşen öğrencilerin GYS skorlarının pozitif (1&2) olma olasılığı anlamlı ölçde yüksek bulundu (OR: 3.9 (1.3-11.7); p=0.0156). Bununla birlikte, geçmiş diş tedavisi deneyimi bulunan öğrenciler anlamlı olarak daha fazla 4&5 FIS puanı ifade etmişlerdir(OR: 4.38 (2.17-8.85); p < 0.001).

Sonuç: Cinsiyetten bağımsız olarak, beyaz diş hekimi önlüğü 9 yaşındaki öğrencilerde olumlu bir algı yaratmış, ancak geçmiş diş hekimliği deneyiminin ve özellikle lokal anestezi altında diş çekimi öyküsünün varlığı çalışmada olumsuz algıya neden olmuştur

Anahtar Kelimeler: Dental anksiyete, çocuk, beyaz önlük, diş çekimi, görsel yüz skalası

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INTRODUCTION

Dental anxiety is a common problem which develops mostly in childhood and adolescence seen in the dental operation room, and especially challenging for pediatric dentists.[1] Approximately half of children have 10%-20% high levels of dental anxiety.[2,3] It should be recognized that the nature of a child's dental anxiety can vary significantly and some children present with fears or phobias in relation to previous dental and medical experiences, frequency of dental visits, type of a dental procedure and specific dental stimuli (e.g. needle or drill)[4-6] and other children report more generalized anxiety associated with the dental setting and dental attire.[7-8]

Children with high dental anxiety have been found to have poorer oral health status and less dental visits.[9,10]

Measurement of dental anxiety by scales is important not just for delivery of high quality clinical care but also for research in children. Understanding the factors and level of anxiety before examination or treatment will provide the dentist information to identify the anxious child in order to have better anxiety management.[11] The Facial Image Scale (FIS, **Figure 1**) is a self-reported dental anxiety scale commonly used in children. It consists of a row of five faces with varying expressions, ranging from very unhappy to very happy. Children are asked to indicate which face they feel most like at the present moment, with the idea that the face they choose represents their level of dental anxiety. The FIS is a simple and easy-to-use tool that can help dental professionals assess a child's anxiety level quickly and effectively. It has been shown to be a reliable and valid measure of dental anxiety in children, and it can be used in clinical and research settings to evaluate the effectiveness of interventions designed to reduce dental anxiety.[12,13]

Since community oral health education programs might help to prevent occurrence and/or recurrence of dental anxiety beforehand,[14,15] 9-year-old primary school students were evaluated in the present study in terms of dental anxiety levels during school education programs implemented by the Ministry of National Education of Türkiye in 2022 within the nation-wide project called "10,000 Schools in Basic Education Project". The Ministry is implementing a project to improve equal opportunities in education by providing supportive programs to improve the basic skills of students in primary schools. By targeting all primary schools included in the project, the Ministry is working to ensure that all students have access to these programs and can benefit from them equally. Strengthening basic skills is a key foundation for academic success, so this initiative has the potential to positively impact students' education and future opportunities.

The aim of the study is to assess the level of dental anxiety experienced by students during oral dental health education sessions conducted by instructors in a classroom environment. The study intends to use the Facial Image Scale (FIS) to measure the level of dental anxiety perceived by students and compare the results to predetermined categories of anxiety.

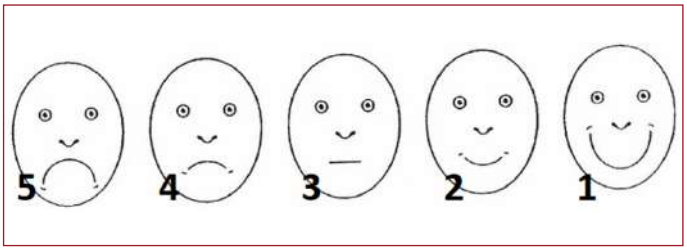

Figure 1: Picture of Facial Image Scale (FIS; From 5-Negative to 1-Positive) used for dental anxiety scoring in the study. From Buchanan ve Niven (2002)[13]

MATERIAL AND METHOD

The study was conducted withing the permission of the Ministry of National Education of Türkiye the nation-wide project entitled "10,000 Schools in Basic Education Project" and approved by the Bursa Governorship Provincial Health Directorate (Doc #: E-95210709319)

Second year faculty students (n=9) were participated within the scope of social responsibility and volunteering course in Bursa Uludag University, Faculty of Dentistry. The written approvals were obtained from the Provincial Directorate of the Ministry of National Education and the faculty education commission. Faculty students who were pre-educated about the Facial Image Scale (FIS) and received basic dental health education on topics such as oral hygiene instructions and cariogenic diet. The study then proceeded with standardized instructors who were divided into two groups who had white dental attire (n=5) and no attire (n=4).

The third-grade primary schoolers (n=163) in Gorukle Dumlupinar Primary School and their parents were informed by their school teachers in advance. An informed consent sheet was obtained from the parents for the education and the forms to be implemented.

On April 2022, children were randomly divided into two groups and received video-assisted dental health instructions in an interactive way. They informed about tooth brushing, the use of dental floss, the ingredients (especially fluoride) and amount of toothpaste and cariogenic foods that cause dental caries. The method of tooth brushing was shown to the children interactively.

Dental anxiety levels were measured using the validated FIS. The FIS scores of the students were recorded simultaneously by the other faculty students during the instruction and analyzed by coinciding with the dental experience form filled by their parents beforehand.

Statistical analysis

Data was analysed using SPSS program (Statistical Package for the Social Sciences, version 28,SPSS Inc, Chicago, Ill, USA). Percentages and descriptive statistics was calculated. The odds ratio (OR), its standard error and 95% confidence interval are calculated. $p < 0.05$ was considered statistically significant.

RESULTS

This study enrolled 163 third-grade primary school students with a mean age of 9.05±0.54. The overall anxiety level in children was 46.01%, with no significant difference observed between genders (p=0.4593). The study found that students who were educated by instructors wearing dental attire were more likely to have a positive FIS score (OR: 3.9 (1.3-11.7); p=0.0156) (**Table 1**).

Students who had past dental experience expressed significantly more negative FIS scores (p<0.001). Children who received only oral prophylaxis in their past dental experience showed significantly higher positive FIS scores in the study. Extraction with local anesthesia also increased the probability of negative FIS scores in the study (p<0.0001). Regardless of whether the dental treatment visit was single or multiple, the FIS scores of children did not differ significantly (p=0.6557).

Table 1: Relation between the FIS scores of the participants and predetermined categories of anxiety.

	FIS Scores*			
	1&2 -Positive- n(%)	3 -Neutral- n(%)	4&5 -Negative- n(%)	OR (95 % CI)**
Gender				
Boy	26 (34.6)	17 (22.6)	32 (42.6)	1.32 (0.63-2.75) p=0.4593
Girl	27 (30.6)	18 (20.4)	43 (48.8)	
Instructors with dental attire				
Yes	59 (69.4)	4 (4.4)	22 (25.9)	3.9 (1.3-11.7) p=0.0156
No	17 (21.8)	8 (10.2)	53 (67.9)	
Past dental experience				
Yes	29 (28.4)	13 (12.7)	60 (58.8)	4.38 (2.17-8.85) p<0.001
No	21 (64.4)	25 (40.9)	15 (24.6)	
Purpose of dental visit				
Oral prophylaxis	15 (51.7)	1 (7.6)	4 (6.7)	15.0 (4.30- 52.29) p<0.0001
Dental treatment (single visit)	7 (24.1)	7 (53.8)	12 (20.0)	1.27 (0.44 - 3.67) p=0.6557
Dental treatment (multiple visit)	4 (13.8)	2 (15.4)	17 (23.3)	0.40 (0.12 - 1.34) p=0.1381
Extraction with local anaesthesia	3 (10.3)	3 (23.0)	27 (45.0)	0.14 (0.04 - 0.52) p=0.0031

*Percentiles (%) with no decimal rounding; **Odds ratios (OR) and 95% confidence intervals (CI)

DISCUSSION

It is important to note that a FIS scores 1 and 2 indicates a lower level of dental anxiety, while a FIS score 4 and 5 indicates a higher level of dental anxiety. Therefore, the study's findings suggest that past dental experience and extraction with local anesthesia may increase anxiety in children participated in present study. However, instructors wearing white dental attire may help reduce anxiety levels in students.

The facial image scale is a commonly used tool for assessing pain in children and has been validated for use in a variety of age groups. The present study used validated facial image scale Bucharan et al.[13] to assess the dental anxiety attending to oral health program.

Evaluating the 'gender' as a contributing factor in dental anxiety in children Alsadat et al. reported that girls aged 6-12 years had more dental fears compared to their boy peers.[16] Likewise, Gaber et al. evaluated dental anxiety in 126 children aged 6-10 years and stated higher percentage of girls (30.5%) compared to boys (15.0%) were anxious.[17] In our study, dental anxiety was assessed using the FIS, and it was found that 48% of girls and 42% of boys indicated scores of 4 or 5, which did not differ significantly between the two groups in accordance with the studies indicating no significant relation between gender and dental anxiety.[18,19] Differences in methodology, sample size, and other factors can also influence the results obtained from different studies.

Examining the effect of dentists' attire on dental anxiety, there are studies reporting that child-friendly coat may be appropriate in anxious children.[20,21] The meta-analysis included data from 3706 children across various studies examining the children's perceptions of dentists's attire and environment indicated no significant difference between white coat and child-friendly attire on children's dental anxiety. In accordance with our study, Similarly, there are studies white coat attire might be more appropriate for anxious children.[22,23] Thus, Kamavaram Ellore et al. reported %70 of examined children had favored traditional white coat attire.[23]

Vishwnath et al. stated that negative attitudes and dental anxiety might be be influenced by previous negative experiences, such as painful or traumatic dental procedures.[24] However, a study examining a dental fear by visual analogue scale in a 1303 French childred aged 5-11 years (mean: 8.12 years) were stated past dental experience of the dental setting can act as a positive component of dental fear.[25] The present study reports higher anxiety levels in children having past dental experience.

In addition, extraction procedure with local anesthesia can be a source of anxiety individuals. However, local anesthesia is a safe and effective way to numb the area around the tooth being extracted, which can help minimize discomfort and pain during the procedure. In a recent study, anxiety was significantly with needle-related treatments.[26] In accordance, the present study reported significant relation between dental anxiety and extraction experience with local anesthesia, however other dental treatments regardless being performed in a single or multiple visits were not significantly associated with the reported dental anxiety levels by FIS.

CONCLUSION

By evaluating the level of dental anxiety experienced by third-grade primary schoolers during oral dental health education, the study may provide valuable insights about the patterns related to dental anxiety. Regardless of gender, white dental attire created a positive perception in 9-year-old students, but the presence of past dental experience and a history of tooth extraction especially under local anesthesia caused negative perception in the study.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of the Bursa Governorship Provincial Health Directorate (Doc #: E-95210709319).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article / Orijinal Araştırma

The Effect of Alpha-Lipoic Acid against Methotrexate on Testicular Damage in Rats

Metotreksat'a Karşı Alfa-Lipoik Asit'in Sıçanlarda Testis Hasarına Etkisi

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Abstract

Aim: The toxic effects of methotrexate, a chemotherapeutic, on the testicles is an important side effect. Methotrexate impairs spermatogenesis and fertility and causes oligospermia. In this study, we aimed to minimize the testicular toxicity, those being the side effects of methotrexate, by using the probable protective effects of α-lipoic acid, a potent antioxidant.

Material and Method: Twenty-eight male Sprague Dawley rats that we employed in this research were separated into three groups as control (0.09% PS) (n=8), methotrexate (20 mg/kg) (n= 10), and methotrexate (20 mg/kg) + α-lipoic acid (100 mg/kg) (n= 10). We performed a histochemical analysis on the testicular tissue of rats using hematoxylin-eosin and Masson's trichrome. We performed an immunohistochemical analysis using inducible nitric oxide synthase (iNOS) and tumor necrosis factor-alpha (TNF-α) primer ab.

Results: The histochemical evaluation revealed a significant decrease in the methotrexate-induced testicular toxicity in the α-lipoic acid-treated groups. On the other hand, TNF-α and iNOS immunostaining results were also observed to support these results.

Conclusion: The treatment use of α-lipoic acid succeeded in protecting against methotrexate-induced testicular damage through an α-lipoic acid-mediated antioxidant and anti-inflammatory mechanisms. α-lipoic acid can be used in combination with methotrexate as a protector against side effects during anticancer therapy. In the present study, it was shown that α-lipoic acid can be used in combination with methotrexate as a protector against side effects during anticancer treatment.

Keywords: Alpha-lipoic acid, anti-inflammatory, methotrexate, testicular damage, oxidative stress

Öz

Amaç: Bir kemoterapötik ajan olan metotreksatın, testisler üzerindeki toksik etkisi önemli bir yan etkidir. Metotreksat, oligospermiye neden olup spermatogenezi ve fertiliteyi bozar. Bu çalışmada, güçlü bir antioksidan olan α-lipoik asidin olası koruyucu etkilerini kullanarak metotreksat'ın yan etkisi olan testiküler toksisiteyi en aza indirmeyi amaçladık.

Gereç ve Yöntem: Araştırmada kullandığımız 28 adet erkek Sprague Dawley cinsi ratlar kontrol (0.09% SF) (n= 8), metotreksat (20 mg/kg) (n= 10) ve metotreksat (20 mg/kg) + α-lipoik asit (100 mg/kg) (n= 10) olmak üzere üç gruba ayrıldı. Ratların testis dokusunda Hematoksilen-Eozin ve Masson Trikrom boyama yöntemlerini kullanarak histokimyasal analizler yapıldı. İndüklenebilir nitrik oksit sentaz (iNOS) ve tümör nekrozis faktörü-alfa (TNF-α) primer antikoru kullanılarak immünohistokimyasal analizler yapıldı.

Bulgular: Histokimyasal değerlendirmede, α-lipoik asitle tedavi edilen gruplarda metotreksat'ın neden olduğu testiküler toksisitede önemli bir azalma olduğu ortaya çıkarıldı. Öte yandan TNF-α ve iNOS immünboyama değerlendirme sonuçlarının da bu sonuçları desteklediği gözlendi.

Sonuç: Tedavide α-lipoik asidin kullanımı, α-lipoik asit aracılı antioksidan ve antiinflamatuvar mekanizmalar yoluyla metotreksat'ın neden olduğu testis hasarına karşı koruma sağladı. α-lipoik asit, kanser tedavisi sırasında yan etkilere karşı koruyucu olarak metotreksat ile birlikte kullanılabilir. Mevcut çalışmada, kanser tedavisi sırasında, α-lipoik asid ve metotreksat'ın kombine kullanımı yan etkilere karşı koruyucu bir ajan şeklinde kullanılabileceği gösterilmiştir.

Anahtar Kelimeler: Alfa-lipoik asit, antiinflamatuvar, metotreksat, testis hasarı, oksidatif stres

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INTRODUCTION

The methotrexate (MTX) drug, folic acid analogue from the point of structure, is a potent cytotoxic substance and has been extensively used against many malignancies as a chemotherapeutic, but the side effects of MTX limit its clinical uses.[1] It is known that the use of chemotherapeutics causes acute toxic effects in several organs, and one of them is the testes. People can become infertile when they receive cancer treatment for other organs not involved in reproduction.[2] This is not an acceptable outcome. Thus, this side effect of MTX should be reduced or destroyed. In our study, we aimed to minimize or eliminate the side effects of MTX.

The complete mechanisms underlying MTX testicular toxicity are unclear. It is known that MTX inhibits cytosolic forms of nicotinamide adenosine diphosphate (NADP)-dependent dehydrogenase enzymes.[3] NADPH is the oxidatively reduced form of NADP. Being an antioxidant enzyme that resides in cytoplasm, glutathione reductase, utilizes NADPH to conserve level of the reduced form of cellular glutathione and keeps safe against the effects of reactive oxygen species (ROS). If their level is not kept under control, damages biomolecules like lipids, proteins, and DNA and eventually leads to cell death.[4-6] In the animals treated by MTX, antioxidant levels reduced and oxidant levels enhanced, a situation that contributes to MTX-induced oxidative stress.[7,8] MTX is used in low doses as anti-inflammatories in diseases such as anti-rheumatic diseases. However, it has an inflammatory effect when used at high doses as a chemotherapeutic to treat various malignancies, including lymphoma, head and neck carcinoma, osteosarcoma, breast carcinoma and acute lymphoblastic leukemia due to the toxic effect of the tissue.[9-11] As a result, MTX induces oxidative stress and inflammation in tissues. Inducible NO synthase (iNOS) is a predominant parameter of ROS-mediated tissue damage due to produce NO which is free oxygen radical.[12] iNOS is also known as an inflammatory cytokine-like tumor necrosis factor-α (TNF-α), meaning it increases inflammatory conditions.[13]

α-Lipoic acid (ALA) is found in some foods and is synthesized de novo in the body. It is a natural antioxidant, which is why it is so important in terms of easy retrieval. Lipoic acid, which prevents free radical damage, is unique among antioxidants in terms of its ability to dissolve in oil and water.[14] Its reduced form, Dihydrolipoic acid, is more biologically active. Many studies have mentioned that ALA has antioxidant and anti-inflammatory effects, and it creates these properties with different mechanisms.[15] The antioxidant effects of ALA are due to increase iNOS expression. It provides the formation of NO which is one of the free oxygen radical. ALA also shows this effect by increasing antioxidant enzymes, namely glutathione reductase, the restorating of the reduced / oxidized glutathione ratio, and lowering the NADP level. ALA's anti-inflammatory effects are attributable to repression of inflammatory activities from IL-6, TNF-α and NF-kB and elevation of anti-inflammatory proteins, like nuclear erythroid 2- related factor (Nrf2).[16]

In this experiment, a rat model was conceived to evaluate whether ALA had any protective effects opposed to ROS-mediated damage caused via MTX-induced testicular injury. For that purpose, immunohistochemical receptor activity and histopathological changes were evaluated in MTX-administered animals with and without ALA treatment. We believe that the outcomes of this research will play a significant role in showing that it is probable to reduce testicular toxicity, which is one of the most crucial side-effects that restrict the effective usage of a chemotherapeutic drug for instance MTX, with antioxidant and anti-inflammatory agents such as ALA.

MATERIAL AND METHOD

Chemicals

Hematoxylin (HX86017674), eosin (HX378237), ethanol 96% (1009712500), ksilen (1086612500), entellan (HX87112361), and hydrochloric acid (143007) were obtained from Merck. iNOS antibody (ab15323) and TNF-α (ab66579) were obtained Abcam. Hydrogen peroxide (H2O2) (ThermoFisher Scientific, FSH40069), acetic acid (Fluka, 52220) were used. Phosphate buffered saline (PBS999), SensiTek HRP Anti-Polyvalent (SHP125) and DAB chromogen (ADK125) were obtained from ScyTek. Picric acid (P6744), biebrich scarlet-acid solution (HT151), fosfotungistik acid solution (HT152), fosfomolibdik acid solution (HT153), aniline blue (HT154) solution were obtained from Sigma-Aldrich.

Experimental Animals

Twenty-eight male-gendered Sprague-Dawley stock rats were employed in the experiment. The rats were obtained from the Experimental Animals Research Laboratory Production Unit of the School of Medicine, Süleyman Demirel University. The rats were maintained in regulated conditions of temperature (22±2°C) and humidity (50±10%) on a 12 h light/dark cycle during the experiment. All rats had ad libitum access to food and water (Standard Issue Rat Chow of Animal Food Institution). This study was approved by the Local Ethical Committee on Animal Research of Süleyman Demirel University, Isparta and was performed according to ethical rules (Protocol number: 15.09.2022 06/70).

Experiment Protocols

Animals were split into three groups at random. Group number 1 (C) was the control group consisting of eight animals (n=8). In this group, for ten days starting from the first day, animals have taken intraperitoneally (i.p.) administered physiologic saline (PS) (0.09% NaCl) in approximately the equal volume same as the drugs given to the other groups on the similar day. Group number 2 (MTX) was the methotrexate group consisting of ten animals (n=10). In this group, on the fourth day of the study, MTX (Methotrexate® available in 50 mg/ml, Koçak Farma, injectable solution) was administered in a single dose of 20 mg/kg i.p.[17] PS was given i.p. on days when the drug was not administered. Group number 3 (MTX

+ ALA) was the methotrexate + α-lipoic acid group consist of ten animals (n=10). In this group, MTX was administered only on the fourth day of the study, same as in Group number 2. ALA (Thioctacid® 600T available in 600 milligrams in 24 milliliters per ampoule, MEDA Pharma, injectable solution) was administered in a dose of 100 mg/kg i.p.[18] for ten days starting on the first day.

Sample Collection and Preparation

At the conclusion of the experiment (the eleventh day), rats were anesthetized under a mixture of xylazine hydrochloride (10 mg/kg) + ketamine hydrochloride (90 mg/kg) and then euthanized. Subsequently, the bilateral testes were isolated, the fat was separated, and the testes were weighed and subsequently fixed in 10% neutral formalin for 24 h for histochemical and immunohistochemical investigations. After fixation, the testes were dehydrated stepwise with an ethanol series, at least 1 h for each step. Then, testicular tissues were embedded in paraffin, sectioned at 3-4 μm thickness, mounted on microscope slides, and dried overnight at room temperature.

Histochemical Analysis

The next preparations were deparaffinized and dehydrated. After deparaffinization and dehydration, the testis sections were stained with routine hematoxylin and eosin (H-E) stain for a general histochemical evaluation and for an examination of cellular toxicity. Sections were stained with Masson's trichrome to better observe the interstitial area. The prepared slides were examined and imaged with a camera-equipped light microscope (DM500, Leica, Germany). Testicular specimens were evaluated for typical histopathological features associated with MTX-induced testicular toxicity (spermatogenetic and interstitial degeneration), and these parameters were scored as 0-3 damage score (0=no, 1=mild, 2=moderate, 3=severe).

iNOS and TNF-α Immunoreactivity Analysis

To detect iNOS and TNF-α immunoreactivity in the testes, we have done the following immunohistochemistry procedure. Paraffin-embedded testicular tissues were cut into thin (3-4 μm) sections with a sliding microtome (SM2000R, Leica, Germany) and sections mounted on poly-l-lysine coated glass slides. The next preparations were deparaffinized and dehydrated. Then, the testis sections were first immersed in 3% hydrogen peroxide (ready-to-use, Thermo Scientific) to incapacitate intrinsic peroxidase activity and then in super-block (ready-to-use, ScyTek) to prevent non-specific antibody attachment. They were later incubated overnight at 4°C with the rabbit polyclonal to iNOS antibody (Abcam, Cambridge, USA) diluted 1:50 in antibody diluent and the rabbit polyclonal to TNF-α antibody (Abcam, Cambridge, USA) diluted 1:100 in antibody diluent. After washing in phosphate-buffered saline (PBS), the spot of the immunoreaction was visualized through incubating the sections sequentially with a biotinylated goat anti-polyvalent antibody, horseradish peroxidase-conjugated streptavidin (ready-to-use ScyTek Laboratories, Logan, UT), and 3,3′-diaminobenzidine solution (5.6% ml, ScyTek Laboratories, Logan, UT). Biotinylated goat anti-polyvalent antibody (ready-to-use, ScyTek Laboratories, Logan, UT) was used instead of the anti-iNOS and anti-TNF-α antibody for a negative control. After washing with the PBS, sections were lightly counterstained with hematoxylin solution. Next, the sections were passed through a graded series of ethanol, cleared by xylene, and covered with entellan. These specimens were evaluated according to the staining intensity and scored as 0-3. The sections were then examined and imaged with a camera-equipped light microscope (DM500, Leica, Germany).

Statistical Analysis

The statistical analysis was accomplished by using a Windows® compatible SPSS® 16.0 program. Results were examined as histological measurements. For these histopathological findings, the Kruskal-Wallis's test was the nonparametric test, and the Mann–Whitney U test was used for comparisons of measurements among the two groups. The level of significance was taken as $p < 0.05$. The values were conveyed as mean±standard deviation.

RESULTS

Effect of ALA Treatment on Testicular Histochemistry

As a result of a histochemical investigations of testicular tissue, a usual histological structure was found in the control group. Effects of MTX treatment and the intervention of ALA on the cellular alterations were investigated by a histological evaluation of the testes. The spermatogenetic degeneration (loss of spermatozoa in the lumen and vacuolization of germinal epithelium), interstitial degeneration (interstitial fibrosis, interstitial congestion, and interstitial mononuclear cell infiltration) were considered in the assessment of the testicular toxicity. Significant regression of these parameters was identified in the ALA treatment group.

Histopathological analysis of the testes was done based on spermatogenetic degeneration and the interstitial degeneration score (**Figure 1, 2**). A significant rise in the testicular damage was marked with the one with treatment of MTX 20 mg/kg compared to the control group ($p < 0.05$). While the mean spermatogenetic damage score was 2.80±0.42, interstitial damage was 2.70±0.67 in the MTX-treated group, they were respectively 0.44±0.72 and 0.55±0.72 in the ALA-treated group. It seems that ALA treatment significantly reduced MTX-induced testicular damage (**Table 1**).

Table 1. Scoring of histopathological changes in groups of testicular tissue

	Control	MTX	ALA-treated
Spermatogenetic degeneration	0.25±0.46	2.80±0.42[a]	0.44±0.72
Interstisial degeneration	0.12±0.35	2.70±0.67[a]	0.55±0.72

Values are expressed in mean±SD, [a]Significantly changed when compared with the control group ($p < 0.05$)

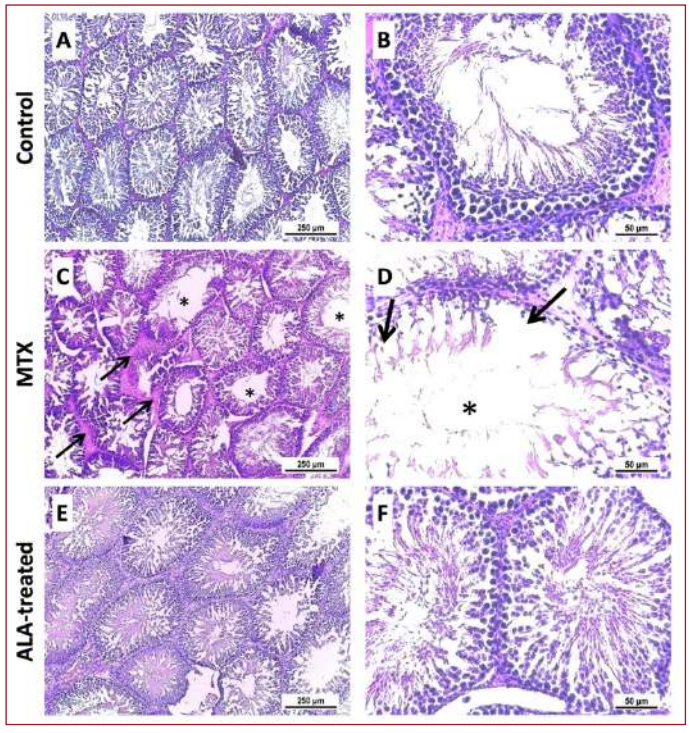

Figure 1: Effect of methotrexate (MTX), alpha lipoic acid (ALA) on testicular tissue. Histopathological sections from control group (A, B) and ALA-treated group (E, F) showed near-normal testis histology. Whereas, sections from the MTX group (C, D) showed extensive testicular damage, such as loosing of spermatozoa in the lumen (black asterix), vacuolization of germinal epithelium (thin arrow), interstitial fibrosis (thick arrow). H–E staining, scale bar 250 µm (×100 magnification), and scale bar 50 µm (×400 magnification).

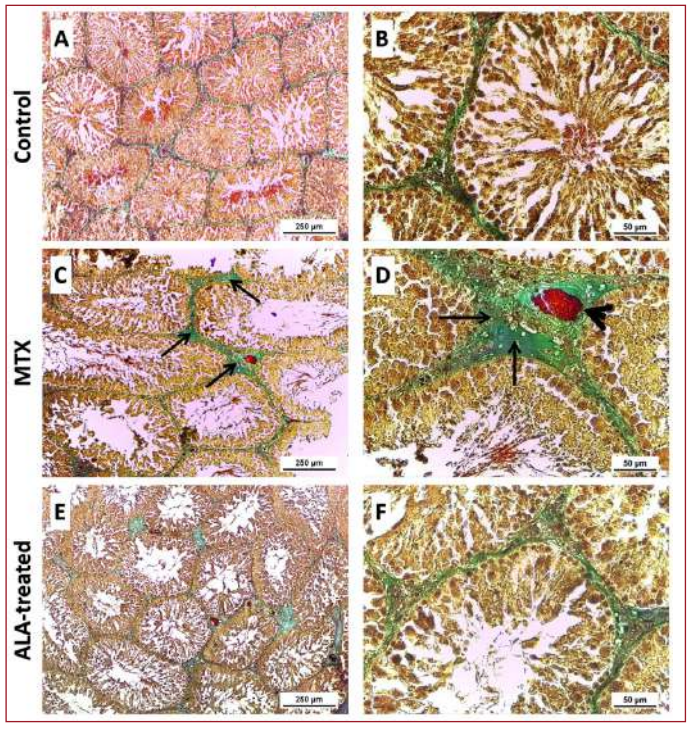

Figure 2: Effect of Methotrexate (MTX), alpha lipoic acid (ALA) on testicular tissue. Histopathological sections are stained with a stain that shows connective tissue well. Control group (A, B) and ALA-treated group (E, F) showed near-normal testis histology. Whereas sections of the MTX group (C, D) showed extensive testicular damage, such as interstitial fibrosis (arrow), interstitial congestion (arrowhead). Masson thricrome staining, scale bar 250 µm (×100 magnification), and scale bar 50 µm (×400 magnification).

Effect of ALA Treatment on iNOS and TNF-α Immunoreactivity

Strong iNOS and TNF-α staining were noted in the testes of the rats that took MTX treatment as compared to the control group ($p < 0.05$). The ALA treatment led to a significant reduction in iNOS and TNF-α positive receptors compared to the MTX group (**Figure 3**, **Table 2**).

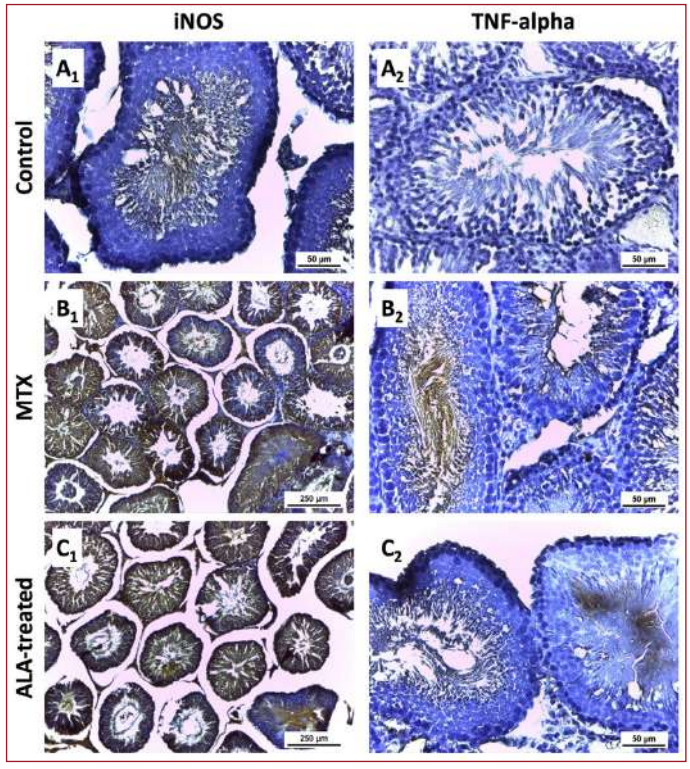

Figure 3: Effect of methotrexate (MTX), alpha lipoic acid (ALA) on testicular tissue. Immunohistological sections from control group (A_1, A_2) showed less staining intensity and ALA-treated (C_1, C_2) group showed moderate staining intensity for both iNOS and TNF-α. On the contrary, sections of the MTX group (B_1, B_2) showed strong staining intensity. iNOS and TNF-α immunostaining, scale bar 250 µm (×100 magnification), and scale bar 50 µm (×400 magnification).

Table 2. iNOS and TNF-α immunoreactivity grades in groups of testicular tissue

	Control	MTX	ALA-treated
iNOS immunoreactivity	(+)	(+++)[a]	(++)
TNF-α immunoreactivity	(-/+)	(+++)[a]	(+)

Values are expressed in mean±SD, [a]Significantly changed when compared with the control group ($p < 0.05$)

DISCUSSION

MTX, an antagonist of folate, is broadly utilized in the therapy of psoriasis, malignancies and rheumatoid arthritis.[19] Toxicities of MTX in different organs in the gastrointestinal, urinary and lung systems have already been reported.[20-22] Additionally, the testicular toxicity of MTX is a significant adverse effect and may lead to infertility. MTX impairs spermatogenesis and fertility and causes oligospermia.[2,7] While some sources mention that this damage returns after the use of cytotoxic drugs, some sources recommend

sperm-freezing before treatment against possible irreversible damage.[23] Various theories have been proposed for the machineries behind MTX toxicity, involving the interference of oxidative stress,[24] inflammation, and apoptosis.[25] In this study, we aimed to inspect whether there will be a difference in the iNOS immunoreactivity of the MTX-administrated animal group and also whether application of ALA could forestall these effects. In this study, MTX clearly caused alteration in the immunohistochemical findings confirmed by the distorted histological image of testes specimens such as spermatogenetic and interstitial degeneration, which was in accord with previous studies.[26-28] Mechanisms that are participating in MTX-induced testicular damage were studied, and our findings demonstrated MTX-induced oxidative stress, marked with increased iNOS, which was in concurrence with previous studies.[20] Additionally, recent studies have shown that MTX leads to iNOS expression and thus increases NO level which causes oxidative stress.[29,30] In another study, it is mentioned that 20 mg/kg single dose MTX administration increases NO and TNF-α expressions in the liver.[31] This imbalance between oxidant/antioxidant and inflammatory signaling in other organs may also be the cause of MTX-induced testicular toxicity. Here, we showed that ALA, with well-documented powerful antioxidant properties,[32] confers protection against MTX-induced testicular damage. MTX has been reported to induce iNOS in the small intestine,[33] the brain,[34] and liver and kidney (20). Here, we observed that MTX can also be caused ROS-mediated testicular damage, shown through an increase in iNOS immunoreactivity. Additionally, in this study, we showed that MTX elevated the expression of TNF-α as did the expression of iNOS. It has been reported that methotrexate increases TNF-α levels in rat brains and the findings are associated with cognitive and behavioral disorders.[35] Earlier researches have stated that MTX increased the levels of TNF-α in kidney and liver tissues.[36-38] To the very best of our understanding, no validation of MTX stimulation of iNOS and TNF-α expression in testis tissue was proposed so far.

The ALA-treated group that received 100 mg/kg ALA for 10 days and 20 mg/kg MTX administered in a single dose on the fourth day showed improved spermatogenesis and also testis histology when compared with MTX alone. In this research, we also showed that ALA ameliorates MTX-induced testis toxicity, enhancing the histology of the testis when compared with MTX alone. Although ALA, also known as thioctic acid, is an endogenous compound, it can also be obtained exogenously from some plant foods. It has complementary roles in damaged tissue associated with a strong antioxidant and anti-inflammatory effect.[39,40] The processes participating included reversing MTX-induced testicular damage, as marked with decreased iNOS and TNF-α immunoreactivity. In addition, ALA reversed MTX-induced inflammatory hints because it notably reduced the level of TNF-α, which is consistent with the earlier stated antioxidant/anti-inflammatory effects of ALA.[20] Other studies have also shown that ALA has anti-inflammatory effect by decreasing the level of TNF-α.[41,42] Furthermore, ALA reversed MTX-induced ROS-mediated damage, inflammation, and disruption in the testis as evidenced by decreasing iNOS and TNF-α immunoreactivity and histopathological improvement compared with MTX alone, which is in agreement with prior studies.[20] Here, we showed that the ALA-treated decline in NO levels was attributable to downregulation of iNOS expressions in the testis. We recently reviewed the interaction among apoptotic, inflammatory, nitrosative and oxidative pathways[43] and are thus cognizant of difficult it is to ascertain whether the association among these pathways is a reason or a result of one another. Mitochondria is the primary organelle that produces ROS and is the main target of ROS-mediated damage[44] This is supported by the fact that Kolli et al. mention MTX-induced intestinal damage, including oxidative stress and mitochondrial dysfunction.[45] The improvement effect of ALA on mitochondrial performance is mentioned in the literature and it can be said that it minimizes an MTX-induced damage by this mechanism. ALA acts as an enzymatic cofactor that can regulate mitochondrial biogenesis. The antioxidant capacity of ALA is associated with two thiol groups that can be oxidized or reduced, and its effect on improving mitochondrial performance may be explained by this.[46] In conclusion, we postulate that MTX is initiating the testis damage through the stimulation of oxidative stress and an inflammatory process. ALA grants protection from MTX-induced toxicity through blocking these mechanisms.

In this experiment, we studied the molecular machinery behind protective effects of ALA against MTX-induced toxicity. iNOS is in charge for the generation of NO and is considered to be the primary stage originator of toxicity beneath the oxidative stress.[47] Increased levels of iNOS were detected in MTX-received rats. To the best of our understanding, it is the first time such a finding has been reported in MTX-induced testicular damage. ALA caused a noteworthy decline in iNOS and TNF-α levels, countering the MTX effect. The mechanism which ALA down-regulated iNOS is unclear, but this may be owing to the reduction in TNF-α establishment or because of its direct scavenging activity on NO through its carboxyl group. We also examined the expressions of TNF-α, a proinflammatory cytokine, in reply to MTX exposure, and we noticed an important increase in its expression on testicular tissue. TNF-α is found in the testis at seminiferous tubules and is highly up-regulated in pathophysiological conditions.[48,49] ALA might prevent reactive intermediates participating in testicular damage by reducing TNF-α formation.[50]

CONCLUSION

Treatment use of ALA has been successful in preserving from MTX-induced testicular damage through ALA-mediated anti-inflammatory and antioxidant processes. In the present study, it was shown that ALA can be used in combination with MTX as a protector against side effects during anticancer treatment.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was approved by the Local Ethical Committee on Animal Research of Süleyman Demirel University, Isparta and was performed according to ethical rules (Protocol number: 15.09.2022 06/70).

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article / Orijinal Araştırma

The Cost Effectiveness of the Respiratory Virus Panel in Childhood Febrile Neutropenia

Çocukluk Çağı Febril Nötropenisinde Solunum Virüsü Panelinin Maliyet Etkinliği

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Abstract

Aim: The aim of this study is to analyze the clinical utility and cost of the respiratory virus panel test in the febrile neutropenia (FN) episode in children undergoing chemotherapy.

Material and Method: From 2014 to 2018, 180 episodes of FN in 93 children with cancer were retrospectively analyzed. The patients were divided into those with (Group A) and without respiratory virus panel (Group B). The demographic and clinical features and cost analysis of the groups A and B were noted.

Results: Of these FN episodes, 46 were in Group A (25.5%) and 134 were in Group B (74.5%). We found positivity in 45 (97.8%) of 46 episodes in Group A. While treatment modification was required in 14 FN episodes (30.4%) in Group A, modification was required in 35 FN episodes (26.1%) in group B. The difference was not statistically significant (p=0.570). In Group A, only 5 (10.8%) were modified according to the respiratory virus panel. The respiratory virus panel prices were $72.43 (interquartile range, $38.8). The ratio of respiratory virus panel cost to the total cost was 9.67% (interquartile range 11.6). The median total cost of group A was $663.18 (interquartile range, 850.1), while that of group B was $596.24 (interquartile range, 723.81). The difference was not statistically significant (p=0.141).

Conclusion: The respiratory virus panel may contribute to the preference of antibiotics by giving rapid results in FN attacks. However, no effect on modification rates was observed, and only a small percentage of patients underwent antibiotic modification according to respiratory virus panel.

Keywords: Febrile neutropenia, respiratory virus panel, children

Öz

Amaç: Bu çalışmanın amacı, kemoterapi alan çocuklarda febril nötropeni (FN) atağında respiratuar virüs panel testinin klinik faydasını ve maliyetini analiz etmektir.

Gereç ve Yöntem: 2014-2018 yılları arasında kanserli 93 çocukta 180 FN epizodu retrospektif olarak analiz edildi. Hastalar solunum virüsü paneli olanlar (Grup A) ve olmayanlar (Grup B) olarak ikiye ayrıldı. Grup A ve B'nin demografik ve klinik özellikleri ile maliyet analizleri not edildi.

Bulgular: Febril nötropeni ataklarının 46'sı Grup A'da (%25,5) ve 134'ü Grup B'de (%74,5) idi. Grup A'da yer alan 46 epizodun 45'inde (%97,8) pozitifilik saptadık. Grup A'da 14 FN atağında (%30,4) modifikasyon gerekirken, B grubunda 35 FN atağında (%26,1) modifikasyon gerekti. Aradaki fark istatistiksel olarak anlamlı değildi (p=0,570). Grup A'daki 46 FN atağından sadece 5'inde (%10,8) solunum virüsü paneline göre tedavisi modifiye edildi. Solunum virüsü paneli fiyatları 72,43$ (çeyrekler arası aralık, 38,8). Medyan solunum virüsü paneli maliyetinin toplam maliyete oranı %9,67'dir (çeyrekler arası aralık 11,6). Grup A'nın medyan toplam maliyeti 663,18$ (çeyrekler arası aralık, 850,1), B grubunun maliyeti ise 596,24$ (çeyrekler arası aralık, 723,81). Fark istatistiksel olarak anlamlı değildi (p=0.141).

Sonuç: Solunum yolu paneli FN ataklarında hızlı sonuç vererek antibiyotik tercihine katkı sağlayabilir. Bununla birlikte, modifikasyon oranları üzerinde herhangi bir etki gözlenmedi ve hastaların sadece küçük bir yüzdesine solunum yolu paneline göre antibiyotik modifikasyonu uygulandı.

Anahtar Kelimeler: Febril nötropeni, solunum yolu paneli, çocuk

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INTRODUCTION

Febrile neutropenia (FN) is one of the most common emergency in children with cancer undergoing chemotherapy.[1] In the last two to three decades, our knowledge about antibacterial and antifungal treatments in FN has increased considerably.[2-7] There is less experience of viral infections than with bacterial or fungal infections on FN. Unfortunately, the proven and possible infection rates in children receiving chemotherapy are low. In a study of 337 FN episodes, the proven infection rate was 25% and the probable infection rate was 22%.[8] In this study, proven infection was detected in 86 episodes. Bacteria were detected in 41 of these, viruses were detected in 29 episodes and fungi were detected in 2 episodes. In children undergoing chemotherapy, the bacteria frequently isolated in the proven infections are viridans streptococci, Pseudomonas spp., and Escherichia coli.[8] Virus agents constitute 34% of proven infections. The viruses that have been detected relatively frequently in children receiving chemotherapy are respiratory viruses, herpes simplex virus, and varicella-zoster virus.[9-16]

Respiratory infections are one of the important health problems in both developed and developing countries. Particularly in children younger than five years of age, viral infections are frequent, its contribution to mortality rates due to acute respiratory tract infections and their complications was found to be 25-33%.[17] In this study conducted in 108 pediatric cancer patients receiving chemotherapy, nasopharyngeal aspirate was analyzed with the "RNA Virus Mini Kit" in 219 episodes. Acute viral respiratory infection was detected in 39.1% of these episodes.[17]

In this study, it was aimed to analyze the clinical utility and cost of the respiratory virus panel test in the febrile neutropenia episode in children undergoing chemotherapy.

MATERIAL AND METHOD

From 2014 to 2018, 183 episodes of FN in 93 children with cancer were retrospectively analyzed. The reason why it was preferred in this period was that the respiratory virus panel was mostly performed in this period. The written consent forms were not obtained from the guardians of all participants. The Declaration of Helsinki and principles of Good Clinical Practice was compiled in this study. Permission for this study was obtained from Selçuk University Faculty of Medicine, Local Ethics Committee with the number 2021/02 dated 27.01.2021.

The definition of febrile neutropenia was made as follows:

- Fever
  - A single oral temperature ≥ 38.3°C, or
  - An oral temperature ≥ 38.0 °C sustained for > one hour
  - An oral temperature ≥ 38.0 °C occurs twice within a 24-hour period
- Neutropenia
  - An absolute neutrophil count < 500/mm³, or
  - An absolute neutrophil count < 1000/mm³ and expected to decrease to < 500/mm³ over the subsequent 48 hours

At the time of FN diagnosis, the patients were divided into those with (Group A) and without respiratory virus panel (Group B).

The patients' demographic features (age, gender, and diagnosis), clinical and laboratory findings at the diagnosis of FN episodes, preferred antimicrobial agents, whether antimicrobial agent modification was made, if it was done, which antimicrobial agent was preferred, the preferred antimicrobial advertisement respiratory virus panel were noted. Also, cost analysis of these FN episodes was made. The total cost (service + drug + material) and the price of the respiratory virus panel were recorded at the discharge of the patient's hospitalization. The total cost and the price of the respiratory virus panel were converted into US dollars at the daily rates of the Central Bank of the Republic of Turkey.

A nasopharyngeal swap sample was taken from the nostril for the respiratory virus panel. During these years, Real-Time PCR-based kits from different companies were used to detect respiratory pathogens in our hospital.

Statistical analysis

IBM Statistical Package for Social Sciences 21.0 software (IBM Corporation, Armonk, NY, USA) was used for the statistical analysis in this study. Frequency and percentage values were used for nominal variables. For continuous variables, mean and standard deviation values were given if the distribution was normal, and the median and minimum and maximum values were given if the distribution was not normal. In the comparison of nominal variables, Chi-square or Fischer-Exact tests were used depending on whether they met the necessary assumptions. The continuous variables were compared with Student's T test or Mann Whitney U test according to whether they met the necessary assumptions. A two-tailed P-value of <0.05 was considered statistically significant.

RESULTS

In this period, 180 FN episodes in 93 children with cancer were included in this study. The demographic characteristics and diagnoses of the patients are given in **Table 1**. The patients' age ranged from one month to 17 years old (median, 7 years). Considering the gender distribution of the patients, 52 patients (56%) were male and 41 patients were female (44%). The most common tumors were central nervous system tumor (n: 19, 20.4%), malignant bone tumor (n: 19, 20.4%), neuroblastoma (n: 15, 16.1%), and rhabdomyosarcoma and other soft-tissue sarcomas (n: 15, 16.1%).

Of these FN episodes, 46 were in Group A (25.5%) and 134 were in Group B (74.5%). Demographic and clinical characteristics, and cost characteristics of Groups A and B are given in **Table 2**. It was determined that 31 (67%) of the respiratory virus panel were obtained at the time of admissions of the FN episode, and 15 (33%) during the follow-up of the FN episode. We found positivity in 45 (97.8%) of

46 patients in Group A. While oropharynx hyperemia was detected in 19 episodes (41.3%) in Group A, it was detected in 34 episodes (25.4%) in Group B. The difference was statistically significant ($X^2(1)=4.184$, p=0.041). While pneumonia findings were detected in 4 episodes in Group A, they were detected in 8 episodes (6%) in Group B. The difference with the Fischer Exact test was not statistically significant (p=0,506). There was no statistical difference between the groups in terms of duration of fever and duration of neutropenia (p values were 0.707 and 0.324, respectively).

Table 1. The patients' demographic and clinic features

Features	n, (%)
Median age, (minimum-maximum values)	7 year, (1 month – 17 years)
Gender	
Male	52, (56%)
Female	41, (44%)
Diagnosis	
Central nervous system tumor	19, (20.4%)
Malignant bone tumors	19, (20.4%)
Ewing sarcoma	15, (16.1%)
Osteosarcoma	4, (4.3%)
Neuroblastoma	15, (16.1%)
Rhabdomyosarcoma and other soft-tissue sarcomas	15, (16.1%)
Lymphomas	12, (13%)
Non-Hodgkin lymphoma	11, (11.9%)
Hodgkin lymphoma	1, (1.1%)
Renal tumors	4, (4.3%)
Germ cell tumor	4, (4.3%)
Retinoblastoma	4, (4.3%)
Langerhans cell histiocytosis	1, (1.1%)

Table 2: The demographic and clinical features and cost characteristic of the groups A and B

	Group A	Group B	P values
Mdn age year, (minimum-maximum values)	7 (0.1-16)	7 (0.3-17)	0.411
Duration of fever, days	2 (1-12)	2 (1-26)	0.707
Duration of neutropenia, days	4 (1-12)	5 (1-17)	0,324
Modification			0.570
No	32 (69.6%)	99 (73.9%)	
Yes	14 (30.4%)	35 (26.1%)	
Mdn total cost ($), IQR	663.18 (850.1)	596.24 (728.81)	0.141

Mdn: median, IQR: interquartile range

While modification of treatment was required in 14 FN episodes (30.4%) in Group A, modification was required in 35 FN episodes (26.1%) in group B. The difference was not statistically significant ($X^2(1)=0.322$, p=0.570). Of the 46 FN episodes in Group A, only 5 (10.8%) were modified according to the respiratory virus panel. In these modifications, clarithromycin (n=2), azithromycin (n=1), sulfamethoxazole trimethoprim (n=1) or oseltamivir (n=1) were added to the antibiotics of the patients.

The microorganisms detected in the respiratory virus panel taken at the time of admission and during the episode are in **Table 3**.

Table 3: Detected microorganisms on the respiratory virus panel

Respiratory Pathogens	At admission	At follow-up period	Total
Virus	24	15	39
Influenza virus	4	3	7
Influenza A	1	2	3
Influenza B	3	1	4
Parainfluenza virus	1	2	3
PIV 1	1	1	2
PIV 2	0	1	1
Respiratory syncytial virus	4	6	10
RSV A	2	3	5
RSV B	2	3	5
Rhinovirus	10	3	13
Coronavirus	3	2	5
CoV 229E	1	1	2
CoV NL63	1	0	1
CoV HKU	1	0	1
Adenovirus	2	0	2
Bacteria	23	7	30
Staphylococcus aureus	8	2	10
Streptococcus pneumoniae	4	2	6
Haemophilus influenzae spp.	5	1	6
Moraxella catarrhalis	6	0	6
Mycoplasma pneumoniae	0	1	1
Chlamydia pneumoniae	0	1	1

Cost features

The respiratory virus panel prices in episodes were $72.43 (interquartile range, $38.8). The ratio of the respiratory virus panel cost to the total cost was 9.67% (interquartile range 11.6). The median total cost of group A was $663.18 (interquartile range, 850.1), while that of group B was $596.24 (interquartile range, 723.81). Mann Whitney U test showed that the difference was not statistically significant (p=0.141).

DISCUSSION

Today, the success of treatment in childhood malignant diseases has increased significantly. Developments in other treatment approaches, especially chemotherapy, and developments in supportive treatments have an important place in this increase in survival. Especially with more intensive chemotherapy applications, there is an increase in the frequency of infection. In children with malignant disease, infection is an important cause of morbidity and mortality. In these patients, FN is one of the most common oncological emergencies. Bacterial infections in children with FN episode have been successfully treated with the use of empirical antibiotic therapy. However, other microorganisms, especially fungal infections, started to come to the forefront as a cause of morbidity and mortality in these patients.[1-3]

Clinically defined infections are seen in 20-30% of FN episodes and only 10-30% of FN cases can be documented microbiologically.[3] Studies showing the role of viruses in the FN episode are few.[17-19] Different rates of virus have been

detected in children with FN. However, the problem here is that it is not known whether these microorganisms are the real cause of infection. Viral study in FN attack, although not a routine study, should be considered in some circumstance: (i) seasonal viruses including especially respiratory syncytial virus, influenza and enterovirus; and (ii) Herpes simplex virus seen in children with mucocutaneous lesions. Since late 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) testing was routinely administered to these children.[3,20]

Respiratory infections have an important place in FN studies investigating viruses. Since the respiratory viral panel is usually performed with real-time PCR-based tests, an expensive test, the issue to be considered is cost-effectiveness. In the study of Aydin-Koker et al.[17] 108 pediatric cancer patients receiving chemotherapy, nasopharyngeal aspirate was analyzed in 219 episodes. They found the acute viral respiratory positivity rate to be 39.1%. The most frequently detected viruses are human rhinovirus, parainfluenza 3 and respiratory syncytial virus. The authors commented that viral upper respiratory tract infections do not increase mortality in cancer patients, but cause significant delays in chemotherapy, which may have an indirect effect on patient survival rates. In the study, the prevalence and clinical outcomes of respiratory viral infection in patients with cancer and FN episodes by Meena et al.[19] were investigated. The authors found a high prevalence of respiratory viral infection in this patient group. They also determined that the number of days with fever and the duration of antibiotic use were prolonged. In the study of Shinn et al.[21] respiratory viral panel positivity and the outcomes of this positivity such as length of hospital stay or intensive care unit and death were examined. The authors emphasized that respiratory viral panel positivity during febrile neutropenia does not impact length of hospital stay or intensive care unit. They also commented that the question of whether respiratory viral panel testing contributes to clinical treatment in this population remains unanswered. In the study of Büyükkapu-Bay et al.[22] they examined 72 episodes in 48 children with cancer. The most common microorganisms were rhinovirus, respiratory syncytial virus, and coronavirus. They used oseltamivir in their patients with whom they had influenza. In their comments, they mentioned that respiratory viral panel test may not be cost-effective for children with cancer and FN, because it would not alter the duration of hospitalization.

In our study, we detected positivity in 45 of 46 episodes in which respiratory viral panel testing was performed. The high rate of this rate can be explained by performing respiratory viral panel only in selected patients in this period. However, the interesting thing was that although the rates of antibiotic modification were slightly higher in Group A, the difference was not statistically significant. Another important finding of ours is modification according to respiratory viral panel in only 6 episodes in Group A. In these modifications, clarithromycin, azithromycin, trimethoprim-sulfamethoxazole or oseltamivir were added to the antibiotics of the patients. Although the difference was not statistically significant, the cost of patients who underwent SVP testing was higher and the ratio of respiratory viral panel to total bill was 9.67%.

CONCLUSION

The respiratory virus panel may contribute to the preference of antibiotics by giving rapid results in FN attacks. However, no effect on modification rates was observed, and only a small percentage of patients underwent antibiotic modification according to respiratory virus panel.

ETHICAL DECLARATIONS

Ethics Committee Approval: Permission for this study was obtained from Selçuk University Faculty of Medicine, Local Ethics Committee with the number 2021/02 dated 27.01.2021.

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article / Orijinal Araştırma

Investigation of the Effect of Demographic and Clinical Characteristics on Mortality of COVID-19 Patients Treated in the Intensive Care Unit: A Retrospective Study

Yoğun Bakım Ünitesinde Tedavi Edilen COVID-19 Hastalarının Demografik ve Klinik Özelliklerinin Mortalite Üzerine Etkisinin İncellenmesi: Retrospektif Çalışma

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Abstract

Aim: COVID-19 can cause clinical pictures ranging from asymptomatic to severe respiratory failure and sudden death. The severity of the disease varies depending on many factors such as comorbidity, vaccination status, as well as demographic characteristics such as age and gender. In this study, it was aimed to investigate the independent risk factors that have an effect on mortality in COVID-19 patients.

Material and Method: In the study, records of 140 patients with a diagnosis of COVID-19 followed in the intensive care unit between 01.01.2021 and 01.01.2022 were examined. Demographic characteristics such as age and gender, comorbidity, vaccination status and clinical course of the patient were investigated and recorded.

Results: In our study, a statistically significant difference was found between mortality and age, and the number of days of total invasive/noninvasive mechanical ventilation support ($p=0.01$, $p=0.25$, $p<0.001$, respectively). It was found that mortality increased statistically significantly with the increase in the CO-RADS score ($p=0.03$). In addition, a statistically significant correlation was found between the vaccination status of the patients and mortality ($p=0.03$).

Conclusion: In conclusion, the findings of the study showed that the mortality rate increased as age, duration of invasive or noninvasive mechanical ventilation support and lung involvement increased. It was also found that COVID-19 vaccines reduce mortality in patients hospitalized in intensive care.

Keywords: COVID-19, intensive care, comorbid disease, COVID-19 vaccine, mortality

Öz

Amaç: COVID-19, asemptomatikten ciddi solunum yetmezliği ve ani ölüme kadar değişen klinik tablolara sebep olabilmektedir. Hastalığın şiddeti yaş, cinsiyet gibi demografik özellikler yanında, komorbidite, aşı durumu gibi birçok faktöre bağlı değişiklik gösterir. Bu çalışmada COVID-19 hastalarında mortalite üzerine etkisi olan bağımsız risk faktörlerinin ortaya konması amaçlandı.

Gereç ve Yöntem: Çalışmada 01.01.2021 ile 01.01.2022 tarihleri arasında yoğun bakım ünitesinde takip edilen COVID-19 tanılı toplam 140 hasta kayıtları incelendi. Hasta yaş, cinsiyet gibi demografik özellikler yanında, komorbidite, aşı durumu ve klinik seyri araştırılarak kaydedildi.

Bulgular: Çalışmamızda mortalite ile yaş, toplam invaziv/noninvaziv mekanik ventilasyon desteği aldığı gün sayısı arasında istatistiksel olarak anlamı bir ilişki bulunmuştur (sırasıyla $p=0,01$, $p=0,25$, $p<0,001$). CO-RADS skorunun artmasıyla mortalitenin istatistiksel olarak anlamlı derece arttığı bulundu ($p=0,03$). Ayrıca hastaların aşı durumu ile mortalite arasında istatistiksel olarak anlamlı bir ilişki bulundu ($p=0,03$).

Sonuç: Sonuç olarak çalışmanın bulguları yaş, invaziv ya da noninvaziv mekanik ventilasyon desteği aldığı toplam gün ve akciğer tutulumu arttıkça mortalitenin de arttığını gösterdi. Ayrıca COVID-19 aşılarının yoğun bakımda yatan hastalardaki mortaliteyi azalttığı bulundu.

Anahtar Kelimeler: COVİD-19, yoğun bakım, komorbit hastalıklar, COVİD-19 aşı, mortalite

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INTRODUCTION

The disease (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first detected on December 31, 2019 in Wuhan, China and spread rapidly around the world, becoming a global problem and recognized as a pandemic by the World Health Organization (WHO). COVID-19 has similar symptoms to severe acute respiratory syndrome (SARS-CoV), which occurred in China between 2002-2003, and Middle East respiratory syndrome (MERS-CoV), which was detected in the Middle East in 2012, but the rate of spread is quite high.[1] COVID-19 is a pathogen that can cause serious health problems by attacking the respiratory tract, digestive tract, liver, kidneys, and central nervous system.[1,2] The disease primarily affects the respiratory system and is manifested by fever, dry cough, and difficulty breathing. If the disease progresses, it can lead to death from pneumonia and acute respiratory distress syndrome.[1,2] Transmission of COVID-19 occurs mainly through droplets in the air we breathe (coughing or sneezing), close contact with an infected person, and touching surfaces or objects exposed to the droplets. As a result, most affected countries have taken numerous measures to contain the spread of infection, such as restricting social activities and travel, quarantining suspected persons, and isolating infected individuals.

Despite numerous global efforts, COVID-19, which is highly contagious, continues to spread rapidly and cause serious illness and death.[3] Various measures have been taken to prevent transmission, and vaccines have been produced, but a specific and effective treatment for infected individuals has not been found to date.[3]

COVID-19 can lead to clinical pictures ranging from asymptomatic to severe respiratory failure and sudden death.[1,2] The severity of the disease can vary depending on many factors such as comorbidity, vaccination status, and demographic characteristics such as age and sex. Some studies have found that chronic lung disease such as asthma and chronic obstructive pulmonary disease (COPD); diabetes; hypertension; renal, hepatic, and cardiac disease; a history of smoking and drug use; age older than 60 years; and male sex cause an increase in morbidity and mortality.[1,3] Some studies have found that chronic lung disease such as asthma and chronic obstructive pulmonary disease (COPD), diabetes, hypertension, organ failure (renal, hepatic, and cardiac), a history of smoking and drug use, age older than 60 years, and male sex cause an increase in morbidity and mortality.[1,3] People with one or more of these characteristics often have a weakened immune system that increases their risk for infection, severe disease, and death. It has been reported that the admission rate of COVID-19 patients to the intensive care unit ranges from 3% to 90%, and the mortality rate ranges from 6% to 86%.[3,4] Many factors such as patient demographic characteristics, comorbidity, and vaccination status affect the wide range of intensive care unit (ICU) admission and mortality rate. For this reason, it is very important to know the independent risk factors, identify those at risk for severe illness, and determine the necessary precautions and treatment methods.

The factors affecting the clinical course in COVID-19 patients have been investigated in many studies, and conflicting results have been reported, so further studies are needed. It is very important to study the risk factors of patients treated in the ICU, especially to determine their effects on mortality and morbidity. In this study, we aimed to retrospectively investigate the impact of demographic characteristics, coordination, and vaccination status of patients admitted to the ICU with a diagnosis of COVID-19 in the last year.

MATERIAL AND METHOD

This retrospective observational study was performed on patients admitted to the intensive care unit because of COVID -19. Institutional review board approval (22-KAEK-025) was obtained from the clinical research ethics committee before starting the study. The study was carried out in accordance with the principles of the Helsinki Declaration. In our study, the records of 140 patients treated in the intensive care unit of COVID-19 between 01/01/2021 and 01/01/2022 01.01.2021 and 01.01.2022 were retrospectively analyzed. Demographic characteristics such as age and sex, comorbidity, vaccination status, and clinical course of the patient were examined and recorded. Non COVID-19 patients treated in the intensive care unit or suspected patients with an unconfirmed diagnosis of COVID-19 were not included in the study.

Nasal high-flow oxygenation, time of onset and duration of noninvasive mechanical ventilation (MV), developing complications, developing organ failure, length of ICU stay, and outcome were recorded on the day patients were admitted to the ICU for COVID-19 from the onset of symptoms. In addition, patient's chest computed tomography (CT) was assessed and recorded when they were admitted to the ICU according to the COVID-19 reporting and data system (CO-RADS) classification.[5]

In the classification of CO-RADS: CO-RADS 1: Very unlikely. CT is normal or there are noninfectious findings suggestive of disease such as congestive heart failure, sarcoidosis, histoplasmosis, malignancy, fibrotic changes. CO-RADS 2: Suspicion is low. No typical COVID-19 symptoms. The CT picture shows bronchiectasis and thickening of the bronchial walls, and there are no obvious opacities. CO-RADS 3: Uncertain. There are central baseline opacities, interlobular septal thickening suggestive of pulmonary edema, or pleural effusions. CO-RADS 4: Suspicion is high. Suspicious CT findings are present. Unilateral vitreous multifocal consolidations without other typical findings are findings suspicious for COVID -19 an underlying lung disease. CO-RADS 5: Most likely. Bilateral and multifocal consolidations or base line vitreous opacities are typically present at COVID-19. CO-RADS 6: Certainly. Patient with bilateral ground-glass areas with positive PCR and CT.

Biostatistical Analysis

Statistically, the conformity of data to the normal distribution was assessed using the Kolmogorov-Smirnov test for one sample. Qualitative data were presented as numbers and percentages, normally distributed quantitative data as mean and standard deviation, and non-normally distributed quantitative data as median (minimum-maximum). The chi-square test was used to analyze qualitative data when comparisons were made between groups. Student's t test was used when quantitative data were normally distributed and Mann-Whitney U test when they were not. The relationship between demographic variables and mortality was analyzed by logistic regression analysis. The Statistical Package for Social Sciences (version 20.0, SPSS Inc, Chicago, IL, USA) was used to analyze all data. The statistical significance value was accepted as p<0.05 in the analysis of the data.

RESULTS

140 patients were evaluated for the study. Of the 140 patients, 79 (56.4%) were female and 61 (43.6%) were male. The age of the patients ranged from 23 to 96 years and the mean was 76 years. Hypertension in 82 (58.6%) patients, diabetes in 59 (42.1%), cardiovascular disease in 44 (31.4%) patients and chronic respiratory disease in 23 (16.4%) patients , patients, 18 (12.9%) had cancer and 10 (7.1%) had cerebrovascular disease (**Table 1**).

Tablo 1. Comorbidity and Demographic Characteristics of the Patients

	Yes (%)	No (%)	Mortalite (p)
Hypertension	82 (58.6)	58 (41.4)	0.08[a]
Diabetes	59 (42.1)	81 (57.9)	0.58[a]
Cardiovascular disease	44 (31.4)	96 (68.6)	0.11[a]
Chronic lung disease	23 (16.4)	117 (83.6)	0.10[a]
Cancer	18 (12.9)	122 (87.1)	0.45[a]
Cerebrovascular disease	10 (7.1)	130 (92.9)	0.56[a]
Gender: male (43.6%), female (56.7%)			0.66[a]
CO-RADS score			0.03[a]*
Immunisation status			0.04[a]*

CO-RADS: COVID-19 Reporting and Data System, a: Pearson Chi-Square test (p<0.05 was considered significant), *: Statistically significant.

In our study, a statistically significant difference was found between patients' age and mortality (p=0.01), (**Table 2**). Mortality was found to statistically increase with increasing age (p=0.01).

Clinically, 137 (97.9%) patients had respiratory symptoms and 3 (2.1%) had gastrointestinal symptoms. The thorax CT classification of patients before admission to the ICU is shown in **Table 3**. The CO-RADS score was 4 in 74 (52.9%) patients, 5 in 32 (22.9%) patients, and 3 in 28 (20%) patients (**Table 3**). The outcome of patients according to the CO-RADS score is shown in **Table 4**. It was found that mortality increased statistically significantly with the increase in CO-RADS score (p=0.03), (**Table 1**).

Table 2. Clinical Course of Patients

	Minimum	Maximum	Median	Mortality (p)
Age	23	96	76	0.01 a *
Days of hospitalization in the ICU (after diagnosis)	1	21	3	0.3 a
Total days of ICU stay	3	21	9	0.1 a
Pulse prednol start day	1	16	4	0.5 a
Noninvasive MV start day	0	21	3	
Invasive MV start day	0	36	6	
Total days of noninvasive MV support	0	14	4	0.02 a *
Total days of invasive MV support	0	36	4	<0.001 a *

ICU: intensive care unit, MV: mechanical ventilation, a: Mann-Whitney U test (p<0.05 was considered significant), *: Statistically significant.

Table 3. CO-RADS Classification of Patients Before Admission to the ICU

CO-RADS Score	Person	Percent (%)
CO-RADS 1	1	0.7
CO-RADS 2	2	1.4
CO-RADS 3	28	20
CO-RADS 4	74	52.9
CO-RADS 5	32	22.9
CO-RADS 6	3	2.1

CO-RADS: COVID-19 Reporting and Data System

Table 4. Fate of Patient According to CO-RADS Score

CO-RADS Score	Person (percent)		
	Discharged to Service	Exitus	Total Person
CO-RADS 1	1 (100%)	0	1 (100%)
CO-RADS 2	1 (50%)	1 (50%)	2 (100%)
CO-RADS 3	16 (57.1%)	12 (42.9%)	28 (100%)
CO-RADS 4	32 (43.2%)	42 (56.8%)	74 (100%)
CO-RADS 5	8 (25%)	24 (75%)	32 (100%)
CO-RADS 6	0	3 (%100)	3 (100%)

CO-RADS: COVID-19 Reporting and Data System

Patients in our study were hospitalized in the intensive care unit between days 1 and 21 after diagnosis, with a median of 3 days (**Table 2**). Invasive MV support was required in 58 (41.4%) patients and 82 (58.6%) patients, respectively (**Table 2**). Noninvasive MV support was found to be used between days 1 and 21 (median value=4) and invasive MV support was used between days 1 and 24 (median value=8) after COVID-19 diagnosis (**Table 2**). Patients remained in the ICU for 1 to 39 days (median=9) (**Table 2**). fifty-eight (41.6%) patients were admitted to the emergency department, and 82 (58.4%) died.

In our study, a statistical difference was found between the duration of non-invasive or invasive MV support and mortality (p=0.02, p<0.001), (**Table 2**). It was found that with increasing duration of MV support, mortality rate increased statistically significantly.

The vaccination status of the patients is shown in **Table 5**. 60 (42.9%) of the patients had not received vaccination. The outcome of patients according to their vaccination status is shown in **Table 6**. A statistically significant difference was found between the vaccination status of the patients in our study and mortality (p=0.03), (**Table 1**).

Table 5. Vaccination Status of Patients

Vaccination	Person	Percent (%)
Yok	60	42.9
Sinovac (1 dose)	9	6.4
Sinovac (2 dose)	35	25
Sinovac (3 dose)	16	11.4
Biontech (1 dose)	1	0.7
Biontech (2 dose)	12	8.6
Sinovac (1 dose), Biontech (1 dose)	2	1.4
Sinovac (2 dose), Biontech (1 dose)	5	3.6

Table 6. Fate of the Patient According to Vaccination Status

Vaccination	Person (%)		
	Discharged to Service	Exitus	Total
No	16 (26.7%)	44 (73.3%)	60 (100%)
Sinovac (1 dose)	3 (33.3%)	6 (66.6%)	9 (100%)
Sinovac (2 dose)	17 (48.6%)	18 (51.4%)	35 (100%)
Sinovac (3 dose)	10 (62.5%)	6 (37.5%)	16 (100%)
Biontech (1 dose)	0	1 (100%)	1 (100%)
Biontech (2 dose)	7 (58.3%)	5 (41.7%)	12 (100%)
Sinovac (1 dose), Biontech (1 dose)	1 (50%)	1 (50%)	2 (100%)
Sinovac (2 dose), Biontech (1 dose)	4 (80%)	1 (20%)	5 (100%)

When the relationship between logistic regression analysis and demographic data on mortality was examined in our study, it was found that age and vaccination were important factors. (**Table 7**).

DISCUSSION

Many factors such as patient demographic characteristics and concomitant disaeses affect the severity of COVID-19. The presence of various factors such as increasing age, smoking, hypertension, diabetes, cardiovascular disease, chronic respiratory disease, renal disease, malignancy has been associated with disease severity, need for critical care, morbidity, and mortality.[4-6] In addition, high levels of white blood cells, neutrophils, D-dimers, ferritin, and low levels of platelets and lymphocytes in the blood have been shown to influence mortality.[6] While some studies have found an association between gender, body mass index, and cerebrovascular disease and mortality, other studies have reported that this association does not exist.[4-8]

Susceptibility to infections in males has been shown to be increased in association with the X chromosome, which plays an important role in immunity.[8] Male sex has been reported to significantly increase mortality in COVID-19 patients.[1,9] In our study, 79 (56.4%) of 140 patients were female and 61 (43.6%) were male. However, no statistically significant association was found between gender and mortality in our study.

Table 7. Demographic Data on Mortality

	B	S.E	Wald	Df	Sig.	Eks(B)
Gender (female)	-20.689	7795.265	.000	1	.998	.000
Age	.039	.016	5.825	1	.016*	1.040
Vaccination status (yes)	1.076	0.404	7.021	1	.008*	2.934
Hypertension	11.634	7922.580	.000	1	.999	112823.157
Diabetes	-22.699	6705.220	.000	1	.997	.000
Cardiovascular disease	-40.611	4995.517	.000	1	.994	.000
Chronic respiratory disease	6.058	27754.303	.000	1	1.000	427.696
Cerebrovascular disease	10.821	43358.827	.000	1	1.000	50068.808
Cancer	6.446	17658.745	.000	1	1.000	630.460
Sinovac (1 dose)	43.016	75562.892	.000	1	1.000	4.805E+18
Sinovac (2 dose)	-10.203	77930.097	.000	1	1.000	.000
Sinovac (3 dose)	20.221	77389.412	.000	1	1.000	605434579.732
Sinovac (1 dose), Biontech (1 dose)	43.360	72914.581	.000	1	1.000	6.777E+18
Sinovac (2 dose), Biontech (1 dose)	18.656	522817.071	.000	1	1.000	126590021.112
Biontech (1 dose)	-31.195	74967.863	.000	1	1.000	.000
Biontech (2 dose)	55.011	79937.998	.000	1	.999	7.782E+23
Cough	45.282	50020.755	.000	1	.999	4.632E+19
Dyspnea	4.376	28024.497	.000	1	1.000	79.528
Temperature	69.038	50482.488	.000	1	.999	9.616E+29
CO-RADS 1	58.504	814169.143	.000	1	1.000	2.558E+25
CO-RADS 2	41.240	38971.552	.000	1	.999	8.132E+17
CO-RADS 3	61.415	33287.888	.000	1	.999	4.700E+26
CO-RADS 4	31.124	33395.694	.000	1	.999	3.290E+13
Pulse prednol day	.106	1334.990	.000	1	1.000	1.112

CO-RADS: COVID-19 Reporting and Data System, Logistic Regression Analysis, *: Statistically significant.

The age of the patients in our study ranged from 23 to 96 years, and the median age was 76 years. Advanced age has been shown to increase morbidity and mortality in COVID-19 patients.[1,10] In addition to the occurrence of many comorbidities with advancing age, the decline in the functions of cells such as T and B cells, which play a role in immunity, also affects the course of COVID-19 disease.[4,11] Our study confirms this, and we found that age is a statistically significant risk factor for mortality.

The two most common comorbidities in patients admitted to the ICU with a diagnosis of COVID-19 were hypertension (47.7%) and diabetes (26.9%), followed by cardiovascular disease (12.9%), chronic respiratory disease (5%, 5), renal disease (5.3%), and liver disease.[1,3-6] Similar to the literature, the most common comorbidities in our patients were hypertension and diabetes. Hypertension in 82 (58.6%) patients, diabetes in 59 (42.1%), cardiovascular disease in 44 (31.4%) patients and chronic respiratory disease in 23 (16.4%) patients, 18 (12.9%) had cancer and 10 (7.1%) had cerebrovascular disease. Hypertension and diabetes are also the most common comorbidities in patients treated in the ICU for reasons other than COVID-19.[12,13] For this reason, the incidence might be increased in COVID-19 patients hospitalized in the ICU. However, hypertension can suppress the immune system by decreasing the level of angiotensin converting enzyme 2 (ACE2).[14,15] It has been reported to increase morbidity and mortality rates by increasing the infectivity and susceptibility of COVID-19.[14,16] On the other hand, diabetes has been reported to increase susceptibility to acute respiratory distress syndrome (ARDS) and to increase the risk of hospitalization of COVID-19 patients in the intensive care unit and follow-up with invasive MV.[2]

Fever (78.8%), cough (53.9%), and malaise (37.9%) are the most common symptoms in COVID -19 patients.[17] In contrast to other viral respiratory infections, rhinorrhea (7.5%) is less common.[17] The frequency of gastrointestinal symptoms ranged from 3% to 39.6%, with diarrhea in 7.5% to 18.1% of patients, nausea 4.5% to 8.3%, vomiting 1.3% to 5.9%, abdominal pain in 0.5% to 4.53%.[17-19] Shortness of breath occurs in about half (48.96%) of severe cases and only in 13.6% of cases with mild symptoms.[17] While the patients in our study were admitted to the ICU, most (97.9%) had respiratory symptoms and some (2.1%) had gastrointestinal symptoms. While the patients in our study were admitted to the ICU, most had respiratory symptoms.

It has been reported that severe disease develops in 22.9% of COVID-19 patients, leading to death in approximately 5.6%.[17] It has been shown that mortality in hospitalized COVID-19 patients is 17.62%.[15] It was reported that 10.96% of COVID-19 patients were admitted to the intensive care unit and 7.1% required support MV.[17] It has been reported that mortality in COVID-19 patients admitted to the ICU varied regionally from 10.6% to 61.9%, with an average of 41.6%.[7] In contrast, some studies report mortality rates in ICU patients, particularly in underdeveloped countries, to be much higher than the reported rates.[20] The mortality rate in COVID-19 patients treated in the intensive care unit in Turkey ranges from 27% to 84%.[21,22] The mortality rate in our study was 58.4% and was similar to the mortality rate of COVID -19 patients treated in the intensive care unit in Turkey.

Many studies have reported that mortality rates are higher in COVID-19 patients who require MV support.[4,12,22] In addition, it has been reported that the mortality rate increases with increasing duration of MV support.[1,7,23] Similar to the literature, our study also found that the need for MV support was significantly higher in the mortality group, regardless of whether it was non-invasive or invasive support. Mortality rates have been shown to increase with lung involvement (CO-RADS) in COVI-19 patients admitted to the ICU on the chest CT.[4,13,22,23] Our study was also similar to the literature. Although no association with mortality was found in logistic regression analysis, it showed that mortality was statistically significantly higher when CO-RADS score increased (p=0.039), (**Table 4**). The CO-RADS score is useful for identifying patients at risk and has been reported to be a good guide for predicting mortality in the early phase.[22,23]

One of the most important steps in the fight against COVID-19 is vaccination. Vaccines have been produced that provide effective protection in a short time, and the results are satisfactory.[24] There are many vaccines that have been approved by WHO based on studies. In our country, the vaccines used are Sinovac, Biontech, and Turkovac. Biontech is an mRNA vaccine that does not contain antigen but provides the genetic information for the antigen and is synthesized in the cells of those vaccinated with antigen. Sinovac is an inactivated vaccine that confers cellular immunity with a non-replicating viral antigen. mRNA vaccines have only recently been used in humans, but inactivated vaccines have been used for years to prevent many diseases and their safety has been extensively studied. The Biontech vaccine, based on the new mRNA technology, has been shown to be superior to Sinovac in preventing symptomatic disease. However, it has been reported that there is no significant difference between the two vaccines in terms of effective protection against severe disease.[24] In another study, the whole vaccine was found to provide 95% effective protection with Biontech and 65.7% with Sinovac.[25] Overall, a single dose of vaccine for COVID-19 provided effective protection of 41% for infection prevention, 52% for symptomatic illness, 66% for hospitalization, 45% for ICU admission, and 53% for mortality. Two doses of vaccine provided effective protection of 85% for infection prevention, 97% for symptomatic illness, 93% for hospitalization, 96% for ICU admission, and 95% for mortality.[25] This demonstrates the importance and necessity of full-dose vaccination.[25] There are many studies that have investigated vaccines, and the general opinion is that mRNA vaccines are more effective than inactivated virus vaccines.[25-28] In accordance with the literature, a statistically significant correlation was found between the vaccination status of the patients in our study and mortality (p=0.03). In our study, relavation between the vaccination status and mortality was similar to the literature.

CONCLUSION

We found that mortality increased with the total number of days of mechanical ventilatory support and pulmonary involvement in patients followed up in the ICU because of COVID-19, regardless of age, invasive or noninvasive course, and that vaccination with COVID-19 decreased mortality in patients treated in the ICU. We believe that patients of advanced age are at risk for mortality associated with COVID-19 and that prophylactic vaccination is important. However, we believe this should be confirmed with more patient populations.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Tokat Gaziosmanpaşa University Clinical Research Ethics Committee (Date: 04.03.2022, Decision No: 22-KAEK-025).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Original Article / Orijinal Araştırma

Frequency and Seasonal Distribution of Adenovirus and Rotavirus in Children Diagnosed with Acute Gastroenteritis: A Single Centre Experience

Akut Gastroenterit Tanısı Konulan Çocuklarda Adenovirüs Ve Rota Virüs Sıklığı Ve Mevsimsel Dağılımı: Tek Merkez Deneyimi

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Abstract

Aim: We aimed to investigate the relationship between age, gender, and season with respect to the frequency of rotavirus and adenovirus antigens in stool specimens obtained from children one month to 18 years of age who were diagnosed with acute gastroenteritis.

Material and Method: The records of stool specimen analyses for 1960 patients with diagnosis of acute gastroenteritis at our hospital from January 2017 to August 2022 were retrospectively examined. The patient's admission year, season, gender, age, and stool viral antigen test results were retrospectively analysed from the file records.

Results: Viral antigen was detected in stool specimen in 272 (13.8%) of the patients included in the study, while 92 (4.7%) of them were rotavirus and 180 (9.1%) were adenoviruses. In our study, it was determined that both rotavirus and adenovirus were most common in the one month-2 years of age group. Rotavirus was detected most frequently in the winter months and adenovirus in the spring months. Twenty-six (28.2%) patients with rotavirus gastroenteritis and 68 (37.7%) patients with adenovirus gastroenteritis were hospitalized and treated.

Conclusion: In our study, rotavirus and adenovirus, which are viral gastroenteritis agents, were detected at high rates in acute gastroenteritis in children. Enteric adenovirus and rotavirus were detected more frequently in winter and spring. The frequency of viral agents should be considered in the clinical evaluation and treatment planning of children with acute gastroenteritis. Demonstrating viral antigens in stool will prevent unnecessary antibiotic use.

Keywords: Adenovirus, children, gastroenteritis, prevalence, rotavirus

Öz

Amaç: Akut gastroenterit tanısı konulan 1 aylık-18 yaş arası çocuklardan alınan dışkı örneklerinde rota virüs ve adeno virüs antijenlerinin yaş, cinsiyet ve mevsim ile ilişkisini araştırmayı amaçladık.

Gereç ve Yöntem: Hastanemize Ocak 2017-Ağustos 2022 tarihleri arasında akut gastroenterit tanısı konulan 1960 hastanın dışkı örneği analiz kayıtları retrospektif olarak incelendi. Hastanın başvuru yılı, mevsimi, cinsiyeti, yaşı ve gaitada viral antijen testi sonuçları dosya kayıtlarından retrospektif olarak incelendi.

Bulgular: Çalışmaya alınan hastaların 272'sinde (%13.8) dışkı örneğinde viral antijen saptanırken, bunların 92'sinde (%4.7) rotavirüs ve 180'ninde (%9.1) adenovirüs saptandı. Çalışmamızda hem rota virüs hem de adenovirüsün bir ay-2 yaş grubunda en yaygın olduğu saptandı. Rota virüs en sık kış aylarında, adenovirüs ise ilkbahar aylarında tespit edilmiştir. Yirmi altı (%28.2) hasta rota virüs gastroenteritiyle ve 68 (%37.7) hasta adenovirüs gastroenteritiyle hastaneye yatırılarak tedavi edildi.

Sonuç: Çalışmamızda viral gastroenterit etkenleri olan rota virüs ve adeno virüs çocuklarda, özellikle iki yaşın altındaki çocuklarda akut gastroenteritlerde yüksek oranda saptanmıştır. Enterik adeno virüsler ilkbaharda rota virüsler kış ayında daha sık saptanmıştır. Akut gastroenteritli çocukların klinik değerlendirme ve tedavi planlamasında viral etkenlerin sıklığı göz önünde bulundurulmalıdır. Gaitada viral antijenlerin gösterilmesi gereksiz antibiyotik kullanımını önleyecektir.

Anahtar Kelimeler: Adeno virüs, çocuklar, gastroenterit, prevalans, rota virüs

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INTRODUCTION

The American Academy of Pediatrics (AAP) defines acute gastroenteritis as a diarrheal disease of rapid onset, with or without additional symptoms and signs, such as nausea, vomiting, fever, or abdominal pain.[1] Viruses are the most important etiology and are responsible for approximately 70% of the episodes of acute gastroenteritis in children. There are over 20 different types of viruses that have been identified as etiological agents. In the United States, rotavirus and noroviruses are the most common viral agent that causes diarrhea, followed by enteric adenoviruses, sapovirus, calicivirus and astroviruses.[2] Bacterial infection accounts for 10% to 20% of all the acute gastroenteritis. The most common bacterial reasons are, Salmonella species, Campylobacter species, Shigella species and Yersina species. Giardia lamblia is the most common protozoal infection that causes gastroenteritis. Other protozoa include Entamoeba histolytica and Cryptosporidium species.[3]

Viral gastroenteritis displays abruptly with vomiting and watery diarrhoea, often accompanied by low-grade fever and abdominal cramps. Clinical appearance of viral gastroenteritis ranges from an asymptomatic infection to severe dehydrating diarrhoea. Dehydration is a severe complication that can lead to hypovolaemic shock, coma, and death.[4] Treatment of viral gastroenteritis is based primarily on replacement of fluid and electrolytes. The AAP, Centers for Disease Control and Prevention, European Society for Pediatric Gastroenterology and Nutrition, and the World Health Organization all strongly support the use of oral rehydration therapy as the first-line therapy for the treatment of acute gastroenteritis, except in cases of severe dehydration.[3] Abundant vomiting, worsening dehydration with altered consciousness, or severe acidosis, hypovolaemic shock, abdominal distension, and ileus are indications for intravenous rehydration.[5] Rotaviruses primarily affect young children, are responsible for 200,000 deaths worldwide, with most deaths occurring in developing countries. The proportion of rotavirus infections range from 8–10% of diarrhoea episodes of all severity to almost 35–40% of diarrhoea episodes requiring hospital admission worldwide.[4] In developing countries, especially in the pediatric age group, acute gastroenteritis morbidity and mortality is quite high.[6] Rotaviruses worldwide endemic, especially in countries with a temperate climate, mostly in winter and under 2 years old seen in children.[7] However, highly effective rotavirus vaccine has prevented severe gastroenteritis cases as well as reduced the attributable mortalities.[8] Acute gastroenteritis due to adenoviruses is most common in countries with a hot climate.[9] The rate of enteric adenovirus 40 and 41 varies from 1–8% in developed countries to 2–31% in developing countries.[10] Adenovirus infected patients normally present clinical symptoms such as diarrhea, vomiting and complications of the respiratory system.[11] Using mathematical modeling, the Global Burden of Diseases study estimated that in 2016, enteric adenovirus infections caused 75 million episodes of diarrhea globally among children <5 years of age, with an associated attributable fraction for mortality of 11.8%.[12]

In adenovirus and rotavirus gastroenteritis, leucocytosis, neutrophilia, and high C-reactive protein can be detected without secondary bacterial infection.[13,14] Therefore, detection of viral antigens in stool will prevent unnecessary antibiotic use. The distribution of viral agents may differ according to age and season. Different results have been obtained in previous studies on this subject. In this study, we aimed to investigate the relationship between age, gender, and season with respect to the frequency of rotavirus and adenovirus antigens in stool specimens obtained from children one month to 18 years of age who were diagnosed with acute gastroenteritis.

MATERIAL AND METHOD

Rotavirus and adenovirus antigen results in stool specimen were obtained retrospectively in the hospital automation system, with a diagnosis of gastroenteritis in children one month to 18 years of age who were admitted to our hospital's Pediatrics Clinic between January 2017 and August 2022. The 2026 patients were diagnosed with acute gastroenteritis. In 1960 patients, stool adenovirus and rotavirus were examined. In the absence of the kit, 66 patients whose stool examination could not be performed and whose information could not be reached were excluded from the study. The patient's admission year, season, gender, age, and stool viral antigen test results were retrospectively analysed from the file records. The patients were divided into five age groups: one month to 2, 2-4, 4-6, 6-10 and 10-18 years of age. Application dates of patients were classified according to the seasons.

Detection of the presence of adenovirus and rotavirus antigen in stool samples taken from patients was made using the LJ 2000 Automatic Stool Parasite Analyzer (Jinan Lanjie Biotechnology Co., Ltd, China) with high resolution CCD camera support. Ethics committee approval for our study was obtained from the ethics committee of Karatay University Medical Faculty Hospital (approval number 2023/008).

Statistical analysis

Statistical analyzes in our study were performed using the Statistical Package for Social Sciences (SPSS) version 22 (IBM Corp. Armonk, NY, USA) program. Kolmogorov Smirnov and Shapiro Wilk tests were used to check whether the numerical measurements in the study group provided the assumption of normal distribution. In descriptive statistics, mean±standard deviation was used for parametric data if it fit the normal distribution, or median (minimum-maximum) if it did not fit the normal distribution, and frequency and percentage values were used for categorical data. Pearson chi-square test was used to compare categorical measures between groups. In the comparison of parametric measurements between the groups, the independent groups T test was used for the variables conforming to the normal distribution, and the Mann Whitney U test was used for the variables not conforming to the normal distribution of the groups. Significance level was accepted as $p<0.05$.

RESULTS

Evaluation of the Demographic Data, Frequency and Seasonal Distribution of Rotavirus Gastroenteritis in Children

The 92 of the 1960 patients (4.7%) who were examined for rotavirus were positive, and 180 (9.1%) of the 1960 patients who were tested for adenovirus were positive. In total, 272 (13.8%) of all patients were positive for rotavirus or adenovirus viral antigen in stool examination. While the mean age of the 272 patients was 3.15±2.94 years (median, 2.1 years, minimum-maximum 0.1-17.9 years).

Of the patients diagnosed with acute gastroenteritis included in the study, 1123 (57.2%) were boy and 837 (42.8%) were girl. While the mean age of the patients was 5.01±4.50 years (median, 3.4 years, minimum-maximum 0.1-17.9 years), the mean age of the boys was 5.01±4.63 years and the mean age of the girls was 5.02±4.32 years. Of the patients who were positive for rotavirus antigen, 66.3% (n=61) were boy. The mean age of the patients with rotavirus was 3.62±3.12 years (median, 2.55 years, minimum-maximum 0.5-17.4 years).

Co-infection of adenovirus with rota virus was not detected in any of our patients. When the mean age of the patients participating in the study was compared in terms of gender, no statistically significant difference was found (p:0.38). The seasonal distribution of rotavirus positive patients was 25 (1.3%) in spring, 8 (0.4%) in summer, 19 (1%) in autumn and 41 (2%) in winter, respectively. When the frequency of rotavirus in the stool specimen of the participants was examined according to months and seasonal distribution, the most common month was December, while the most common season was winter (**Table 1**, **Figure 1,2**).

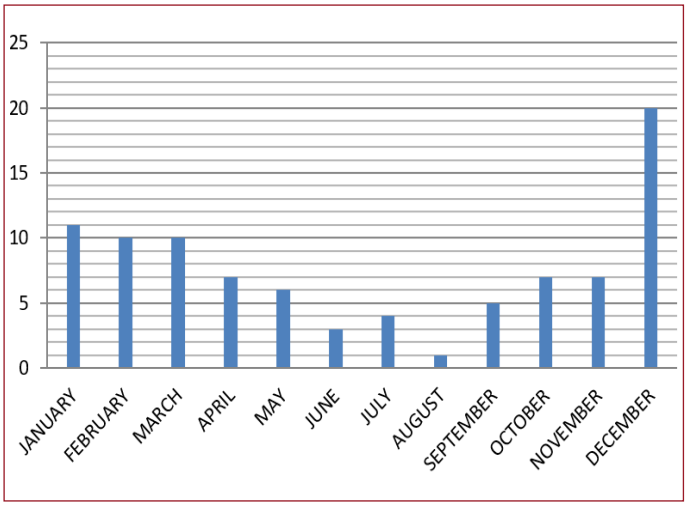

Figure 1. Distribution of patients with positive rotavirus antigen in stool specimen by months

Table 1: Distribution of patients with 92 positive rotavirus antigens in stool specimen by months

Months	n	%
January	11	0,54%
February	10	0,49%
March	10	0,49%
April	7	0,35%
May	6	0,30%
June	3	0,15%
July	4	0,20%
August	1	0,05%
September	5	0,25%
October	7	0,35%
November	7	0,35%
December	20	0,99%

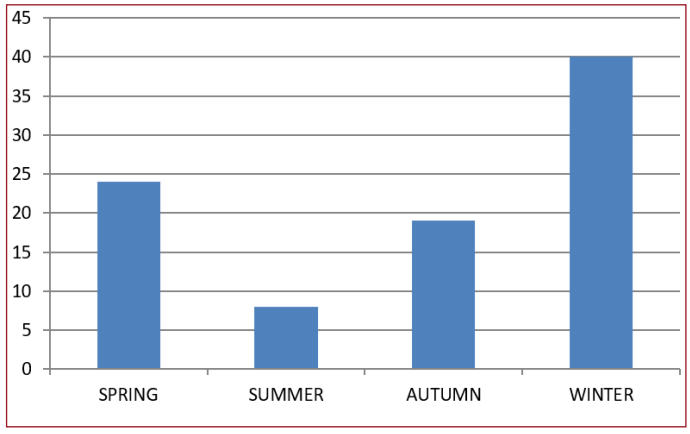

Figure 2. Seasonal distribution of patients with positive rotavirus antigen in stool specimen

Evaluation of the Demographic Data, Frequency and Seasonal Distribution of Adenovirus Gastroenteritis in Children

The frequency of rotavirus was found to be statistically significantly higher in December and winter season when compared to other months and seasons (p<0.0001). Twenty-six (28.2%) of the patients with rotavirus gastroenteritis were hospitalized and treated.

Enteric adenovirus antigen in stool specimen was positive in 180 (9.1%). Enteric adenovirus antigen in stool specimen was positive in 88 (4.4%) boys and 92 (4.7%) girls. While the mean age of the patients with adenovirus was 2.88±2.77 years (median, 1.9 years, minimum-maximum 0.1-17.9 years). When compared in terms of gender, there was no statistically significant difference in the frequency of detecting enteric adenovirus in stool specimen (p:0.295). When the frequency of adenovirus in the stool specimen of the participants was examined according to months and seasonal distribution, the most common month was March, while the most common season was spring (**Table 2, Figure 3,4**). The frequency of adenovirus was found to be statistically significantly higher in March and spring season when compared to other months and seasons (p<0.0001 for both). Of the patients with adenovirus gastroenteritis, 68 (37.7%) were hospitalized and treated.

Table 2: Distribution of patients with 180 positive adenovirus antigens in stool specimen by months

Months	n	%
January	6	0,30%
February	30	1,48%
March	38	1,88%
April	18	0,89%
May	9	0,44%
June	10	0,49%
July	11	0,54%
August	4	0,20%
September	13	0,64%
October	15	0,74%
November	15	0,74%
December	11	0,54%

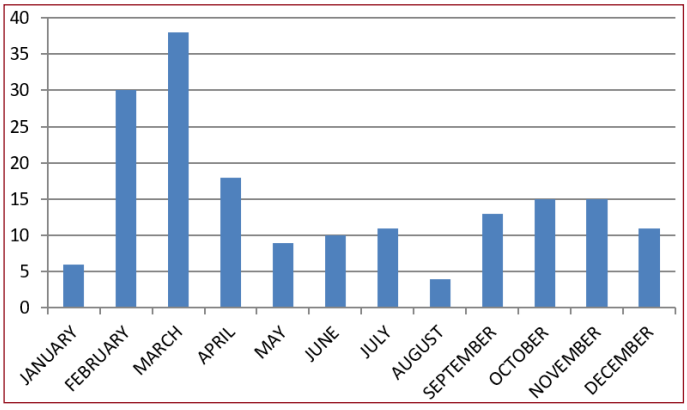

Figure 3. Distribution of patients with positive adenovirus antigen in stool specimen by months

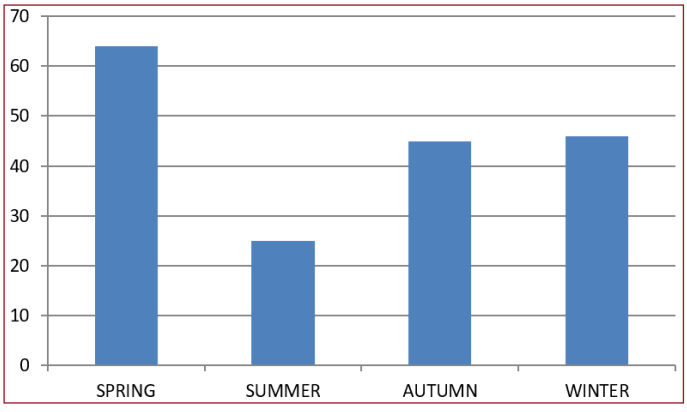

Figure 4. Seasonal distribution of patients with positive adenovirus antigen in stool specimen

Frequency of Adenovirus And Rotavirus in Children with Acute Gastroenteritis by Age Groups

The patients one month to 2 years of age were the most common age group. A statistically significant difference was found when the patients were compared with age groups in terms of gender (p:0.024). The rotavirus positivity rate by age groups was 1.9% in the group one month to 2 years of age, 1.2% in the group 2-4 years of age, 0.9% in the group 4-6 years of age, 0.5% in the group 6-10 years of age, and 0.3% in the group 10-18 years of age, respectively. Rotavirus antigen positivity rates in stool were found to be statistically significantly higher in the group one month to 2 years of age when compared with other age groups (p:0.001). Distribution of patients with positive stool rotavirus and adenovirus antigen was showed in **Figure 5**.

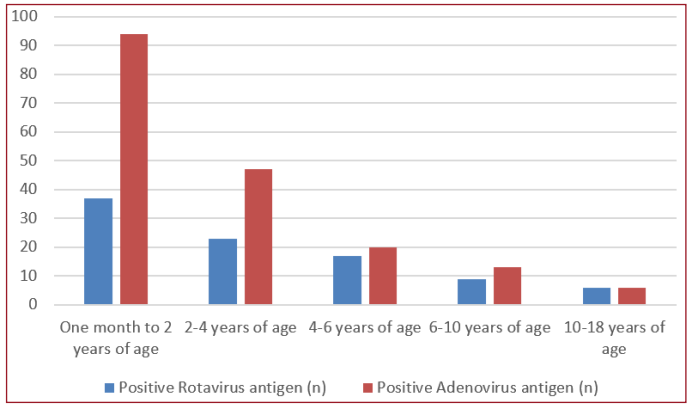

Figure 5. Distribution of patients with positive stool rotavirus and adenovirus antigen by age groups

According to age groups, adenovirus positivity rate was 4.8% in the group one month to 2 years of age, 2.4% in the group 2-4 years of age, 1.1% in the group 4-6 years of age, 0.7% in the group 6-10 years of age, and 0.3% in the group 10-18 years of age, respectively. Adenovirus antigen positivity rates in stool were found to be statistically significantly higher in the group one month to 2 years of age when compared with other age groups (p<0.0001).

DISCUSSION

Viruses, including rotaviruses, enteric adenovirus, norovirus, astrovirus, and calicivirus, are known to be the most important etiological agents responsible for about 70.0% of cases of gastroenteritis infection in children.[15] In both developing and developed countries, viral gastroenteritis is the most common cause of hospitalization for infants and older children with severe dehydration resulting from diarrhea; it is also a cause of infant mortality. Rotavirus infections are the most common reasons in these patients.[16] Nowadays, studies show that the frequency of rotavirus and adenovirus varies between countries, and in different geographic regions of the same country, according to years and age groups. In a study conducted across many countries with different socioeconomic development levels, rotavirus and adenovirus prevalence varied 4.8%-45% and 1.5%-17.6%, respectively.

In developed countries, the prevalence of viruses in patients with enteric infections is reported to be 3.1-5.0%.[17-19] However, it has been reported that enteric adenovirus is highly variable in developing countries. In a study conducted in Iraq, the prevalence of adenovirus was reported to be approximately 15%.[20]

In Turkey, the prevalence of rotavirus is reported to vary between 6.7% and 20.2% and the prevalence of adenovirus between 0.96% and 7.6%.[21] According to studies conducted in Turkey, rotavirus infections constituted 9.8–39.8% of viral

gastroenteritis infections, and adenovirus infections 7.8-10.0%.[22-25] In the study conducted by Öner et al.[21] ,which included a 4-year period published in 2022, on children and adults, rotavirus antigen was found to be 8.2%, adenovirus antigen was 2.2% in stool samples. In a recent study conducted in our country, while rotavirus antigen screening was performed in 1,359 children younger than 5 years, adenovirus antigens was done in 1,270 children younger than 5 years. In this study, rotavirus antigen was detected in 194 (14.3%) of all stool specimen tested, and adenovirus antigen was detected in 39 (3.1%).[6] Viral antigens were detected in 884 (17.1%) of a total of 5156 stool specimens examined with the diagnosis of acute gastroenteritis in a previous study conducted in children in Konya. Of those who were found positive, 764 (14.8%) were determined as rotavirus and 120 (2.3%) as adenovirus.[26] In a previous study in children under 5 year of age, viral antigen was detected in 53 of 96 stool specimens. Rotavirus was detected in 39.6%, adenovirus 10.4%, and norovirus 5.2%.[27] In our study, we detected rotavirus and adenovirus most frequently in children younger than two years old.

In another study, viral antigens were observed in 348 (25%) of 1358 stool specimens in children. Among the positive results, the incidence of rotavirus was 23.7%, adenovirus 1.5%, and the incidence of co-existence of both viruses was found to be 0.4%.[28] In a study in the South eastern Anatolia region of our country, 597 of 3607 patients diagnosed with acute gastroenteritis were found to be positive for rotavirus antigen.[29] In another study, rotavirus was detected in 14 (12.5%) children and adenovirus was detected in 5 (4.5%) children in a total of 112 children under 6 years of age with diarrhea, and dual infection was detected in one case (0.9%).[30] Some studies have reported on co-infection of adenovirus with other viruses, including rotavirus, which can be associated with more severe symptoms.[31] Rotavirus-adenovirus co-infection was determined in 21.7% of cases in the study by Shams in Iran.[15] In our study, co-infection of adenovirus with rota virus was not detected.

The records of stool specimens of 772 patients under 4 years of age who were followed up with the diagnosis of acute gastroenteritis in Ankara were reviewed retrospectively, and rotavirus antigen was found positive in 174 (22.5%).[32] In a study in Istanbul, rotavirus antigen was investigated by immunochromatographic method in 3618 stool specimens taken from children with pre-diagnosis of acute gastroenteritis and sent for rotavirus search, and rotavirus antigen was found in 745 (20.6%) of the samples.[33] In another study, viral antigen was detected in 988 (14.04%) out of 7037 patients. Rotavirus was detected in 750 (10.7%) patients, and adenovirus in 238 (3.3%) patients.[9] In our study, we detected viral antigen including rotavirus and adenovirus in the stool specimen of 13.5% of the 1960 children examined. Adenovirus antigen positivity was detected in 9.1% of the patients and rotavirus positivity in 4.7% of the children.

In study by Turkdagi et al. viral antigen was detected in 338 (12.1%) of 2795 patients. Rotavirus was found to be positive in 273 (9.8%) of these samples, adenovirus in 36 (1.3%) and rotavirus and adenovirus in 29 (1.0%) samples. It was determined that 154 (45.6%) of the patients with viral antigen were girl, 184 (54.4%) were boy, and 198 (58.6%) were in the under 2 years of age.[34] In the study conducted in Çorum, viral antigen was detected in 706 (22.1%) of 3189 children. Rotavirus was detected in 17.5% of stool specimens, adenovirus in 3.3%, and rotavirus and adenovirus in 1.3%. It was observed that there was no statistical difference between the genders in terms of the frequency of detection of rotavirus and adenovirus.[35] In our study, we also did not find a significant difference when the prevalence of rotavirus and adenovirus was compared in terms of gender.

Rotavirus and adenovirus infections are seen throughout the year. Rotavirus infection is common in many countries in winter and spring. Öner et al. found that the rotavirus infections increased from December to April and then decreased until September. The adenovirus infection was observed in January with the highest positive rate. The seasonal pattern of the rotavirus varies according to the climate zone and is also associated with local weather.[21] In study in China revealed that adenovirus was detected throughout the year and there was no seasonal pattern or any peak in frequency of adenovirus through the year.[36] Furthermore, enteric adenoviral gastroenteritis in most parts of the world was documented throughout the entire year and does not display seasonal distribution.[37] However, another study showed an increase in rotaviruses infections in autumn-winter season.[38] In the study in Van, viral antigens were determined in 205 (21%) of 955 patients; rotavirus was positive in 124 (13%), adenovirus in 81 (8.5%), and rotavirus and adenovirus together in 43 (4.5%). Viral antigen-positive cases were most frequently seen in autumn and winter months.[39] In our study, rotavirus was most commonly detected in December and winter, while adenovirus was detected in March and spring.

It is known that rotavirus gastroenteritis has a more severe clinical course and requires more hospitalization and intravenous fluid therapy.[40] In our study, approximately one third of the children diagnosed with rotavirus gastroenteritis were hospitalized and treated while approximately 38% of the children diagnosed with adenovirus gastroenteritis were hospitalized.

Limitations of the Study

Since it is a retrospective study, detailed data on the clinical signs and symptoms of the patients could not be reached. The reason why the frequency of enteric adenovirus was higher than the frequency of rotavirus in our study may be due to rotavirus vaccination in children. However, as this is a retrospective study, we could not learn about the vaccination status.

CONCLUSION

Our study showed that rotavirus and adenovirus, which are the agents of viral gastroenteritis, are seen at a substantial rate. Especially in children less than the two years of age, viral agents are more likely to be detected. Enteric adenovirus and rotavirus were detected more frequently in winter and spring. We suggest that viral gastroenteritis agents should be considered and examined in children with suspected gastroenteritis. Demonstration of viral antigens in stool samples will prevent unnecessary antibiotic use, as it can mimic bacterial infection clinically and laboratory.

ETHICAL DECLARATIONS

Ethics Committee Approval: Ethics committee approval for our study was obtained from the Ethics Committee of Karatay University Medical Faculty Hospital (approval number 2023/008).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article / Orijinal Araştırma

Clinical, Demographic and Echocardiographic Characteristics of Pediatric Chest Deformities

Pediatrik Göğüs Deformitelerinin Klinik, Demografik ve Ekokardiyografik Özellikleri

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Abstract

Aim: We aimed to retrospectively evaluate the clinical, demographic and echocardiographic findings of children diagnosed with chest deformity in the pediatric cardiology clinic.

Material and Method: This study enrolled children under the age of 18 years who were referred with chest deformity to our pediatric cardiology unit, over a period of six years (January 2017-December 2022).

Results: The mean age of the patients was 9.9 ± 5.2 years, median 11 years (0-18 years old). 89 patients with abnormal echocardiographic findings: 42 (9.56%) mitral valve prolapse, 18 (4.1%) atrial septal defect, 9 (2%) ventricular septal defect, 8 (1.82%) bicuspid without aortic valve stenosis aortic valve, 5 (1.1%) patent ductus arteriosus, 2 (0.45%) pulmonary stenosis, 2 (0.45%) great artery transposition, 2 (0.45%) hypertrophic cardiomyopathy, subaortic ridge 1 (% 0.27). Cardiac compression was present in 13.4% of the cases with pectus excavatum. 13(%3) patients were operated by a thoracic surgeon. Marfan Syndrome was diagnosed in 7 patients and Noonan Syndrome was diagnosed in 2 patients who applied to our clinic with chest deformity.

Conclusion: We suggest that echocardiographic examination in patients with chest deformity is important in the diagnosis of congenital heart diseases, early diagnosis and treatment of heart compression finding.

Keywords: Children, chest deformity, echocardiography

Öz

Amaç: Çocuk kardiyoloji kliniğinde göğüs deformitesi tanısı alan çocukların klinik, demografik, klinik ve ekokardiyografik bulgularını retrospektif olarak değerlendirmeyi amaçladık.

Gereç ve Yöntem: Bu çalışmaya altı yıl boyunca (Ocak 2017-Aralık 2022) pediatrik kardiyoloji birimimize göğüs deformitesi ile başvuran 18 yaş altı çocuklar dahil edildi.

Bulgular: Hastaların yaş ortalaması 9,9 ± 5,2 median:11 yaş (0-18 yaş) idi. Anormal ekokardiyografik bulguları olan 89 hasta: 42 (%9,56) mitral kapak prolapsusu, 18 (%4,1) atriyal septal defekt, 9 (%2) ventriküler septal defekt, 8 (%1,82) aort kapak darlığı olmayan biküspid aort kapak, 5 (%1,1) patent duktus arteriozus, 2 (%0,45) pulmoner darlık, 2 (%0,45) büyük arter transpozisyonu, 2 (%0,45) hipertrofik kardiyomiyopati, subaortik ridge 1 (%0,27) idi. Pektus ekskavatumlu olguların %13.4'ünde kardiyak bası vardı. 13(%3) hasta göğüs cerrahisi tarafından opere edildi. Göğüs deformitesi ile kliniğimize başvuran 7 hastada Marfan Sendromu, 2 hastada Noonan Sendromu teşhis edildi.

Sonuç: Göğüs deformitesi olan hastalarda ekokardiyografik incelemenin doğumsal kalp hastalıklarının tanısında, kalp sıkışması bulgusunun erken tanı ve tedavisinde önemli olduğunu düşünüyoruz.

Anahtar Kelimeler: Çocuklar, göğüs deformitesi, ekokardiyografi

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INTRODUCTION

Congenital chest wall deformities can be classified as pectus excavatum, pectus carinatum, Poland syndrome, and sternal defects.[1] The most common deformation of the anterior chest wall is pectus excavatum.[1] It constitutes 90% of all chest wall deformities.[2] Pectus excavatum is seen in approximately one in 400 live births.[3] The rates are four times higher in boys according to girls.[3]

Chest wall deformities are accompanied by other malformations of the skeletal system, cardiovascular, gastrointestinal and genitourinary anomalies.[4] Pectus excavatum and carinatum are important clinical findings in Marfan syndrome.[4]

The degree of recession in the pectus excavatum determines the clinical findings. When there is moderate and severe inward collapse, pressure on the heart and lungs occurs. With exertion, chest pain and shortness of breath develop. In patients with pectus excavatum, a decrease in cardiac output occurs in the supine position and during exertion. Patients tend to avoid movement. Mitral valve prolapse is the most common cardiac anomaly in patients with pectus excavatum.[4] Mitral valve prolapse as a result of anterior compression of the heart thought to be developing. Atrial septal defect, and ventricular septal defect and aortic coarctation have also been reported in patients with pectus excavatum.[4]

The second most common chest wall deformity is pectus carinatum. It constitutes approximately 16% of all chest deformities.[6] It is more common in men than women. Approximately 25% of patients with pectus carinatum have a positive family history.[7]

There is protrusion of the sternum and chondrocostal joints in the pectus carinatum. Protrusion in pectus carinatum can be symmetrical or asymmetrical. Although it is more common in pre-adolescence and adolescence, it can also be seen in infancy. There is an increase in the severity of pectus carinatum during periods.

Approximately 18% of cases with pectus carinatum have a congenital heart disease.[8] The most important reasons for patients to apply to the clinic are chest pain and exertional dyspnea. Echocardiographic examinations of the patients not only evaluate the mitral valve, but also provide detailed information about other congenital heart diseases. Pectus carinatum may be accompanied by Marfan or Noonan syndromes.

We aimed to retrospectively evaluate the clinical, demographic, clinical and echocardiographic findings of children diagnosed with chest deformity in the pediatric cardiology clinic.

MATERIAL AND METHOD

We retrospectively analysed the demographic, clinical and echocardiographic data of patients with chest deformities.

Study Population

This study enrolled children under the age of 18 years who were admitted with chest deformity to our paediatric cardiology unit, over a period of six years (January 2017-December 2022). Pectus deformities were identified by physical examination of the chest.

Echocardiographic Examination

Echocardiographic examination was performed with the Philips EPIQ 7C (USA) device, by taking multiple orthogonal parasternal, apical and subcostal images of the patients lying in the left lateral decubitus position by the same pediatric cardiologist. Traditional echocardiographic evaluation includes measurements of left ventricular end-diastolic and end-systolic diameter, septal and left ventricular posterior wall thicknesses in diastole and systole, left ventricular ejection fraction (EF) and left ventricular fractional shortening (FS) from the parasternal long-axis view. In four-chamber imaging, it was evaluated whether there was any external pressure on the heart.

Statistical Analysis

Descriptive statistics were applied to the obtained data, and their distribution by age and gender was examined. SPSS 21.0 Software Program was used for statistical analysis in the study.

Ethics Committee Approval

The study was approved by Local Ethic Committee (Decision No: 2022/533). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

RESULTS

During the study period, 439 patients presented or referral to our unit with chief complaint of chest wall deformity. There were 337 (76.8%) boys and 102 (23.2%) girls. The mean age of the patients was 9.9 ± 5.2 years with a median of 11 years and a range of 0-18 years. The patients with chest wall deformities were included, 189 (43%) pectus excavatum, 132 (30%) pectus carinatum and 118 (27%) were both, pectus excavatum and carinatum.

Echocardiograpy were performed in all patients. 350 (79.72%) patients with pectus deformity revealed normal echocardiography. Echocardiographic diagnoses included 42 (9.56%) mitral valve prolapsus, 18 (4.1%) atrial septal defect, 9 (2%) ventricular septal defect, 8 (1.82%) bicuspid aortic valve without aortic valve stenosis, 5 (1.1%) patent ductus arteriozus, 2 (0.45%) pulmonary stenosis, 2 (0.45%) transposition of the great arteries, 2 (0.45%) hypertrophic cardiomyopathy, subaortic ridge 1 (0.27%). **Table 1** summarizes echocardiographic findings of patients with chest deformity. **Figure 1** and **Figure 2** show the appearance and echocardiographic evidence of right ventricular compression of our patient diagnosed with pectus excavatum as cardiac compression finding.

13.4% of the cases with pectus excavatum had cardiac compression. 13(%3) patients were operated by a thoracic surgeon.

Marfan Syndrome was diagnosed in 7 patients and Noonan Syndrome was diagnosed in 2 patients who applied to our clinic with chest deformity. Pectus excavatum deformity was accompanying in patients with Marfan syndrome and Noonan syndrome.

Table 1. Echocardiographic findings of patients with chest deformity

	n	%
Mitral Valve Prolapse	42	9.56
Atrial Septal Defect	18	4.1
Ventricular Septal Defect	9	2
Bicuspid Aortic Valve	8	1.82
Patent Ductus Arteriozus	5	1.1
Pulmonary Stenosis	2	0.45
Transposition of Great Arteries	2	0.45
Hypertrophic Cardiomyopathy	2	0.45
Subaortic Ridge	1	0.27

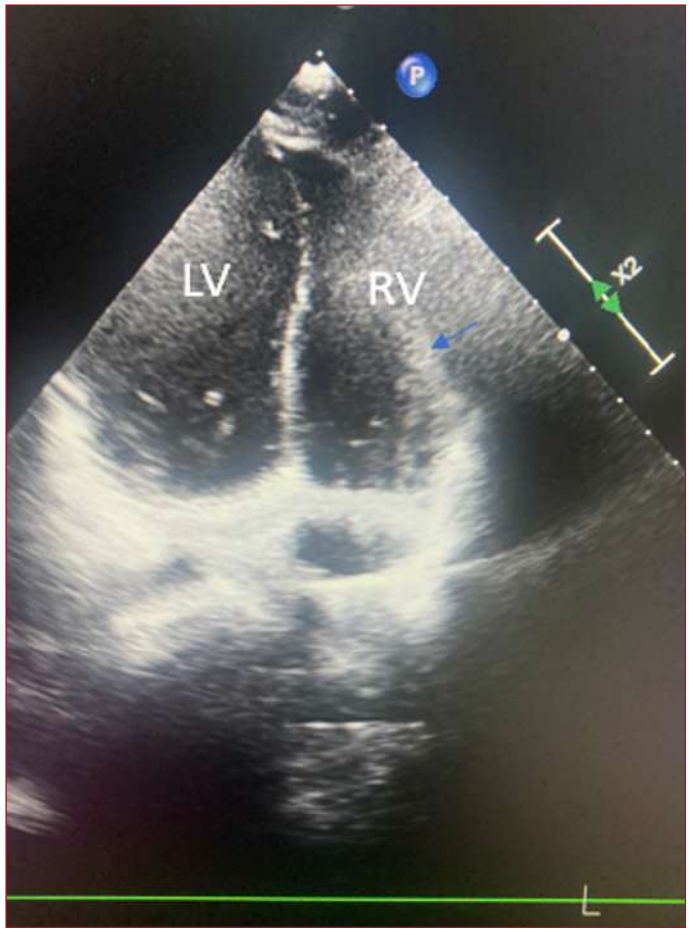

Figure 1: Arrow indicates right ventricular compression. RV: Right ventricul, LV: Left ventricul

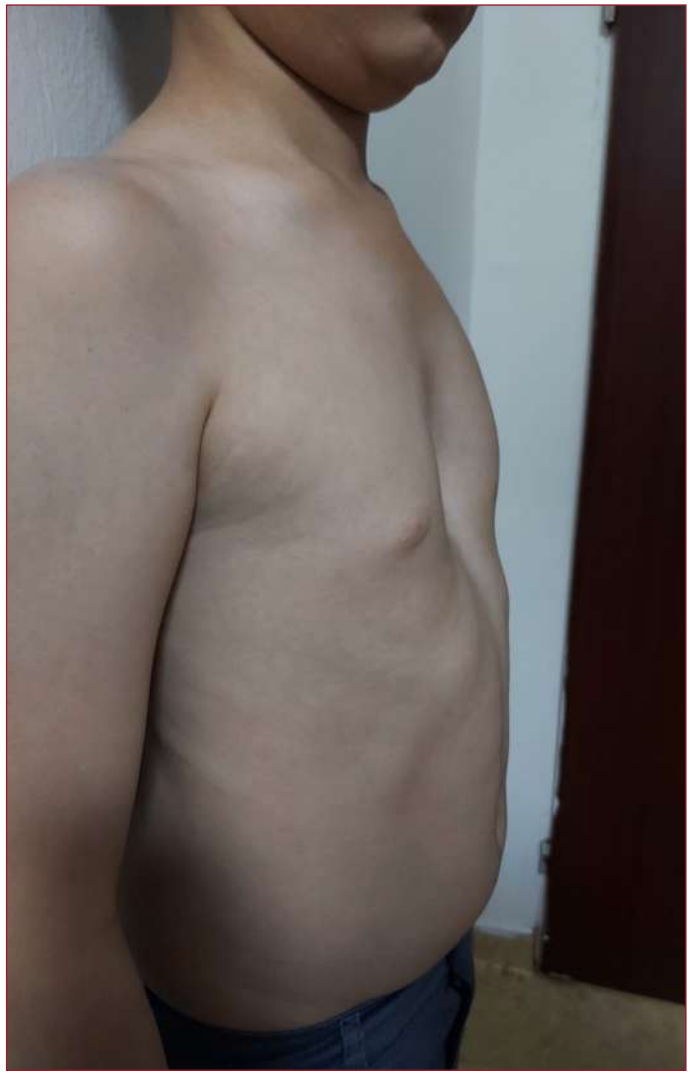

Figure 2: Appearance of the patient with pectus excavatum

DISCUSSION

Chest deformities are 4 times more common in boys than in girls.[9] In our study, similar to the literature, 76.2% of the patients with chest deformity were boys.

Congenital heart defects with pectus deformity is relatively common. Birth prevalence of congenital heart disease is estimated to be 8 cases per 1000 live births.[9] We found 20.8% congenital heart disease in the patients with pectus deformities.

The most common cardiac pathologies accompanying chest deformities are mitral valve prolapse and atrial septal defect. Park et al. found the incidence of mitral valve prolapse in the patients with pectus excavatum was 23%.[11] We found mitral valve prolapse (9.56%) as the most common cardiac pathology accompanying chest deformity. Mitral valve prolapse is thought to develop as a result of chest deformity pressing on the heart. Coln et al. found that in 123 patients, while 54 patients had preoperative mitral valve prolapse,

only seven patients had mitral valve prolapse after pectus correction surgery.[12] The improvement in a significant proportion of patients with mitral valve prolapse after pectus surgery supports the hypothesis that mitral valve prolapse develops due to cardiac compression.

Atrial septal defect is more common in children with chest deformity than in the normal population. The rate of atrial septal defect in patients with chest deformities was reported 2.1%, 2.8% and 15%, by Simsek et al, Akcalı et al and Sanchez et al respesctively.[13-15] We found the frequency of atrial septal defect in patients with chest deformity to be 4.1%. In patients with chest deformity, the evaluation of the atrial septum with transthoracic echocardiography can be difficult. We think that in selected cases transesophageal echocardiography can be performed.

Cyanotic congenital heart dissease is uncommon with pectus deformities. Kikuchi et al. reported pectus excavatum in a patient with tetralogy of Fallot.[16] We found transposition of great arteries in 2 patients with pectus deformity.

In chest deformities, cosmetic reasons, psychological reasons, lung and heart compression are surgical indications. Patients with a diagnosis of pectus excavatum should be carefully evaluated in terms of cardiac compression during echocardiographic examination. Zhao et al found that exercise capacity was limited as a result of reduced filling of he right heart by the compressive effects of pectus excavatum. [17] Cahill et al found an increase in cardiac stroke volume after surgical correction in patients with chest deformities. [18] Kowalewski et al found statistically significant increases in right ventricular volume indices after surgery.[19]

In our study, 13.4% of the cases with pectus excavatum had cardiac compression. Patients with cardiac compression were consulted with the thoracic surgery department. 13 (3%) patients were operated by a thoracic surgeon. After surgery, echocardiographic examination should be performed in terms of pressure on the heart. All patients were asymptomatic after surgery.

Chest deformities may occur as part of various syndromes. [20] Andrescu et al found that pectus excavatum and pectus carinatum were associated with Marfan and Noonan syndrome.[21]

Marfan syndrome involves the skeletal, ocular, and cardiovascular systems. Patients with Marfan syndrome present with tall stature, ectopia lentis, and a positive family history. Skeletal manifestations are the primary signs of Marfan syndrome. Pectus carinatum and pectus excavatum can be seen frequently in Marfan syndrome. Mitral valve prolapse, enlargement of the aortic root, rupture of sinus valsalva may be seen in patients with Marfan syndrome.[22]

Noonan syndrome is characterized by characteristic facies, short stature, congenital heart defect, pectus carinatum or pectus ekskavatum and developmental delay of variable degree.[23] We diagnosed Marfan syndrome in 7, Noonan Syndrome in 2 of the patients who applied to our clinic with chest deformity.

Since chest wall deformities may be associated with a genetic disorder or syndrome, a thorough family history taking, along with appropriate pediatric and genetic investigations, can provide valuable information.

CONCLUSION

Chest deformities can have a significant cardiac impact depending on the type and severity of the deformity. Cardiac compression, especially in pectus excavatum, which is the most common chest deformity, is an indication for surgery. Echocardiographic examination in patients with chest deformity is important in the diagnosis and treatment of the congenital heart diseases and heart compression. Echocardiographic evaluation should be performed in these patients, and they should be investigated for cardiac compression and congenital heart diseases. The patient's follow-up plan should be made with the thoracic surgery department by sharing the examinations of cardiac involvement.

ETHICAL DECLARATIONS

Ethics Committee Approval: Approval for this study was obtained from the Local Ethics Committee of Selçuk Medical Faculty Ethics Committee, 2022/533.

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article / Orijinal Araştırma

Biochemical, Hematological, Peripheral Smear and Weight Evaluation of Low Dose Epigallocatechin Gallate in Diethylnitrosamine-Administered Rats

Dietilnitrozamin Uygulanan Sıçanlarda Düşük Doz Epigallocatechin Gallate'nin Biyokimyasal, Hematolojik, Periferik Yayma ve Ağırlık Değerlendirilmesi

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Abstract

Introduction: Currently, with the development of technology, the use of many chemicals especially Diethylnitrosamine (DEN) in agriculture and industry has increased. The polyphenolic compounds of Epigallocatechin gallate (EGCG) is the active ingredient of green tea. It has been reported that green tea has antioxidant effects. In this preliminary study, effects of low dose EGCG against exposure of DEN administered rats.

Material and Method: As a group, groups were divided into five groups of ten rats for the application as Control, Sham, DEN, EGCG and DEN+EGCG. The parameters analyzed are hemogram, biochemical, peripheral smear and weight.

Results: DEN injection has significantly increased Lactate dehydrogenase (LDH), Aspartate amino transferase (AST), Alanine aminotransferase (ALT) and Alkaline Phosphatase (ALP) values, Which are signs of hepatocyte injuries. The number of White Blood Cell Count (WBC)s increased in the EGCG group. In terms of High-density lipoprotein (HDL) and total cholesterol (Tchol) levels, the group in which DEN+EGCG were applied together was found to be the highest and Trigliseride (TG) and Low-density lipoprotein (LDL) levels were found to be lowest. The current study will be a comprehensive study demonstrating the effects of low-dose EGCG against DEN-administred rats.

Conclusion: These results indicate that consumption of low-dose EGCG polyphenolic compound in green tea may be effective against DEN administered rats.

Keywords: Biochemical, diethylnitrosamine, epigallocatechin gallate, hematological, peripheral smear, rat

Öz

Giriş: Günümüzde teknolojinin gelişmesiyle birlikte başta Dietilnitrozamin (DEN) olmak üzere birçok kimyasalın tarım ve sanayide kullanımı artmıştır. Epigallocatechin gallate'nin (EGCG) polifenolik bileşikleri, yeşil çayın aktif bileşenidir. Yeşil çayın antioksidan etkileri olduğu bildirilmiştir. Bu ön çalışmada, düşük doz EGCG'nin DEN uygulanan sıçanlara maruz kalmaya karşı etkileri incelenmiştir.

Gereç ve Yöntem: Gruplar uygulama için Kontrol, Sham, DEN, EGCG ve DEN+EGCG olmak üzere onar (n=10) rattan oluşan beş gruba ayrıldı. Parametreler olarak hemogram, biyokimyasal, periferik yayma ve ağırlık analiz edildi

Bulgular: DEN enjeksiyonu, hepatosit hasarlanmalarının belirtileri olan Laktat dehidrogenaz (LDH), Aspartat amino transferaz (AST), Alanin amino transferaz (ALT) ve Alkaline Fosftaz (ALP) değerlerini önemli ölçüde artırdı. EGCG grubunda beyaz kan hücre (WBC) sayısı arttı. Yüksek dansite lipoprotein (HDL) ve Total kolesterol (TChol) düzeyleri açısından DEN+EGCG'nin birlikte uygulandığı grup en yüksek, trigliserit (TG) ve düşük dansite lipoprotein (LDL) düzeyleri ise en düşük bulundu.

Sonuç: Bu sonuçlar, yeşil çayda düşük dozlu EGCG polifenolik bileşiğinin tüketilmesinin, DEN uygulanan sıçanlara karşı etkili olabileceğini göstermektedir.

Anahtar Kelimeler: Biyokimyasal, diethylnitrosamine, epigallocatechin gallate, hematolojik, periferik kan smear, sıçan

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INTRODUCTION

Diethylnitrosamine (DEN) is an environmental carcinogen found in many products such as smoked, cured foods, nitrite-processed meats such as salami, dairy products, alcoholic beverages, and tobacco smoke.[1] [DEN is frequently used in animal studies and is a liver-damaging agent.[2,3] The intermediates and end products of DEN mediate the binding of tumor initiation sites by covalently binding to DNA with one or two oxidizing electrons.[4-6] As a result, it causes the proliferation of silent hepatocyte cells that carry DEN-induced mutations.[7] DEN has been shown to be a potent compound for the treatment of cancer.

The polyphenolic compounds of epigallocatechin gallate (EGCG) in green tea are consumed and produced in about 30 countries around the world. About two-thirds of the world's population, mainly in India and China, consume green tea. [8,9] Some studies have found that green tea has protective effects against cardiovascular diseases, hypertension, gastrointestinal diseases, some cancers, liver diseases, and arthritis, and apoptosis properties, especially antioxidant properties, have been evaluated.[8-12] The use of EGCG in green tea has been reported in many studies.

Since there are not enough studies on DEN and EGCG, this study aimed to investigate the hematological, biochemical, peripheral smear, and weight effects of the protective property of low-dose EGCG, which has been shown to have many health benefits and is the active ingredient of green tea, in rats treated with DEN.

MATERIAL AND METHOD

In this study, 50 Wistar albino rat, 3 months old,190-225g were used, which were obtained from the Van Yüzüncü Yıl Üniversity Experimental Application and Research Center of Ethics Committee (Project number: 2017/01 TYL 6343). The rats were housed in rooms that had 12 h dark/light for a period of 10 days and the temperature of which had been set as 22±2 °C. Feeding was given with the standard normal feed ad libitum and free access to water. The animals were weighed and their weights were recorded every day before and during the study. As a group, they were divided into five groups as ten in each group for application: The Control, Sham, DEN (Sigma, St. Louis, MO, USA) EGCG (Sigma, St. Louis, MO, USA) and DEN+EGCG. The experimental groups were organized as follows.

Group I (Control): Randomly selected ten rats were separated and was no additional application performed. Animals were allowed for their routine life.

Group II (Sham): On the first day, saline 0.5 ml/kg/i.p. single dose was administered.

Group III (DEN): On the first day, a single dose of 150 mg/kg/i.p. DEN was injected.[13]

Group IV (EGCG): EGCG 10 mg/kg/day were given orally by gavage every day for 10 days from the first day.[14]

Group V (DEN+EGCG): On the first day, a single dose of 150 mg/kg/day DEN i.p. was administered. EGCG 10 mg/kg/day was given by each gavage for 10 days from the first day.

At the end of the 10 days, blood was taken from the hearts of the animals with an injector for blood samples. A drop of blood taken from the rats was spread directly on the slide at an angle of 15°. It was left to dry for 30 minutes at room temperature. Then, it was determined and stained for 5 minutes with May-Grunwald Giemsa Staining. It was then passed through distilled water and allowed to dry for 10 minutes. After drying, Giemsa's pH 7.0 diluted with 10% distilled water was dyed with azur-eosin methylene blue dye for 10-15 minutes.

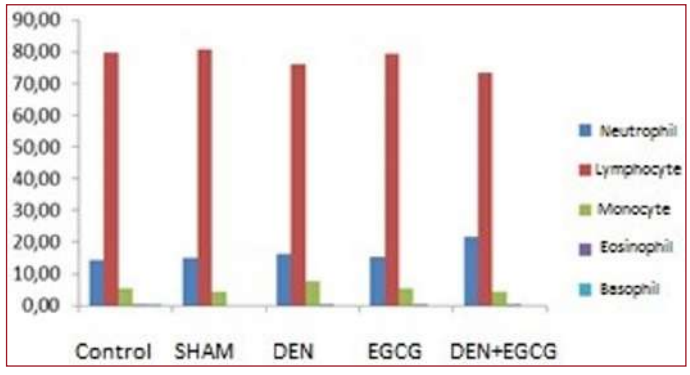
K2-Ethylene Diamine Tetra Acetic Acid (K2-EDTA) Nihon Kohden Celltac G Automatic Hematology Analyzer in blood tubes was studied with Hemolynac-310 and Hemolynac-510 Lysing reagent commercial kit in MEK-9100 device.

Biochemical parameters were determined on Abbott ARCHITECT C 16200, adapted to Abott device, and were studied by spectrophotomeric method with commercial kits. The erythropoietin (EPO) test was studied by the immunoassay method with a commercial EPN kit adapted to the Immulite 2000 device.

All of data were expressed with mean, standard errors, minimum and maximum values. Statistically, the values of the groups were compared using Kruskal-Wallis and Mann Whitney -U tests.

RESULTS AND DISCUSSION

The peripheral smear results of chemicals given in our study show that no statistically significant difference occurred in the control and other groups (**Graphic 1**, **Figure 1-3**). In this case, it can be thought that DEN and EGCG do not have significant effects on platelets, which are the most important cellular element in the coagulation process, at the specified dose and time.[15]



Graph 1. Peripheral smear results belonging to the all of the groups.
DEN: Diethylnitrosamine, EGCG: Epigallocatechin gallate,
DEN+EGCG: Diethylnitrosamine+Epigallocatechin gallate.

The fact that haemotological values were observed in all groups (**Table 1**). The lowest Hemoglobin (HGB) and Hematocrit (HTC) values observed in the control group can be explained similarly.

[16] The highest value in the WBC parameter is seen in the EGCG group, and it is seen that EGCG, the active ingredient of green tea, has an increasing effect on the number of WBCs (**Table 2**). It supports that EGCG may have an effect on the immune system. However, the application of EGCG together with DEN reduces this value to a value close to the control. Ramesh et al. (2010) examined the effects of EGCG in rabbits on atherosclerotic diet and observed that EGCG significantly increased WBC compared to the control group.[17] In terms of neutrophil value, EGCG, DEN and DEN+EGCG application caused a significant increase in the Neutrophil parameter compared to the control (**Table 2**). This suggests that neutrophils, one of the indicators of acute inflammation, are increased by these chemicals. The mechanism of the changes in DEN hemogram parameters is not clear. It may be related to immune-mediated mechanisms, non-immunological mechanisms, and bone marrow suppression.[18] The highest monocyte value was observed in the Sham group and the lowest in the DEN+EGCG group (**Table 2**). Similarly, Sham application was observed as the group with the highest increase in Basophil value. The fact that no significant difference was observed in the Control, Sham, DEN, EGCG and DEN+EGCG groups in terms of the platelet parameters examined suggests that the chemicals in question did not affect the platelet values (**Table 3**). However, the specific application of DEN+EGCG application for 10 days increased Red Blood Cell (RBC), HGB and HCT. Hematological parameters can be used to predict DEN damage.

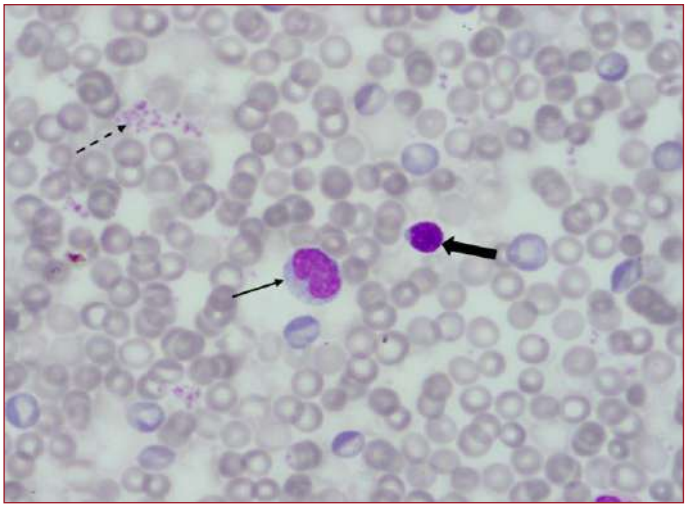


Figure 1. General view of thick arrow sign with lymphocyte, thin arrow sign with monocyte and dashed arrow sign with platelets from peripheral blood cells (May Grunwald-Giemsa, 100x).

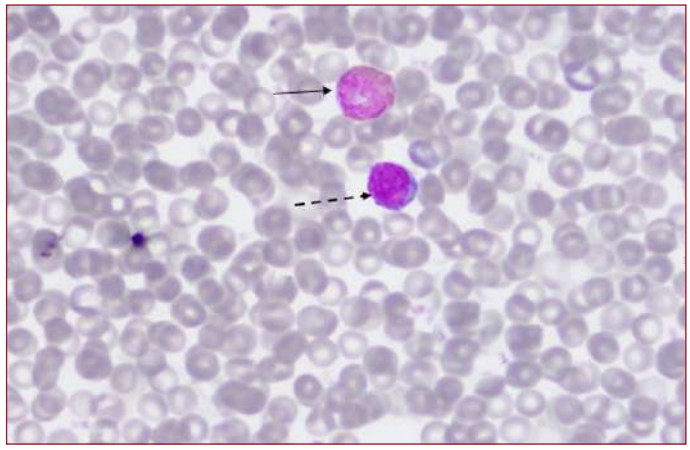


Figure 2. Smooth arrow sign with eosinophil and dashed arrow sign with lymphocyte in peripheral blood (May Grunwald-Giemsa, 100 x).

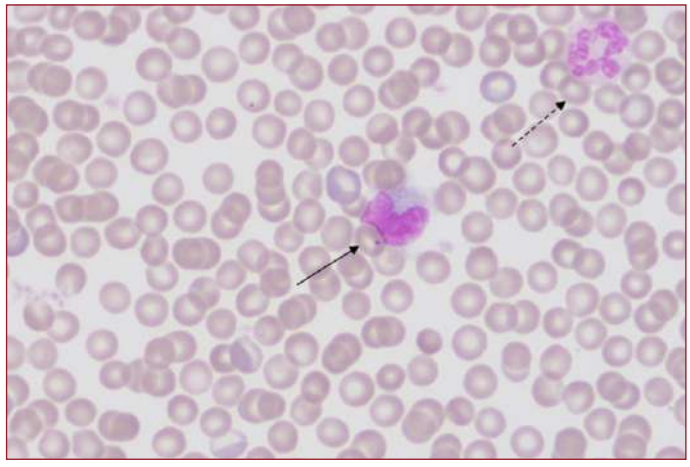


Figure 3. Dashed arrow sign with neutrophil and fine arrow sign with monocyte in peripheral blood (May Grunwald-Giemsa, 100x).

Table 2. Leukocyte numbers of the groups.

	WBC	NE	LY	MO	EO	BA
Control	8,65^{bc}	2,57^{b}	5,35^{a}	0,24^{ab}	0,03^{b}	0,45^{b}
Sham	10,23^{ab}	3,48^{ab}	5,69^{a}	0,33^{a}	0,06^{a}	0,67^{a}
DEN	10,78^{ab}	4,24^{a}	5,73^{a}	0,26^{ab}	0,04^{b}	0,51^{ab}
EGCG	11,21^{a}	3,87^{a}	6,51^{a}	0,27^{ab}	0,06^{a}	0,52^{ab}
DEN+EGCG	7,74^{c}	4,10^{a}	3,14^{b}	0,14^{b}	0,02^{b}	0,34^{b}

DEN: Diethylnitrosamine, EGCG: Epigallocatechin gallate, DEN+EGCG: Diethylnitrosamine+ Epigallocatechin gallate. Different letters indicate significant difference between groups p<0.05. WBC: White Blood Cell Count, NE: Neutrophils, LY: Lymphocytes, MO: Monocytes, EO: Eosinophils, BA: Basophils.

Table 1. Hematological parameters of erythrocyte in groups.

	RBC	HGB	HCT	MCV	MCH	MCHC	RDW-CV	RDW-SD
Control	7,33^{b}	14,33^{b}	47,08^{b}	64,59^{a}	19,63^{a}	30,47^{a}	17,97^{a}	46,34^{a}
Sham	8,24^{a}	15,94^{a}	51,50^{a}	62,63^{a}	19,39^{a}	30,94^{a}	18,66^{a}	46,72^{a}
DEN	7,94^{a}	15,80^{a}	50,19^{a}	63,19^{a}	19,65^{a}	31,07^{a}	18,39^{a}	46,47^{a}
EGCG	8,27^{a}	15,72^{a}	51,37^{a}	62,10^{a}	19,00^{a}	30,60^{a}	18,91^{a}	46,99^{a}
DEN+EGCG	8,40^{a}	15,85^{a}	51,06^{a}	60,82^{a}	18,90^{a}	31,05^{a}	18,96^{a}	46,10^{a}

DEN: Diethylnitrosamine, EGCG: Epigallocatechin gallate, DEN+EGCG: Diethylnitrosamine+Epigallocatechin gallate. Different letters indicate significant difference between groups p<0.05. RBC: Red blood cell, HGB: Hemoglobin, HCT; Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, RDW-CV: Red blood cell distribution width-coefficient of variation, RDW-SD: Red blood cell distribution width- standard deviation.

Table 3. Platelets belonging to the groups.

	PLT	PCT	MPV	PDW	P-LCR
Control	797,77[a]	0,51[a]	6,37[a]	15,33[a]	18,35[a]
Sham	737,68[a]	0,48[a]	6,44[a]	15,51[a]	18,99[a]
DEN	774,15[a]	0,51[a]	6,36[a]	15,29[a]	17,92[a]
EGCG	793,89[a]	0,51[a]	6,47[a]	15,50[a]	19,42[a]
DEN+EGCG	795,79[a]	0,50[a]	6,23[a]	15,36[a]	17,20[a]

DEN: Diethylnitrosamine, EGCG: Epigallocatechin gallate, DEN+EGCG: Diethylnitrosamine+ Epigallocatechin gallate. Different letters indicate significant difference between groups p<0.05. PLT: Platellet, PCT: Platelet crit, MPV: Mean platelet volume, P-LCR: Platelet large cell ratio.

In the study, transferrin, iron and iron binding values were examined in order to evaluate the effect of DEN, EGCG and these two chemicals on iron metabolism of the hematopoietic system (**Table 4**). The fact that there is no statistical difference in these parameters examined suggests that DEN and EGCG do not have a significant effect on these parameters. Although there is no statistical significance, the highest values in Transferrin, Iron binding and Iron values compared to the control were observed in the DEN+EGCG group, although it was not observed in a short time or, suggesting that significant differences may occur with the use of these chemicals in the long term.[19] In research studies, it was reported that after drinking 3-4 cups of tea a day, anemia problem associated with iron deficiency did not occur. In a study conducted by Suliburska et al. (2012), it was found that the glucose and iron levels of the individuals in the group consuming green tea were lower than the control group.[20]

Table 4. Transferrin, iron and iron binding values of the groups.

	Transferrin	Iron binding	Iron
Control	0,95[a]	186,70[a]	112,90[a]
Sham	0,89[a]	198,10[a]	89,20[a]
DEN	0,93[a]	200,40[a]	100,30[a]
EGCG	0,90[a]	182,89[a]	101,67[a]
DEN+EGCG	1,02[a]	216,50[a]	119,00[a]

DEN: Diethylnitrosamine, EGCG: Epigallocatechin gallate, DEN+EGCG: Diethylnitrosamine+ Epigallocatechin gallate. Different letters indicate significant difference between groups p<0.05.

The fact that no significant difference was observed between the groups in terms of glucose value was interpreted as that the applied chemicals had no direct effect on glucose metabolism (**Table 5**). In the study of Gubur (2015), glucose levels in rats fed with fructose and in the control group were higher than in the green tea group (p <0.05).[21] DEN+EGCG group High-density lipoprotein (HDL) value increased significantly (p <0.05). In the DEN+EGCG group Trigliseride (TG) value was low compared to the other groups but statistical unsignificance (**Table 5**). In Gubur's study have demonstrated no difference in HDL, Low-density lipoprotein (LDL) and total cholesterol (Tchol) levels, but TG levels in the fructose group were significantly higher than in the control and green tea groups (p<0.05).[20] EGCG, which we tried to protect against DEN, increased HDL levels. Suliburska et al. (2012) found that individuals in the group consuming green tea had high HDL Tchol levels consistent with our study.[21,22] The protective effects of EGCG may be related to its phenolic content.[23]

Hepatocellular damage causes release of Aspartate amino transferase (AST) and Alanine aminotransferase (ALT). Increased levels of AST and ALT are an indicator of cellular infiltration and functional disturbance of liver cell membranes. In our study, hepatocellular function enzymes of ALT and AST values were found to be significantly higher in DEN and DEN+EGCG groups compared to EGCG, Sham and Control groups. In EGCG group administered with DEN, it did not decrease ALT value (**Table 5**). This situation reveals that DEN has a negative effect on liver functions and induced hepatic injury. In many studies, in accordance with our study, it was determined that DEN application caused an increase in liver enzyme activity.[24-29]

The erythropoietin (EPO) hormone increases in conditions where oxygen intake is reduced, such as hypoxia, chronic obstructive pulmonary disease (COPD) and climbing to high altitudes.[30] In our study, the amount of EPO was tried to be measured, but could not be measured because it was outside the limits that can be measured by the device.

After the applications, rats gained weight in all groups (**Table 6**). However, there was no significant difference between the groups in terms of weight gain. This situation suggested that the animals were well cared for in the short study period. But previous studies have been shown that a period of EGCG treatment helps to lose the body weight as well as waist circumference and to improve fecal lipid concentration in most rat experiments.[31]

Table 6. Weight records of the groups.

Weight	Control	Sham	DEN	EGCG	DEN+EGCG
Initially	199,60	194,80	193,20	200,40	200,60
SD	21,04	30,31	21,95	31,96	30,75
Before op	220,00	235,40	225,00	224,40	225,00
SD	19,11	26,25	15,78	19,93	19,89

DEN: Diethylnitrosamine, EGCG: Epigallocatechin gallate, DEN+EGCG: Diethylnitrosamine+ Epigallocatechin gallate. Different letters indicate significant difference between groups p<0.05. SD=Standard Deviation.

Table 5: Biochemical parameters of the groups.

	TG	Tchol	LDL	HDL	Glucose	AST	ALT	LDH	ALP
Control	73,50[a]	31,80[b]	15,11[a]	18,74[b]	132,00[a]	54,50[a]	19,30[c]	1237,10[a]	267,80[bc]
Sham	66,40[a]	27,60[b]	15,45[a]	16,29[b]	119,60[a]	49,60[a]	21,00[bc]	1158,50[a]	289,10[abc]
DEN	70,10[a]	30,70[b]	14,43[a]	19,60[b]	106,60[a]	61,70[a]	25,10[ab]	1475,00[a]	327,10[ab]
EGCG	80,78[a]	35,00[ab]	17,80[a]	20,37[b]	115,38[a]	58,56[a]	21,56[bc]	1267,38[a]	226,38[c]
DEN+EGCG	56,50[a]	42,00[a]	16,98[a]	25,91[a]	136,30[a]	77,30[a]	29,20[a]	986,40[a]	339,40[a]

DEN: Diethylnitrosamine, EGCG: Epigallocatechin gallate, DEN+EGCG: Diethylnitrosamine+Epigallocatechin gallate. Different letters indicate significant difference between groups p<0.05. TG: Trigliseride, Tchol; Totol Cholesterol, LDL: Low-density lipoprotein, HDL: High-density lipoprotein. AST: Aspartate amino transferase, ALT: Alanine aminotransferase, LDH: Lactate dehydrogenase, ALP: Alkaline Phosphatase. Different letters indicate significant difference between groups p<0.05.

CONCLUSION

DEN is an environmental carcinogen that produces toxic effects primarily in the blood and liver.[3,4] In different studies, EGCG has anti-inflammatory, antimicrobial, antiviral and antioxidant effects, important protective role in a lot of diseases.[32-33] In this study, consumption of the polyphenolic compound of low-dose EGCG in green tea may be protective against DEN administered rats. There are also some limitations in this study. For example, we did not comprehensively evaluate parameters such as biochemical antioxidant parameters. Therefore, future studies are still needed to investigate such parameters. However, it is thought that studies with different doses and different durations are required for clinical use.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Van Yüzüncü Yıl University Ethics Committee (Date: 26.12.2023, Decision No: 6343).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article / Orijinal Araştırma

The Effect of Estimated Glomerular Filtration Rate on Mortality in the Elderly COVID-19 Patients in the Intensive Care Unit

Yoğun Bakımda Yatan Yaşlı COVID-19 Hastalarında Tahmini Glomerüler Filtrasyon Hızının Mortaliteye Etkisi

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Abstract

Aim: Acute kidney injury (AKI) has been reported in patients with COVID-19 pneumonia and associated with higher mortality. Our study aimed to determine the relationship of eGFR during admission to the intensive care unit with mortality and clinical outcomes in the elderly COVID-19 patients.

Material and Method: This study in which the elderly patients were included was retrospectively performed in a single-center intensive care unit (ICU).

Results: A total of 152 patients including 75 female and 77 male patients were included in the study. Mean age of the patients was 74.3±7.3 years. The number of patients was 92 (60.5%) in eGFR Stage 1-2, 15 (9.9%) in Stage 3a, 26 (17.1%) in Stage 3b, and 19 (12.5%) in Stage 4-5. The rate of patients who received invasive mechanical ventilation was 40.8% and hospital mortality rate was 48.7%. According to the multivariate logistic regression analysis, eGFR, LDH, Charlson score, and duration of stay in the intensive care unit were effective on mortality. Compared to eGFR Stage 1-2 patients, the mortality risk was 4.836 times higher in Stage 3a patients, 12.233 times higher in Stage 3b patients and 10.242 times higher in Stage 4-5 patients.

Conclusion: Our results revealed that COVID-19 patients' eGFR during admission to the intensive care unit, LDH, Charlson score, and duration of stay in the intensive care unit were effective on mortality.

Keywords: COVID-19, intensive care unit, acute kidney injury, mortality

Öz

Amaç: Akut böbrek hasarı(AKI), COVID-19 pnömonisi olan hastalarda bildirilmiştir ve daha yüksek mortalite ile ilişkilidir. Çalışmamızın amacı yaşlı COVID-19 hastalarında yoğun bakıma yatıştaki eGFR ile mortalite ve klinik sonuçlar arasındaki ilişkiyi belirlemektir.

Gereç ve Yöntem: Yaşlı katılımcıların dahil edildiği bu çalışma, tek merkezli bir yoğun bakım ünitesinde (YBÜ) retrospektif olarak yapıldı.

Bulgular: Çalışmaya 75 kadın ve 77 erkek olmak üzere toplam 152 hasta dahil edildi. Hastaların yaş ortalaması 74,3±7,3 yıl idi. eGFR için Evre 1-2'deki hasta sayısı 92 (%60,5), Evre 3a'daki hasta sayısı 15 (%9,9), Evre 3b'deki hasta sayısı 26 (%17,1) ve Evre 4-5'deki hasta sayısı 19'dur (%12,5). İnvaziv mekanik ventilatör uygulanan hastalar %40,8, hastane mortalitesi %48.7 idi. Yapılan multivariate logistic regression analizine göre eGFR, LDH, Charlson skoru ve yoğun bakım yatış süresi mortalite üzerine etkili bulunmuştur. eGFR Evre 1-2'de olan hastalara göre mortalite riski Evre 3a hastalarında 4.836 kat, Evre 3b hastalarında 12.233 kat ve Evre 4-5 hastalarında 10.242 kat fazladır.

Sonuç: Bulgularımız, COVID-19 hastalarının yoğun bakım ünitesine başvuru anında eGFR, LDH, Charlson skoru ve yoğun bakım yatış süresi mortalite üzerine etkili bulunmuştur.

Anahtar Kelimeler: COVID-19, yoğun bakım ünitesi, akut böbrek hasarı, mortalite

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INTRODUCTION

Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), commonly known as "COVID-19", was accepted as a public health emergency of international concern by the World Health Organization (WHO) on the 11th of March 2020.[1] Acute kidney injury (AKI) is one of the most common extrapulmonary complications of this disease and according to a study performed on patients hospitalized with COVID-19, AKI occurred in variable severity in 46% of these patients.[2] Pathophysiology of AKI reveals that it is a result of both direct and indirect effects of SARS-CoV-2 infection including systemic inflammatory responses, activation of Renin-Angiotensin-Aldosterone-System (RAAS), endothelial dysfunction, and coagulation.[3]

The estimated glomerular filtration rate (eGFR) is a calculation based on serum creatinine, age, race, gender, and body size and used as a measurement of kidney function.[4] It has been defined as the sign of mortality in COVID-19 patients and non-COVID-19 patients who had both acute and chronic kidney disease (CKD).[5]

Kidney failure represents a para-physiological case secondary to aging with an annual decrease of approximately 1 mL/min in GFR. Additionally, metabolic comorbidities such as diabetes mellitus and hypertension negatively affect renal function and cause a more rapid decrease in GFR.[6]

Our study aimed to determine the relationship of eGFR during admission to the intensive care unit with mortality and clinical outcomes including the duration of hospital stay and duration of stay in the intensive care unit in the elderly COVID-19 patients.

MATERIAL AND METHOD

This study was retrospectively performed in a single-center intensive care unit (ICU) between August 2020 and February 2021. Inclusion criteria of the study were as follows: being diagnosed with COVID-19 (positive SARS-CoV-2 reverse transcriptase-polymerase chain reaction (RT-PCR) test and thorax CT compatible with COVID-19 infection) and being at the age of 65 and above.

Patients under the age of 65, patients with chronic renal failure, patients with advanced malignancy, patients with hematologic malignancy, patients with acute myocardial infraction, and patients with acute ischemic or hemorrhagic stroke were excluded from the study. This study was performed in accordance with the principles of the Declaration of Helsinki and approved by the institutional ethics committee (Date: 20/12/2022, Number: 766).

Data were collected from medical records of patients. Demographic characteristics, presence of comorbidity, eGFR, troponin, WBC, lymphocyte, neutrophil, platelet count, INR, D-dimer, ferritin, lactate dehydrogenase (LDH), lactate, PaO_2/FiO_2 ratio, disease severity scores, Glasgow Coma Scale (GCS), Charlson Comorbidity Index (CCI), Sequential Organ Failure Assessment (SOFA), Acute Physiology and Chronic Health Evaluation II (APACHE II), need for Mechanical Ventilation (MV) (Invasive), number of days with ventilation, duration of hospital stay, duration of stay in the ICU, hospital mortality, and need for dialysis were recorded during admission to the intensive care unit. Patients were divided into two groups as surviving and non-surviving patients. The two groups were compared according to their demographic data, comorbidities, characteristics during admission, lab results, eGFR value, need for invasive MV, need for dialysis, duration of hospital stay, and duration of stay in the intensive care unit.

eGFR Measurement

eGFR value of the participants were obtained from their medical records. eGFR was calculated based on the Modifcation of Diet in Renal Disease (MDRD) formula.[7]

Kidney function was assessed as eGFR during admission to the intensive care unit and categorized as follows: Stage 1 and 2 (from normal kidney function to mildly decreased kidney function, eGFR ≥ 60 ml/min/1.73m²); Stage 3a (mildly to moderately decreased kidney function, eGFR ≥ 45-59 ml/min/1.73m²); Stage 3b (moderately to severely decreased kidney function, eGFR 30-44 ml/min/1.73m²); and Stage 4 and 5 (severely decreased kidney function to very severe kidney failure, eGFR 1-29 ml/min/1.73m²).[8]

Statistical Analysis

Data were assessed on IBM SPSS Statistics Standard Concurrent User V 26 (IBM Corp., Armonk, New York, USA) and MedCalc® Statistical Software version 19.6 (MedCalc Software Ltd, Ostend, Belgium) programs. Descriptive statistics were expressed as number of units (n), percentile (%), mean±standard deviation (mean±sd), median (M), minimum (min), maximum (max), and interquartile range (IQR) values. Normal distribution of data of the numerical variables was assessed with Shapiro–Wilk normality test. Normally distributed numerical data of the exitus and discharged patients according to the hospital outcomes were compared with independent samples t test and non-normally distributed numerical data were compared with Mann-Whitney U test. Comparison of the exitus and discharged patients according to the hospital outcomes was performed with Pearson's chi-square or Fisher's exact test. Performance of eGFR in predicting mortality was assessed with the Receiver Operating Characteristics (ROC) curve analysis. $p<0.05$ was accepted as the statistically significant value.

RESULTS

A total of 152 patients including 75 (49.3%) female and 77 (50.7%) male patients were included in the study. Mean age of the patients was 74.3±7.3 years and their ages ranged from 65 to 93 years. As comorbidities, 59 patients (38.8%) had diabetes, 85 (55.9%) had hypertension and 35 (23.0%) had chronic pulmonary disease. The number of patients was 92 (60.5%) in

Stage 1-2 for eGFR, 15 (9.9%) in Stage 3a, 26 (17.1%) in Stage 3b, and 19 (12.5%) in Stage 4-5. The number of patients who received invasive mechanical ventilation was 62 (40.8%) and hospital mortality rate was 48.7% (74 patients) (**Table 1**).

There was no statistical difference between the exitus and discharged patients in terms of gender, age and BMI values. For comorbidities, hypertension distributions were statistically different according to hospital outcomes. Thirty (50.8%) of patients with hypertension and 26 (38.8%) of patients without hypertension were exitus. The rate of patients who were exitus was higher among patients with hypertension than among patients without hypertension. eGFR distributions were statistically different according to hospital outcomes. Mortality rate was statistically higher in Stage 3a, Stage 3b and Stage 4-5 than in Stage 1-2. Troponin values during admission were statistically higher in exitus patients than in discharged patients. There was no statistical difference between exitus and discharged patients in terms of neutrophil and platelet values. However, N/L and INR values were statistically higher in exitus patients (**Table 2**).

Table 1: Descriptive Characteristics of Patients (N=152)

Variables	Statistics
Gender, n (%)	
Female	75 (49.3)
Male	77 (50.7)
Age, (year)	74.3±7.3 (65-93)*
BMI, (kg/m²)	27.71±4.68
Comorbidities, n (%)	
Diabetes	59 (38.8)
Hypertension	85 (55.9)
CAD	31 (20.4)
CVD	23 (15.1)
COPD	35 (23.0)
Liver Disease	4 (2.6)
Malignancy	3 (2.0)
eGFR, n (%)	
Stage 1-2	92 (60.5)
Stage 3a	15 (9.9)
Stage 3b	26 (17.1)
Stage 4-5	19 (12.5)
Troponin during admission	21.0 (32.9)
WBC	11.65 (7.11)
Lymphocyte	0.68 (0.61)
Neutrophil	10.38 (7.35)
Platelet	245.5 (144.7)
INR	1.13 (0.21)
D-Dimer	1700 (2696)
Ferritin	643 (861)
LDH	469 (272)
Lactate	1.70 (1.30)
PaO_2/FiO_2	84.0 (114.2)
GCS during admission	15 (3)
CHARLSON score	4 (2)
SOFA during admission	5 (3)
APACHE II during admission	14 (9)
Mechanical ventilation, n (%)	
Yes	90 (59.2)
No	62 (40.8)
Number of days with ventilation	7 (8)
Duration of hospital stay	15 (16)
Duration of stay in the ICU	10 (9)
Hospital outcome - ex, n (%)	74 (48.7)

Numerical data are given as mean±standard deviation or median (interquartile range) values. *(minimum-maximum) values. BMI:Body Mass Index, CAD:Coronary Artery Disease, CVD:Verebro Vascular Disease,COPD:Chronic Obstructive Pulmonary Disease; WBC: white Blood Cell, INR: International Normalized Ratio,LDH: Lactate Dehydrogenase,PaO_2/FiO_2: Ratio of Arterial Oxygen Partial Pressure to Fractional İnspired Oxygen,GCS: Glasgow Coma Score,SOFA: sequential Organ Failure Assessment, APACHE II: Acute Physiology and Chronic Health Evaluation II, ICU: Intensive Care Unit

Table 2: Comparison of Variables according to the Hospital Outcomes

	Hospital Outcome		Test Statistics	
	Exitus	Discharged	Test value	p value
Gender, n (%)			2.146	0.143†
Female	32 (42.7)	43 (57.3)		
Male	42 (54.5)	35 (45.5)		
Age, (year)	75.1±7.2	73.6±7.4	1.245	0.215&
BMI, (kg/m²)	28.09±4.9	27.34±4.37	0.979	0.329&
Comorbidities, n (%)				
Diabetes			0.181	0.671†
No	44 (47.3)	49 (52.7)		
Yes	30 (50.8)	29 (49.2)		
Hypertension			4.680	0.031†
No	26 (38.8)	41 (61.2)		
Yes	48 (56.5)	37 (43.5)		
CAD			1.372	0.242†
No	56 (46.3)	65 (53.7)		
Yes	18 (58.1)	13 (41.9)		
CVD			0.294	0.655†
No	64 (49.6)	65 (50.4)		
Yes	10 (43.5)	13 (56.5)		
Chronic Pulmonary Disease			1.302	0.335†
No	54 (46.2)	63 (53.8)		
Yes	20 (57.1)	15 (42.9)		
Liver Disease			0.003	>0.999†
No	72 (48.6)	76 (51.4)		
Yes	2 (50.0)	2 (50.0)		
Malignancy			0.289	>0.999†
No	73 (49.0)	76 (51.0)		
Yes	1 (33.3)	2 (66.7)		
eGFR, n (%)			42.103	<0.001†
Stage 1-2	26 (28.3)[a]	66 (71.7)		
Stage 3a	10 (66.7)[b]	5 (33.3)		
Stage 3b	21 (80.8)[b]	5 (19.2)		
Stage 4-5	17 (89.5)[b]	2 (10.5)		
Troponin during admission	27.0 (43.7)	17.8 (19.2)	2.768	0.006‡
WBC	11.17 (7.79)	11.89 (7.80)	0.190	0.849‡
Lymphocyte	0.64 (0.45)	0.74 (0.92)	2.038	0.042‡
Neutrophil	9.83 (7.22)	10.08 (7.62)	0.219	0.827‡
NLR	15.07 (17.02)	13.17 (12.66)	2.072	0.038
Platelet	220.0 (161.5)	252.00 (141.25)	1.301	0.193‡

%: Row percent, Numerical data are given as mean±standard deviation or median (interquartile range) values, †: Chi square test, &: Independent samples t test, ‡: Mann-Whitney U test. a and b superscripts indicate differences between categories in each column. There is no statistically significant difference between groups with the same superscripts.BMI:Body mass index, CAD:Coronary artery disease, CVD:cerebrovascular disease, eGFR:Estimated Glomerular Filtration Rate, WBC: white blood cell, NLR: Neutrophil-to-lymphocyte ratio

Ferritin, LDH, AST, procalcitonin, CHARLSON score, SOFA score during admission and APACHE scores during admission were statistically higher in exitus patients than in discharged patients. Lymphocyte, PaO_2/FiO_2 and PO_2 values and duration of hospital stay were statistically lower in exitus patients than in discharged patients. The rate of patients who were exitus was statistically higher in patients who received invasive Mechanical ventilation compared to the patients who did not receive MV. The rate of patients who were exitus was statistically higher among patients who needed dialysis compared to the patients who did not need dialysis (**Table 2**).

In single-variable comparisons performed according to hospital outcomes in **Table 2**, the variables with p<0.25 value were analyzed with multivariate binary logistic regression analysis. Backward Wald elimination method was used to determine terminal factors effective on mortality. According to **Table 3**, eGFR, LDH, Charlson score, and duration of stay in the intensive care unit were effective on mortality. Compared to eGFR Stage 1-2 patients, the mortality risk was 4.836 times higher in Stage 3a patients, 12.233 times higher in Stage 3b patients and 10.242 times higher in Stage 4-5 patients. The mortality risk increased as LDH, Charlson score and duration of stay in the intensive care unit increased. According to Hosmer-Lemeshow test, the established model provided the goodness-of-fit (p=0.417). Variables in the model revealed the mortality rate as 54.1% (**Table 3**).

Table 2: Comparison of Variables according to the Hospital Outcomes

	Hospital Outcome		Test Statistics	
	Ex	Discharged	Test value	p value
INR (continued)	1.17 (0.28)	1.08 (0.16)	3.140	0.002‡
D-Dimer	2210 (2781)	1473 (2176)	1.346	0.178‡
Ferritin	782 (1235)	511 (649)	3.197	0.001‡
LDH	557 (350)	399 (266)	3.989	<0.001‡
Lactate	1.70 (1.33)	1.50 (1.13)	1.715	0.086‡
PaO_2/FiO_2	77.2 (31.2)	150.0 (144.7)	4.633	<0.001‡
Hemoglobin	12.75 (3.15)	12.30 (2.03)	0.382	0.703‡
Fibrinogen	6440 (1880)	5700 (2930)	1.070	0.285‡
AST	39.00(26.0)	27.5 (20.2)	3.533	<0.001‡
ALT	22.0 (20.5)	22.0 (23.2)	0.680	0.497‡
GGT	41.5 (42.7)	37.5 (54.7)	0.900	0.368‡
CRP	103.5 (138.9)	86.7 (132.0)	1.513	0.130‡
Procalcitonin	0.32 (0.66)	0.16 (0.44)	3.167	0.002‡
PH	7.40 (0.13)	7.42 (0.11)	1.796	0.072‡
PaO_2	63.0 (27.5)	68.0 (20.5)	1.994	0.046‡
GCS during admission	15 (5)	15 (2)	1.410	0.159‡
CHARLSON score	5 (3)	4 (3)	3.762	<0.001‡
SOFA during admission	6 (4)	4 (1.2)	5.355	<0.001‡
APACHE II during admission	16 (12)	12 (6.2)	4.342	<0.001‡
Mechanical Ventilation, n (%)				
No	18 (20.0)	72 (80.0)	72.668	<0.001†
Yes	56 (90.3)	6 (9.7)		
Number of Days with Ventilation	7 (8)	7 (16)	0.066	0.957‡
Need for Dialysis, n (%)				
No	61 (44.9)	75 (55.1)	7.513	0.008†
Yes	11 (84.6)	2 (15.4)		
Duration of hospital stay	13.0 (15.5)	17.5 (16.2)	1.992	0.046‡
Duration of stay in the ICU	10.0 (12.5)	9.5 (8.5)	1.372	0.170‡

%: Row percent, Numerical data are given as mean±standard deviation or median (interquartile range) values, †: Chi square test, &: Independent samples t test, ‡: Mann-Whitney U test.INR: International Normalized Ratio, LDH: Lactate dehydrogenase, PaO_2/FiO_2: Ratio of arterial oxygen partial pressure to fractional inspired oxygen, AST: aspartate aminotransferase, ALT: alanine aminotransferase, GGT:Gama Glutamil Transferaz, CRP: C-reactive protein, GCS: Glasgow coma score, SOFA: sequential organ failure assessment, APACHE II: Acute Physiology and Chronic Health Evaluation II, ICU: Intensive Care Unit;

Table 3: Assessment of factors affecting mortality with Multivariate Binary Logistic Analysis

	Regression Coefficients						
	β	Standard Error	Wald statistics	p	Exp(β)	95% Confidence Interval for exp(β)	
						Lower Bound	Upper Bound
Constant	-5.988	1.230	23.713	<0.001	0.003		
eGFR							
Stage 1-2	Ref						
Stage 3a	1.576	0.737	4.569	0.033	4.836	1.140	20.517
Stage 3b	2.504	0.768	10.633	0.001	12.233	2.716	55.107
Stage 4-5	2.327	0.959	5.884	0.015	10.242	1.563	67.115
LDH	0.005	0.001	11.984	0.001	1.005	1.002	1.008
Charlson score	0.358	0.156	5.260	0.022	1.431	1.053	1.943
Duration of stay in ICU	0.096	0.033	8.516	0.004	1.101	1.032	1.175

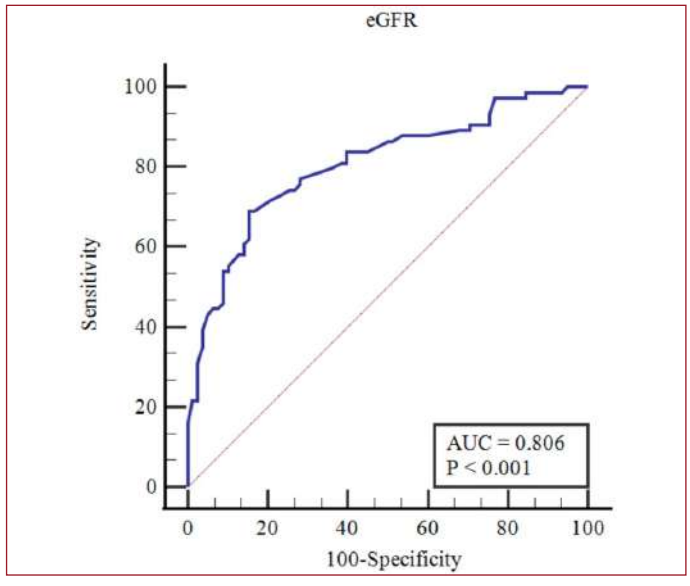
Variables in the model: gender, age, hypertension, CAD, eGFR, Troponin during admission, lymphocyte, platelet, INR, D_dimer, ferritin, LDH, Lactate, PaO_2/FiO_2, glucose, CRP, procalcitonin, PH, PO_2, GCS_ during admission, CHARLSON_score, SOFA_ during admission, APACHE_ during admission, Mech. vent, duration of hospital stay, duration of stay in ICU, need for dialysis. Model Statistics: Hosmer and Lemeshow Test $\chi 2$=8.166; p=0.417; Nagelkerke R2=0.541 Elimination Method: Backward Wald. eGFR:Estimated Glomerular Filtration Rate, LDH: Lactate dehydrogenase, ICU: Intensive Care Unit

When the performance of eGFR in predicting mortality was assessed by ROC Curve Analysis, it had 68.9% sensitivity and 84.6% specificity when eGFR was ≤60.0 in predicting mortality (**Table 4**, **Graph 1**).

Table 4: Assessment of the performance of eGFR in predicting mortality with ROC Curve Analysis

	AUC (95.0% CI)	p	Cutoff	Sensitivity (95.0% CI)	Specificity (95.0% CI)
eGFR	0.806 (0.734-0.865)	<0.001	≤60.0	68.9 (57.1-79.2)	84.6 (74.7-91.8)

AUC: Area under the curve, CI: Confidence interval, eGFR:Estimated Glomerular Filtration Rate



Graph 1: ROC curve for eGFR in predicting mortality

DISCUSSION

In a study including 152 patients above the age of 65 who were followed up with severe COVID-19 in the intensive care unit of a tertiary care hospital, hospital mortality rate was 48.7% and eGFR was <60 ml/min/1.73m² in 39.5% of the patients. Most of the previous studies used a paired comparison to compare Stage 1-2 with Stage 3a, 3b, 4 and 5 and revealed the relationship between eGFR and mortality.[9,10] A more detailed classification was performed in our study.

In our study, mortality rate was higher among patients with hypertension. In addition, ferritin, LDH, AST, procalcitonin, Charlson score, SOFA score during admission, and APACHE score during admission were higher and lymphocyte and PaO_2/ FiO_2 ratio were lower in patients who were exitus. In an international multicenter study including a total of 758 adult COVID-19 patients, 8.5% of the patients had chronic renal failure history and kidney dysfunction was reported in 30% of the patients during admission (eGFR < 60 mL/min/1.73m²). It was reported in the multivariate analysis that age, hypertension, renal dysfunction, oxygen saturation<92%, and high LDH during admission independently predicted all-cause mortality, which is similar to the findings in our study.[9]

In our study, mortality rate was higher in patients who needed invasive MV and dialysis. In a multi-center retrospective study performed on critical COVID-19 patients, 85.1% of 1286 patients had AKI and kidney replacement therapy was used in 9.8% of them. Advanced age, obesity, higher APACHE II score, and use of mechanical ventilation in the 1st day of intensive care unit stay were associated with the increasing risk of AKI. All AKI stages were associated with ICU mortality in the multivariate analysis.[11] In our study, logistic regression analysis revealed that eGFR, LDH, Charlson score, and duration of stay in the intensive care unit were effective on mortality and compared to eGFR Stage 1-2 patients, the mortality risk was 4.836 times higher in Stage 3a patients, 12.233 times higher in Stage 3b patients and 10.242 times higher in Stage 4-5 patients. The mortality risk increased as LDH, Charlson score and duration of stay in the intensive care unit increased.

Pathophysiology of AKI in critical COVID-19 is multifactorial. Acute tubular injury is the most common histologic finding; however, collapsing glomerulopathy and thrombotic microangiopathy were observed in this population. Other rare findings such as anti-neutrophil cytoplasmic antibody vasculitis, anti-glomerular basement membrane disease and podocytopathies were also reported.[12-14] As well as endothelial dysfunction, complement activation and local and systemic inflammatory responses may also play role. Renal tropism of SARS-CoV-2 with direct invasion of kidney is recommended, but it is controversial.[15] In critical COVID-19, indirect factors such as presence of hypoxemia, hypotension, hypo or hypervolemia and also use of high positive end expiratory pressure mechanical ventilation or high inspiration pressure and nephrotoxic drugs may also have contribution.[12,14]

In a study performed by Aukland et al. on 361 COVID-19 patients in Norway, AKI was detected during admission to the intensive care unit in 32.0% of patients. Age, acute circulatory failure during admission to hospital and AKI during admission to ICU were reported as determinants of both 30-day and 90-day mortality.[16] In systematic review and meta-analysis of 54 studies, AKI occurred in about 30% of the patients who were hospitalized with COVID-19, the course of >45% of patients who needed intensive care became complicated and 1 out of 5 patients who were admitted to the intensive care unit received kidney replacement therapy.[17]

In a study by Fisher et al., the incidence of acute kidney injury was compared in patients who were hospitalized with COVID-19 and without COVID-19 and the incidence of acute kidney injury was found higher in COVID-19 patients compared to the control group (56.9% vs. 25.1% respectively).[18] In a meta-analysis by Fabrizi et al., the incidence rate of acute kidney injury in patients with severe COVID-19 was 53% (95% CI: 42.7-63.3%).[19] Similar results were found in the meta-analysis by Hansrivijit et al.. They reported that the incidence of acute kidney injury was higher in critical patients compared to the other COVID-19 patients (7.3% vs. 19.9%).[20]

Chan et al. found in their study that in-hospital mortality rate was 50% in COVID-19 patients with acute kidney injury and 8% in those without acute kidney injury.[2] Lin et al. revealed in their meta-analysis that acute kidney injury in patients who were hospitalized with COVID-19 was associated with a significant increase in the mortality risk.[21] Similar results were found in the study performed by Fisher et al. on 3,345 patients with COVID-19. According to their study, mortality rate was significantly higher in patients with COVID-19 and acute kidney injury compared to the patients who were hospitalized with COVID-19 (33.7% vs. 9.3% respectively).[18] In the meta-analysis of 142 studies performed by Fu et al. on 49,048 patients who were hospitalized with COVID-19, a significant increase was observed in mortality risk in patients with accompanying acute kidney injury.[22] In our study, when eGFR was evaluated by ROC Curve Analysis, it had 68.9% sensitivity and 84.6% specificity when eGFR was ≤60.0 in predicting mortality.

A total of 152 patients who were admitted to the intensive care unit were included in this retrospectively performed single-center study, which limits the study to be generalized. Only electronic health record systems were used to define AKI in this study. AKI was classified using only eGFR for main analysis, not the urinary criteria, which may have caused AKI rate to seem lower. In addition, we did not have the data of patients after their discharge. Therefore, we could not assess the effects of COVID-19 on long-term survival and kidney function.

CONCLUSION

Our data revealed that COVID-19 patients' eGFR value during admission to the intensive care unit, LDH, Charlson score and duration of stay in the intensive care unit were effective on mortality. Therefore, clinicians in developing countries should increase their awareness on kidney disease in severe COVID-19 patients and focus on increasing primary prevention and population training in order to use preventive measures against COVID-19. It is also recommended to perform further similar studies with larger sample size.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Kayseri City Training and Research Hospital Clinical Researches Ethics Committee (Date: 20/12/2022, Number: 766).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

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

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Original Article / Orijinal Araştırma

Is Upper Gastrointestinal Tract Screeing Necessary in Kidney Transplant Candidates?

Böbrek Nakil Adaylarında Üst Gastrointestinal Sistem Taraması Gerekli Mi?

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Abstract

Aim: The aim of this study is to present and discuss upper gastrointestinal tract endoscopy findings in renal transplant candidates with a high incidence of gastrointestinal system diseases.

Material and Method: Between January 2014 and December 2019, patients over the age of 18 who were on dialysis for chronic renal failure and renal transplant candidates at Karadeniz Technical University Farabi Hospital were included in the study. Upper gastrointestinal endoscopic findings and pathology results (atrophy, *Helicobacter pylori* and intestinal metaplasia) of the patients were retrospectively evaluated by scanning from the electronic archive system of the hospital.

Results: The study included 105 patients. 53 (50.5%) of the patients were male. The mean age of the patients was 44.09±14.16 years and there was no statistically significant difference between male and female sexes (p=0.961). The most common endoscopic findings were pangastritis (44.8%), antral gastritis (27.6%) and esophagitis (16.2%). Only 4 (3.8%) patients had duodenal ulcer. Atrophy was positive in 3 (13%) of 23 patients, HP was positive in 19 (27.1%) of 67 patients and IM was positive in 17 (24.3%) of 56 patients.

Conclusion: Patients who are renal transplant candidates should be reviewed for indication of routine upper endoscopic examination before transplantation to prevent upper GI tract complications that may develop after transplantation.

Keywords: Endoscopy, renal transplant candidate, chronic kidney disease

Öz

Amaç: Çalışmadaki amacımız gastrointestinal sistem hastalık insidansı yüksek olan renal nakil adayı olan hastalarda üst GIS endoskopi bulgularını sunmak ve tartışmaktır.

Gereç ve Yöntem: Ocak 2014-Aralık 2019 tarihleri arasında Karadeniz Teknik Üniversitesi Farabi Hastanesi'nde kronik böbrek yetmezliği nedeniyle diyalize giren, renal nakil adayı olan 18 yaş üstündeki hastalar çalışmaya dâhil edildi. Hastaların üst gastrointestinal endoskopik bulguları ve patoloji sonuçları (atrofi, *Helicobacter pylori* ve intestinal metaplazi) retrospektif olarak hastanenin elektronik arşiv sisteminden taranarak değerlendirildi.

Bulgular: Çalışmaya 105 hasta dâhil edildi. Hastaların 53'ü (%50,5) erkek idi. Hastaların ortalama yaşı 44,09±14,16 idi, kadın ve erkek cinsiyet arasında yaş açısından istatistiksel olarak anlamlı farklılık saptanmadı (p=0,961). En sık görülen bulgular endoskopik bulgular pangastrit (%44,8), antral gastrit (%27,6) ve özofajit (%16,2) idi. Sadece 4 (%3.8) olguda duodenal ülser belirlendi. Atrofi değerlendirilmesi yapılabilen 23 hastanın 3'ünde (%13) atrofi, HP değerlendirmesi yapılan 67 hastanın 19'unda (%27,1) HP ve intestinal metaplazi (IM) değerlendirmesi yapılan 56 hastanın 17'sinde (%24,3) IM pozitif saptandı.

Sonuç: Renal nakil adayı olan hastalar nakil sonrası gelişebilecek üst GI sistem komplikasyonlarının önlenmesi amacıyla nakil öncesi rutin üst endoskopik inceleme endikasyonu gözden geçirilmelidir

Anahtar Kelimeler: Endoskopi, renal nakil adayı, kronik böbrek hastalığı

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INTRODUCTION

Chronic kidney disease (CKD) is a progressive and irreversible disease characterized by a decrease in the number of nephrons and renal function, usually progressing to end-stage renal failure. Uremia is a syndrome caused by renal failure, affecting the gastrointestinal system and other organs, with clinical and laboratory findings.[1] Gastrointestinal (GI) disorders are among the leading chronic diseases seen in patients with end-stage renal disease (ESRD).[2]

GI symptoms have been reported in more than 80% of dialysis patients.[3] These symptoms may increase as kidney disease progresses.[4] GI diseases are often not detected by clinical findings and diagnostic tests.[5] Therefore, endoscopic screening in patients with CKD is essential to detect GI diseases.

According to many studies, the most common upper GI diseases in patients with CKD are gastritis, erosive duodenitis and esophagitis, peptic ulcer, H.pylori infections and bleeding. The causes of GI tract damage include medications, metabolic disorders, gastrin, gastroesophageal reflux disease and infection due to *Helicobacter pylori* (HP).[2]

Patients who are preparing for kidney transplantation, either from a living donor or a cadaveric donor, need to know their GI system in advance and make the necessary preparations because they will undergo a major surgery that will affect them metabolically and psychologically.

Patients are at high risk for GI tract complications because they will receive high dose corticosteroid therapy and other immunosuppressive therapies after renal transplantation (G9). According to many studies, there is no consensus regarding upper GI tract endoscopy before renal transplantation.[6] Many studies in kidney transplant candidates have found high incidences of GI tract diseases.[7]

The aim of this study was to investigate the necessity of pre-transplant screening of the upper GI tract in patients who will be under great metabolic and psychological stress after kidney transplantation and whether there is a need to take precautions for complications such as gastric ulcer/bleeding that may develop due to changes in the treatment plan after transplantation.

MATERIAL AND METHOD

Between January 2014 and December 2019, patients over the age of 18 who were on dialysis for chronic renal failure and renal transplant candidates at Karadeniz Technical University Farabi Hospital were included in the study. Upper gastrointestinal tract endoscopic findings and pathology results (atrophy, *Helicobacter pylori* and intestinal metaplasia) of the patients were retrospectively scanned from the electronic archive of the hospital.

Upper GI tract endoscopy was performed under sedoanalgesia after at least 8 hours of fasting. Two biopsies were taken from at least one region of the antrum, corpus, fundus and/or cardia of the stomach and placed in 5 ml formalin-containing containers. The biopsy materials were evaluated by Giemsa method for HP and PAS/AB pH 2.5 histochemical reaction for IM.This study was designed and conducted according to the principles of the Declaration of Helsinki. The study was carried out with the permission of Karadeniz Technical University Faculty of Medicine Clinical Researches Ethics Committee (Date: 24.11.2021, Decision No: 24237859-849). Since the study was retrospective, informed consent was not obtained from the patients.

Statistical Analysis

SPSS Windows version 22 program was used for statistical tests. Continuous variables were evaluated in terms of normal distribution by histogram, Q-Q graph and Shaphiro-Wilk or Kolmogorov-Smirnov tests according to the number of variables. Continuous variables with normal distribution were presented as mean±standard deviation throughout the study and independent-variables t-test was used to compare two groups. Categorical variables were presented as frequency and percentage. Tests with a p value of 0.05 or less at the 95 percent confidence interval were considered statistically significant.

RESULTS

The study included 105 patients. 53 (50.5%) of the patients were male and 52 (49.5%) were female. The mean age of the patients was 44.09±14.16 years and there was no statistically significant difference in age between male and female (p=0.961) (**Table 1**).

Table 1. Demographic characteristics of the patients

Variable		p
Male / Female, n (%)	53 (50.5) / 52 (49.5)	
Age, mean±SD (years)	44.09±14.16	
Male	44.02±14.76	0.961
Female	44.15±13.66	

Endoscopic findings were normal in 23 patients (21.9%) and abnormal in 82 patients (78.1%). The most common findings were pangastritis (44.8%), antral gastritis (27.6%) and esophagitis (16.2%) (**Table 2**).

Table 2. Endoscopic findings of the patients

Variable.	n (%)
Esophagus	
Esophagitis	17 (16.2)
LA Grade A	14
LA Grade B	3
Hiatal hernia	6 (5.7)
Laxity of the LES	2 (1.9)
Varicose veins	1 (1)
Stomach	
Antral gastritis	29 (27.6)
Erosive antral gastritis	11
Non-erosive antral gastritis	18
Pangastritis	47 (44.8)
Erosive pangastritis	1
Non-erosive pangastritis	46
Fundic gland polyp	3 (2.9)
Duodenum	
Bulbit	29 (27.6)
Erosive bulbit	6
Non erozive bulbit	23
Ulcer	4 (3.8)

*LA: Los Angeles. LES: Lower esophageal sphincter

Biopsies were obtained in 70 of 105 patients (66.7%). Of the patients who underwent biopsy, 47 (67.1%) had non-active chronic gastritis, 17 (24.3%) had active chronic gastritis, and 6 (8.6%) had normal pathology findings (**Table 3**).

Table 3. Pathology findings of the patients

Variable	n (%)
Normal	6 (8.6)
Gastritis	64 (91.4)
Non-active chronic gastritis	47
Active chronic gastritis	17
Atrofi	3 (13)
HP	19 (27.1)
IM	17 (24.3)

*HP: *Helicobacter pylori*, IM: Intestinal metaplasia

Atrophy was positive in 3 of 23 patients (13%), HP was positive in 19 of 67 patients (27.1%) and intestinal metaplasia (IM) was positive in 17 of 56 patients (24.3%) (**Table 3**).

DISCUSSION

The prevalence of CKD and ESRD has been steadily increasing over the last 10 years.[8]

Peptic ulcer and gastric cancer have been associated with chronic HP infection.[9,10] There are studies showing that the frequency of HP infection in patients with CKD is higher than in the general population.[11] Studies have reported HP infection rates between 49% and 66% in CKD patients.[7,11] Netto et al. found the HP positivity rate to be 58.3% in renal transplant candidates in a study conducted in Brazil.[12] The HP positivity rate in our study was found to be 27.1%, which is lower compared to the literature.

Weak immunity and a relatively more frequent history of antibiotic use due to susceptibility to infections in patients with ESRD may explain this difference. However, the HP positivity rate in our CKD patient population was similar to the HP positivity rate of 27.8% in another study in which a total of 2530 patients including the general population admitted to our endoscopy unit were evaluated.[13] Although it is below the average in Turkey, it is similar to the current HP positivity rate in our endoscopy population, suggesting that the difference with the literature may be explained by local prevalence. Most of the HP prevalence rates reported in the literature are based on older studies and the idea that HP prevalence decreases with both treatment and hygiene standards remains a problem that needs to be tested.

Gastric intestinal metaplasia is a precancerous lesion involved in Correa's cascade, a model for gastric cancer development.[14] Netto et al. found intestinal metaplasia positivity in 8.3% of renal transplant candidates.[12] In our study, the IM positivity rate of renal transplant candidates was 24.3%. This rate is similar to the IM rate of 26.8% in our study cited above.[13]

Acute upper GI tract bleeding is higher in patients with ESRD compared to the general population.[15] Because of the high mortality risk, upper GI tract ulcers and erosions should be recognized and treated without bleeding in patients who will receive high-dose corticosteroid therapy after renal transplantation. In our study, duodenal ulcer was observed in 4 patients, while erosions were observed in the stomach in 12 patients and in the bulbus in 6 patients.

Although upper GI tract endoscopy does not require any preparation other than fasting and taking a biopsy does not pose a significant additional risk in renal transplant candidates, the indication for routine upper endoscopic examination of renal transplant candidates should be reviewed because pathologic findings that are statistically different from the general population were not detected. Our retrospective and single-center study has limitations. Multicenter and well-designed studies are needed to develop appropriate screening and treatment protocols for renal transplant candidates.

CONCLUSION

Patients who are renal transplant candidates should be reviewed for indication of routine upper endoscopic examination before transplantation to prevent upper GI tract complications that may develop after transplantation..

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Karadeniz Technical University Faculty of Medicine Clinical Researches Ethics Committee (Date: 24.11.2021, Decision No: 24237859-849).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Erratum / Düzeltme

Erratum to: The Burnout Levels of Caregivers and Caregiver Burden of the Patients with Declined Active Participation in the Community Mental Health Center During the COVID-19 Pandemic

Düzeltme: COVID-19 Pandemisinde Hastaların Toplum Ruh Sağlığı Merkezi'ne Aktif Katılımının Azalmasıyla Bakım Verenlerde Artan Tükenmişlik ve Bakıcı Yükü Düzeyleri

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The author forwarded the following statement to the Editorial Board of the Journal of Contemporary Medicine and requested a correction to be published. "I had an article published in your journal. There is an error in the 'MATERIAL AND METHOD' section of this article that I later noticed. I would like to correct this error."

Wrong Sentence:

"Both interviews were conducted face-to-face by inviting caregivers to the CMHC."

Corrected Sentence:

"The second interview was held face-to-face by inviting the caregivers to the CMHC. The data in the first interview were obtained from a previous interview archive made with caregivers at CMHC, retrospectively, before the pandemic."

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