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Correspondence Address:

Editor in Chief

Burçin NALBANTOĞLU, Prof., M.D.

Tekirdağ Namık Kemal University Faculty of Medicine, Department of Child Health and Diseases, Tekirdağ, Turkey

E-mail: bnalbantoglu@nku.edu.tr

Phone: +90 (282) 250 56 32

Editor

Erdoğan Selçuk ŞEBER, MD

Tekirdağ Namık Kemal University Faculty of Medicine, Division of Medical Oncology, Tekirdağ, Turkey

Phone: +90 282 250 50 00

E-mail: nkmj@nku.edu.tr

Publisher Corresponding Address

Galenos Publishing House

Address: Molla Gürani Mah. Kaçamak Sk.

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ORIGINAL ARTICLE

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The Turkish Validity and Reliability of the Pediatric Vestibular Symptom Questionnaire

Pediatrik Vestibüler Semptom Ölçeği'nin Türkçe Geçerlik ve Güvenirliğinin Araştırılması

Büşra ALTIN[1], Songül AKSOY[2]

[1]*Hacettepe University Faculty of Health Sciences, Department of Audiology, Ankara, Turkey*
[2]*Lokman Hekim University Faculty of Health Sciences, Department of Audiology, Ankara, Turkey*

ABSTRACT

Aim: The Pediatric Vestibular Symptom Questionnaire (PVSQ) identifies and measures subjective vestibular symptoms such as dizziness and imbalance in children. The aim of this study was to investigate the validity and reliability of the Turkish PVSQ in children with dizziness and balance disorders.

Materials and Methods: Fifty-two children (10.9±3.28 years) with complaints of dizziness and 40 healthy children (9.65±2.45 years) aged 6-17 years in the control group were included in the study. The scale normalized score ranges from 0-3. The reliability of the scale was evaluated by Cronbach's alpha coefficient, and validity was evaluated by confirmatory factor analysis (CFA). ROC curve was used to calculate the sensitivity and specificity of PVSQ in discriminating healthy children from those with vestibular symptoms.

Results: The mean PVSQ score of the children included in the study group was 9.81±6.16, and the control group was 1.10±3.12, and a statistically significant difference was found between the mean scores of the two groups ($p<0.001$). As a result of CFA, it was seen that the model was compatible. Turkish PVSQ has a high degree of internal consistency (Cronbach's alpha=0.890). The cut-off point of the PVSQ was 0.35, and the maximum sensitivity of the scale was 0.885, and the specificity was 0.950 at this point.

Conclusion: Turkish version of the PVSQ, used in the diagnosis and evaluation of dizziness and balance disorders in children, is a reliable, valid, easy to use, and brief measurement tool in the evaluation and severity of vestibular symptoms in children.

Keywords: Vertigo, dizziness, pediatrics, questionnaire

ÖZ

Amaç: Pediatrik Vestibüler Semptom Ölçeği (PVSÖ), 6-17 yaş arası çocuklarda dizziness, dengesizlik gibi subjektif vestibüler semptomları belirlemekte ve ölçmektedir. Bu çalışmada baş dönmesi ve denge bozukluğu problemi olan çocuklarda Türkçe PVSÖ'nün geçerlilik ve güvenilirliğinin araştırılması amaçlanmıştır.

Gereç ve Yöntem: Çalışmaya 6-17 yaş arası baş dönmesi/dengesizlik şikayeti olan 52 çocuk (10,9±3,28 yaş) ve kontrol grubuna sağlıklı 40 çocuk (9,65±2,45 yaş) alınmıştır. Ölçek normalize puanı 0-3 arasında değişmektedir. Ölçeğin güvenirliği Cronbach's alfa katsayısı, geçerliği doğrulayıcı faktör analizi (DFA) ile değerlendirilmiştir. PVSÖ'nün sağlıklı çocuklarla vestibüler belirtileri olanları ayırmada duyarlılığını ve özgüllüğünü hesaplamak ve kesim noktasını belirlemek için ROC eğrisi kullanılmıştır.

Bulgular: Çalışma grubuna alınan çocukların PVSÖ puan ortalaması 9,81±6,16, kontrol grubunun 1,10±3,12 olarak elde edilmiş ve iki grubun ortalamaları arasında istatistiksel açıdan anlamlı fark bulunmuştur ($p<0,001$). DFA sonucunda modelin uyumlu olduğu görülmüştür. Türkçe PVBÖ yüksek derecede iç tutarlığa sahiptir (Cronbach's alfa=0,890). PVSÖ'nün kesim noktası 0,35 puan, bu noktada ölçeğin maksimum duyarlılığı 0,885, özgüllüğü 0,950 olarak elde edilmiştir.

Sonuç: Çocuklarda baş dönmesi ve denge bozuklukları problemlerinin tanılanması ve değerlendirilmesinde kullanılan PVSÖ'nün, Türkçe sürümü çocuklarda vestibüler belirtilerin değerlendirilmesinde ve şiddetinin belirlenmesinde güvenilir, yüksek geçerliğe sahip, uygulaması kolay ve kısa süreli bir ölçüm aracıdır.

Anahtar Kelimeler: Baş dönmesi, dengesizlik, pediatri, ölçek

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INTRODUCTION

Vestibular problems are the most common cause of dizziness in childhood and its prevalence is thought to be between 5% and 15%[1-3]. In addition to dizziness and imbalance complaints in children, vestibular disorders can negatively affect educational success and quality of life and cause psychological symptoms and avoidance behaviors[3]. While the leading causes of vestibular problems are benign paroxysmal vertigo and vestibular migraine in childhood, head trauma is the third most common cause of vertigo in children[4]. In addition to these, dizziness associated with otitis media (OM), psychogenic vertigo, vestibular neuronitis, Meniere's disease and central vertigo can also be seen[5,6].

Despite the diversity and incidence in pediatric vestibular diseases, it is noteworthy that when compared to adults, vertigo and imbalance complaints in children have received less attention in the literature. However, it often remains undiagnosed, as health professionals may describe these symptoms in children as a behavioral disorder or "clumsiness"[7]. The reason for this situation may be that children usually cannot express or describe their symptoms without asking appropriate questions, cannot easily accept the symptom terminology suggested by an adult, and may show behaviors such as hugging their parents while experiencing dizziness or lightheadedness[8]. A detailed medical history is the most important parameter of the diagnostic decision-making process to identify the symptoms, triggers, and process of the disease[9]. Therefore, in order to determine the etiology of the problem and make a diagnosis, it is extremely important to help the child describe his symptoms by providing different descriptors to express his complaints.

Although there are a number of questionnaires/scales to evaluate the presence, severity, and impact of vestibular symptoms in adults, there is no Turkish validity and reliability study for the pediatric population. The Pediatric Vestibular Symptom Questionnaire (PVSQ) is a scale developed by Pavlou et al.[10] in 2016 to determine and measure subjective vestibular symptoms such as dizziness and imbalance in children aged 6-17 years. The aim of this study is to investigate the usability of the PVSQ by conducting validity and reliability studies in children with dizziness and balance disorder in Turkish society[10]. Thus, it will be possible to better analyze the problems of children with dizziness and balance disorders and to determine diagnosis-specific treatment and rehabilitation options.

MATERIALS AND METHODS

Participants

The study included 52 children aged 6-17 years, who met the inclusion criteria and were followed up in Hacettepe University Hospital Dizziness and Balance Disorders Research and Application Center and Ear Nose and Throat Department, with the complaints of dizziness and balance disorder between March 2018 and December 2019. Forty children who did not complain of dizziness/imbalance were included as the control group. Participation in the study was on a voluntary basis and written informed consent of the children and their families was obtained. The study was conducted in accordance with the principles of the Declaration of Helsinki and permission was obtained from the Hacettepe University Non-interventional Studies Ethics Committee (decision no: GO 18/162-23, date: 13.02.2018). Twenty-nine girls and 23 boys, with a mean age of 10.9±3.28 (6-17 years), were involved in the study group, and 21 girls and 19 boys, with a mean age of 9.65±2.45 (6-17 years), were involved in the control group. Diagnostic and demographic information of the children are shown in Table 1.

Inclusion criteria for the study;

- Complaints of dizziness and/or balance disorder,

- Being at the age range of 6-17 years.

Exclusion criteria for the study;

- Presence of cognitive and/or orthopedic problems,

- Presence of central disorder other than vestibular migraine.

Table 1. Socio-demographical characteristics of the participants

Variables	Patient group (n=52)	Healthy group (n=40)
Age	10.9±3.28	9.65±2.45
Gender	29 F (55.77%) 23 M (44.23%)	21 F (52.5%) 19 M (47.5%)
Diagnosis		
BPV	n=8 (15.38%)	
VM	n=10 (19.23%)	
Dizziness following OM	n=10 (19.23%)	
BVH	n=3 (5.76%)	
UVH	n=3 (5.76%)	
VN	n=2 (3.84%)	
MD	n=2 (3.84%)	
MS	n=2 (3.84%)	
BHL (using CI)	n=8 (15.38%)	
BHL (using HA)	n=7 (13.46%)	
PVSQ score (mean±SD)	9.81±6.16	1.10±3.12

BPV: Benign positional vertigo, VM: Vestibular migraine, OM: Otitis media, BVH: Bilateral vestibular hypofunction, UVH: Unilateral vestibular hypofunction, VN: Vestibular neuronitis, MD: Meniere's disease, MS: Motion sickness, BHL: Bilateral hearing loss, CI: Cochlear implant, HA: Hearing aid, PVSQ: Pediatric Vestibular Symptom Questionnaire, SD: Standard deviation, F: Female, M: Male

Methods

Volunteer children or parents/caregivers who met the inclusion criteria were asked to complete the PVSQ. For the Turkish adaptation of the scale, Marousa Pavlou, who developed the scale, was contacted and necessary permissions were obtained. The scale was translated into Turkish independently by two translators, one with clinical experience and the other with good command of the language, and then the two translations were compared and turned into a single translated text. Then, the text was re-examined by considering the problems encountered by field experts and independent translators and cultural differences, and was translated back into its original language, English, by two translation experts who had not seen the scale before. This form was compared with the original form and it was accepted that it was equivalent to the Turkish form and it was put into practice.

Pediatric Vestibular Symptom Questionnaire

The PVSQ identifies and measures subjective vestibular symptoms such as dizziness and imbalance in children aged 6-17 years[10]. The scale has two factorial structures: dizziness and balance. Dizziness is a nonspecific symptom defined as "rotational, positional or recurrent dizziness with vestibular vertigo, nausea, gaze instability and/or postural instability". The implementation of PVSQ takes approximately 10 minutes. There are four answer options in the scale, each item is scored between 0 (never) and 3 (most of the time), and there is an option of "I don't know". There are a total of 11 questions in the scale and the total score varies between 0 and 30. Normalization is achieved by dividing the total score by the number obtained by subtracting the number of "I don't know" answers from the total number of questions [total score / (total number of questions - "I don't know" answers)]. Ten questions are used in the normalization equation. For example, if the participant scored seven of the 10 questions between 0 and 3 and got 20 points, and gave the answer "I don't know" to the remaining three, the participant's score obtained as a result of normalization is 20/(10-3)=20/7=2.85. The eleventh question asks whether symptoms prevent participation in activities and, if so, what activities. Higher scores from the scale indicate an increase in symptom severity.

Statistical Analysis

Descriptive analyses are given using mean (X) and standard deviation (SD). In order to examine the normality assumptions of continuous variables, before determining the construct validity of the scale, the correlation matrix was examined with the Bartlett's Test of Sphericity and whether the determinant was close to zero or not was evaluated with the Kaiser-Meyer-Olkin (KMO) coefficient. In order for the factor analysis to give reliable results, the KMO should be higher than 0.60 and the Bartlett's test should be significant at the $p<0.001$ significance level[11]. Afterwards, confirmatory factor analysis was performed and the Cronbach's alpha analysis was used to determine the internal reliability of the scale. The ROC (receiving operating characteristics) curve was used to calculate the sensitivity and specificity of PVSQ in distinguishing healthy children from those with vestibular symptoms. In all analyses, $p<0.05$ was accepted as the level of significance. Statistical Package for the Social Sciences (SPSS) V23.0 and AMOS 23.0 (IBM SPSS Statistics, USA) statistical programs were used for data analysis.

RESULTS

There was no statistically significant difference between the mean ages of the participants in the study group and in the control group ($p=0.082$). The mean PVSQ score of the children included in the study group was 9.81 ± 6.16 and 1.10 ± 3.12 in the control group and a statistically significant difference was found between the mean scores of the two groups ($p<0.001$).

Validity of the Scale

Construct Validity

As a result of the analyses, the KMO coefficient was determined as 0.872. The Bartlett's test of sphericity χ^2 value was determined as 528,329 (SD=10, $p<0.001$). The scale originally had two sub-dimensions. Among the parameters used to determine the fit adequacy of the model tested in confirmatory factor analysis (CFA), the chi-square goodness test was obtained as 49.462, the Goodness of Fit Index as 0.906, the Adjusted Goodness of Fit Index as 0.844, the Comparative Fit Index as 0.959, the Normed Fit Index as 0.889, and the Root Mean Square Error of Approximation (RMSEA) as 0.74. The value of RMSEA below 0.8 and other values above 0.9 indicates that the adaptability of the scale is appropriate. The value of chi-square/degrees of freedom (χ^2/SD) was found as 1.49. In Figure 1, the path diagram showing the distribution according to dizziness and balance factors is given.

Reliability

Internal Consistency Reliability

In this study, the reliability of the PVSQ was calculated with the internal consistency coefficient (Cronbach's alpha). This value was found to be 0.896, which is above 0.70 and indicates that the scale has a high degree of internal consistency. The sub-dimensions of the scale and the total Cronbach's alpha coefficients are given in Table 2. When the item was deleted, there was no significant change in Cronbach's alpha scores (range, 0.87-0.89). All items have a significant correlation with the total score and a high level of consistency with each other (Table 3).

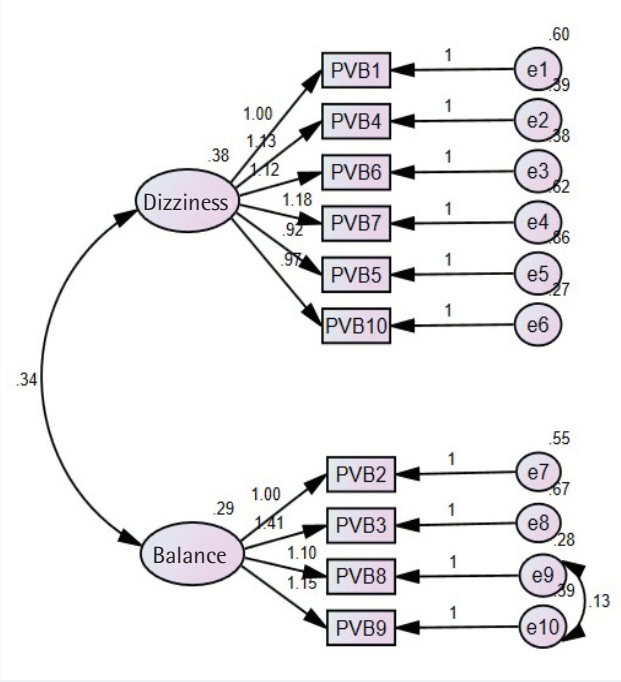

Figure 1. Confirmatory analysis path diagram of PVSQ in Turkish

PVSQ: Pediatric Vestibular Symptom Scale

Sensitivity and Specificity

The ROC curve of the PVSQ total score of children with vestibular problems is given in Figure 2. The area under the ROC curve calculated using the Youden index was 0.957, p<0.001. The cutoff point was 0.35, and at this point, the maximum sensitivity of the scale was 0.885 and the specificity was 0.950.

DISCUSSION

In the present study, which aimed to establish the Turkish validity and reliability of the PVSQ, 92 children aged 6-17 years were included. The Turkish version of the scale was found to have high sensitivity and specificity. While the cut-off point of the scale was 0.68 out of 3, with 95% sensitivity and 85% specificity in the original scale, it was calculated as 0.35 with 88% sensitivity and 95% specificity in our study. The cut-off point was only compared with the original scale, as there was no other PVSQ validity study to which the scale could be compared. However, while children from a primary school were randomly included in the study in the original of the scale, in our study, children who did not have the complaints of dizziness and imbalance were included in the study group. Since the prevalence of vertigo and dizziness in school-age children is approximately 15%[2], the possibility that randomly selected children may also have undiagnosed

Table 2. Internal validity coefficient values of PVSQ and its sub-dimensions (Cronbach's alpha)

Sub-dimensions	Items	Mean±SD	Cronbach's alpha	ICC
Dizziness	I1, I4, I5, I6, I7, I10	3.66±4.31	0.835	0.829
Balance	I2, I3, I8, I9	2.55±2.92	0.796	0.784
Total		6.21±6.86	0.896	0.892

I: Items, ICC: Intraclass correlation coefficient, SD: Standard deviation, PVSQ: Pediatric Vestibular Symptom Questionnaire

Table 3. Mean scores, item/total correlation and alpha coefficient values of PVSQ items

Item	Mean±SD	Scale average when item was deleted	Item/total correlation	Alpha coefficient when item was deleted	Adjusted total item correlation
1. Feeling that objects are spinning or moving around	0.65±0.83	5.47	0.369	0.883	0.572
2. Unsteadiness so bad that you actually fall	1.24±0.85	5.49	0.340	0.884	0.549
3. Feeling sick	1.70±0.87	4.98	0.488	0.878	0.656
4. A light-headed or swimmy feeling in the head	1.82±0.83	5.48	0.577	0.875	0.687
5. Feeling of pressure in the ear(s)	1.75±0.89	5.24	0.270	0.890	0.500
6. Blurry vision, difficulty seeing things clearly, and/or spots before the eyes	1.07±0.92	5.48	0.503	0.875	0.683
7. Headache or feeling of pressure in the head	1.24±0.92	5.26	0.479	0.879	0.626
8. Unable to stand or walk without holding on to something or someone	1.82±0.86	5.64	0.660	0.876	0.694
9. Feeling unsteady, about to lose balance	1.09±1.00	5.48	0.626	0.877	0.670
10. A fuzzy or cotton wool feeling in the head	0.92±0.94	5.57	0.662	0.874	0.722

PVSQ: Pediatric Vestibular Symptom Scale, SD: Standard deviation

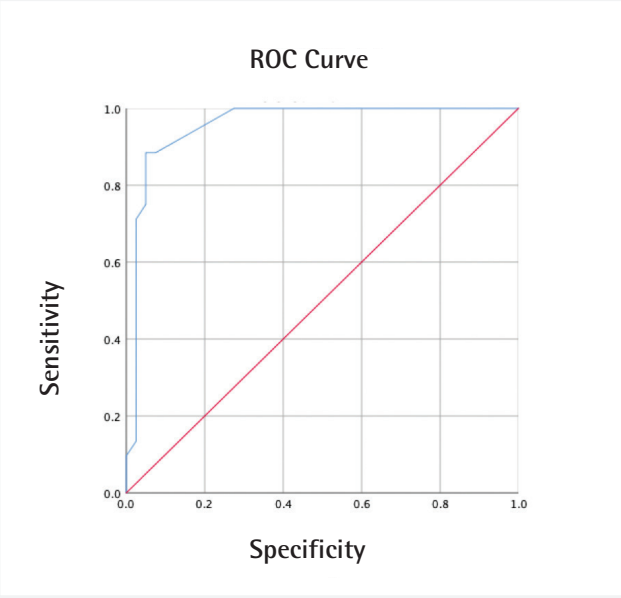

Figure 2. PVSQ ROC curve

PVSQ: Pediatric Vestibular Symptom Scale, ROC: Receiving operating characteristics

vestibular problems is thought to be one of the reasons for this higher rate. The internal consistency coefficient of the Turkish PVSQ was found to be 0.892. Since this ratio is close to 1, it is seen that the scale has a high degree of internal consistency. When the item was deleted, there was no significant change in Cronbach's alpha scores (range, 0.87-0.89). All items have a significant correlation with the total score and a high level of consistency with each other. The corrected total item correlation of >0.5 indicates that each item has discriminative capacity. It was stated that the original scale had high internal consistency (Cronbach's alpha=0.88)[10].

There are two factor structures in the scale, namely dizziness and balance. While "dizziness" is defined as a non-specific symptom, "vestibular vertigo" is defined as rotational, positional or recurrent dizziness with nausea, gaze and/or postural instability[10]. While the items 1, 4, 6, 7, and 10 were defined in the dizziness sub-factor and the items 2, 3, 5, and 8 were defined in the balance sub-factor in the original scale, item 9 (feeling of imbalance or feeling of being out of balance) was taken into two factor structures due to factor loads. However, CFA was performed in our study and the fit indices were not obtained at normal values. The items were evaluated in terms of CFA results and significance, and the 5th item was included in the dizziness, and the 10th item was included in the balance sub-factor. As a result, the items 1, 4, 5, 6, 7, and 10 were taken into the dizziness sub-factor and the items 2, 3, 8, and 9 into the balance sub-factor, and the CFA results show that the construct validity of the Turkish version of the scale is appropriate. The value of RMSEA below 0.8 and other values above 0.9 show that the adaptability of the scale is appropriate. However, it is seen that some items of the scale indicate both dizziness and balance complaints, so it is thought that it would be more appropriate to evaluate the scale in one dimension.

Item 11 of the scale ("Do any of these symptoms keep you from doing what you want to do? If your answer is "yes", please specify") was not included in these analyses as it was an open-ended question. In the study of Pavlou et al.[10], 87.5% of the participants answeredi to this question, while they reported the symptoms of headache and a feeling of pressure (79.6%), a feeling of lightness in the head (65%), and a feeling of rotation or movement of objects (57%), nausea (42.9%), and loss of balance (40.8%). In our study, 65.38% (n=34) of the participants answered "yes" to this question, 29.4% (n=10) of these children had headache, 44.11% (n=15) had loss of balance, 47.05% (n=16) complained of the sensation of objects turning or moving, 44.11% (n=15) of them complained of nausea, and 5.88% (n=2) had the complaint of the sense of pressure.

In a review study with ten articles covering a total of 724 subjects, benign paroxysmal vertigo (18.7%) and migraine-related vertigo (17.6%) in childhood were shown as the two main conditions associated with vertigo and dizziness in children, and head trauma was found to be the third most common cause of vertigo (14%)[5]. Similarly, in our study, 19.23% of the participants had a diagnosis of VM and 15.38% of them had a diagnosis of benign positional vertigo.

In addition, 15.38% of the children who participated in our study had the complaints of dizziness/imbalance after OM. It is thought that this situation can be explained by the fact that children with conductive hearing loss due to OM have more oculomotor abnormalities than their peers with normal hearing[12]. Casselbrant et al.[13] stated that OM attacks may affect children's balance, make them more clumsy and prone to accidents, and possibly impair motor development. In addition to conductive hearing loss, it is stated that vestibular function is also impaired in children with sensorineural hearing loss, and children with hearing loss have worse balance performance compared to children with normal hearing[14]. In our study, it is seen that approximately 29% of children with dizziness and balance disorder have hearing loss and use hearing aids such as cochlear implants and hearing devices.

Study Limitations

The study has some limitations. First of all, correlation with other scales could not be evaluated since there is no other Turkish scale evaluating dizziness/imbalance in children. Since there is no adaptation study in the literature other than the

original language of the scale, validity, reliability and cut-off point values in other languages could not be compared. While determining the number of participants, it was planned to take 10 participants for each item, considering the number of scale items, but since 100 participants could not be reached, a study was conducted with 92 participants. The desired sample size could not be reached due to the faster recovery of vertigo problems in children compared to adults and fewer clinical applications. Further studies with more participants are needed.

CONCLUSION

Dizziness and balance disorders are important problems affecting motor and cognitive development and quality of life in childhood. In this study, the Turkish validity and reliability of the PVSQ, which is used in the diagnosis and evaluation of dizziness and balance disorders in children, was investigated. Turkish PVSQ is a reliable, highly valid, easy-to-apply and short-term measurement tool for the evaluation and severity of vestibular symptoms in children. It is thought that the use of such scales will contribute to the early intervention and rehabilitation process by determining the balance problems experienced by children and the degree of disability.

Ethics

Ethics Committee Approval: The study was approved by the Hacettepe University Non-interventional Studies Ethics Committee (decision no: GO 18/162-23, date: 13.02.2018).

Informed Consent: Participation in the study was on a voluntary basis and written informed consent of the children and their families was obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: B.A., Concept: S.A., Design: B.A., S.A., Data Collection or Processing: B.A., Analysis or Interpretation: B.A., S.A., Literature Search: B.A., Writing: B.A., S.A.

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. Riina N, Ilmari P, Kentala E. Vertigo and imbalance in children: a retrospective study in a Helsinki University otorhinolaryngology clinic. Arch Otolaryngol Head Neck Surg. 2005;131:996-1000.
2. Russell G, Abu-Arafeh I. Paroxysmal vertigo in children--an epidemiological study. Int J Pediatr Otorhinolaryngol. 1999;49:S105-7.
3. Humphriss R, Hall A, May M, Macleod J. Balance ability of 7 and 10 year old children in the population: results from a large UK birth cohort study. Int J Pediatr Otorhinolaryngol. 2011;75:106-13.
4. McCaslin DL, Jacobson GP, Gruenwald JM. The predominant forms of vertigo in children and their associated findings on balance function testing. Otolaryngol Clin North Am. 2011;44:291-307.
5. Gioacchini FM, Alicandri-Ciufelli M, Kaleci S, Magliulo G, Re M. Prevalence and diagnosis of vestibular disorders in children: a review. Int J Pediatr Otorhinolaryngol. 2014;78:718-24.
6. Gedik-Soyuyuce O, Gence-Gumus Z, Ozdilek A, Ada M, Korkut N. Vestibular disorders in children: A retrospective analysis of vestibular function test findings. Int J Pediatr Otorhinolaryngol. 2021;146:110751.
7. Casselbrant ML, Mandel EM. Balance disorders in children. Neurol Clin. 2005;23:807-29.
8. Rine RM, Wiener-Vacher S. Evaluation and treatment of vestibular dysfunction in children. NeuroRehabilitation. 2013;32:507-18.
9. Niemensivu R, Kentala E, Wiener-Vacher S, Pyykkö I. Evaluation of vertiginous children. Eur Arch Otorhinolaryngol. 2007;264:1129-35.
10. Pavlou M, Whitney S, Alkathiry AA, Huett M, Luxon LM, Raglan E, et al. The Pediatric Vestibular Symptom Questionnaire: A Validation Study. J Pediatr. 2016;168:171-7.
11. Fidell S, Silvati L, Howe R, Pearsons KS, Tabachnick B, Knopf RC, et al. Effects of aircraft overflights on wilderness recreationists. J Acoust Soc Am. 1996;100:2909-18.
12. Rehagen SK, Valente M, Lieu JEC. Vestibular Screening in Pediatric Patients with Otitis Media. J Am Acad Audiol. 2020;31:209-16.
13. Casselbrant ML, Furman JM, Rubenstein E, Mandel EM. Effect of otitis media on the vestibular system in children. Ann Otol Rhinol Laryngol. 1995;104:620-4.
14. Maes L, De Kegel A, Van Waelvelde H, Dhooge I. Association between vestibular function and motor performance in hearing-impaired children. Otol Neurotol. 2014;35:e343-7.

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Comparison of Sociodemographic and Clinical Characteristics of Patients with Bipolar Disorder with and without Guardianship Decision

Vesayet Kararı Alan ve Almayan Bipolar Bozukluk Tanılı Hastaların Sosyodemografik ve Klinik Özelliklerinin Karşılaştırılması

Filiz KULACAOĞLU[1], Filiz İZCİ[2], Metin ASLAN[2], Hilal KOLSUZ[2], Sümeyye ÇALLI[2], Yağmur SEVER FİDAN[2]

[1]*University of Health Sciences Turkey, Prof. Dr. Mazhar Osman Mental and Neurological Diseases Training and Research Hospital, Clinic of Psychiatry, İstanbul, Turkey*

[2]*University of Health Sciences Turkey, Erenköy Mental and Neurological Diseases Training and Research Hospital, Clinic of Psychiatry, İstanbul, Turkey*

ABSTRACT

Aim: We aimed to compare the sociodemographic and clinical variables of the patients with bipolar disorder (BD), who were required guardianship by Mental Health Hospital's Health Board.

Materials and Methods: This retrospective study consisted of 201 patients with BD-1, aged between 18 and 65 years, who were sent to medical health board in order to prepare a report on whether guardianship was required by the courts. Sociodemographic and clinical variables data form including age, marital status, education, employment, the number of episodes, the history of electroconvulsive therapy (ECT), the type of medication, the number of hospitalization, and the history of suicide were used.

Results: One-hundred (49.75%) patients with BD were required guardianship decision (GD). The mean age of the group with GD (43.57±11.53 years) was significantly higher than the group without GD (39.54±10.73 years). There was a significant relationship between GD and marital status and employment. The group with GD had significantly higher number of total and manic episodes, duration of hospitalization, and duration of illness than the group without GD. A significant relationship was found between GD and medical comorbidity, history of ECT, the presence of psychotic delusion, and treatment with antipsychotic, lithium and valproic acid. Paranoid-persecution, reference, and bizarre types of delusion were found to be related to GD.

Conclusion: A significant relationship was found between GD and marital status, employment, duration of illness, number and duration of hospitalizations, number of total and manic episodes, medical comorbidity, and history of ECT, presence of psychotic delusions, and type of treatment. Clinicians should be aware of these variables during the decision of guardianship for patients with BD.

Keywords: Bipolar disorder, guardianship, restriction

ÖZ

Amaç: Bu çalışmada Ruh Sağlığı Hastanesi Sağlık Kurulu tarafından vesayet altına alınan bipolar bozukluk (BB) hastalarının sosyodemografik ve klinik değişkenlerinin karşılaştırılması amaçlandı.

Gereç ve Yöntem: Bu retrospektif çalışma, mahkeme tarafından vesayetin gerekli olup olmadığı konusunda rapor hazırlanmak üzere sağlık kuruluna gönderilen 18-65 yaşları arasındaki BB-1 olan 201 hastadan oluştu. Hastaların dijital kayıtlarından hastaneye yatış dosyaları taranarak bilgi elde edildi. Yaş, medeni durum, eğitim, çalışma, hastalık öyküsü, atak sayısı, elektrokonvülsif (EKT) tedavi öyküsü, ilaç türü, hastaneye yatış sayısı, intihar öyküsü gibi sosyodemografik ve klinik değişkenler için veri formu kullanıldı.

Bulgular: Tüm katılımcılar arasından 100 (%49,75) BB tanılı hastaya vesayet kararı (VK) çıkarılmıştır. VK olan grubun yaş ortalaması (43,57±11,53), VK olmayan gruba (39,54±10,73) göre anlamlı olarak daha yüksekti. VK ile medeni durum ve iş durumu arasında anlamlı bir ilişki vardı. VK alan grupta toplam atak sayısı, manik atak sayısı, hastanede yatış süresi ve hastalık süresi, VK almayan gruba göre anlamlı olarak daha yüksekti. VK ile

Address for Correspondence: Filiz KULACAOĞLU MD, University of Health Sciences Turkey, Prof. Dr. Mazhar Osman Mental and Neurological Diseases Training and Research Hospital, Clinic of Psychiatry, İstanbul, Turkey
Phone: +90 505 775 35 55 **E-mail:** fkulaca@gmail.com **ORCID ID:** orcid.org/0000-0001-9800-4971
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tıbbi komorbidite, EKT tedavi öyküsü, psikotik sanrı varlığı, antipsikotik, lityum ve valproik asit tedavisi arasında anlamlı bir ilişki bulundu. Ayrıca vesayet ile paranoid-persekütif, referans ve bizar sanrı türleri arasında anlamlı bir ilişki bulundu.

Sonuç: BB hastalarında VK ile medeni durum, çalışma durumu, hastalık süresi, yatış sayısı ve süresi, toplam ve manik atak sayısı, tıbbi komorbidite ve EKT öyküsü, psikotik sanrıların varlığı ve tedavi şekli arasında anlamlı bir ilişki bulundu. Klinisyenler BB olan hastalara VK verirken bu değişkenlerin farkında olmalıdırlar.

Anahtar Kelimeler: Bipolar bozukluk, vesayet, kısıtlama

INTRODUCTION

The Civil Code (CC) defines the conditions for a person's entitlement to and exercise of the rights, as well as guarantees those rights in cases where those rights are obstructed or restricted. From the civil rights point of view, while the competence of the person is prioritized, the rights are kept at the highest level and the least restrictions are imposed on the person[1]. "Capacity to act" provides a legal consequence to human actions. According to the CC, the capacity to act is defined as the ability to act rationally and to comprehend the causes and consequences of their actions. Capacity to act is a mental function such as understanding, discerning, reasoning, making decisions, and reaching conclusions. Evaluation of capacity to act should be specific to the person, situation, and event[1,2].

Courts restrict the civil rights of people and transfer their obligation to exercise their rights to their legal representative, thereby putting such a person under guardianship. Guardianship is defined as "an institution that protects adults who are incapable of self-management for some reason, in terms of personal and financial interests, and ensures their representation"[3]. CC art. 405 governs the total deprivation of a person's civil rights if they have a mental illness or weakness. Legal consultant is assigned to adults who do not have sufficient reasons to be restricted, but who benefit from the limitation of their capacity to act in some respects[4]. With the concept of guardianship, it is aimed to attach a legal consequence to the actions of the person and to protect herself and the society from the consequences of the actions of the person who has no capacity to act. Treatment and protection of civil rights of patients who are placed under guardianship in psychiatry clinics are provided through courts[5]. Guardianship may be essential for the patients with psychiatric disorders, such as schizophrenia, bipolar disorder, and developmental disorder, due to their lack of decision-making capacity. It is appropriate to recommend guardianship to those who have insufficient insight, frequent episodes, and those who do not respond to treatment or have a severe disease that will make their daily life difficult[1,6]. Melamed et al.[7] investigated guardianship of 60 people with a mental disorder in Israel. According to the results of this study, the majority of the patients with guardianship decisions (GD) were psychotic disorders and dementia while 1% of them included patients with BD. According to a previous study that investigated guardianship reports given by mental health hospital's medical board in Turkey, 31% of those individuals had dementia, 28% of them had a psychotic disorder, 15.2% of them had mental retardation and 8.1% of them had BD[8]. Although a GD is rare for patients with BD than other chronic psychiatric disorders, BD is one of the important psychiatric illnesses that require a GD. It is considered to offer guardianship to patients with BD who have insufficient insight, become chronic, do not respond to treatment, and have a severe illness that will make their daily life difficult[7]. Recently, Akıncı et al.[9] investigated clinical and sociodemographic characteristics of patients with BD with the decision of a legal representative. According to this study, the total number of episodes, number of manic and mixed episodes, hospitalizations, presence of psychotic symptoms, and alcohol and substance use were found higher in the group with the GD than in the group without GD.

There are few studies on the need for guardianship in the literature, and they generally focus on patients with dementia. There are limited data for the GD of patients with BD. In this study, we aimed to compare the sociodemographic and clinical variables of the patients with BD according to their GD which was given by Mental Health Hospital's Health Board.

MATERIALS AND METHODS

In this retrospective study, we evaluated the digital medical records of patients with BD, who were sent to medical health board of Erenköy Research and Training hospital for Psychiatry and Neurological disease between 01/01/2015 and 12/31/2021 in order to prepare a report on whether guardianship was required by the courts. Patients with a diagnosis of BD according to Diagnostic and Statistical Manual of Mental Disorder-5 and aged between 18 and 65 years were included. Patients with mental retardation and those with missing files were excluded from the study. Finally, 201 patients with BD were included in the study. Sociodemographic and clinical data form including history of disease, number of episodes, history of ECT, type of medication, number of hospitalization, history of suicide were used. Approval for the study was granted by the Erenköy Research and Training Hospital for Mental Health and Neurological Diseases Ethical Committee with approval number 34, dated October 04, 2021.

Statistical Analysis

The data were analyzed using the Statistical Package for the Social Sciences 22 program. A frequency table was created for sociodemographic questions. In order to see the differences in the group means of the decision variable, independent sample t-test was applied for the measurement values with normal distribution, and the Mann-Whitney U analysis was applied for the measurement values that did not show normal distribution. Chi-square analysis was performed to see the relationship between the decision variable and the categorical variables. A p value less than 0.05 was considered as significant.

RESULTS

A total of 201 participants were included in our study. Among all of the participants, 100 patients with BD required GD and 101 patients with BD did not require GD. A comparison of the sociodemographic characteristics of the participants with GD and without GD was shown in Table 1. The mean age of the group with GD was 43.57±11.53 years and the mean age of group without GD was 39.54±10.73 years. It was determined that the group with (GD) had a higher age (t=-2.562, p=0.011) than the group without GD.

There was a significant relationship between GD and marital status (x^2=7.391, p=0.025) and employment (x^2=17.889, p=0.000). Of the patients with GD, 42% were single, 28% were married, and 30% were divorced. Of the patients without GD, 30.7% were single, 46.5% were married, and 22.8% were divorced. Of the group with GD, 76% were unemployed, 20% were workers, and 3% were retired. On the other hand, of the group without GD, 47.5% were unemployed, 41.6% were working, and 10.9% were retired. No relationship was found between GD and economic income (x^2=2.923, p=0.404), gender (x^2=0.259, p=0.611), education level (x^2=2.902, p=0.234), and the presence of children (x^2=1.792, p=0.181).

Comparison results of clinical variables for two groups were presented in Table 2. There was no significant difference between two groups in terms of age of onset (z=-0.058, p=0.954) and number of depressive episodes (z=-0.969, p=0.332). However, there were significant differences between two groups in terms of duration of illness (z=-2.433, p=0.016), number of total episodes (z=-3.919, p=0.000), number of manic episodes (z=-4.048, p=0.000), and duration of hospitalization (z=-5.838, p=0.000).

22.8% of those who did not require GD had no medical comorbidity and 39% of those who required GD had medical

Table 1. Comparison of sociodemographic variables of the participants

		Guardianship decision (+) group (n=100)	Guardianship decision (-) group (n=101)	p
		Mean (±SD) n (%)		
Age (years)		43.57±11.53	39.54±10.73	**0.011***
Gender (female)		59 (59%)	56 (55.4%)	0.611
Marital status	Single	42 (42%)	31 (30.7%)	**0.025***
	Married	28 (28%)	47 (46.5%)	
	Divorced	30 (30%)	23 (22.8%)	
Education	Primary	49 (49.0%)	43 (42,6%)	0.234
	Secondary	35 (35.0%)	32 (31,7%)	
	Bachelor	16 (16.0%)	26 (25,7%)	
Employment	Retired	3 (3.0%)	11 (10.9%)	**0.000**
	Civil servant-worker	21 (21.0%)	42 (41.6%)	
	Unemployed	76 (76.0%)	48 (47.5%)	
Economic income	<2,000 TL	46 (46.0%)	41 (40.6%)	0.404
	2,000-3,000 TL	32 (32.0%)	27 (26.7%)	
	3,000-5,000 TL	17 (17.0%)	26 (25.7%)	
	>5,000 TL	5 (5%)	7 (6.9%)	
Children	Absent	53 (53%)	44 (43.6%)	0.181
		47 (47%)	57 (56.4%)	

p<0.05.
SD: Standard deviation, TL: Turkish Liras

comorbidity. A significant relationship was found between GD and medical comorbidity (x^2=11.892, p=0.018). 12% of those with GD had psychiatric comorbidity and 7% of those without GD had psychiatric comorbidity. No significant relationship was found between GD and psychiatric comorbidity (x^2=1.509, p=0.219). A significant relationship was found between the history of ECT (x^2=7.691, p=0.006) and GD. While 52% of those with GD had a history of ECT, 32.7% of those that did not require GD had a history of ECT. No significant relationship was found between GD and alcohol use disorder (x^2=0.198, p=0.656), substance use disorder (x^2=0.213, p=0.645), and the history of suicide (x^2=0.336, p=0.562).

The comparison of the delusion types and drugs used by patients in the decision of guardianship was shown in Table 3. There was a significant relationship between psychotic delusion and decision of guardianship (x^2=23.565, p=0.000). While 39.6% of those who did not take GD did not have psychotic delusions, 60.4% of them had psychotic delusions. 10% of the patients with GD did not have psychotic delusions and 90% of them had psychotic delusions. A significant relationship was found between the decision of guardianship and paranoid-persecution type of delusion (x^2=10.321, p=0.001), delusion of reference (x^2=6.065, p=0.014), and bizarre delusion (x^2=4.787, p=0.029). No significant relationship was found between the decision of guardianship and grandiose delusion (x^2=2.441, p=0.118) and mystical delusion (x^2=0.107, p=0.744).

When it comes to drug used by the patients, a significant relationship was found between valproic acid (VPA) and the decision of guardianship (x^2=12.943, p=0.000). 37.6% of those who did not take a GD used VPA whereas 63% of those who required GD used VPA. A significant relationship was found between lithium and the decision of guardianship (x^2=7.907, p=0.005). 69% of those who required GD did not use lithium whereas 49.5% of those who did not require GD did not use lithium. A significant relationship was detected between antipsychotic and the decision of guardianship (x^2=16.113, p=0.000). 71.3% of those who did not require GD used antipsychotic and 93% of those who required GD used antipsychotic medication.

Table 2. Comparison of clinical variables of the participants

		Guardianship decision (+) group (n=100)	Guardianship decision (-) group (n=101)	t, χ^2, z	p
		Mean±SD n (%)			
Age of onset (years)		26.78±10.61	25.37±6.48	-0.058	0.954
Duration of illness (years)		17.25±10.05	13.92±9.33	-2.433	**0.016***
Number of total episodes		6.54±5.26	4.28±2.66	-3.919	**0.000***
Number of manic episodes		5.04±4.51	3.09±2.53	-4.048	**0.000***
Number of depressive episodes		0.45±0.83	0.52±0.85	-0.969	0.332
Duration of hospitalization (weeks)		19.56±17.51	9.26±9.95	-5.838	**0.000***
Medical comorbidity	None	61 (61.0%)	78 (77.2%)	11.892	**0.018**
	Hypertension/coroner artery disease	16 (16.0%)	4 (4.0%)		
	Diabetes mellitus	8 (8.0%)	3 (3.0%)		
	Hypothyroidism	6 (6.0%)	8 (7.9%)		
	Other	9 (9.0%)	8 (7.9%)		
Psychiatric comorbidity	Present	12 (12%)	7 (6.9%)	1.509	0.219
	Absent	88 (88%)	94 (93.1%)		
History of ECT	Present	52 (52%)	33 (32.7%)	7.691	**0.006**
	Absent	48 (48%)	68 (67.3%)		
Alcohol use disorder	Present	24 (24%)	27 (26.7%)	0.198	0.656
	Absent	76 (76%)	74 (73.3%)		
Substance use disorder	Present	13 (13%)	11 (10.9%)	0.213	0.645
	Absent	87 (87%)	90 (89.1%)		
History of suicide	Present	20 (20%)	17 (16.8%)	0.336	0.562
	Absent	80 (80%)	84 (83.2%)		

ECT: Electroconvulsive therapy, SD: Standard deviation

Table 3. Comparison of the delusion types and medication in the decision of guardianship

		Guardianship decision (+) group (n=100)	Guardianship decision (-) group (n=101)	χ^2	p
Delusion	Present	90 (90%)	61 (60.4%)	23.565	**0.000***
	Absent	10 (10%)	40 (39.6%)		
Paranoid-persecution delusion	Present	68 (68%)	46 (45.5%)	10.321	**0.001***
	Absent	32 (32%)	55 (54.5%)		
Grandiose delusion	Present	37 (37%)	27 (26.7%)	2.441	0.118
	Absent	63 (63%)	74 (73.3%)		
Reference delusion	Present	17 (17%)	6 (5.9%)	6.065	**0.014***
	Absent	83 (83%)	95 (94.1%)		
Mystical delusion	Present	6 (6%)	5 (5%)	0.107	0.744
	Absent	94 (94%)	96 (95%)		
Bizarre delusion	Present	9 (9%)	2 (2%)	4.787	**0.029***
	Absent	91 (91%)	99 (98%)		
Valproic aside	Present	63 (63%)	37 (37.6%)	12.943	**0.000***
	Absent	37 (37%)	63 (62.4%)		
Lithium	Present	31 (31%)	51 (50.5%)	7.907	**0.005***
	Absent	69 (69%)	50 (49.5%)		
Antipsychotic	Present	93 (93%)	72 (71.3%)	16.113	**0.000***
	Absent	7 (7%)	29 (28.7%)		

*p<0.05

DISCUSSION

In this retrospective study, we evaluated medical records of 201 patients with BD, who were sent to medical board by the courts in order to prepare a report on whether legal representative was required. According to our results 100 patients (49.75%) with BD had been required the decision of guardianship. In our study, the mean age of the group with GD was 43.57±11.53 years, the mean of the duration of the illness was 17.25±10.05 years, and both of them were significantly higher than in the group without GD. Our results supported the results of previous study. According to a study in Israel, the mean age of the patients with mental disorder who required guardianship was 48 years and the duration of their illness was 20 years. However, 80% of patients who required guardian were schizophrenia, 5% of them were dementia, and only 1% of them were BD[7]. In a recent study that investigated the legal representative reports in Türkiye, the mean duration of illness and the mean age of all patients were reported to be 11.8 years and 55 years, respectively. Moreover, it was determined that 39.2% of those patients were dementia, 27.7% of them were schizophrenia and other psychotic disorders, and 4% of them were BD[8]. Similar to our study, Akıncı et al.[9] found that the mean age of patients with BD who required legal representative was 45.8 years. These results can suggest that longer disease duration increases the decision of guardianship.

According to our results, marital status and employment were found to be related to GD. Of the patients with GD, 28% were married, 30% were divorced, and 42% were single. Similar to our results, it has been reported that divorcement is common among people with mental disorder, and divorce and separation are two to three times more probable in people with BD than in general population[10]. Our results support the idea that BD is associated with higher divorce rate and poor marital adjustment. However, poor marital adjustment can lead to relapses and worse prognosis in BD and this may be related to the higher rates of GD in our study. However, similar to our results, a recent study that investigated 61 adult patients with GD showed that 37% of the patient group were single whereas only 9% of this group were married. This result can be interpreted as GD can lead to poor social support[11].

Of the group with GD, 76% were unemployed. According to a meta-analysis, BD damages employment outcome in the longer term, but up to 60% of people may be in employment[12]. According to our results, of the group without GD, 47.5% were unemployed, which was lower than in the group with GD. The magnitude of functional losses associated with bipolar disorder is large. In the light of our results, the lower functionality indicates unemployment and this may be also related to the need for guardianship in the patients with BD. One of our study's results was that there was no relationship between

GD and economic income, gender, education level, and the presence of children. Akıncı et al.[9] found no difference among sociodemographic variables such as gender, educational status, marital status, employment, and social income concerning the recommendations for the requirement of legal representative.

In our study, we found no significant difference between two groups in terms of age of onset, and depressive episodes. However, the number of manic episodes, duration of illness, and duration of hospitalization were higher in the group with GD. It has been reported that periods of relapse lead to progressive dysfunctions, and higher number of episodes was linked with poor prognosis in BD. Moreover, the number of episodes increases the number of hospitalizations as well. Previously, Akıncı et al.[9] found significantly greater number of manic and mixed episodes and higher duration of hospitalization in BD patients who were recommended for a guardian. Previous researches also reported a high number of episodes and their high recurrence rate to be important for the restriction decision[1,5,13].

The present study found that the presence of medical comorbidity was related to GD. 39% of those that required GD had medical comorbidity in our study. Among those patients, 16% of them had hypertension/coroner artery disease, 8% of them had diabetes mellitus, 6% of them had hypothyroidism. According to a recent review, BD is associated with chronic low-grade inflammation and several medical comorbidities such as cardiovascular disease, diabetes mellitus, and obesity in patients with BD[14]. However, metabolic illness and obesity are related to greater symptom severity and poor treatment responses. Thus, a decreased life expectancy is seen in BD[14]. We can interpret these results in the way that medical comorbidity is related to poor prognosis in BD and poor prognosis is related to higher rates of requirement of guardianship in those patients.

It is critical to consider coexisting psychiatric disorders when deciding whether to appoint a guardian in patients with BD. According to our study, 12% of those with GD had psychiatric comorbidity and 7% of those without GD had psychiatric comorbidity. Moreover, no significant relationship was found between GD and psychiatric comorbidity, alcohol and substance use disorder. Vieta et al.[15] reported that the rate of psychiatric comorbidity in BD was 31% and psychiatric comorbidity affected the treatment response and prognosis in a negative way. Alcohol and substance use disorder is a common comorbidity for BD. Contrary to the results of our study, Akıncı et al.[9] found higher rates of alcohol and substance use disorder in the patients with BD who required guardian and the presence of psychiatric comorbidity increased the likelihood of assigning a guardian 11-fold. The difference in the results of our study and Akıncı et al.'s[9] study may be explained by the retrospective design of our study. We could not make an interview with the patients and data of the patients may have been lost due to its retrospective design.

The psychotic features of the last episodes of the patients were listed in Table 3. According to our results, 90% of the patients with GD had psychotic delusion in the last episode of their hospitalization, and it was found to be related to the decision of guardianship. In BD, the presence of a psychotic symptoms indicates a poor prognosis and lower functionality[16]. According to our results, there was a relationship between the decision of guardianship and paranoid-persecution, reference, and bizarre types of delusion. No relationship was demonstrated between mystical and grandiose types of delusion. A recent study showed that a lifetime history of psychotic symptoms was present in 73.8% of patients with BD and delusions were seen in 68.9% of those patients. However, patients with psychotic symptoms showed younger age of disease onset and higher number of hospitalizations with manic episodes[17]. Thus, we can say that psychotic features in BD may affect the restriction decision, and our study also supports the literature in this respect.

We found a significant relationship between GD-lithium treatment and GD-VPA treatment. According to our results, 69% of the patients who required guardianship did not use lithium whereas 63% of them used VPA. Previous studies showed that treatment with lithium lowers the risk of relapse in BD. According to a meta-analysis, long-term lithium treatment has been suggested due to the chronic persistent impairment secondary to relapses. Thus, long-term lithium treatment should be started earlier rather than later[18]. In this present study, the majority of the patients with GD did not use lithium. We can interpret these results as the patients who required GD relapsed more frequently by using less lithium and adversely affected the prognose in this way.

Study Limitations

This study has certain strengths and limitations. One of the important strengths of our study is that there are very rare researches on the GD for psychiatric disorders, especially bipolar disorder. Our hospital is a mental health hospital and the medical board is specialized for forensic psychiatry. Thus, we think that the participants of our study guide in terms of bipolar population in Turkey. One of the limitations is the study's retrospective design. The assessment of the patients was done by scanning the hospital files. We could not evaluate the patients face to face. Second, our sample size was relatively small. Prospective studies are needed to examine many variables in the process of GD.

CONCLUSION

In conclusion, we found a significant relationship between the requirement of guardianship and marital status, employment, duration of illness, number and duration of hospitalizations, number of total and manic episodes, medical comorbidity, and history of ECT, presence of psychotic delusions, and type of treatment. We believe that our study will guide further studies and help clinicians in the examination process regarding the restriction decision of patients with BD.

Ethics

Ethics Committee Approval: Approval for the study was granted by the Erenköy Research and Training Hospital for Mental Health and Neurological Diseases Ethical Committee with approval number 34, dated October 4, 2021.

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: F.K., F.İ., M.A., H.K., S.Ç., Y.S.F., Concept: F.K., F.İ., Design: F.K., F.İ., Data Collection or Processing: F.K., M.A., H.K., S.Ç., Y.S.F., Analysis or Interpretation: F.K., M.A., H.K., S.Ç., Y.S.F., Literature Search: F.K., F.İ., M.A., H.K., S.Ç., Y.S.F., Writing: F.K., F.İ., M.A., H.K., S.Ç., Y.S.F.

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REFERENCES

1. Sercan M: Yurttaşlık Hukukunda (Medeni Hukuk) Adli Psikiyatri. Adli Psikiyatri Uygulama Kılavuzu, Ankara: Türkiye Psikiyatri Derneği, Tuna Matbaacılık, 2007.
2. Türkcan S: Yeşilbursa D: Adli Psikiyatri. Psikiyatri Temel Kitabı. (Eds Köroğlu E, Güleç C): 937-44. Ankara, Hekim Yayın Birliği, 2007.
3. Soysal H: Adli Psikiyatri Güncellenmiş Basım, İstanbul, Özgür Yayınları, 2012.
4. Kaçak N: Yeni İçtihatlarla Türk Medeni Kanunu (2nd ed). Ankara, Seçkin Yayıncılık, 2007.
5. Özden SY: Adli Psikiyatri, İstanbul, 3P PharmaPublication Planning, 2007.
6. Duman ÖY, Göka E. Yeni Türk Medeni Yasası ve psikiyatri. Psikiyatri Psikoloji Psikofarmakoloji (3P) Dergisi. 2002;10:25-32.
7. Melamed Y, Doron I, Shnitt D. Guardianship of people with mental disorders. Soc Sci Med. 2007;65:1118-23.
8. Gokceimam PS, Sunbul EA, Gokbakan AM, Ozel ME, Kurban RD, Askin R. Bir Psikiyatri Dal Hastanesi Saglik Kuruluna vesayet uygunlugu acisindan gonderilen kisilerin degerlendirilmesi. Anadolu Psikiyatri Derg. 2019;20:627-35.
9. Akıncı E, Öncü F, Ger C, Sabuncuoğlu M, Kırmızı A, Eradamlar N. Clinical and Sociodemographic Characteristics That Affect the Recommendation for Assignment of A Legal Representative in Patients With Bipolar Disorder. Turk Psikiyatri Derg. 2017;28:144-55.
10. Rusner M, Carlsson G, Brunt DA, Nyström M. The paradox of being both needed and rejected: the existential meaning of being closely related to a person with bipolar disorder. Issues Ment Health Nurs. 2012;33:200-8.
11. Marwaha S, Durrani A, Singh S. Employment outcomes in people with bipolar disorder: a systematic review. Acta Psychiatr Scand. 2013;128:179-93.
12. Ricotta DN, Parris JJ, Parris RS, Sontag DN, Mukamal KJ. The Burden of Guardianship: A Matched Cohort Study. J Hosp Med. 2018;13:595-601.
13. Balcıoğlu İ, Başer SZ. Türkiye'de sık karşılaşılan psikiyatrik hastalıklar hekimlerin karşılaştığı adli ve psikiyatrik problemler. Sempozyum Dizisi. 2008;62:41-8.
14. Sinha A, Shariq A, Said K, Sharma A, Jeffrey Newport D, Salloum IM. Medical Comorbidities in Bipolar Disorder. Curr Psychiatry Rep. 2018;20:36.
15. Vieta E, Colom F, Corbella B, Martínez-Arán A, Reinares M, Benabarre A, et al. Clinical correlates of psychiatric comorbidity in bipolar I patients. Bipolar Disord. 2001;3:253-8.
16. Hua LL, Wilens TE, Martelon M, Wong P, Wozniak J, Biederman J. Psychosocial functioning, familiality, and psychiatric comorbidity in bipolar youth with and without psychotic features. J Clin Psychiatry. 2011;72:397-405.
17. van Bergen AH, Verkooijen S, Vreeker A, Abramovic L, Hillegers MH, Spijker AT, et al. The characteristics of psychotic features in bipolar disorder. Psychol Med. 2019;49:2036-48.
18. Geddes JR, Burgess S, Hawton K, Jamison K, Goodwin GM. Long-term lithium therapy for bipolar disorder: systematic review and meta-analysis of randomized controlled trials. Am J Psychiatry. 2004;161:217-22.

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Puerarin Protects from Methotrexate Induced Hepatotoxicity in AML-12 Cells

Puerarin AML-12 Hücrelerinde Metotreksatın Neden Olduğu Hepatoksisiteden Korur

Melek AKINCI[1], Çağatay OLTULU[2], Elvan BAKAR[3], Zatiye Ayça ÇEVİKELLİ YAKUT[4]

[1]*Trakya University Faculty of Pharmacy, Department of Pharmacology, Edirne, Turkey*
[2]*Trakya University Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Edirne, Turkey*
[3]*Trakya University Faculty of Pharmacy, Department of Basic Pharmacy, Edirne, Turkey*
[4]*Trakya University Faculty of Pharmacy, Department of Pharmacognosy, Edirne; Marmara University Faculty of Pharmacy, Department of Pharmacology, İstanbul, Turkey*

ABSTRACT

Aim: The purpose of this study was to look into the effects of puerarin (PR) on methotrexate (MTX)-induced hepatotoxicity *in vitro*.

Materials and Methods: We designed our research with four groups in the AML-12 cell line: control, PR, MTX, and PR+MTX groups. Administered concentration levels to the cell lines were determined with the MTT test. To investigate oxidative stress, the expression levels of glutathione, superoxide dismutase, and catalase were determined with quantitative real-time-polymerase chain reaction (qRT-PCR) analysis. To evaluate the role of apoptosis pathways in MTX induced hepatotoxicity and the hepatoprotective effects of PR, gene expressions of caspase 3 (Cas-3), Cas-9, apoptotic protease activating factor-1, Bcl-2, Bax, p53, second mitochondria-derived activator of caspase/direct inhibitor of apoptosis-binding protein (smac/DIABLO), topoisomerase (Top) I, and Top II were investigated with qRT-PCR.

Results: MTX impaired the antioxidant defense through SOD but elevated the expression of catalase and glutathione due to an increase in free radicals. In the PR+MTX group, SOD expression increased and catalase and glutathione expression decreased compared to the MTX group. Cas-9, Apaf-1, and Top I gene expression levels were reduced in group PR. In the group of PR+MTX, PR application increased the expression of Bax, p53, and smac/DIABLO while decreasing the expression of Bcl-2, which resulted in the elimination of damaged structures by apoptosis.

Conclusion: PR alleviated the hepatotoxicity caused by MTX with its antioxidant effects and positive effects on apoptosis pathways. However, different dose studies are needed because PR could not prevent double-strand damage in DNA due to MTX and there is an increase in Top I expression in the PR group.

Keywords: Methotrexate, puerarin, hepatoprotective, oxidative stress, apoptosis

ÖZ

Amaç: Puerarinin (PR) metotreksat (MTX) nedenli hepatotoksisite üzerindeki etkilerinin incelenmesi hedeflenmiştir.

Gereç ve Yöntem: Çalışmamız AML-12 hücre hattında kontrol, PR, MTX, PR+MTX olacak şekilde dört grup olarak planlandı. Hücre hatlarına uygulanacak madde konsantrasyonları MTT yöntemi ile belirlendi. Oksidatif stresi irdelemek amacıyla süperoksid dismutaz (SOD), katalaz ve glutatyon ekspresyon düzeyleri kantitatif gerçek zamanlı-polimeraz zincir reaksiyonu (qRT-PZR) analizi ile ölçüldü. MTX'in neden olduğu hepatotoksisitede ve PR'nin hepatoprotektif etkilerinde apoptoz yolaklarının rolünü değerlendirmek amacıyla qRT-PZR analizi ile kaspaz-3 (Cas-3), Cas-9, apoptotik proteaz aktive edici faktör 1, Bax, Bcl-2, p53, ikinci mitokondri türevli kaspaz aktivatörü/doğrudan apoptoz bağlayıcı protein inhibitörü (smac/DIABLO), topoizomeraz (Top) I, Top II gen ekspresyonları incelendi.

Bulgular: MTX SOD üzerinden antioksidan savunmayı zayıflattı, fakat serbest radikal artışı nedeni ile katalaz ve glutatyon ekspresyonunu artırdı. PR+MTX grubunda, MTX grubuna göre, SOD ekspresyonu arttı, katalaz ve glutatyon ekspresyonları azaldı. PR grubunda, Cas-9, Apaf-1 ve Top I gen ekspresyon düzeyleri azaldı. PR+MTX grubunda PR uygulaması Bax, p53 ve smac/DIABLO ekspresyonunu artırarak ve Bcl2 ekspresyonunu azaltarak hasarlı yapıların apoptozla ortadan kaldırılmasını sağladı.

Address for Correspondence: Melek AKINCI MD, Trakya University Faculty of Pharmacy, Department of Pharmacology, Edirne, Turkey
Phone: +90 505 896 45 58 **E-mail:** melektamer@trakya.edu.tr **ORCID ID:** orcid.org/0000-0003-3879-4232
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Sonuç: PR, MTX'in neden olduğu hepatotoksisiteyi antioksidan etkileri ve apoptoz yolaklarındaki olumlu etkileri ile hafifletmiştir. Fakat PR MTX'e bağlı gelişen DNA'daki çift iplik hasarını önleyemediği ve PR grubunda Top I ekspresyonunda artış geliştiği için, farklı doz çalışmalarına ihtiyaç duyulmaktadır.

Anahtar Kelimeler: Metotreksat, puerarin, hepatoprotektif, oksidatif stres, apoptoz

INTRODUCTION

Cancer, which is the second-leading cause of death in the world, is a heterogeneous disease characterized by irreversible disruption of cellular homeostasis and function. Cancer treatment choices include surgery, chemotherapy, radiation, and palliative care[1]. Chemotherapy is the use of chemotherapy medications to cure cancer. Methotrexate (MTX) is an antineoplastic and immunosuppressive agent used for the treatment of different malignancies and autoimmune disorders such as psoriatic dermatomyositis and rheumatoid arthritis[2]. MTX, a folate antagonist, affects nucleic acid synthesis by competitively inhibiting dihydrofolate reductase[3].

Chemotherapeutic medicines, which are effective and commonly used cancer treatments, lead to cytotoxic side effects. Serious adverse effects such as hepatotoxicity, nephrotoxicity, testicular dysfunction, and cardiotoxicity have been documented in individuals receiving low-dose MTX[2]. Alcohol intake, abnormal liver enzyme levels, a history of liver disease, and the presence of chronic disorders such as obesity, diabetes, and hyperlipidemia all raise the risk of MTX-induced hepatotoxicity[4]. MTX is known to raise blood aminotransferase levels, and long term MTX treatment has been related to the onset of fibrosis, fatty liver disease, and cirrhosis[5]. Although the mechanism of hepatotoxicity induced by MTX has not been fully understood, several recent studies have proposed that oxidative stress and inflammation are key factors involved. Studies have indicated that an excess of reactive oxygen species and nitric oxide, coupled with insufficient antioxidant defenses, play a crucial role in the development of MTX-induced liver damage[2]. Hence, it has been suggested that natural products possessing antioxidant and anti-inflammatory properties could potentially mitigate the hepatotoxicity caused by MTX[2]. Puerarin (PR), an isoflavone glycoside, is the primary antioxidant present in *Pueraria lobata*, which is commonly used for treating liver illnesses in traditional Chinese medicine. Positive effects of PR on the liver have been found in various investigations. For example, PR demonstrated hepatoprotective effects in an experimental liver injury model induced with carbon tetrachloride in rats[6]. In another experimental study conducted on rats by Chen et al.[7] (2013), the PR was reported to have repaired liver damage triggered with chronic alcohol consumption. Based on this knowledge, we also investigated the effects of PR on liver damage in the AML-12 cell line with MTX in our research, taking into consideration oxidative stress, apoptosis, and DNA damage.

The protective impact of PR on the hepatotoxic effects of MTX was examined in our research. Glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD) enzymes, and genes encoding Caspase-3 (Cas-3), Caspase-9 (Cas-9), apoptotic protease activating factor-1 (Apaf-1), Bcl-2, Bax, p53, second mitochondria-derived activator of caspase/direct inhibitor of apoptosis-binding protein with low pI (smac/DIABLO), Topoisomerase I and II (Top I, Top II) proteins, were investigated. Thus, the interactions between the pathways implicated in apoptosis were investigated, as well as their impacts on nuclear gene expression.

METARIALS AND METHODS

Groups

Our study consisted of 4 groups as the control group, PR group, MTX group, and PR+MTX group.

Chemicals

Eagle's Minimum Essential Medium (EMEM) (320-026-CL), HAMS F 12 (318-010-CL), and trypsin/EDTA (325-542-EL) were purchased from Multicell (Wisent Bioproducts, St-Bruno, QC, Canada). Dulbecco's modified Eagle's medium (DMEM) (320-026-CL), penicillin-streptomycin (Gibco 15070063), fetal bovine serum (FBS) (Gibco 26140079), and L-glutamine (Gibco 25030081) were supplied from Gibco (Thermo Fisher Scientific, Waltham, MA, USA). Thiazolyl Blue Tetrazolium Bromide (MTT) was taken from Biocompare (New York, USA). Dimethyl sulfoxide (DMSO) (Merck 67-68-5) and Phosphate buffered saline (PBS) (Merck 524650) were obtained from Merck-Millipore (Darmstadt, Germany). The PureLink RNA Mini Kit (121-830-18A) was taken from Invitrogen (Thermo Fisher Scientific, Waltham, MA, USA). SYBR Select Master Mix and high capacity cDNA reverse transcription kit (8368814) were supplied from Applied Biosystems (Thermo Fisher Scientific, Waltham, MA, USA).

The PR and MTX solutions were prepared in an aqueous solution containing 0.01% DMSO, and the PR+MTX mixture was prepared in a 1:1 ratio.

Cell Culture

AML-12 (alpha mouse liver 12) cells are hepatocytes isolated from the normal liver of a 3-month-old mouse. AML-12 (ATCC®, CRL-2254™), 5% heat-inactivated FBS; nutrient medium contains 100 IU/mL penicillin, 10 mg/mL streptomycin and 1%

L-glutamine, 1:1 ratio of EMEM, DMEM, HAMS F12 are seeded in flasks and they are placed in the incubator that contains 95% moisture and 5% CO_2 at 37 °C. Our study started in the 5th passage and ended in the 12th passage.

Dose Determination with MTT Method

180 µL AML-12 cells were seeded in 96 well plates to have $1x10^6$ cells in each well to determine IC_{50} values of all groups to be used in the study. They were left to incubate for 24 hours to enable the cells to adhere onto the plate wells. All substances were administered to all groups except for the control group and doses are shown at Figure 1 (in a volume of 20 µL). Then, all groups were left in the incubator (37 °C, 5% CO_2) for 24 hours. An aqueous solution containing 0.01% DMSO was applied to the control group. Aqueous solutions of PR and MTX containing 0.01% DMSO were prepared. In a 1:1 (v/v) ratio, PR and MTX were combined. Each well received 20 µL of MTT (5 mg/mL) solution. To dissolve formazan crystals 200 µL of 0.01% DMSO were added after 3 hours. The absorption value was calculated using a microplate scanner at 492 nm (Thermo Scientific Multiskan Go). The control group was regarded 100% alive and the IC_{50} dose was calculated by probit analysis. MTT test was run in four replicates in all groups.

RNA Isolation and cDNA Synthesis

AML-12 cells were seeded 3 times in culture plates to have $3x10^6$ cells in each well. After 24 hours, AML-12 cells were administered the chemical applications of the experimental groups at the dose of IC_{50} for 24 hours. RNA was isolated (PureLink RNA Mini Kit) from the obtained cells. Concentrations and purity values of the obtained RNA samples were determined with nanodrop (NaNoQ OPTIZEN). cDNA synthesis was carried out from RNA samples (high capacity cDNA reverse transcription kit).

qRT-PCR Analysis

Quant Studio 6 Flex device of SYBR Select Master Mix was used for quantitative real-time-polymerase chain reaction (qRT-PCR) analysis of enzyme expressions of the cells associating with SOD, CAT, GSH and gene expressions of the cells associating with Cas-3, Cas-9, Apaf-1, Bax, Bcl-2, p53, smac/DIABLO, Top I, Top II. PCR conditions were determined as follows: 1 cycle was 2 minutes at 50 °C and 10 minutes at 95 °C, afterwards 50 cycles for denaturation were 15 seconds at 95 °C and 1 second at 60 °C for annealing and extension. mRNA expression levels were analyzed by comparative cycle threshold (2-ΔΔCt) method (User Bulletin 2, Applied Biosystems). To obtain a

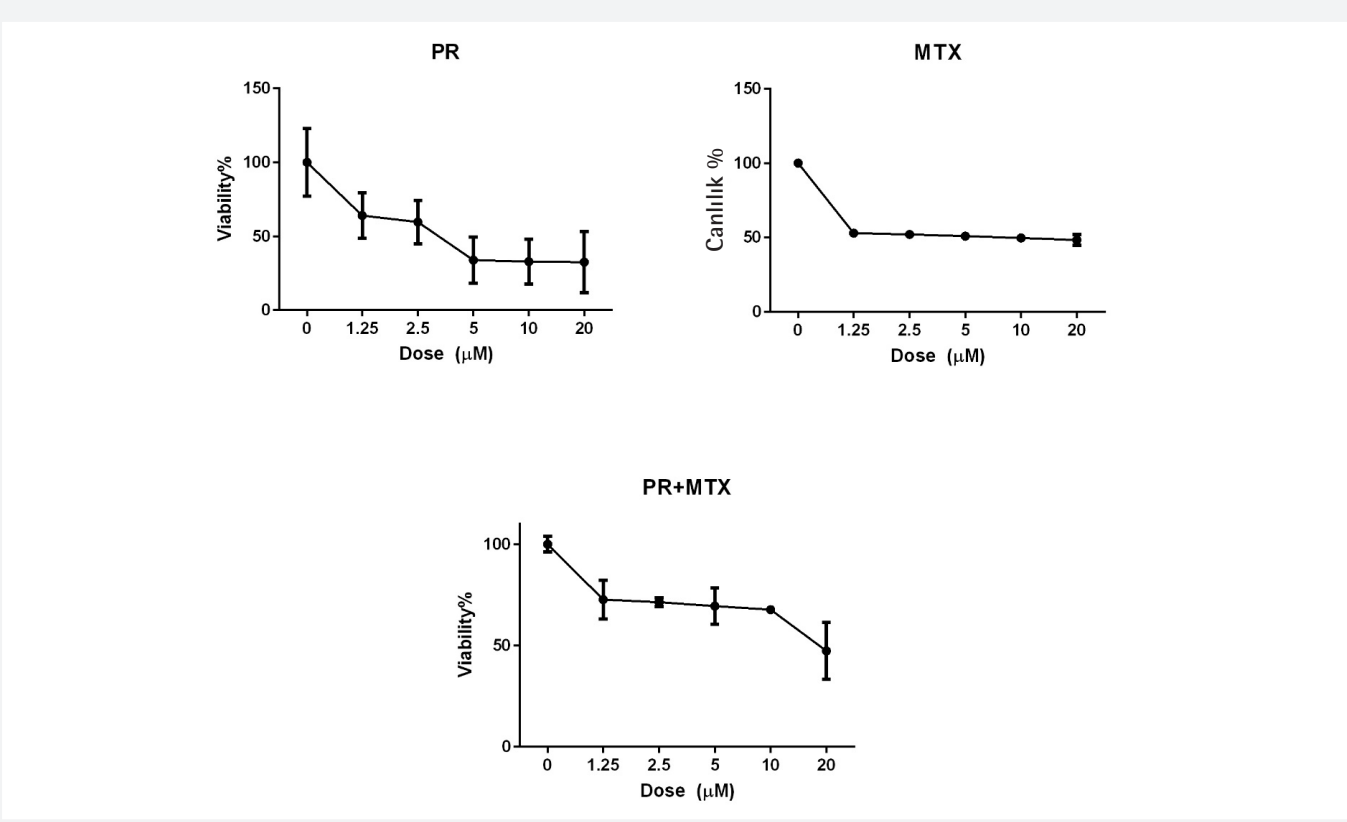

Figure 1. MTT assay results of each treatment group. Vertical bars represent standard deviation (n=4, mean±standard deviation) (viability %=Sample absorbance average/control absorbance average × 100)

PR: Puerarin group, MTX: Methotrexate group, PR+MTX: Puerarin+methotrexate combination group

copy of the GSH gene sequences was selected "Nucleotide" from the National Center for Biotechnology Information. After that, relevant organism/gene name was entered in the search box, and FASTA was determined and the relevant genes were designed. Relative fold-changes in gene expression were calculated by comparing the experimental groups to the control group and were normalized to the expression of β-actin mRNA (Table 1).

Statistical Analysis

IC_{50} value was calculated by applying probit analysis to percent viability data obtained by MTT test. After the application of the AML-12 cell line at IC_{50} doses for 24 hours to the AML-12 cells, one-way ANOVA test and post-hoc Tukey were administered to the relative fold-change values of gene expressions. Values at $p<0.05$ were accepted to be significant. Probit analysis and ANOVA test were done with Statistical Package for the Social Sciences 20 software (IBM).

RESULTS

To evaluate the effect of PR, MTX, and the PR+MTX combination on the viability of AML-12 cell lines, we conducted MTT assays for a duration of 24 hours. MTT assay results indicated that exposure of the AML-12 cell line to different concentrations of PR, MTX, and the PR+MTX combination for 24 hours caused a reduction in cell viability that depended on the dose, as shown in Figure 1. IC_{50} doses were determined as 3.28 µM in PR, 7.97 in MTX and 18.98 µM in PR+MTX.

Table 1. Primer sequences of analyzed genes for qRT-PCR analysis

Gene	Primer sequences (forward/reverse)
SOD	F: AGCTGCACCACAGCAAGCAC[8] R: TCCACCACCCTTAGGGCTCA
CAT	F: TCCGGGATCTTTTTAACGCCATTG[9] R: TCGAGCACGGTAGGGACAGTTCAC
GSH	F: ACTTGGCACTCCTCTCCTGA R: AGGCACTAGAACCTGCTGGA
Cas-3	F: GGTATTGAGACAGACAGTGG[10] R: CATGGGATCTGTTTCTTTGC
Cas-9	F: GAGTCAGGCTCTTCCTTTG[10] R: CCTCAAACTCTCAAGAGCAC
Apaf-1	F: GATATGGAATGTCTCAGATGGCC[11] R: GGTCTGTGAGGACTCCCCA
Bax	F: TTCATCCAGGATCGAGCAGA[10] R: GCAAAGTAGAAGGCAACG
Bcl-2	F: ATGTGTGTGGAGAGCGTCAA[10] R: ACAGTTCCACAAAGGCATCC
p53	F: CACGAGCGCTGCTCAGATAGC[10] R: ACAGGCACAAACACGCACAAA
Smac/DIABLO	F: CTCTGTGGCTGAGGGTTGAT[12] R: TTGTAGATGATGCCCACAGG
Top I	F: TCATACTGAACCCCAGCTCC[10] R: GTCCTGCAAGTGCTTGTTCA
Top II	F: CTTCTCTGATATGGACAAACATAAGATTCC[10] R: GGACTGTGGGACAACAGGACAATAC

qRT-PCR: Quantitative real-time-polymerase chain reaction, Cas: Caspase

PR administration significantly elevated SOD expression levels in comparison to the control group ($p<0.0001$). Although MTX administration led to a significant increase in SOD expression compared to the control group ($p<0.01$), it was significantly lower than in the PR group ($p<0.0001$). When compared to the PR group, the PR+MTX group had significantly lower SOD expression levels ($p<0.0001$). While there was no significant difference between the PR+MTX and MTX groups, SOD expression was higher in PR+MTX, as shown in Figure 2A.

Comparisons of CAT expression levels among the control, MTX, and PR groups revealed a statistically significant increase in CAT expression in the MTX group ($p<0.0001$). Compared to the control and PR groups, the PR+MTX group exhibited significantly higher levels of CAT expression ($p<0.0001$ and $p<0.001$, respectively), as shown in Figure 2B.

Statistical analysis of GSH expression levels revealed an increase in the MTX-administered groups compared to both the control and PR groups ($p<0.0001$). With respect to the control group, PR administration increased GSH expression levels ($p<0.01$), as shown in Figure 2C.

Cas-3 gene expression was higher in the PR ($p<0.0001$), MTX ($p<0.05$), and PR+MTX ($p<0.0001$) groups compared to the control group. When compared to the PR group, MTX administration significantly reduced Cas-3 gene expression ($p<0.0001$). Cas-3 gene expression was determined higher in the PR+MTX group than in the PR group ($p<0.0001$) (Figure 2D).

With MTX administration, Cas-9 gene expression increased significantly compared to the control group and PR group ($p<0.0001$). Although Cas-9 gene expression decreased in the PR+MTX group compared to the MTX group, that difference was not significant. Cas-9 gene expression was higher in the PR+MTX group than in the PR group ($p<0.0001$). When PR was administered alone, Cas-9 gene expression increased significantly compared to the control group ($p<0.001$) (Figure 2E).

In comparison to the control and PR groups, MTX administration significantly raised the Apaf-1 gene expression ($p<0.0001$). Although expression of Apaf-1 gene reduced in the PR+MTX group compared to the MTX group, that difference was not significant. Compared to the control and PR groups, Apaf-1 gene expression was higher in the PR+MTX group ($p<0.0001$) (Figure 2F).

Bax gene expression increased significantly with PR administration compared to the control group (p<0.0001). MTX administration decreased Bax gene expression compared to the PR group (p<0.0001). Bax gene expression in the PR+MTX group was higher compared to the control group (p<0.05), but significantly lower compared to the PR group (p<0.0001) (Figure 3A).

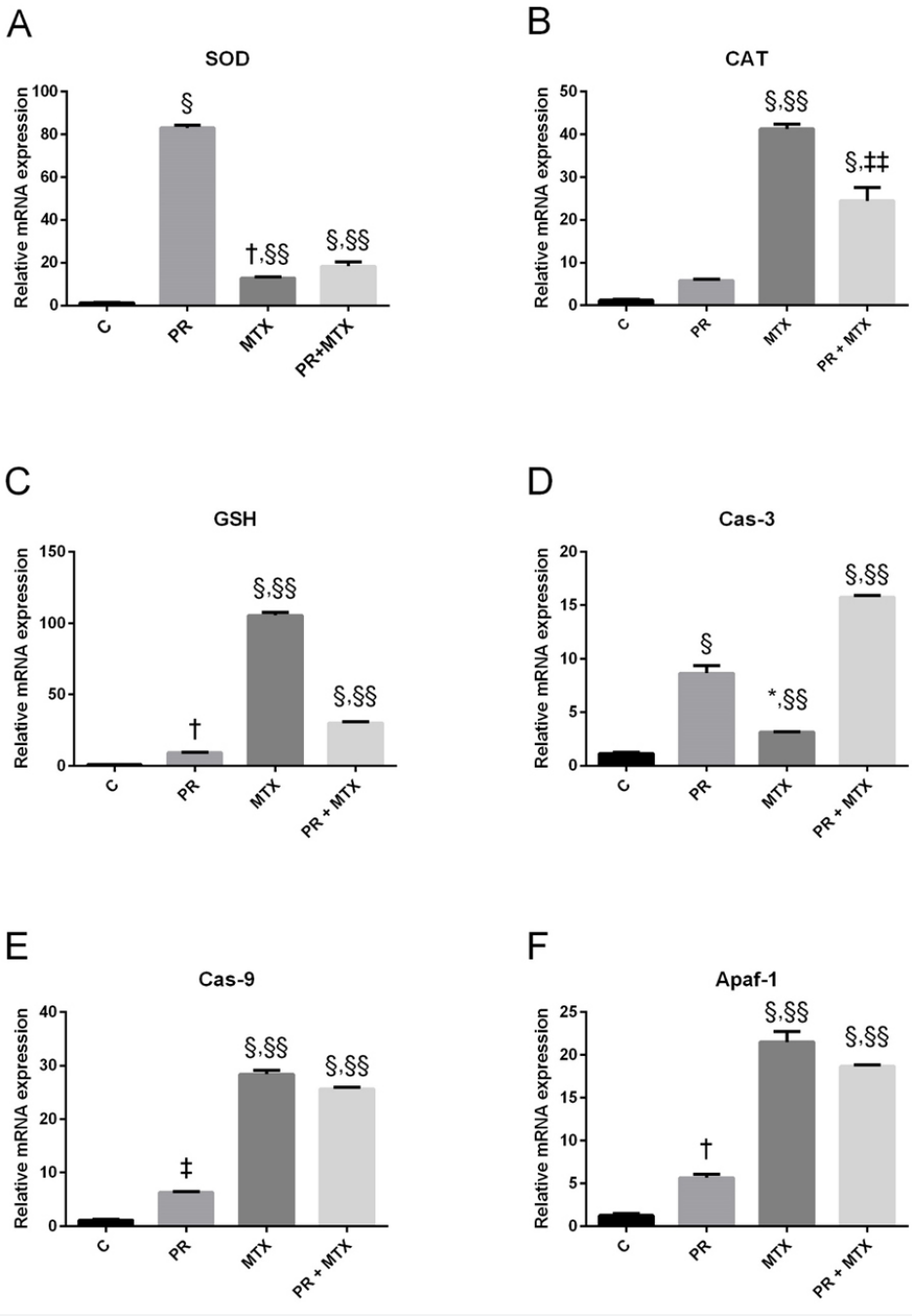

Figure 2. SOD (A), CAT (B), GSH (C), Cas-3 (D), Cas-9 (E), and Apaf-1 (F) relative mRNA expression

*p<0.05, †: p<0.01, ‡: p<0.001, §: p<0.0001 compared to the control group.

**p<0.05, ††: p<0.01, ‡‡: p<0.001, §§: p<0.0001 compared to the PR group

C: Control group, PR: Puerarin group, MTX: Methotrexate group, PR+MTX: Puerarin+methotrexate combination group

MTX administration significantly increased Bcl-2 expression compared to the PR group ($p<0.001$). Bcl-2 expression was significantly lower in the PR group compared to the control group ($p<0.05$) (Figure 3B).

PR and MTX administrations elevated p53 gene expression significantly compared to the control group. Statistical significance of that increase was determined as $p<0.001$ in the PR group and MTX group, and $p<0.0001$ in the PR+MTX group. Gene expression on p53 was found to be higher in the PR+MTX group than in the PR group and control group ($p<0.0001$) (Figure 3C).

PR administration increased smac/DIABLO gene expression level compared to the control group ($p<0.0001$). Expression of smac/DIABLO gene level in the PR+MTX group was significantly higher than in the control group ($p<0.001$). The smac/DIABLO gene expression level in the PR+MTX group was lower than in the PR group ($p<0.0001$). When PR was administered with MTX, the smac/DIABLO gene expression level was found to be higher than in the MTX group, although it was not statistically significant (Figure 3D).

Top I gene expression increased significantly with MTX administration compared to the control group and PR group ($p<0.001$ and $p<0.05$). Decrease in the expression of Top I was observed in the PR+MTX group compared to the MTX group, which was not found statistically significant. In the PR group, Top I gene expression was elevated when compared to the control group ($p<0.01$) (Figure 3E).

PR and MTX administrations increased Top II gene expression compared to the control group. Statistical significance of this increase was determined as $p<0.01$ in the PR group, $p<0.001$ in the MTX group, and $p<0.0001$ in the PR+MTX group. Top II expression was higher in the PR+MTX group than in the PR group ($p<0.001$) (Figure 3F).

DISCUSSION

According to our results, the PR administration has a protective effect against MTX-induced liver damage *in vitro*. Our study is the first to investigate the effects of PR on the development of MTX-related liver damage. Furthermore, our research retains its originality by casting light on the mechanisms underpinning MTX-caused liver damage.

SOD and CAT are antioxidant enzymes found in cells. SOD removes the superoxide anion by converting it to H_2O_2, which is reduced to H_2O by CAT. Previous research has revealed that MTX causes oxidative stress by reducing SOD levels[3]. In our study, while PR administration raised SOD expression by demonstrating an antioxidant effect, MTX administration significantly reduced SOD levels compared to the PR group. Supportively, according to literature, PR provides antioxidant and antiinflammatory activities in many diseases such as nervous system diseases, respiratory diseases, osteoporosis, and liver diseases[13]. In addition, PR alleviated renal damage in MTX induced experimental study[14].

According to literature, when the amount of superoxide anion in the cell surpasses the detoxification capacity, CAT activity rises to enhance antioxidant defense and CAT contributes to GSH formation by raising GSH reductase activity[15]. It is also known that exposing cells to hydrogen peroxide enhances CAT function[16]. In our study, MTX administration increased the cell's exposure to free radicals and increased CAT expression. PR administration with MTX attenuated this increase in CAT levels.

GSH is a tripeptide with a low molecular weight that acts as an antioxidant. When GSH combines with free radicals, it produces oxidized glutathione (GSSG) and other disulfides[3]. GSH levels could rise in response to elevated amounts of free radicals in order to avoid oxidative stress from causing disease[17-19]. In the study, GSH levels were found to be significantly higher in the MTX group. This situation developed in response to the free radicals whose levels increased with MTX administration, and the cell was able to increase GSH levels, which had a protective effect against oxidative stress. High CAT expression in this group also contributed to increasing GSH levels[15]. In the PR+MTX group, depending on the protective effects of PR against oxidative stress, GSH levels were found to be lower than in the group administered MTX alone. When cells are continuously exposed to free radicals, their amounts may drop as a consequence of the conversion of reduced GSH to disulfides. In experimental studies on animals, it has been shown that MTX administration reduces the level of GSH in liver tissue. It is believed that GSH deficiency contributes to MTX-induced liver tissue injury[3].

Apoptosis refers to the programmed cell death ensuring homeostasis in living organisms. In addition to genes, RNA, protein synthesis and energy also play a key role in regulating the apoptosis. It is known that abnormalities in the apoptotic pathway occur in many diseases, such as diabetes, infection, and tumor, which are affected by oxidative stress and inflammation. Both the internal (mitochondrial) and external pathways contribute to the development of apoptosis. DNA damage and hypoxia are intracellular signals that trigger apoptosis. Chemotherapeutic drugs, external factors such as radiation, and the activation of death receptors could be shown as the examples of extracellular signals[20]. MTX induces apoptosis in various of tissues[3]. The apoptosis induced by MTX has a beneficial impact in cancer treatment by enabling cancer cells to be eliminated. However, abnormalities in apoptosis pathways could have negative impacts in a variety of organs, including the liver. For instance, the administration of MTX

to rats has resulted in liver tissue damage through apoptosis mechanisms in a number of experimental investigations[3,21].

Caspases that activate each other in a proteolytic way are known as cysteine proteases. While Cas-9 is one of the initiator caspases, Cas-3 could be given as an example of effector caspase. They inactivate enzymes involved in DNA repair and replication[20]. When a cell undergoes apoptosis, cytochrome-c release from the mitochondria rises. Cytochrome-c, Apaf-1, and ATP create a complex known as "apoptosome" in the cytosol.

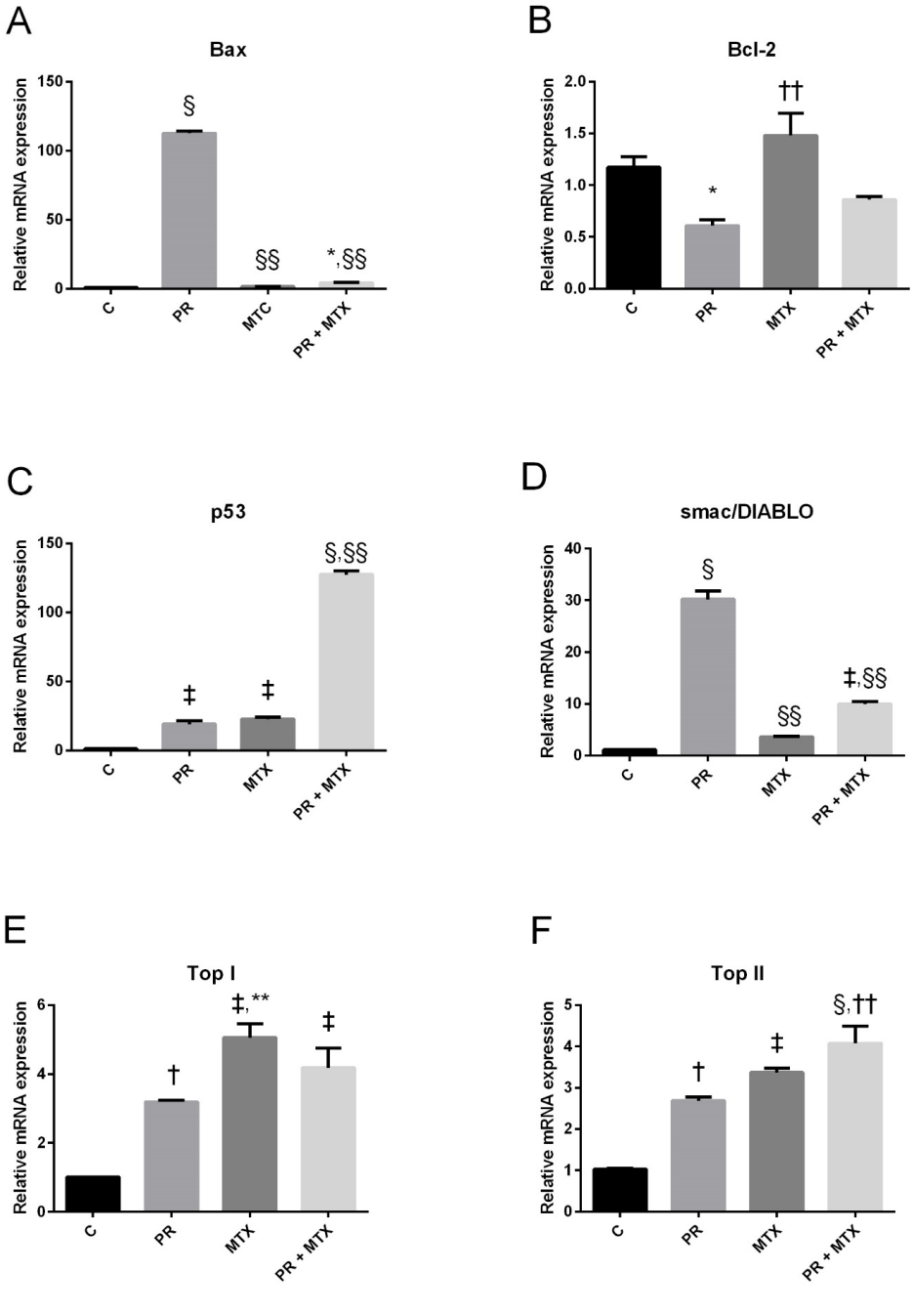

Figure 3. Bax (A), Bcl-2 (B), p53 (C), smac/DIABLO (D), Top I (E), and Top II (F) relative mRNA expression

*p<0.05, †: p<0.01, ǂ: p<0.001, §: p<0.0001 compared to the control group.

**p<0.05, ††: p<0.01, ǂǂ: p<0.001, §§: p<0.0001 compared to the PR group.

C: Control group, PR: Puerarin group, MTX: Methotrexate group, PR+MTX: Puerarin+methotrexate combination group

Cas-9 is activated by the apoptosome and Cas-3 is activated by Cas-9. Supportively, Apaf-1 and Cas-9 expression levels were detected to be elevated in the MTX group in our research. The fact that the expression level of Cas-3 was not high in this group indicates that the pathway has not progressed up to Cas-3. An experimental liver damage model generated with MTX administration in rats has demonstrated that MTX causes apoptosis in liver tissue by increasing Cas-9 and Cas-3 activity[22]. In our study, when PR was administered along with MTX, the decrease in both Apaf-1 and Cas-9 gene expression compared to the MTX group indicated the protective effect of PR on hepatotoxicity caused by MTX.

Proteins such as the Bcl-2 family, caspases, p53, and cytotome-c regulate apoptosis. The Bcl-2 family includes both proapoptotic and antiapoptotic proteins. One of the parameters we investigated in our study is that Bax has a proapoptotic effect, while Bcl-2 is one of the antiapoptotic proteins[20]. In our study, PR administration along with MTX increased Bax gene expression compared to the control group and induced apoptosis of damaged structures. According to Chen et al.[23] (2012), PR treatment in ischemic pulmonary artery smooth muscle cells raised Bax levels, which in turn increased apoptosis. Recent research has demonstrated that isoflavones and flavone glycosides promote the production of the protein Bax, which contributes to the destruction of cancer cells[24,25].

In our study, as an indicator of cell damage, Bcl-2 gene expression levels elevated in the MTX group to prevent cell death. This result is supported by the fact reported in previous studies that in individuals with chronic hepatitis, Bcl-2 expression rises in paralel with blood transaminase levels[26]. Furthermore, it has been determined that the Bcl-2 expression level is correlated with inflammation in the liver of patients with autoimmune hepatitis[27]. Additionally, the increase in the Bcl-2 levels is involved in the hepatocarcinogenesis development[28]. In a clinical research, it was found that Bcl-2 expression dropped with antiviral therapy in hepatitis patients[26]. In our study, Bcl-2 gene expression was observed to be lower in the MTX+PR group than in the MTX group. P53 is a transcription element that gives cells the opportunity to fix damaged DNA. When the harm is irreparable, it induces apoptosis by raising the production of Bax and Apaf-1 while suppressing Bcl-2[20]. In our study, p53 levels increased as a result of a defense mechanism against DNA damage in the PR+MTX group. The experimental hepatotoxicity model caused by acetaminophen in animals has shown that p53 has a protective impact against liver injury, which is consistent with our results[29,30].

Smac/DIABLO, a recently identified apoptosis protein inhibitor (IAP) binding protein, is released from mitochondria during apoptosis and potentiates apoptosis by attenuating IAPs inhibition on caspases[31]. In our research, we hypothesize that enhanced smac/DIABLO expression with PR administration has a protective impact on removing damaged structures. Bcl-2 reduces smac/DIABLO release[32]. The findings of our research show that Bcl-2 and smac/DIABLO transcript levels in the groups are inversely proportional.

DNA-topoisomerases are crucial enzymes in metabolism and regulation of DNA structure, including DNA replication, transcription, and chromosome segregation. Top I enzymes usually consist of a monomer and break the single strand of the DNA double helix. Top II enzymes with two or more subunits could break both of the double helix of DNA[33]. The level of topoisomerases rises as a cellular response to various conditions that result in cellular damage, such as infection, inflammation, or oxidative stress[34]. In our study, when PR was administered with MTX, the decrease in Top I level compared to the MTX group indicates that single strand damage in DNA is diminished. The increase in the Top I level in PR group indicates that the dose of PR applied is high for this cell. In our research, the amount of PR that was administered along with MTX was inadequate to repair the double strand of DNA damage. As a result, Top II level in the PR+MTX group was determined to be elevated.

CONCLUSION

Our research has clarified that PR could be effective in hepatotixicity of MTX, which is a widely used treatment option in various cancer diseases. PR mitigated the oxidative stress, abnormalities in apoptotic pathways, and DNA single strand damage in the liver tissue due to MTX administration. Our study would guide the experiments in animals and clinical studies. Since PR could not prevent double-strand DNA damage caused by MTX and there was an increase in Top I expression in the PR group, different dose studies are needed.

Ethics

Ethics Committee Approval and Informed Consent: Since our study is a cell culture study, ethics committee approval and informed consent are not required.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: M.A., Ç.O., E.B., Concept: M.A., Ç.O., E.B., Design: M.A., Ç.O., E.B., Data Collection or Processing: M.A., Ç.O., E.B., Analysis or Interpretation: M.A., Ç.O., E.B., Z.A.Ç.Y., Literature Search: M.A., E.B., Z.A.Ç.Y., Writing: M.A., Z.A.Ç.Y.

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. McCahill LE, Krouse RS, Chu DZ, Juarez G, Uman GC, Ferrell BR, et al. Decision making in palliative surgery. J Am Coll Surg. 2002;195:411-22.
2. Alfwuaires MA. Galangin mitigates oxidative stress, inflammation, and apoptosis in a rat model of methotrexate hepatotoxicity. Environ Sci Pollut Res Int. 2022;29:20279-88.
3. Ali N, Rashid S, Nafees S, Hasan SK, Sultana S. Beneficial effects of Chrysin against Methotrexate-induced hepatotoxicity via attenuation of oxidative stress and apoptosis. Mol Cell Biochem. 2014;385:215-23.
4. Bath RK, Brar NK, Forouhar FA, Wu GY. A review of methotrexate-associated hepatotoxicity. J Dig Dis. 2014;15:517-24.
5. Methotrexate. LiverTox: Clinical and Research Information on Drug-Induced Liver Injury [Internet]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK548219/
6. Xia DZ, Zhang PH, Fu Y, Yu WF, Ju MT. Hepatoprotective activity of puerarin against carbon tetrachloride-induced injuries in rats: a randomized controlled trial. Food Chem Toxicol. 2013;59:90-5.
7. Chen X, Li R, Liang T, Zhang K, Gao Y, Xu L. Puerarin improves metabolic function leading to hepatoprotective effects in chronic alcohol-induced liver injury in rats. Phytomedicine. 2013;20:849-52.
8. Tam NN, Gao Y, Leung YK, Ho SM. Androgenic regulation of oxidative stress in the rat prostate: involvement of NAD(P)H oxidases and antioxidant defense machinery during prostatic involution and regrowth. Am J Pathol. 2003;163:2513-22.
9. Dkhil MA, Moneim AE, Al-Quraishy S. Indigofera oblongifolia Ameliorates Lead Acetate-Induced Testicular Oxidative Damage and Apoptosis in a Rat Model. Biol Trace Elem Res. 2016;173:354-61.
10. Oltulu C, Akıncı M, Elvan B. Antitumor Activity of Etoposide, Puerarin, Galangin and Their Combinations in Neuroblastoma Cells, International Journal of Life Sciences and Biotechnology. 2022;5:407-23.
11. Yakovlev AG, Ota K, Wang G, Movsesyan V, Bao WL, Yoshihara K, et al. Differential expression of apoptotic protease-activating factor-1 and caspase-3 genes and susceptibility to apoptosis during brain development and after traumatic brain injury. J Neurosci. 2001;21:7439-46.
12. Tokatlı C, Doğanlar O, Doğalar ZB. Meriç Delta Balıklarında Çevre Kirliliğinin Genotoksik Etkileri: Antioksidan Savunma, Isı Şok Protein Sinyali ve DNA Hasar-Onarım Mekanizmaları. Journal of Limnology and Freshwater Fisheries Research. 2020;6:14-24.
13. Liu Q, Liu Z, Huo X, Wang C, Meng Q, Sun H, et al. Puerarin improves methotrexate-induced renal damage by up-regulating renal expression of Oat1 and Oat3 in vivo and in vitro. Biomed Pharmacother. 2018;103:915-22.
14. Wang D, Bu T, Li Y, He Y, Yang F, Zou L. Pharmacological Activity, Pharmacokinetics, and Clinical Research Progress of Puerarin. Antioxidants (Basel). 2022;11:2121.
15. Aguiló A, Tauler P, Fuentespina E, Tur JA, Córdova A, Pons A. Antioxidant response to oxidative stress induced by exhaustive exercise. Physiol Behav. 2005;84:1-7.
16. Kenney MC, Chwa M, Atilano SR, Tran A, Carballo M, Saghizadeh M, et al. Increased levels of catalase and cathepsin V/L2 but decreased TIMP-1 in keratoconus corneas: evidence that oxidative stress plays a role in this disorder. Invest Ophthalmol Vis Sci. 2005;46:823-32.
17. Chakravarthi S, Jessop CE, Bulleid NJ. The role of glutathione in disulphide bond formation and endoplasmic-reticulum-generated oxidative stress. EMBO Rep. 2006;7:271-5.
18. Sthijns MM, Weseler AR, Bast A, Haenen GR. Time in Redox Adaptation Processes: From Evolution to Hormesis. Int J Mol Sci. 2016;17:1649.
19. Chowdhury FA, Mahboob S, Saha A, Jahan A, Islam MN. Effect of oxidative stress on glutathione reductase activity of Escherichia coli clinical isolates from patients with urinary tract infection. J Immunol Clin Microbiol. 2017:2.
20. Coşkun G, Özgür H. Apoptoz ve Nekrozun Moleküler Mekanizması. Arşiv. 2011;20:145-58.
21. Khalaf MM, Hassanein EHM, Shalkami AS, Hemeida RAM, Mohamed WR. Diallyl Disulfide Attenuates Methotrexate-Induced Hepatic Oxidative Injury, Inflammation and Apoptosis and Enhances its Anti-Tumor Activity. Curr Mol Pharmacol. 2022;15:213-26.
22. Ali N, Rashid S, Nafees S, Hasan SK, Shahid A, Majed F, et al. Protective effect of Chlorogenic acid against methotrexate induced oxidative stress, inflammation and apoptosis in rat liver: An experimental approach. Chem Biol Interact. 2017;272:80-91.
23. Chen C, Chen C, Wang Z, Wang L, Yang L, Ding M, et al. Puerarin induces mitochondria-dependent apoptosis in hypoxic human pulmonary arterial smooth muscle cells. PLoS One. 2012;7:e34181.
24. Li Y, Kong D, Bao B, Ahmad A, Sarkar FH. Induction of cancer cell death by isoflavone: the role of multiple signaling pathways. Nutrients. 2011;3:877-96.
25. Abusaliya A, Ha SE, Bhosale PB, Kim HH, Park MY, Vetrivel P, et al. Glycosidic favonoids and their potential applications in cancer research: a review. Molecular & Cellular Toxicology. 2022;18:9–16.
26. Anatol P, Danuta P, Janusz D, Bozena P. Expression of bcl-2 protein in chronic hepatitis C: effect of interferon alpha 2b with ribavirin therapy. World J Gastroenterol. 2005;11:2949-52.
27. Yachida M, Kurokohchi K, Arima K, Nishioka M. Increased bcl-2 expression in lymphocytes and its association with hepatocellular damage in patients with autoimmune hepatitis. Clin Exp Immunol. 1999;116:140-5.
28. MaS, Chen GG: Lai PBS: Bcl-2 Family Members in Hepatocellular Carcinoma (HCC) – Mechanisms and Therapeutic Potentials. In: Chen GG, Lai PBS (eds), Apoptosis in Carcinogenesis and Chemotherapy. Springer. 2009;219-35.
29. Huo Y, Yin S, Yan M, Win S, Aung Than T, Aghajan M, et al. Protective role of p53 in acetaminophen hepatotoxicity. Free Radic Biol Med. 2017;106:111-7.
30. Sun J, Wen Y, Zhou Y, Jiang Y, Chen Y, Zhang H, et al. p53 attenuates acetaminophen-induced hepatotoxicity by regulating drug-metabolizing enzymes and transporter expression. Cell Death Dis. 2018;9:536.
31. Roberts DL, Merrison W, MacFarlane M, Cohen GM. The inhibitor of apoptosis protein-binding domain of Smac is not essential for its proapoptotic activity. J Cell Biol. 2001;153:221-8.
32. Adrain C, Creagh EM, Martin SJ. Apoptosis-associated release of Smac/DIABLO from mitochondria requires active caspases and is blocked by Bcl-2. EMBO J. 2001;20:6627-36.
33. Mastrangelo S, Attina G, Triarico S, Romano A, Maurizi P, Ruggiero A. The DNA-topoisomerase Inhibitors in Cancer Therapy. Biomedical and Pharmacology Journal. 2022;15:553-62.
34. Rialdi A, Campisi L, Zhao N, Lagda AC, Pietzsch C, Ho JSY, et al. Topoisomerase 1 inhibition suppresses inflammatory genes and protects from death by inflammation. Science. 2016;352:aad7993.

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The Results of Urodynamics and Pressure Flow Study of Patients with Neurological Disease in a Single Center for 12 Years: Neurogenic Bladder Etiology

Tek Merkez, Nörolojik Hastalığı Olan Hastaların 12 Yıllık Ürodinami ve Basınç Akım Çalışması Sonuçları: Nörojenik Mesane Etiyolojisi

Çağrı DOĞAN[1], Murat AKGÜL[1], Cenk Murat YAZICI[1], Arzu MALAK[2], Enes ALTIN[1], Serkan ŞERAMET[1], Hulusi Sıtkı DAYISOYLU[1], Erdem Can TOPKAÇ[1]

[1]*Tekirdağ Namık Kemal University Faculty of Medicine, Department of Urology, Tekirdağ, Turkey*
[2]*Tekirdağ Namik Kemal University Faculty of Health Sciences, Division of Nursing, Tekirdağ, Turkey*

ABSTRACT

Aim: Generally, urodynamic-pressure flow study (U-PFS) is performed on patients with lower urinary tract symptoms (LUTS) for verifying the diagnosis and evaluating the rate of response for treatment. The aim of the study was to assess the results of the U-PFS of patients according to the etiology of neurological disorders.

Materials and Methods: The data of 2,489 patients who underwent U-PFS in our clinic between 2010-2022 were analyzed retrospectively. A total of 535 patients with LUTS and neurogenic disorder were included in the study. Patients were divided into subgroups according to their diagnosis. The patient's age, gender, and U-PFS data (sensation of first urine, maximum cystometric capacity (MSC), maximum detrusor pressures in the filling phase, presence of urgency, and bladder compliance status) were evaluated and compared according to neurological disorders.

Results: Cervical and lumbar disc disorder was found in 204 (38.1%) patients, multiple sclerosis (MS) in 103 (19.2%), and cerebrovascular incidents in 74 (13.8%) patients (SVI), spinal cord injury in 48 (8.9%), polyneuropathy in 43 (8.0%), Parkinson's disease (PD) in 30 (5.6%), diabetic neuropathy in 18 (3.4%), and operated spine bifida (oSB) in 15 (2.8%) was detected. Detrusor pressures in the filling phase were compared according to neurological disorders, and detrusor pressures were statistically significantly higher in patients with oSB and PD (52.66±40.78 mmHg; 45.30±34.43 mmHg, respectively; p<0.001). When the MSCs were compared, it was observed that the bladder capacity was significantly lower in PD and ASD patients, whereas bladder capacity was relatively increased in lomber and servical disc disorder, spinal cord injury and polyneuropathy patients (respectively 308.71±190.25 mL, 264.81±140.25 mL, 491.90±167.49, 474.52±182.92, 447.67±168.03, p<0.001).

Conclusion: These specific patient groups (oSB and spinal cord injury) are hazardous groups for the development of end-stage kidney failure. Clinicians should take into consideration that patients and their relatives have to be informed about possible long-term complications.

Keywords: Neurogenic bladder, urodynamic study, epidemiology

ÖZ

Amaç: Çalışmamızda, kliniğimize alt üriner sistem semptomları (AÜSS) ile başvuran ve nörolojik hastalığı olan hastaların ürodinami-basınç akım çalışması (Ü-BAÇ) sonuçlarının belirlenmesi ve bu bulguların nörolojik hastalıkların etiyolojisine göre karşılaştırılması amaçlanmıştır.

Gereç ve Yöntem: Kliniğimizde 2010-2022 yılları arasında Ü-BAÇ yapılan 2.489 hastanın verileri retrospektif olarak incelendi. AÜSS ve nörojenik hastalığı olan toplam 535 hastanın verileri çalışmaya dahil edildi. Hastalar tanılarına göre alt gruplara ayrıldı. Hastaların yaş, cinsiyet, Ü-BAÇ verileri [ilk idrar hissi, maksimum sistometrik kapasitesi (MSK), dolum fazında maksimum detrüsor basınçları, ani sıkışma hissi varlığı ve mesane kompliyansı] değerlendirmeye alınarak mevcut nörolojik hastalıklara göre karşılaştırıldı.

Address for Correspondence: Çağrı DOĞAN MD, Tekirdağ Namık Kemal University Faculty of Medicine, Department of Urology, Tekirdağ, Turkey
Phone: +90 555 890 88 05 **E-mail:** drcagridogan@gmail.com **ORCID ID:** orcid.org/0000-0001-9681-2473
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Bulgular: Kliniğimizde nörolojik hastalığı olan ve Ü-BAÇ yapılan hastaların 204'ünde (%38,1) servikal ve lomber disk bozukluğu, 103'ünde (%19,2) multipl skleroz (MS), 74'ünde (%13,8) serebrovasküler olay, 48'inde (%8,9) spinal kord yaralanması, 43'ünde (%8,0) polinöropati, 30'unda (%5,6) Parkinson hastalığı (PH), 18'inde (%3,4) diyabetik nöropati ve 15'inde (%2,8) opere spina bifida (oSB) olduğu tespit edildi. Dolum fazındaki detrüsor basınçları karşılaştırıldığında oSB ve MS hastalarında detrüsor basınçları istatistiksel olarak belirgin yüksek olduğu saptandı (sırasıyla 52,66±40,78 mmHg; 45,30±34,43 mmHg; p<0,001). MSK'lar karşılaştırıldığında, PH ve oSB hastalarında mesane kapasitesinin belirgin düşük, lomber disk bozuklukları, opere lomber, servikal fraktür ve polinöropati hastalarında ise mesane kapasitesinin görece artmış olduğu gözlendi (sırasıyla 308,71±190,25 mL, 264,81±140,25 mL, 491,90±167,49, 474,52±182,92, 447,67±168,03, p<0,001).

Sonuç: oSB ve spinal kord yaralanması olan hastaların ürodinamik verileri değerlendirildiğinde, bu hasta gruplarının son dönem böbrek yetmezliği açısından üst üriner sistemin etkilenmesi açısından çok yüksek risk altında olduğu görülmektedir. Klinisyenler özellikle bu iki nörojenik hastalık grubunda takip protokolünü oluştururken bunları göz önüne almalı ve olası gelişebilecek yakın ve uzun dönem komplikasyonları hastalarla paylaşmalıdır.

Anahtar Kelimeler: Nörojen mesane, ürodinamik çalışma, epidemiyoloji

INTRODUCTION

Lower urinary tract symptoms (LUTS) due to neurological diseases may vary depending on the neurological disease or the localization of the neurological disease. For example, neurogenic detrusor overactivity is expected in neurological diseases with suprapontine involvement, while underactive detrusor or acontractile detrusor come to the fore in diseases with peripheral nervous system involvement. Even patients with the same neurological disease may apply to the clinic with different LUTS.

In some neurological diseases, LUTS may appear before the development of neurological findings, and patients may be diagnosed with neurological disease after the occurrence of LUTS. Especially 10% of multiple sclerosis (MS) patients can apply to a physician with only LUTS[1]. Therefore, the possibility of neurological disease in patients with LUTS or LUTS in patients with neurological disease should be questioned. In the evaluation of LUTS in patients with neurological disease, urodynamic testing, which is an invasive test, and, if necessary, a pressure flow study is recommended[2].

Although there are many studies in the literature on LUTS in neurological diseases, there are not enough data on urodynamic findings in these patient groups. In our study, it was aimed to determine the results of urodynamic-pressure flow study of patients being admitted to our clinic with LUTS and having neurological disease, and to compare these findings according to neurological pathology.

MATERIALS AND METHODS

After getting approval from the Tekirdağ Namık Kemal University Local Ethics Committee (no: 2022.180.10.04, date: 25.10.2022), the data of 2,489 patients who underwent urodynamic evaluation in our clinic between 2010 and 2022 were evaluated retrospectively. Patients with neurological diagnoses at the time of urodynamic analysis were identified and included in the study. Patients without neurological disease or repeated urodynamics for the same patient despite having neurological disease were excluded from the study. Initial urodynamic reports were taken as reference from recurrent urodynamic results. Neurological diagnoses of the patients were compared with the data in the automation program (Enlil, v3.23.01.1, 2015, Turkey) and in the neurology clinic, and patients with incompatible diagnoses were excluded from the study. Among the urodynamic findings, first urine sensation, maximum bladder capacity, filling phase overactivity, filling phase maximum detrusor pressure, bladder compliance (>30 cmH_2O for non-neurogenic, >40 cmH_2O for neurogenic is considered nomocompliant; <10 cmH_2O for neurogenic, <30 cmH_2O for neurogenic were considered hypocompliant) and, if any, evacuation phase parameters (maximum detrusor pressure and flow velocity during the evacuation phase) were evaluated[2].

Since urodynamic evaluation is an invasive procedure, informed consent forms were obtained from all patients before urodynamic evaluation. Before the procedure, the patients were performed urine culture and those with growth in the culture were treated with the appropriate antibiotic according to the antibiogram, and all urodynamics were performed with sterile urine. In order for the abdominal pressure catheter to work optimally, fleet enemas were routinely applied to the patients one day before the procedure. Urodynamic evaluations were performed with the Aymed brand (version: 19050010-03, 2019, Turkey) aqueous system urodynamic device, accompanied by a trained urodynamic nurse. In adult patients, during the filling phase, saline fluid warmed to body temperature was sent into the bladder at a rate of 25 mL/hour. In pediatric cases, the infusion rate was adjusted to be one tenth of the patient's expected bladder capacity. The procedure was terminated by performing a pressure flow study in the patients deemed necessary. The amount of residual urine before and after each procedure was evaluated using a catheter. All urodynamic data used in the study were performed in the same clinic by an experienced urodynamic team, in accordance with international urodynamic standards.

Statistical Analysis

After the descriptive analysis of the data in the study, they were given as mean and standard deviation. To compare the quantitative data of the two groups, the normality test was performed with the Shapiro-Wilk test. Normally distributed parametric data were evaluated with the Student's t-test, and non-parametric data were evaluated with the Mann-Whitney U test. The chi-square test was used to compare the qualitative data within the groups. The results were within the 95% confidence interval, and the $p<0.05$ value was considered as statistically significant.

RESULTS

A total of 598 patients with a diagnosis of neurological disease at the time of the first urodynamics in our clinic, whose history and diagnoses were compatible with the patient registry system, were included in the study. The urodynamic data of patients with diseases (tethered cord disease, vascular dementias, Arnold Chiari malformation, neuromyelitis optica, Guillain-Barré syndrome, cauda equina syndrome, autoimmune encephalitis, etc.) with a total number of patients below 10 were excluded from the study. As a result, urodynamic data of a total of 535 patients were evaluated. The mean age of the patients was 52.69±15.86 years, and 321 (60.0%) were female and 214 (40.0%) were male. When the neurological diagnoses of the patients were evaluated, cervical and lumbar disc disorder was detected in 204 (38.1%) patients, MS in 103 (19.2%) patients, Cerebrovascular event (CVE) in 74 (13.8%) patients, spinal cord injury (SCI) in 48 (8.9%) patients, polyneuropathy in 43 (8.0%) patients, Parkinson's disease (PD) in 30 (5.6%) patients, diabetic neuropathy in 18 (3.4%) patients, and operated spina bifida (oSB) in 15 (2.8%) patients. The clinical and demographic characteristics of the patients are shown in Table 1. When the patients were evaluated according to their age, it was observed that the most advanced age group was in Parkinson's patients (65.03±8.62 years), while the youngest patients (45.75±16.04) were in the SCI group.

In the evaluation of urodynamic filling stage data according to neurological disease groups, it was observed that the first urine sensation of the patients did not differ according to the disease variety, but there was a statistical difference in maximum cystometry capacity (MCC) and maximum detrusor pressures. In the subgroup analyses, it was noted that the difference in maximum cystometry capacity was due to oSB patients and Parkinson's patients, and it was lower than in other patient groups ($p<0.001$). Among the existing neurological diseases, oSB was observed to have the lowest maximum cystometric capacity. When the maximum detrusor pressures were evaluated according to the neurological diseases, it was observed that the maximum detrusor pressures of the oSB patients reached the highest level among the existing neurological diseases, while the MS patient group was at the second highest level (Table 1).

In the evaluation of compliance disorder, which is an important variable for upper urinary tract damage, it was determined that 66.7% of the patients with a history of oSB had hypocompliance, while this rate was followed by Parkinson's patients with the rate of 40% (Table 2).

Considering the filling phase detrusor activity, it was found that neurogenic detrusor overactivity was at the highest rate in patients with oSB (80.0%) and at the lowest rate in patients with polyneuropathy (23.3%). Neurogenic detrusor overactivity was observed in 50% of diabetic patients with both peripheral and central nervous system damage. When the pressure flow studies of the patients were evaluated, the presence of acontractile detrusor was detected in 104 (17.4%) patients. Although acontractile detrusor was observed in 75 (14.0%) of these patients during the filling phase, neurogenic detrusor overactivity was detected. It was observed that the patient group with the highest rate was the SCI group. Details of the distribution of patients according to their diagnoses are shown in Figure 1.

Table 1. Comparison of urodynamics+pressure flow study according to neurogenic diagnoses

Neurological diseases	Number (n)	Age (year)	First urination sensation (mL)	Maximum cystometric capacity (mL)	Maximum Pdet in filling phase (mmHg)
CVE	74	62.09±12.56	64.81±43.06	385.19±165.98	23.67±24.86
MS	103	45.95±10.55	80.48±82.32	424.45±194.38	45.30±44.34
Diabetic neuropathy	18	62.44±10.26	44.28±29.22	429.22±141.49	17.58±11.52
Parkinson's	30	65.03±8.62	86.00±68.76	308.70±190.25	23.63±24.86
Polyneuropathy	43	60.27±13.94	55.40±39.07	447.67±168.03	17.27±15.80
Operated spina bifida	15	52.66±40.78	63.50±39.63	264.80±140.25	52.66±40.78
Lumbar disc disorders	153	54.41±12.72	65.31±40.53	491.90±167.49	16.39±15.73
Cervical disc disorders	51	59.38±14.21	61.22±38.54	425.58±131.25	18.79±14.18
Operated lumbar and cervical fracture	48	45.75±16.04	59.41±31.34	474.52±186.92	31.00±34.97
p value		<0.001	<0.401	<0.001	<0.001

CVE: Cerebrovascular event, MS: Multiple sclerosis

Table 2. Comparison of neurogenic detrusor overactivity and bladder compliance status data according to the etiology of neurogenic diseases

Neurogenic diseases	Urge attack		Bladder compliance		
	Yes (n, %)	No (n, %)	Hypocompliant (n, %)	Normocompliant (n, %)	Hypercompliant (n, %)
CVE	33 (44.6)	41 (55.4)	11 (15)	60 (80.9)	3 (4.1)
MS	67 (65.1)	36 (34.9)	29 (28.1)	68 (66)	7 (6.9)
Diabetic neuropathy	9 (50)	9 (50)	4 (22.3)	13 (72.2)	1 (5.5)
Parkinson's	17 (56.7)	13 (43.3)	12 (40)	17 (56.7)	1 (3.3)
Polyneuropathy	10 (23.3)	33 (76.7)	9 (20.9)	24 (55.8)	10 (23.3)
Operated spina bifida	12 (80)	3 (20)	10 (66.7)	5 (33.3)	0 (0)
Lumbar and cervical disc disorders	49 (24.01)	155 (75.98)	22 (10.8)	151 (74)	31 (15.2)
Operated lumbar and cervical fracture	26 (54.2)	22 (45.8)	9 (18.8)	32 (66.7)	7 (14.6)
p value	<0.001		<0.001		

CVE: Cerebrovascular event, MS: Multiple sclerosis

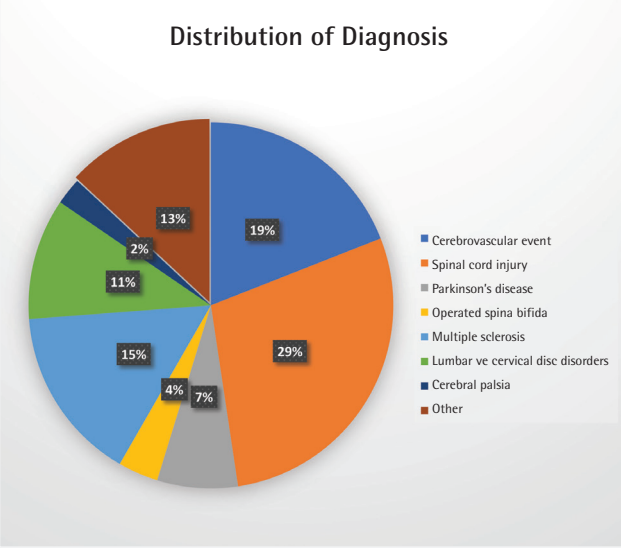

Figure 1. Determining the diagnosis of patients with detrusor overactivity in the filling phase and underactive detrusor in the discharge phase

DISCUSSION

When the neurological diagnoses of the patients who applied to our clinic with LUTS and a concomitant neurological disease were evaluated, it was determined that the patients with disc disorders, MS and CVE were the most common. In a study conducted in the United States, it was reported that the most common factors in patients admitted to the urology clinic due to LUTS associated with neurological disease were CVE, MS, and SCI, respectively[3]. These findings have similar characteristics and show that LUTS is seen quite frequently, especially in patients with MS and SCI. Disc disorders were the most common neurological disease group seen in our series. In the literature review, there is no significant study showing the relationship between disc disorders and LUTS. When the epidemiological studies of neurological diseases are examined, it is seen that lumbar and cervical disc disorders are not often included in the neurological disease groups, and the studies that have been conducted are generally based on pathologies such as MS, CVE, and SCI[4,5]. In this context, we think that our series provides important data in terms of showing that disc disorders can also cause LUTS.

Detrusor pressures in the filling phase are important both for the clinical findings of the patients and for possible upper urinary tract damage. It was observed that the disease groups with the highest maximum detrusor pressure in the filling phase consisted of OSD and MS patients, respectively. Maximum detrusor pressures were found to exceed 40 cmH_2O in these patient groups. Similar findings have also been shown in studies[6]. In addition to high detrusor pressure, bladder compliance is also important in terms of upper urinary system function. In a patient with low bladder compliance, intravesical pressures may remain high and this may cause upper urinary system dysfunction. In our study, it was determined that the group with the most common hypocompliant bladder was oSB patients, followed by the PD group. oSB patients were found to be the riskiest group in terms of upper urinary system due to both high detrusor pressures and low compliance. Similar data have been shown in other studies, and it has been reported that the development of end-stage renal disease in oSB patients is approximately 8 times higher than in the normal population[7]. It has been shown that patients with SCI have high bladder pressures and low compliance, and in this patient group, it is known that the cause of death was related to end-stage renal failure at a rate of approximately 50% in the past years and it has decreased to below 10% today with the development of treatment options[8-10]. In our study, hypocompliance was detected in approximately 20% of patients with SCI, while

maximum detrusor pressures were observed to increase to an average of 31 cmH_2O. These findings were seen at a lower rate compared to similar studies, and we think that this is due to the early evaluation of patients with SCI (3-12 months) in our clinic and therefore, the first urodynamic evaluation was performed at a time when bladder compliance was not yet affected. Although maximum detrusor pressures were found to be high in patients with MS, only about one-third of them were observed to have impaired compliance. Studies have shown that although detrusor pressures are high in MS patients, the upper urinary system is not affected[7]. We think that this may be due to the fact that compliance is not deteriorated in MS patients and that high detrusor pressures do not occur in a long time.

It has been found that detrusor pressures are low in patients with polyneuropathy manifested by peripheral nerve damage, and compliance is impaired in only 10% of cases. Among the neurological disease groups, the group in which hypercompliance is most common includes the patients with peripheral nerve damage. In this patient group, the risk of upper urinary tract damage is less, and we believe that this may be related to low intra-bladder pressure and not significantly affected compliance. Detrusor overactivity was observed in half of the patients with diabetic neuropathy, while normoactive bladder was detected in the other half. This shows that a pathology that is normally expected to cause peripheral nervous system damage also has a central effect.

Study Limitations

Although the retrospective nature of our study is one of the main limitations, we believe that prospective collection of urodynamic data in our clinic will reduce this limitation. Since the total number of patients with a group of diseases was less than 10, the exclusion of these groups from the study can be considered as another limitation. However, we think that these diseases are not sufficient to make scientific interpretations due to the lack of numbers. The fact that the urodynamic data used in the study belong to studies conducted in the same clinic by an experienced urodynamic team in accordance with international urodynamic standards represents the strength of the study. In addition, we think that our study is valuable in terms of presenting regional data, since there are not enough data on this subject in our country.

CONCLUSION

Since patients with oSB and patients with SCI are at high risk for end-stage renal disease, clinicians should take these into consideration when creating a follow-up protocol, especially in these two disease groups, and share possible near and long-term complications with patients and their relatives.

Ethics

Ethics Committee Approval: The study was approved by the Tekirdağ Namık Kemal University Local Ethics Committee (no: 2022.180.10.04, date: 25.10.2022).

Informed Consent: Informed consent forms were obtained from all patients before urodynamic evaluation.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Ç.D., M.A., C.M.Y., A.M., E.A., Concept: Ç.D., M.A., C.M.Y., A.M., S.Ş., H.S.D., E.C.T., Design: Ç.D., C.M.Y., E.A., S.Ş., E.C.T., Data Collection or Processing: Ç.D., M.A., E.A., S.Ş., H.S.D., E.C.T., Analysis or Interpretation: Ç.D., M.A., C.M.Y., A.M., H.S.D., Literature Search: Ç.D., A.M., E.A., S.Ş., H.S.D., E.C.T., Writing: Ç.D., M.A.

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REFERENCES

1. Blok B, Castro-Diaz D, Del Popolo G, Groen J, Hamid R, Karsenty G, Kessler TL, et al. European Association of Urology. Non-Oncology Guidelines. Neuro-Urology. Available from: https://uroweb.org/guidelines/neuro-urology
2. D'Ancona C, Haylen B, Oelke M, Abranches-Monteiro L, Arnold E, Goldman H, et al. The International Continence Society (ICS) report on the terminology for adult male lower urinary tract and pelvic floor symptoms and dysfunction. Neurourol Urodyn. 2019;38:433-77.
3. Hall SA, Curto TM, Onyenwenyi A, Lemack GE, Tennstedt SL, Link CL, et al. Characteristics of persons with overactive bladder of presumed neurologic origin: results from the Boston Area Community Health (BACH) survey. Neurourol Urodyn. 2012;31:1149-55.
4. Ruffion A, Castro-Diaz D, Patel H, Khalaf K, Onyenwenyi A, Globe D, et al. Systematic review of the epidemiology of urinary incontinence and detrusor overactivity among patients with neurogenic overactive bladder. Neuroepidemiology. 2013;41:146-55.
5. Jaggi A, Fatoye F. Real world treatment patterns in the neurogenic bladder population: a systematic literature review. Transl Androl Urol. 2017;6:1175-83.
6. Ahmad I, Granitsiotis P. Urological follow-up of adult spina bifida patients. Neurourol Urodyn. 2007;26:978-80.
7. Lawrenson R, Wyndaele JJ, Vlachonikolis I, Farmer C, Glickman S. Renal failure in patients with neurogenic lower urinary tract dysfunction. Neuroepidemiology. 2001;20:138-43.
8. Hackler RH. A 25-year prospective mortality study in the spinal cord injured patient: comparison with the long-term living paraplegic. J Urol. 1977;117:486-8.
9. Lidal IB, Snekkevik H, Aamodt G, Hjeltnes N, Biering-Sørensen F, Stanghelle JK. Mortality after spinal cord injury in Norway. J Rehabil Med. 2007;39:145-51.
10. Soden RJ, Walsh J, Middleton JW, Craven ML, Rutkowski SB, Yeo JD. Causes of death after spinal cord injury. Spinal Cord. 2000;38:604-10.

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Immediate Reconstruction of Distal Thumb Amputations Using Reverse Homodigital Dorsoradial Flap and the Amputated Part of the Phalanx

Başparmak Distal Uç Ampütasyonlarının Revers Homodijital Dorsoradial Flep ve Falanksın Ampüte Kısmı Kullanılarak Erken Rekonstrüksiyonu

Necmi CAM[1], Muharrem KANAR[1], Ömer Faruk KÜMBÜLOĞLU[2], Hacı Mustafa ÖZDEMİR[1]

[1]University of Health Sciences Turkey, Şişli Hamidiye Etfal Training and Research Hospital, Clinic of Orthopedics and Traumatology, İstanbul, Turkey
[2]University of Health Sciences Turkey, Şişli Hamidiye Etfal Training and Research Hospital, Clinic of Orthopedics and Traumatology, Division of Hand Surgery, İstanbul, Turkey

ABSTRACT

Aim: The contribution of the thumb to hand function is indisputable. Treatment after an amputation injury should be aimed to restore the length, sensation, function and appearance of the thumb. This study aimed to present the surgical details and clinical outcomes of the reconstruction of the distal thumb amputations with a reverse homodigital dorsoradial flap using the amputated phalanx as a graft in heavy-duty workers, where functional gains are more prominent than appearance.

Materials and Methods: Eight patients who underwent reconstruction using reverse homodigital dorsoradial flap and the amputated part of the distal phalanx due to traumatic amputation at the distal thumb, between 2016 and 2019, were evaluated retrospectively in this study. At the final follow-up, static two-point discrimination, key pinch strength, interphalangeal joint range of motion and Quick Disability of the Arm, Shoulder, and Hand score were measured.

Results: Patients were analyzed. They had a mean age of 42.25 (range, 34-52) years and a mean follow-up of 20.4 (range, 16-24) months. At the final follow-up, the average static two-point discrimination was 7.5 (range, 6-9) mm. The mean injured side key pinch forces was 93% compared to the opposite side (range, 76-110%). All patients returned to their jobs.

Conclusion: It was concluded that the reconstruction of the thumb distal tip amputations with the reverse homodigital dorsoradial flap using the amputated phalanx should be considered as an alternative to other reconstruction methods in patients with low aesthetic expectations, or irreversible damage to the nail bed.

Keywords: Thumb, amputation, reconstructive surgery

ÖZ

Amaç: Başparmağın el fonksiyonlarına katkısı tartışılmazdır. Ampütasyon sonrası uygulanacak olan tedavi, başparmağın uzunluğunu, hissini, işlevini ve görünümünü eski haline getirmeyi amaçlamalıdır. Bu çalışmada, fonksiyonel kazanımların estetik görünümden daha önemli olduğu ağır işlerde çalışan ve başparmak ampütasyonu ile başvuran hastaların tedavisinde, ampüte falanksın greft olarak kullanılarak ters homodijital dorsoradial flep ile rekonstrüksiyon uygulamalarımızın klinik sonuçlarının değerlendirilmesi amaçlandı.

Gereç ve Yöntem: Bu çalışmada 2016-2019 yılları arasında distal falanksın travmatik ampütasyonu nedeniyle ters homodijital dorsoradial flep ve distal falanksın ampüte kısmı kullanılarak rekonstrüksiyon uygulanan sekiz hasta retrospektif olarak değerlendirildi. Son takipte statik iki nokta ayrımı, çimdikleme gücü, interfalangeal eklem hareket açıklığı ve Hızlı Kol, Omuz ve El Sorunları anket skoru ölçüldü.

Address for Correspondence: Necmi CAM MD, University of Health Sciences Turkey, Şişli Hamidiye Etfal Training and Research Hospital, Clinic of Orthopedics and Traumatology, İstanbul, Turkey
Phone: +90 505 646 07 04 **E-mail:** drnecmicam@gmail.com **ORCID ID:** orcid.org/0000-0001-7101-3106
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Bulgular: Hastaların yaş ortalamaları 42,25 (dağılım 34-52) yıl idi. Ortalama takip süresi 20,4 (dağılım, 16-24) aydı. Son takipte, ortalama statik iki nokta ayrım ortalaması 7,5 (dağılım, 6-9) mm idi. Karşı taraf ile yüzdelik olarak karşılaştırılan çimdikleme güç ortalamaları %93 olarak saptandı (dağılım, %76-110). Tüm hastalarımız önceki işlerini fonksiyonel kayıp yaşamadan devam ettirebildiler.

Sonuç: Başparmak distal uç ampütasyonlarının, ampüte falanks greft olarak kullanılarak ters homodijital dorsoradial flep ile rekonstrüksiyonunun, estetik beklentisi düşük veya tırnak yatağında geri dönüşümsüz hasar olan hastalarda diğer rekonstrüksiyon yöntemlerine alternatif olarak değerlendirilmesinin uygun olacağı sonucuna varıldı.

Anahtar Kelimeler: Başparmak, ampütasyon, rekonstrüktif cerrahi

INTRODUCTION

In the treatment of thumb amputations, replantation is considered as the best treatment method. Although many patients have the chance for replantation, some patients do not have arterial and venous structures suitable for replantation, especially in amputations distal to the interphalangeal (IP) joint level. Many reconstruction methods have been described for the treatment of distal thumb amputations when replantation is not possible[1].

Treatment of the amputations distal to the IP joint with reconstruction methods is still controversial. Many factors affect the decision on treatment. In addition to some factors such as the type of injury, patient-related factors and expectations (age, occupation, socioeconomic level, cultural habits of the patient), the surgeon's experience also determines the treatment option[1-5].

For the distal amputations involving the phalanx of the thumb, in order to achieve better functional and aesthetic results, transfer from toe to thumb is one of the most effective reconstructive treatments. This technique allows to achieve the length and sensibility of the thumb and an aesthetic appearance[1,2,5,6]. Another reconstruction method in which the amputated part of the distal phalanx and the nail bed is used as a graft with flaps has been performed for many years[7-10].

The most important point that determines the favorable outcome of the applied treatment for the patients is to decide which treatment method should be chosen in the first place. After a good evaluation of the patients, the treatment should be decided after being informed about the treatment options that can be applied in line with their expectations and needs[6,11].

This retrospective study aimed to present the surgical details and clinical outcomes of the reconstruction of the distal thumb amputations with a reverse homodigital dorsoradial flap using the amputated part of the distal phalanx as a graft in heavy-duty workers where functional gains are more prominent than appearance.

MATERIALS AND METHODS

Eight patients who underwent immediate reconstruction using reverse homodigital dorsoradial flap and the amputated part of the distal phalanx due to traumatic amputation at the distal thumb, between 2016 and 2019, were retrospectively evaluated in this study. The study was approved by the University of Health Sciences Turkey, Şişli Hamidiye Etfal Training and Research Hospital of Local Ethics Committee (protocol no: 2718, date: 10.03.2020).

All of the patients were male and their mean age was 42.25 years (range 34-52 years). Our study group consisted of patients who had no previous hand injury, worked in heavy industry, and had type 1c amputation injuries according to the Merle classification[6,12]. Patients treated with replantation or one of the other treatment options were excluded from the study.

At the final follow-up, static pinch force was measured with key pinch grip using a pinch gauge (Baseline Pinch Gauge, Alimed Corp., Dedham, MA, USA) and the percentage was determined by comparing with the contralateral side. Range of motion of the IP joint was measured clinically with a goniometer. Functional evaluation was performed using the Quick Disabilities of the Arm, Shoulder, and Hand (DASH) score and patients were asked to evaluate their satisfaction with thumb functions subjectively, as excellent, good, fair, or poor. Sensorial return was evaluated by a static two-point discrimination test. Bone healing was evaluated radiologically. Patient data and surgical details are summarized in Table 1.

Surgical Technique

Detailed interviews and briefings were held with all patients, including the treatment options that could be applied in line with their expectations. The type of anesthesia to be applied was decided by the anesthesiologist after the evaluation of the patients. All patients underwent surgery within the first 8 hours after injury.

Firstly, the injury was examined in terms of whether it was suitable for replantation or not. In patients who were not suitable for replantation, the reconstruction procedure was continued. All operations were performed under a pneumatic tourniquet inflated without using an esmarch bandage. Surgical loop magnification was used.

Soft tissues and the nailbed around the amputated phalanx were excised. Then, the amputated part was placed in the stump

and fixed with a K wire (Figures 1, 2). Before determining the flap dimensions, the required pedicle length was calculated by marking the pivot point in the middle third of the proximal phalanx. Subsequently, the flap sizes required for distal phalanx covering were measured and flap was drawn. The incision and lifting of the flap started from the proximal to the distal direction (Figure 3). The sensory collateral branch of the radial nerve, which was located close to the artery, was included in

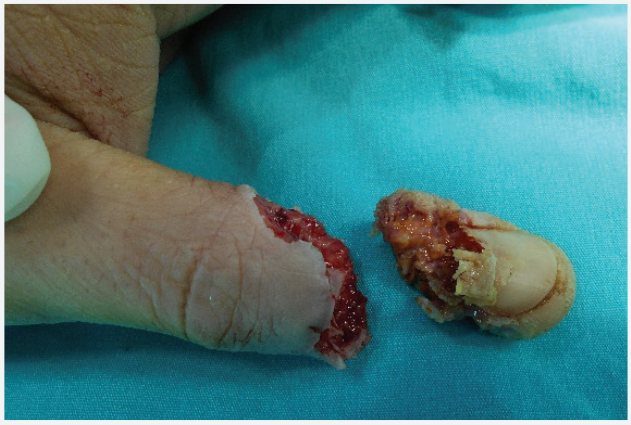

Figure 1. Distal amputation of thumb

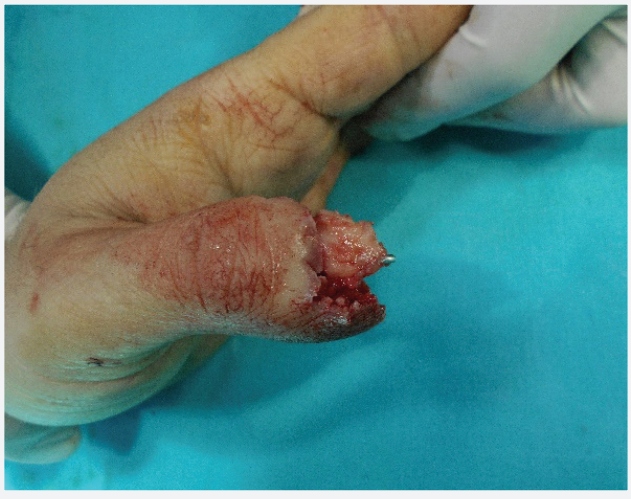

Figure 2. After soft tissues and the nailbed around the amputated phalanx were excised and fixed with a K wire to the stump

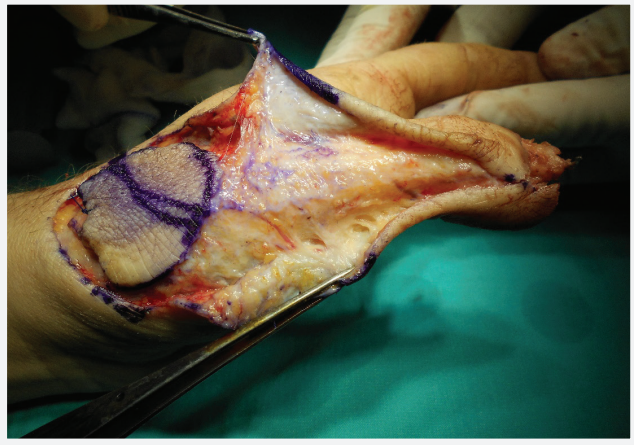

Figure 3. Required flap size drawn and dissection

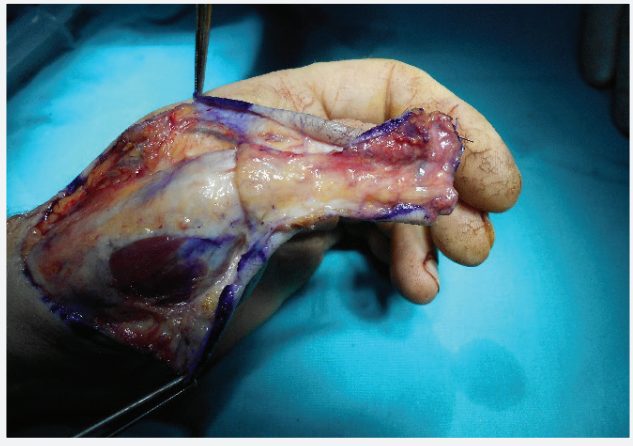

Figure 4. Flap lifting with the sensorial branch

Table 1. Patient data and surgical details

	Age (years)	Side of injury	Type of injury	Amputation level of distal phalanx	Donor site closing
Case 1	43	L	Avulsion amputation	Middle third	Primary
Case 2	48	R	Crush amputation	Base	FTSG
Case 3	38	L	Compression and pulling amputation	Middle third	FTSG
Case 4	52	L	Compression and pulling amputation	Middle third	FTSG
Case 5	45	R	Avulsion amputation	Middle third	FTSG
Case 6	34	L	Compression and pulling amputation	Base	FTSG
Case 7	40	L	Crush amputation	Middle third	FTSG
Case 8	38	R	Chain injury	Middle third	FTSG

FTSG: Full thickness skin graft, L: Left, R: Right

the flap. The skin incision was continued superficially along the pedicle line. Sufficient space was reserved for the pedicle to settle. The flap was lifted from the proximal to the distal direction. The pedicle was raised as wide as possible (Figure 4). At this stage, the tourniquet was opened and hemostasis was achieved. The distal wound was closed with the flap. A full-thickness skin graft was taken from the volar side of the wrist. The pedicle was covered with a full-thickness skin graft. The flap donor site was closed with a full-thickness skin graft or primary closure (Figure 5). Appearance of the thumb at the last control is shown in Figure 6.

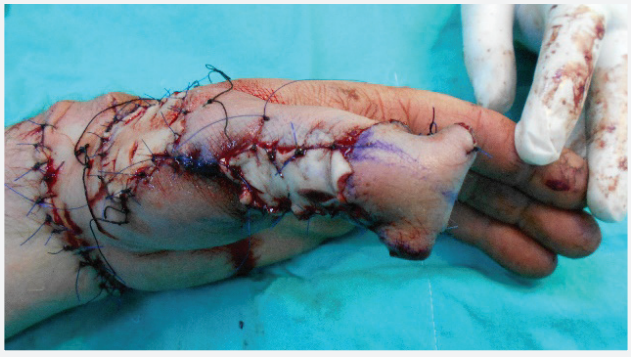

Figure 5. Phalanx was closed with the flap and donor area closed by skin greft

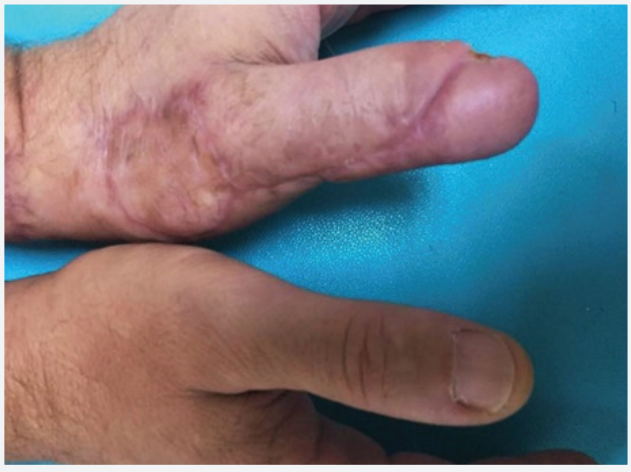

Figure 6. Appearance of the thumb at the last control

Postoperative Care

Postoperatively, a thumb spica splint (the wrist and thumb in neutral position, allows for finger motion) was applied. Oral antibiotics and analgesics were prescribed and the patients were discharged on the first postoperative day. The wound was checked weekly. Plaster splint was terminated in the 3rd week and aluminum finger splint was used to start wrist and metacarpophalangeal joint movement. The K wire was removed at an average of 5 weeks (range, 4-8 weeks) postoperatively. After the removal of the K wires, all patients were referred to rehabilitation. All patients were followed for at least 16 months.

RESULTS

The mean follow-up was 20.4 (range, 16-24) months. The dominant hand of all patients was the right side. Left thumb of 5 patients and right thumb of 3 patients were injured.

In the postoperative period, soft tissue infection developed in 2 patients at the thumb tip wound. Treatments with local debridement, wound care and antibiotic therapy were performed under outpatient clinic conditions. The K wire which was under the skin of a patient was removed with local anesthesia. No patient developed donor site complications and all flaps survived.

At the final follow-up, the mean Quick DASH score was 2.55 (range, 0-6.8). The mean static two-point discrimination was 7.5 (range, 6-9) mm. The operative side key pinch forces were 93% on average compared to the opposite side (range, 76-110%). The mean range of motion of the IP joint was 65^0 flexion and 3.75^0 extension. Bone resorption was observed in three of eight patients (37.5%). Five patients evaluated the

Table 2. The results of the distal thumb reconstruction

	Two point discrimination (mm)	Key pinch strength (% of contralateral side)	QuickDASH score	Range of motion (interphalengeal joint) (flexion/extension)
Case 1	6	90	0	65 / 10
Case 2	9	110	2.3	70 / 0
Case 3	7	85	4.5	60 / 5
Case 4	9	76	6.8	55 / (-5)
Case 5	7	100	4.5	65 / 5
Case 6	8	85	0	70 / 10
Case 7	7	88	0	70 / 5
Case 8	7	110	2.3	65 / 0
Average	7.5	93	2.55	65 / 3.75

Quick DASH: Quick Disabilities of the Arm, Shoulder, and Hand

final functional state of their thumbs as excellent and three patients as good. All patients returned to their jobs. One of the patients complained of pain with percussion and cold intolerance. The surgical results are summarized in Table 2.

DISCUSSION

Approximately 1% of all trauma admissions to the emergency department are amputation injuries and about 69% of all amputations are finger and thumb amputations[13]. Amputation of the thumb can cause up to 40% loss of hand function[14,15]. As a result of injuries, surgeons have focused on regaining this lost function and many methods have been defined over the years for thumb repair or reconstruction[6]. Following the first thumb replantation successfully performed by Komatsu and Tamai[16] in 1968, with the developments in microsurgery, more successful results started to be obtained over time and accordingly, the expectations of the patients increased[17,18].

The thumb is uniquely positioned to perform grasping and pinching movements due to its anatomical position in the hand. In order not to lose this functionality after amputation, durability, sensation, stability, length, and mobility should be gained with treatment. In parallel with patient expectations, aesthetic appearance should not be ignored[15,18].

Amputations involving 1/3 of the thumb, distal to the IP joint, are referred to as the "compensated amputation zone" in the literature. In distal amputations, functional loss can be tolerated more easily than proximal amputations, and for this reason, it is not always necessary to reconstruct the length. It is often sufficient to provide soft tissue coverage without causing further shortening. Secondary healing, full thickness skin grafts, advancement flaps and other homodigital or heterodigital flaps may be preferred depending on the condition of the injury[4,6,18-20].

Although thumb distal amputations are more tolerable, functional and aesthetic deficiencies may occur, especially in injuries with bone loss close to the IP joint. In order not to encounter unfavorable results in people with high aesthetic and functional expectations, treatment should be decided according to patient condition. In such cases, replantation should be preferred first, and in cases where replantation is not possible, treatment with reconstructive methods should be planned[1,5,6,11,18].

Thumb injuries occur in many patients at work. The majority of these patients are manual workers. Grip strength and pinch ability are important for their job and also finger sensation is very important to prevent re-injury[1,5]. The patients in our study were all manual workers. For this reason, it was important not to lose their functional and tactile skills. Replantation was not attempted in any patient, since the amputated part was not suitable for replantation. Reconstruction was performed with a homodigital dorsoradial flap using the amputated phalanx as a graft. Our main aim in realizing this method was to protect the thumb's length as much as possible and to ensure the sensitivity of the thumb tip.

Reconstruction of the distal thumb using a free great toe flap is considered to have functional and aesthetically pleasing results. Many great toe transfer techniques have been described for the distal thumb reconstruction[5,6,21-25]. On the contrary, the necessity of distal thumb reconstructions is still controversial and some of the surgeons have concern about performing such a complicated surgical procedure[2-6,23,26]. Moreover, the risk of free flap failure is the primary reason that the patients do not accept this surgery. Prolonged hospital stay and risk of donor foot functional impairment are among the reasons mentioned[27]. None of the patients in our study group had a hospital stay longer than 24 hours, they had no complaints about the donor site, and necrosis did not develop in any flap.

In another reconstruction method in which the amputated part of the distal phalanx and nail bed are used as grafts, it is aimed to provide revascularization of the dead bone and nail bed with flaps. Good results have been reported in the literature regarding this method that can restore length and appearance. In reconstructions with this method, many different flap techniques have been used to provide revascularization[7-10]. The reason we prefer a reverse homodigital dorsoradial flap is that it contains a sensory branch, it can be taken wide enough to cover the entire bone surface, it does not affect the other fingers, and the donor site can be easily restored[28,29].

Bone resorption is one of the complications that can be seen in reconstructions with avascular bone grafts. Dubert et al.[30] reported that bone resorption developed in all patients in the treatment results of distal tip amputations, which they used amputated nail bed and bone as a graft with homodigital neurovascular anterograde island flap[30]. In this study, cold intolerance was seen at the rate of 67%. Han et al.[9] published the results of distal thumb reconstruction using the amputated part of the thumb with the homodigital dorsoulnar flap. In this method, the amputated nail bed and phalanx were used together as graft for reconstruction and they included the periosteum in the flap to support revascularization to prevent the development of resorption. They reported minimal bone graft resorption according to the 6-month follow-up[9]. In this study, they reported a two-point discrimination mean of 9.9 mm. In our study, bone resorption was seen in three patients of eight (37.5%) and the mean two-point discrimination was 7.5 mm.

Yang et al.[21] reported that bone resorption developed in all 15 patients in the free great toe wrap-around flap method, in which they used bone graft from the iliac crest. As a result of this study, they stated that the probability of bone resorption

increased with longer graft use[21]. The expectations of the patients in our study were not for appearance, but for not experiencing functional loss. Volume of the distal phalanx of the thumb was larger than the other fingers and we have concluded that the probability of bone resorption would be high if the phalanx is used as a graft in thumb injuries where the distal phalanx is amputated close to the level of the IP joint. Based on these, we decided to debride all soft tissues including the nail bed from the amputate phalanx and enclose the bone graft completely with the flap to increase the bone revascularization. The purpose of choosing such an application was to minimize the development of bone resorption. However, bone resorption developed in three of our patients. The phalanx amputation levels of these patients were very close to the IP joint level, so they were reconstructed with a longer phalanx graft. Despite some length loss due to bone resorption over time, the patients did not have any functional complaints while doing their job.

Aesthetic appearance is important among the gains of distal thumb reconstructions. Del Piñal et al.[5] evaluated cosmetic results with visual analog scores in patients who underwent reconstruction with free great toe transfer and achieved an average score of 9.5 (range, 8-10)[5]. To improve aesthetic results, the nail bed must be reconstructed. However, functional outcomes were much more important than aesthetic concerns in our patients. Consequently, we could not perform this because we aimed to cover more bone surfaces with vascularized tissue instead of reconstructing the nail bed. None of our patients complained about their aesthetic appearance. Also, the patients were informed before the surgery that the nail beds would be irreversibly removed at the surgery.

In the evaluations of our patients at their last follow up, all of them were able to return to their pre-injury works. All flaps survived and necrosis was not seen. The mean Quick DASH score was 2.55. Five patients subjectively evaluated the final functional state of their thumbs as excellent and three patients as good.

Study Limitations

The main limitations of our study are the small sample size, retrospective design, and lack of control groups.

CONCLUSION

It was concluded that the reconstruction of the thumb distal tip amputations with the reverse homodigital dorsoradial flap using the amputated phalanx should be considered as an alternative to other reconstruction methods in patients with low aesthetic expectations, or irreversible damage to the nail bed.

Ethics

Ethics Committee Approval: The study was approved by the University of Health Sciences Turkey, Şişli Hamidiye Etfal Training and Research Hospital of Local Ethics Committee (protocol no: 2718, date: 10.03.2020).

Informed Consent: Consent form was filled out by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: N.C., Ö.F.K., Concept: N.C., M.K., Ö.F.K., H.M.Ö., Design: N.C., M.K., Ö.F.K., H.M.Ö., Data Collection or Processing: N.C., M.K., Ö.F.K., Analysis or Interpretation: N.C., M.K., Ö.F.K., H.M.Ö., Literature Search: N.C., M.K., Ö.F.K., Writing: N.C., M.K., Ö.F.K.

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. Friedrich JB, Vedder NB. Thumb reconstruction. Clin Plast Surg. 2011;38:697-712.
2. Graham DJ, Venkatramani H, Sabapathy SR. Current Reconstruction Options for Traumatic Thumb Loss. J Hand Surg Am. 2016;41:1159-69.
3. Morrison WA. Thumb reconstruction: a review and philosophy of management. J Hand Surg Br. 1992;17:383-90.
4. Azari KA, Lee WPA. Thumb reconstruction. In: Wolfe SW, Hotchkiss RN, Pederson WC, Kozin SH (eds), Green's Operative Hand Surgery. 6th ed. Philadelphia: Elsevier; 2011:1839-82.
5. Del Piñal F, Moraleda E, de Piero GH, Ruas JS, Galindo C. Onycho-osteo-cutaneous defects of the thumb reconstructed by partial hallux transfer. J Hand Surg Am. 2014;39:29-36.
6. Adani R, Woo SH. Microsurgical thumb repair and reconstruction. J Hand Surg Eur Vol. 2017;42:771-88.
7. Mantero R, Bertolotti P. Le cross-finger et réimplantation des extrémités digitales [Reimplantation of the finger tips using a cross-finger technique (author's transl)]. Ann Chir. 1975;29:1019-23.
8. Foucher G, Braga Da Silva J, Boulas J. La technique de "reposition-lambeau" dans les amputations digitales distales. A propos d'une série de vingt et un cas. Ann Chir Plast Esthet. 1992;37:438-42.
9. Han D, Sun H, Jin Y, Wei J, Li Q. A technique for the non-microsurgical reconstruction of thumb tip amputations. J Plast Reconstr Aesthet Surg. 2013;66:973-7.
10. Alagoz MS, Uysal CA, Kerem M, Sensoz O. Reverse homodigital artery flap coverage for bone and nailbed grafts in fingertip amputations. Ann Plast Surg. 2006;56:279-83.
11. Kumta SM. Unfavourable results in thumb reconstruction. Indian J Plast Surg. 2013;46:294-302.
12. Merle M. A critical study of thumb reconstruction by second toe transfer. Ann Chir Main Memb Super. 1991;10:517-22.

13. Barmparas G, Inaba K, Teixeira PG, Dubose JJ, Criscuoli M, Talving P, et al. Epidemiology of post-traumatic limb amputation: a National Trauma Databank analysis. Am Surg. 2010;76:1214-22.

14. Slocum DB, Pratt DR. Disability evaluation for the hand. J Bone Joint Surg Am. 1946;28:491-5.

15. Emerson ET, Krizek TJ, Greenwald DP. Anatomy, physiology, and functional restoration of the thumb. Ann Plast Surg. 1996;36:180-91.

16. Komatsu S, Tamai S. Successful replantation of a completely cut-off thumb: case report. Plast Reconstr Surg. 1968;42:374-7.

17. Shale CM, Tidwell JE, Mulligan RP, Jupiter DC, Mahabir RC. A nationwide review of the treatment patterns of traumatic thumb amputations. Ann Plast Surg. 2013;70:647-51.

18. Muzaffar AR, Chao JJ, Friedrich JB. Posttraumatic thumb reconstruction. Plast Reconstr Surg. 2005;116:103-22.

19. Kleinman WB, Strickland JW. Thumb reconstruction. In: Green DP, Hotchkiss RN, Pederson WC (eds), Green's Operative Hand Surgery. 4th ed. Churchill Livingstone: Philadelphia; 1999:2068-170.

20. Elsahy NI. Replantation of a completely amputated distal segment of a thumb. Case report. Plast Reconstr Surg. 1977;59:579-81.

21. Yang K, Zhao Z, Pan Y, Song F, Deng J, Zhu J. Resorption of Iliac Bone Grafts Following Wrap-Around Flap for Thumb Reconstruction: A Follow-Up Study. J Hand Surg Am. 2020;45:64.e1-8.

22. Woo SH, Seul JH. Distal thumb reconstruction with a great toe partial-nail preserving transfer technique. Plast Reconstr Surg. 1998;101:114-9.

23. Woo SH, Lee GJ, Kim KC, Ha SH, Kim JS. Immediate partial great toe transfer for the reconstruction of composite defects of the distal thumb. Plast Reconstr Surg. 2006;117:1906-15.

24. Adani R, Cardon LJ, Castagnetti C, Pinelli M. Distal thumb reconstruction using a mini wrap-around flap from the great toe. J Hand Surg Br. 1999;24:437-42.

25. Adani R, Marcoccio I, Tarallo L, Fregni U. The aesthetic mini wrap-around technique for thumb reconstruction. Tech Hand Up Extrem Surg. 2005;9:42-6.

26. Morrison WA. Thumb reconstruction: a review and philosophy of management. J Hand Surg Br. 1992;17:383-90.

27. Efanov JI, Wong C, Guilbault C, Bou-Merhi J, Harris PG, Izadpanah A, et al. Investigating Patients' Perception of Microvascular Free Toe Flap for Reconstruction of Amputated Thumbs: A Guide for Surgeons during Informed Consent. J Reconstr Microsurg. 2018;34:692-700.

28. Moschella F, Cordova A. Reverse homodigital dorsal radial flap of the thumb. Plast Reconstr Surg. 2006;117:920-6.

29. Hrabowski M, Kloeters O, Germann G. Reverse homodigital dorsoradial flap for thumb soft tissue reconstruction: surgical technique. J Hand Surg Am. 2010;35:659-62.

30. Dubert T, Houimli S, Valenti P, Dinh A. Very distal finger amputations: replantation or "reposition-flap" repair? J Hand Surg Br. 1997;22:353-8.

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Retrospective Analysis of Patients with Basal Cell Carcinoma Diagnosed in Our Clinic

Kliniğimizde Tanı Koyulan Bazal Hücreli Karsinom Olgularının Retrospektif İncelenmesi

Hülya ALBAYRAK[1], Onur RAİMOĞLU[2]

[1]Tekirdağ Namık Kemal University Faculty of Medicine, Department of Dermatology, Tekirdağ, Turkey
[2]Çorlu State Hospital, Clinic of Dermatology, Tekirdağ, Turkey

ABSTRACT

Aim: Basal cell carcinoma (BCC) is the most common malignancy of the skin and its incidence is steadily increasing. The aim of our study was to reveal the clinical and demographic characteristics of BCC.

Materials and Methods: The study included 273 BCC that were histopathologically diagnosed in 256 patients who admitted the Dermatology Clinic of Tekirdağ Namık Kemal University between January 2014 and January 2019. The patients' age, gender, tumor histological subtype, tumor localization, and co-morbid conditions were all assessed retrospectively in the study.

Results: The mean age of the 256 patients included in the study with BCC was statistically 67.67, with the youngest patient being 32 and the oldest patient being 104 years old. Of the patients, 137 were male (56.5%), and 119 were female (46.5%). The most common histopathological subtype observed was the nodular type, accounting for 61.5% (n=168), and the most frequent localization was the head and neck region, accounting for 88.3% (n=241).

Conclusion: BCC is the most common epidermal malignancy of the skin, characterized by a slow growth pattern and a locally invasive nature. It often occurs in individuals aged 50 and above, particularly in the head and neck region where there is intense exposure to ultraviolet radiation. In our study, we also observed that BCC was most frequently seen in the 70-80 age range, primarily in the head and neck region, and predominantly in the nodular subtype. The superficial type was more commonly observed on the trunk. Although the incidence of BCC is similar between males and females, it tends to occur more frequently in females at younger ages.

Keywords: Basal cell carcinoma, histopathological subtype, head-neck, nodular type basal cell carcinoma

ÖZ

Amaç: Bazal hücreli karsinom (BHK) derinin en sık görülen malignitesidir ve görülme sıklığı giderek artmaktadır. Çalışmamızın amacı bölgemizde sık olarak görülen BHK'nın klinik ve demografik özelliklerini ortaya koymaktır.

Gereç ve Yöntem: Tekirdağ Namık Kemal Üniversitesi Dermatoloji Polikliniği'ne Ocak 2014-Ocak 2019 tarihleri arasında başvuran 256 hastada histopatolojik olarak tanısı konulan 273 BHK çalışmaya dahil edildi. Hastaların yaş, cinsiyet, tümör histopatolojik alt tipi, tümör lokalizasyonu, hastaların eşlik eden komorbiditeleri belirlenerek retrospektif olarak incelendi.

Bulgular: Çalışmaya alınan 256 BHK hastasının yaş ortalaması istatistiksel olarak 67,67 idi ve en genç hasta 32, en yaşlı hasta 104 yaşındaydı. Hastaların 137'si erkek (%56,5), 119'u kadındı (%46,5). En sık görülen histopatolojik alt tip %61,5 oranında (n=168) nodüler tip ve en sık görülen lokalizasyon %88,3 oranında (n=241) baş-boyun bölgesiydi.

Sonuç: BHK yavaş büyüme paternine sahip olan, lokal invaziv karakterde en sık görülen epidermal malign kutanöz tümördür. Sıklıkla 50 yaş üzerinde, ultraviyole maruziyetinin yoğun olduğu baş-boyun bölgesinde görülmektedir. Bizim çalışmamızda da; BHK'nın en sık 70-80 yaş aralığında, baş-boyun bölgesinde ve en sık nodüler tipte olduğu görüldü. Gövdede yüzeyel tip daha fazla görülmekteydi. Erkek ve kadınlarda görülme sıklığı aynı olmasına rağmen erken yaşlarda kadınlarda daha sık görülmekteydi.

Anahtar Kelimeler: Bazal hücreli karsinom, histopatolojik alt tipler, baş-boyun, nodüler tip bazal hücreli karsinom

Address for Correspondence: Hülya ALBAYRAK MD, Tekirdağ Namık Kemal University Faculty of Medicine, Department of Dermatology, Tekirdağ, Turkey
Phone: +90 506 715 50 65 **E-mail:** halbayrak@nku.edu.tr **ORCID ID:** orcid.org/0000-0002-2022-578X
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INTRODUCTION

Basal cell carcinoma (BCC) is the most common type of non-melanoma skin cancer and accounts for 75% of all skin cancers[1]. It originates from the undifferentiated cells in the basal cell layer of the epidermis or the outer root sheath of the hair follicle[2]. The logarithmic relationship between age and the risk of BCC development has been proven[3]. Although it can be seen anywhere on the skin, more than 80% occurs in sun-exposed areas such as the head and neck[4]. With the prolongation of the average lifespan, a further increase in the incidence of BCC is expected in the near future[5]. In general, these slowly growing and rarely metastasizing tumors can cause significant morbidity due to their mostly facial involvement, tendency to recur, and the possibility of local invasion and destruction in the tissue[6]. Chronic sun damage is one of the most important risk factors for the development of BCC, there is approximately 15-20 years between ultraviolet B (UVB) damage and the onset of BCC[7]. In our study, it was aimed to create our own clinical data about BCC, which is the most common skin tumor, and compare it with the literature.

MATERIALS AND METHODS

This study, which was planned retrospectively, included 256 patients who applied to Tekirdağ Namık Kemal University Dermatology Clinic between January 2014 and January 2019 and were diagnosed with BCC after clinical evaluation and histopathological examination. Ethical approval was obtained from Tekirdağ Namık Kemal University Ethics Committee with protocol number 2019.67.04.14 and date 23.03.2020.

The patients included in the study were examined in detail. For the patients who were considered to have BCC and exposed to biopsy or excision and then histopathologically diagnosed with BCC, the age, gender, tumor histopathological subtype, tumor localization, and other accompanying diseases were evaluated.

Statistical Analysis

The Shapiro-Wilk test was used to evaluate the conformity of the measured data to normal distribution. Continuous variables were given as mean, standard deviation, median, minimum and maximum values, and categorical variables were presented as n and percentage values. In comparisons between the groups, the Student's t-test was used for the analysis of normally distributed data, and the Kruskal-Wallis test was used for data that did not show normal distribution. The Friedman test was employed to compare repeated measurements. In the presence of a difference between the measurements, the Wilcoxon test was used for pairwise comparisons. For all statistics, the value of $p<0.05$ was considered significant.

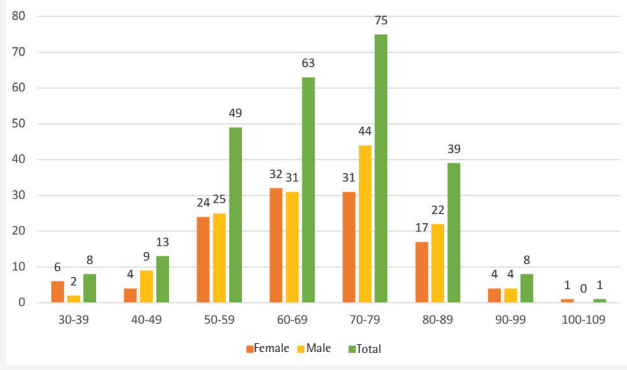
RESULTS

Of the 256 patients included in the study, 137 (53.5%) were male and 119 (46.5%) were female. The youngest patient was 32 years old and the oldest patient was 104 years old. The mean age of the patients was 67.67±13.45 years. The mean age of men was 68.24±12.81 years, and the mean age of women was 67.02±14.18 years. In both sexes, there was no significant difference between the age of the onset of BCC and gender ($p>0.05$). The distribution of patients by age and gender is shown in Graphic 1.

There were 273 BCCs in 256 patients in the study. 94.9% (n=243) of the patients presented with a single lesion. Other patients had multiple lesions.

The most common histopathological subtype was the nodular type with a rate of 61.5% (n=168). Superficial type was the second most common type (n=38) with a rate of 13.9%, and the least common type was keratotic type with a rate of 0.4% (n=1). The most common region of BCC was the head and neck region with a rate of 88.3% (n=241), the following region was the trunk with a rate of 8.1% (n=22), and the least common region was the upper extremity. The most commonly involved area on the face was the nose with a rate of 24.5% (n=67), followed by the cheek area with 14.7% (n=40). 12.1% (n=33) were on the scalp. In the head and neck region, the least lesion was detected in the postauricular region, and the least lesion in all body regions was in the upper extremity. The histopathological types of tumors and their distribution according to 4 main anatomical body regions are given in Table 1.

When we evaluated the histopathological types of tumors according to their frequency in body anatomical localizations, the nodular type was seen most frequently in the head and neck region with a rate of 94% (n=158). Although the superficial type was mostly seen in the head and neck region with a rate of 57.9% (n=22), it was not seen in the upper



Graphic 1. Distribution ranges of patients by age and gender

extremity. Morpheiform type (n=14), infiltrative type (n=9), adenoid type (n=4), keratotic type (n=1), micronodular type (n=2) lesions were in the head and neck region at the rate of 100%. Basosquamous type was most common in the head and neck region with a rate of 85.7% (n=6), while mixed type and pigmented type were most common in the head and neck region with a rate of 90.9% (n=10) and 78.9% (n=15), respectively. When tumor histopathological subgroups were compared within themselves, the tendency of superficial BCC to localize on the trunk and extremities was found to be significantly higher than other histopathological subtypes (p=0.001). The prevalence of tumor histopathological subtypes according to body anatomical localization is given in Table 1.

Tumors of the trunk and lower extremities were more common in males. Considering the distribution of tumor histopathological subtypes by gender in the study, nodular, superficial, morpheiform, keratotic and infiltrative types were seen more frequently in men than in women. While adenoid type was at the same rate in men and women, basosquamous, pigmented, micronodular and mixed types were more common in women. The distribution of tumors in anatomical regions by gender is shown in Table 2.

Considering the accompanying diseases of the patients, the most common comorbidities were hypertension in 68.4% (n=175), chronic obstructive pulmonary disease in 17.5% (n=45) and gonarthrosis in 16% (n=41). When the non-cutaneous malignancies accompanying the patients were evaluated in the study, 8 of 256 patients were accompanied by malignancies other than BCC. For the accompanying malignancy other than BCC, there were lung cancer in 3 patients, breast cancer in 2 patients, chronic lymphocytic leukemia in 1 patient, and rectal cancer in 1 patient. According to the histopathological subtypes, only nodular and basosquamous type BCC were accompanied by non-cutaneous malignancy.

DISCUSSION

Although BCC is mostly seen in the elderly population, it can also be seen in younger age groups. The largest majority of BCC patients in our study were in the age range of 70-80 years, followed by the age range of 60-70 years. In a retrospective study on 797 patients, which was performed by Devine et al.[8], 81% of the patients were reported to be over 65 years of age. The mean age and gender distribution of the patients in our study were found to be consistent with the study of Devine et al.[8]. When the patients in our study were examined according to age groups, 75% (n=6) of the patients under the age of 40 years were seen to be women. The fact that habits such as solarium and sunbathing are more common in the female population suggests that it may be the reason for the increased incidence in female patients at younger ages.

Considering the number of tumors in the patients in the study, 94.9% (n=243) of the patients had 1 tumor and others had more than one tumor at the time of diagnosis. Although most BCCs are a single lesion at diagnosis, more than one tumor may occur simultaneously. In the case report by Kim et al.[9], they stated that BCCs could also be seen multiple in a non-syndromic manner.

Table 1. Histopathological types of tumors and their distribution according to 4 main anatomical body regions

Histopathological type	Head-neck	Trunk	Lower extremity	Upper extremity	Total
Nodular	158 (65.6%)	4 (18.2%)	5 (62.5%)	1 (50%)	168
Superficial	22 (9.1%)	14 (63.6%)	2 (25%)	0 (0%)	38
Morpheiform	14 (5.8%)	0 (0%)	0 (0%)	0 (0%)	14
Infiltrative	9 (3.7%)	0 (0%)	0 (0%)	0 (0%)	9
Adenoid	4 (1.7%)	0 (0%)	0 (0%)	0 (0%)	4
Basosquamous	6 (2.4%)	1 (4.5%)	0 (0%)	0 (0%)	7
Keratotic	1 (0.4%)	0 (0%)	0 (0%)	0 (0%)	1
Mixed	10 (4.1%)	1(4.5%)	0 (0%)	0 (0%)	11
Pigmented	15 (6.2%)	2 (9.1%)	1 (12.5%)	1 (50%)	19
Micronodular	2 (0.8%)	0 (0%)	0 (0%)	0 (0%)	2
Total	241	22	8	2	

Table 2. Distribution of tumors in anatomical regions according to gender

Anatomical region	Male	Female	Total
Head and neck	126 (52.3%)	115 (47.7%)	241 (100%)
Trunk	14 (63.6%)	8 (36.4%)	22 (100%)
Lower extremity	7 (87.5%)	1 (12.5%)	8 (100%)
Upper extremity	1 (50%)	1 (50%)	2 (100%)

In our study, the nodular type was the most common one among BCC tumors, with a rate of 61.2% (n=167). In order of frequency, the others were superficial at the rate of 13.9% (n=38), pigmented at the rate of 7.3% (n=20), and morpheiform at the rate of 5.1% (n=14). Betti et al.[10] conducted a study with 693 patients in Italy, and they reported that the most common type of BCC was the nodular type with a rate of 64.8%, and the second most common type was the superficial type with a rate of 17.5%, as in our study. In the study conducted by Scrivener et al.[11] with 13,457 BCC patients in France in 2002, the most common types were reported to be nodular type (78.7%), superficial type (15.1%) and morpheiform type (78.7%), respectively. The distribution of tumors in our study according to their histopathological subtypes was found to be consistent with these two studies in the literature.

When the lesions were grouped according to their anatomical regions, they were mostly in the head and neck region with a rate of 88.3%, and then on the trunk with a rate of 8.1%. In another retrospective study performed by Souza et al.[12] on 1,042 lesions in Brazil, it was reported that 79% of the tumors were located in the head and neck region and 13% were in the trunk. In the study of Subramaniam et al.[13] in Australia in 2017, they reported that the lesions were mostly located in the head and neck region with a rate of 40.2% according to the body anatomical regions. The head and neck region was followed by the trunk with the rate of 33.9%. The results of our study according to the anatomical distribution were also compatible with the literature. In the study of Subramaniam et al.[13], the reason for the higher rate of lesions on the trunk compared to our study may be due to the different phenotypic characteristics of the patients and sun exposure due to the different geographical characteristics of the regions where the study was conducted. In this study, 27.8% of the lesions in the head and neck region were on the nose, 16.5% on the forehead, and 13.6% on the scalp. When the lesions in different anatomical regions in the head and neck region were examined, it was reported in the study of Souza et al.[12] that they were mostly seen on the nose with a rate of 39.1%, on the chin with a rate of 14.3% and on the forehead with a rate of 12%. As in our study, the nose was the localization where tumor was most commonly seen in the head and neck region. The reason why BCC is mostly seen in the nose in the head and neck region may be that the nose is more exposed to UVB rays due to its anatomical structure. Its high incidence in an anatomically and functionally important organ such as the nose also shows the importance of early diagnosis and treatment without local invasion.

Considering the frequency of tumor histopathological subtypes in different anatomical regions in our study, the most common type in head-neck, lower and upper extremities was nodular type. The most common type was superficial BCC on the trunk, especially on the posterior aspect of the trunk, with a higher incidence. Bastiaens et al.[14] also reported in their study that the most common superficial type was seen on the trunk and extremities.

In our study, when tumors in the anatomical regions of the body were examined, the incidence of lesions in the upper extremity in men and women was equal, and the incidence in other regions was higher in men than in women. In the study of Souza et al.[12], unlike our study, the incidence of lower extremity lesions in women was significantly higher, and the results in other regions were similar to our study.

The rate of non-cutaneous malignancy in the patients was 3%. In the study of Reinau et al.[15], the rate of comorbid malignancy in BCC was found to be 9.2% and it was found to be significant compared to the control group. This rate was found to be low in our study. The fact that the number of our patients is less and the incidence of cancer in the countries where the studies are conducted can change these rates. It is natural that the coexistence of BCC and malignancy is high in patients with the age of occurrence of BCC. However, prospective studies with a larger number of patients are needed to determine the relationship between various malignancies and BCC.

Study Limitations

The limitations of our study are that it is single-centered, retrospective, and the sample size is relatively small.

CONCLUSION

BCC is the most common malignancy in men and women, and there was no significant difference in the age of onset of BCC between the male and female groups. Considering the histopathological subtypes, the most common one was nodular type BCC. Most tumors appeared as a single tumor at the time of diagnosis. Syndromes with multiple BCC have not been identified among tumors that occur simultaneously. There is an increased incidence of BCC tumors seen at early ages, especially in women aged 30-40 years. When we examined the frequency of BCC subtypes according to body anatomical localizations, the most common histopathological subgroup was nodular type in most anatomical regions, while the frequency of superficial BCC in the trunk posterior (back) region was significantly higher than other types. BCC is most commonly found in the head and neck region, and on the nose, cheeks and scalp in the head and neck region, respectively. The fact that it is more common especially in sun-exposed anatomical regions shows that cumulative sun damage plays an important role in its pathogenesis. All individuals should try to protect themselves from BCC by taking precautions such as using sun-protective hats and clothing, especially those living in areas with high sun exposure and working in jobs

that are more exposed to the sun. It is important for the early diagnosis of BCC and other melanocytic and non-melanocytic skin cancers, especially in individuals over 50 years of age and those with risk factors for BCC, to have a skin examination at least once a year at an earlier age.

Ethics

Ethics Committee Approval: Ethical approval was obtained from Tekirdağ Namık Kemal University Ethics Committee with protocol number 2019.67.04.14 and date 23.03.2020.

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: H.A., O.R., Concept: H.A., O.R., Design: H.A., Data Collection or Processing: O.R., Analysis or Interpretation: H.A., O.R., Literature Search: H.A., O.R., Writing: H.A., O.R.

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REFERENCES

1. Goto M, Kai Y, Arakawa S, Oishi M, Ishikawa K, Anzai S, et al. Analysis of 256 cases of basal cell carcinoma after either one-step or two-step surgery in a Japanese institution. J Dermatol. 2012;39:68-71.
2. Soyer HP, Rigel DS, Wurm EMT. Actinic keratosis Basal cell carcinoma and squamous cell carcinoma. In: Callen JP, Cerroni L, Heymann WR, Hruza GJ, Mancini AJ, Patterson JW, Röcken M, Schwarz T, eds. Dermatology. 3th ed. Elsevıer Saunders; Press: 2012;1784-92.
3. Holm AS, Nissen CV, Wulf HC. Basal Cell Carcinoma is as Common as the Sum of all Other Cancers: Implications for Treatment Capacity. Acta Derm Venereol. 2016;96:505-9.
4. Handa Y, Kato Y, Ishikawa H, Tomita Y. Giant superficial basal cell carcinoma of the scrotum. Eur J Dermatol. 2005;15:186-8.
5. Lomas A, Leonardi-Bee J, Bath-Hextall F. A systematic review of worldwide incidence of nonmelanoma skin cancer. Br J Dermatol. 2012;166:1069-80.
6. Verduzco-Martínez AP, Quiñones-Venegas R, Guevara-Gutiérrez E, Tlacuilo-Parra A. Correlation of dermoscopic findings with histopathologic variants of basal cell carcinoma. Int J Dermatol. 2013;52:718-21.
7. Skoda AM, Simovic D, Karin V, Kardum V, Vranic S, Serman L. The role of the Hedgehog signaling pathway in cancer: A comprehensive review. Bosn J Basic Med Sci. 2018;18:8-20.
8. Devine C, Srinivasan B, Sayan A, Ilankovan V. Epidemiology of basal cell carcinoma: a 10-year comparative study. Br J Oral Maxillofac Surg. 2018;56:101-6.
9. Kim DH, Ko HS, Jun YJ. Nonsyndromic Multiple Basal Cell Carcinomas. Arch Craniofac Surg. 2017;18:191-6.
10. Betti R, Inselvini E, Carducci M, Crosti C. Age and site prevalence of histologic subtypes of basal cell carcinomas. Int J Dermatol. 1995;34:174-6.
11. Scrivener Y, Grosshans E, Cribier B. Variations of basal cell carcinomas according to gender, age, location and histopathological subtype. Br J Dermatol. 2002;147:41-7.
12. Souza CF, Thomé EP, Menegotto PF, Schmitt JV, Shibue JR, Tarlé RG. Topography of basal cell carcinoma and their correlations with gender, age and histologic pattern: a retrospective study of 1042 lesions. An Bras Dermatol. 2011;86:272-7.
13. Subramaniam P, Olsen CM, Thompson BS, Whiteman DC, Neale RE; QSkin Sun and Health Study Investigators. Anatomical Distributions of Basal Cell Carcinoma and Squamous Cell Carcinoma in a Population-Based Study in Queensland, Australia. JAMA Dermatol. 2017;153:175-82.
14. Bastiaens MT, Hoefnagel JJ, Bruijn JA, Westendorp RG, Vermeer BJ, Bouwes Bavinck JN. Differences in age, site distribution, and sex between nodular and superficial basal cell carcinoma indicate different types of tumors. J Invest Dermatol. 1998;110:880-4.
15. Reinau D, Surber C, Jick SS, Meier CR. Epidemiology of basal cell carcinoma in the United Kingdom: incidence, lifestyle factors, and comorbidities. Br J Cancer. 2014;111:203-6.

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Factors Associated with Mortality in Hospitalized Older Adults

Hospitalize Edilen Yaşlılarda Mortalite ile İlişkili Olan Faktörler

Pınar TOSUN TAŞAR[1], Sevnaz ŞAHİN[2], Ömer KARAŞAHİN[3], Mevlüt ÜNEŞ[4], Zehra KOSUVA ÖZTÜRK[2], Fisun ŞENUZUN AYKAR[5]

[1]*Atatürk University Faculty of Medicine, Department of Internal Medicine, Clinic of Geriatrics, Erzurum, Turkey*
[2]*Ege University Faculty of Medicine, Department of Internal Medicine, Clinic of Geriatrics, İzmir, Turkey*
[3]*Erzurum Regional Training and Research Hospital, Clinic of Infectious Diseases, Erzurum, Turkey*
[4]*Atatürk University Faculty of Medicine, Department of Internal Medicine, Erzurum, Turkey*
[5]*İzmir Tınaztepe University Faculty of Medicine, Internal Medicine Nursing, İzmir, Turkey*

ABSTRACT

Aim: This study aimed to evaluate mortality risk associated with readily accessible laboratory parameters and underlying conditions in hospitalized older adults.

Materials and Methods: This retrospective study included geriatric patients admitted for inpatient care to the internal medicine wards of two major university hospitals in two different regions of Turkey. Data related to the patients were collected by retrospective review of patient charts and electronic records. Survival data were obtained from the Death Reporting System of the Turkish Ministry of Health. Survival after admission at 30 days and 1 year was noted.

Results: The study included 1.465 hospitalized older adults with a median age of 74 years, of whom 51% were women. Of these patients, 115 (7.8%) died within 30 days and 382 (26.1%) died within 12 months. For 30-day mortality, independent risk factors appeared to be infectious diseases [odds ratio (OR) 2.109, p=0.006], receiving palliative support (OR 5.982, p=0.006), malignancy (OR 2.514, p=0.001), Charlson Comorbidity Index (CCI) (OR 1.219 per unit increase, p<0.001), MPV (OR 1.525 per unit increase, p<0.001), and CRP (OR 1.006 per unit increase, p<0.001). For 12-month mortality, independent risk factors were found to be infectious diseases (OR 1.978, p=0.01), palliative support (OR 6.506, p<0.001), malignancy (OR 2.654, p<0.001), CCI (OR 1.200 per unit increase, p<0.001), and CRP (OR 1.006 per unit increase, p<0.001).

Conclusion: The results of this study show that CCI, CRP, and NLR were associated with higher mortality both at 30 days and 12 months. A one-unit increase in MPV was an independent risk factor for 30-day mortality and increased the odds of mortality by 52.5%.

Keywords: Mortality, elderly, factors

ÖZ

Amaç: Çalışmamızda hospitalize edilen yaşlılarda kolay ulaşılabilir laboratuvar parametreleri ile mortalite açısından risk değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntem: Bu retrospektif çalışma ülkemizin iki bölgesindeki iki büyük üniversitenin dahili servislerinde hospitalize edilen geriatrik hastalar arasında yapıldı. Bilgiler hasta dosyalarından ve elektronik kayıtlardan retrospektif olarak tarandı. Hastaların sağkalım bilgileri Türkiye Cumhuriyeti Sağlık Bakanlığı, Türkiye Halk Sağlığı Kurumu Ölüm Bildirim Sistemi'nden elde edildi. Hastaların 30 günlük ve 1 yıllık sağkalım bilgilerine ulaşıldı.

Bulgular: Bu çalışmaya dahil edilen 1,465 hastanın yaş ortancası 74 yıl idi ve %51'i kadındı. Hastaların 115'inin (%7,8) 30 gün ve 382'sinin (%26,1) 12 ay içeresinde öldüğü görüldü. Otuz günlük mortalite için enfeksiyon hastalıklarının 2,109 kat, palyatif destek alanların 5,982 kat, malignitenin 2,514 kat, Charlson Komorbidite İndeksi'nde (CCI) bir birimlik artışın 1,219 kat, MPV'deki bir birimlik artışın 1,525 kat, C-reaktif proteindeki (CRP) bir birimlik artışın 1,006 kat bağımsız risk faktörleri olduğu görüldü (sırasıyla p=0,006, p=0,006, p=0,001, p<0,001, p<0,001, p<0,001). On iki aylık mortalite için enfeksiyon hastalıklarının 1,978 kat, palyatif desteğin 6,506 kat, malignitenin 2,654 kat, CCI'deki bir birimlik artışın 1,200 kat, CRP'deki bir birimlik artışın 1,006 kat bağımsız risk faktörleri olduğu görüldü (sırasıyla p=0,010, p<0,001, p<0,001, p<0,001, p<0,001).

Address for Correspondence: Pınar TOSUN TAŞAR MD, Atatürk University Faculty of Medicine, Department of Internal Medicine, Clinic of Geriatrics, Erzurum, Turkey
Phone: +90 505 398 89 85 **E-mail:** pinar.tosun@gmail.com **ORCID ID:** orcid.org/0000-0002-2617-4610
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Sonuç: CCI'deki, CRP değerindeki, NLO'daki artışın hem 30 günlük mortalite hem de 12 aylık mortalite riskini artırdığı görülmüştür. MPV'deki bir birimlik artışın 30 günlük mortalite için bağımsız risk faktörü olup %52,5 olasılıkla mortaliteyi artırdığı görülmüştür.

Anahtar Kelimeler: Mortalite, yaşlı, faktör

INTRODUCTION

The older population is steadily growing worldwide. The rate of adults aged 60 years and older in the population is currently approximately 11% and is expected to reach 22% by 2050[1]. This is associated with an increase in hospital admissions among older adults. However, hospitalized older patients face potential loss of functionality[2], prolonged hospital stay, referral to an assisted living facility or nursing home because of increased care needs[3], and excessive health care costs. Therefore, early detection of patients at high risk and rapid initiation of appropriate treatment may shorten hospital stays and prevent indirect losses.

Studies on hospitalization in older adults have generally focused on geriatric syndromes such as cognitive function, falls, functionality, and incontinence[4]. These studies have shown that physical condition and cognitive function are the most important factors at the time of hospital admission[5]. However, there are few studies examining the association between laboratory parameters and mortality. The prognostic value of these parameters may facilitate patient selection, especially in centers with high potential patient volume but limited capacity. Therefore, this study aimed to evaluate mortality risk associated with readily accessible laboratory parameters and underlying conditions in a large patient cohort.

MATERIALS AND METHODS

Inclusion and Exclusion Criteria

This retrospective study included geriatric patients admitted for inpatient care to the internal medicine wards of two major university hospitals in two different regions of Turkey between January 1, 2018 and December 31, 2019.

Patients admitted for hematologic diseases such as leukemia, myelodysplastic syndrome, myelofibrosis, or myeloproliferative disease, those admitted due to trauma or towards other than internal medicine, and those admitted to receive chemotherapy were not included. In addition, patients for whom complete blood count was not performed at the time of admission were excluded from the study.

Data related to the patients' demographic characteristics, underlying conditions, reason for admission (kidney disease, electrolyte imbalance, infection diseases, endocrine diseases, delirium, malnutrition, gastrointestinal bleeding, liver disease, palliative support, general follow-up and examination) and complete blood count values at admission were collected with retrospective review of patient charts and electronic records. The diagnoses of the patients were obtained from the International Statistical Classification of Diseases and Related Health Problems codes and the anamnesis in the files. Their values of white blood cell (WBC), neutrophil, lymphocyte, and platelet (PLT) counts, hemoglobin (Hb) level, mean thrombocyte volume (MPV), and neutrophil to lymphocyte ratio (NLR) were recorded. Complete blood count was measured with an automated cell counter (Sysmex XN-1000). NLR was calculated by dividing the neutrophil count by the lymphocyte count from the same blood sample obtained at admission.

The patients' underlying conditions were evaluated using the Charlson Comorbidity Index (CCI), which is a practical and widely used method for predicting mortality. The CCI was first described in the literature in 1987[6] and was modified in 1992[7].

Survival data were obtained from the Death Reporting System of the Turkish Ministry of Health, General Directorate of Public Health using the patients' citizenship numbers. Survival after admission at 30 days and 1 year was noted.

The study was conducted after obtaining ethical approval from İzmir Tınaztepe University Ethics Committee (decision no: 13, dated: 20/04/2021).

Statistical Analysis

The data were analyzed using the IBM Statistical Package for the Social Sciences statistics version 21.0 package program. Descriptive statistics were presented as median (minimum-maximum) or number and percent distribution. Comparisons between surviving and non-surviving patients were made using the chi-square and Mann-Whitney U tests. A multivariate logistic regression model was created with infectious disease, palliative care, diabetes mellitus (DM), coronary artery disease (CAD), presence of malignancy, CCI, MPV, and C-reactive protein (CRP) value, which were found to be statistically significant among 30-day mortality categorical and time variables. A multivariate logistic regression model was created with the presence of infectious disease, palliative care, DM, CAD, malignancy and CCI, and CRP value, which were found to be statistically significant among the 30-day mortality categorical and time variables (Model: Backward: LR. Entry: 0.05 and Removal: 0.10).

RESULTS

The study included 1.465 hospitalized older adults with a median age of 74 years (range: 60-99), of whom 747 (51.0%) were women. Of these patients, 115 (7.8%) died within 30 days and 382 (26.1%) died within 12 months. Reasons for hospital admission compared according to 30-day and 12-month mortality are presented in Table 1. Both 30-day and 12-month mortality rates were significantly higher among patients hospitalized due to infectious diseases, delirium, malnutrition, and palliative support, and significantly lower in patients admitted for endocrine diseases.

Comparisons of underlying conditions and admission laboratory values according to 30-day and 12-month mortality are presented in Table 2. The 30-day mortality rate was significantly lower in patients with hypertension, DM, and CAD and significantly higher in patients with malignancy. Malignancy, chronic kidney disease, Alzheimer's disease, and chronic liver disease were significantly more common among patients who died within 12 months, while hypertension and DM were significantly less common. Patients who died within 30 days had significantly lower Hb, lymphocyte and PLT counts, and PLT/MPV and significantly higher WBC and neutrophil counts, NLR, MPV, and CRP level. Hb, lymphocyte, WBC, and neutrophil counts, NLR, and CRP levels were also significantly higher among patients who died within 12 months.

Reasons for hospital admission, underlying conditions, and admission laboratory values that were statistically significant in comparisons based on 30-day and 12-month mortality were further evaluated in logistic regression analysis (Table 3). Admission for infectious diseases or palliative support and the presence of malignancy were identified as independent risk factors for both 30-day and 12-month mortality (~6-6.5 times higher odds in patients hospitalized for palliative support, ~2.5 times higher odds for malignancy, and ~2 times higher odds for infectious disease). The presence of DM was associated with significantly lower risk of mortality at both time points (~65% lower), while the presence of CAD was associated with lower odds of mortality only in the first 30 days (~50% lower). A one-unit increase in CCI corresponded to 20% higher odds of both 30-day and 12-month mortality. A one-unit increase in MPV was associated with 52.5% higher odds of 30-day mortality. A one-unit increase in CRP was associated with a small but statistically significant 0.6% increase in the odds of 30-day and 12-month mortality (Table 3).

DISCUSSION

NLR is a systemic marker of inflammation that can be easily obtained from CBC and serves as an indicator of the balance between the natural and acquired immune systems. High NLR has been shown to be associated with mortality in oncology patients[8], including those with lung[9], ovarian[10], and breast[11] cancer, in patients with sepsis and bacteremia[12-14], and after cardiovascular disease, acute coronary syndrome, and stroke[15-17]. Kim et al.[18] reported that high NLR was associated with mortality in patients with ST-elevated myocardial infarction before undergoing primary percutaneous angioplasty. In their study of patients presenting to the emergency department, Hwang et al.[13] found that high NLR was an independent risk factor in patients with sepsis and septic shock. High NLR was also associated with stroke severity in patients diagnosed with acute ischemic stroke[15]. A possible reason for this may be that inflammatory factors released by neutrophils cause vascular degeneration, whereas lymphocytes are believed to have an anti-atherosclerotic role.

Table 1. Causes of hospitalization according to 30-day and 12-month mortality

	Mortality					
	30-day		p	12-month		p
	Yes (n=115)	No (n=1350)		Yes (n=382)	No (n=1083)	
Kidney disease, electrolyte imbalance	48 (41.7)	460 (34.1)	0.097	175 (45.8)	333 (30.7)	<0.001
Infection diseases	33 (28.7)	204 (15.1)	**<0.001**	**101 (26.4)**	**136 (12.6)**	**<0.001**
Endocrine diseases	15 (13.0)	374 (27.7)	**<0.001**	**71 (18.6)**	**318 (29.4)**	**<0.001**
Delirium	8 (7.0)	45 (3.3)	**0.046**	**23 (6.0)**	**30 (2.8)**	**0.003**
Malnutrition	20 (17.4)	90 (6.7)	**<0.001**	**58 (15.2)**	**52 (4.8)**	**<0.001**
GIS bleeding	3 (2.6)	58 (4.3)	0.383	16 (4.2)	45 (4.2)	0.978
Liver disease	9 (7.8)	71 (5.3)	0.245	33 (8.6)	47 (4.3)	0.001
Palliative support	10 (8.7)	13 (1.0)	**<0.001**	**15 (3.9)**	**8 (0.7)**	**<0.001**
General follow-up and examination	45 (39.1)	474 (35.1)	0.387	**134 (35.1)**	**385 (35.5)**	**0.869**

GIS: Gastrointestinal, Kidney disease and electrolyte imbalances: Acute kidney failure, chronic kidney disease, hyponatremia, hypernatremia, uremia, hypervolemia; Infectious diseases: Pneumonia, diabetic foot infection, decubitus ulcer infection, urinary tract infection; Endocrine diseases: Hyperglycemia, hypoglycemia, hypothyroidism, hyperthyroidism, pituitary insufficiency, osteoporosis

Table 2. Comparison of systemic diseases and admission laboratory values according to 30-day and 12-month mortality

	Mortality					
	30-day		p	12-month		p
Comorbidity	Yes (n=115)	No (n=1350)		Yes (n=382)	No (n=1083)	
HT	60 (52.2)	860 (63.7)	**0.014**	211 (55.2)	709 (65.5)	**<0.001**
DM	35 (30.4)	639 (47.3)	**<0.001**	140 (36.6)	534 (49.3)	**<0.001**
CAD	15 (13.0)	326 (24.1)	**0.007**	75 (19.6)	266 (24.6)	0.050
CHF	21 (18.3)	191 (14.1)	0.229	64 (16.8)	148 (13.7)	0.140
Depression	6 (5.2)	40 (3.0)	0.183	15 (3.9)	31 (2.9)	0.305
CRF	34 (29.6)	431 (31.9)	0.602	142 (37.2)	323 (29.8)	**0.008**
RA	2 (1.7)	20 (1.5)	0.526	4 (1.0)	18 (1.7)	0.396
Nephrotic syndrome	1 (0.9)	15 (1.1)	0.638	4 (1.0)	12 (1.1)	0.591
Nephritic syndrome	-	7 (0.5)	0.564	1 (0.3)	6 (0.6)	0.418
Other CTD	1 (0.9)	11 (0.8)	0.627	4 (1.0)	8 (0.7)	0.565
Chronic liver disease	10 (8.7)	81 (6.0)	0.250	33 (8.6)	58 (5.4)	**0.022**
CVE	7 (6.1)	101 (7.5)	0.583	36 (9.4)	72 (6.6)	0.074
Parkinson's disease	2 (1.7)	32 (2.4)	0.493	9 (2.4)	25 (2.3)	0.958
Alzheimer's disease	4 (3.5)	63 (4.7)	0.383	30 (7.9)	37 (3.4)	**<0.001**
COPD	15 (13.0)	139 (10.3)	0.218	46 (12.0)	108 (10.0)	0.257
Malignancy	50 (43.5)	178 (13.2)	**<0.001**	124 (32.5)	104 (9.6)	**<0.001**
Decompensated CHF	4 (3.5)	16 (1.2)	0.065	9 (2.4)	11 (1.0)	0.051
Hyperthyroidism	4 (3.5)	36 (2.7)	0.385	6 (1.6)	34 (3.1)	0.106
Hypothyroidism	10 (8.7)	104 (7.7)	0.703	24 (6.3)	90 (8.3)	0.203
Osteoporosis	6 (5.2)	62 (4.6)	0.447	19 (5.0)	49 (4.5)	0.720
CCI	6 (4-8)	5 (3-6)	**<0.001**	5 (3.7-7)	4 (3-6)	**<0.001**
Laboratory values						
Hb	10.8 (9.3-12.5)	11.7 (9.8-13.5)	**0.002**	10.5 (9.1-12.3)	12.1 (10.1-13.8)	**<0.001**
WBC count	9.55 (6.87-13.99)	8.23 (6.43-10.66)	**0.001**	8.88 (6.72-12.34)	8.11 (6.83-10.47)	**<0.001**
Neutrophil count	7.24 (4.65-11.51)	5.56 (4.02-7.95)	**<0.001**	6.6 (4.58-9.71)	5.11 (3.83-7.79)	**<0.001**
Lymphocyte count	1.06 (0.72-1.46)	1.42 (9.30-2.04)	**<0.001**	1.11 (0.74-1.54)	1.51 (0.99-2.13)	**<0.001**
NLR	7.7 (4.0-12.9)	3.7 (2.3-6.8)	**<0.001**	5.5 (3.5-10.4)	3.5 (2.1-6.4)	**<0.001**
PLT count	228 (155-290)	240 (183-300)	**0.020**	232 (157-317)	240 (187-298)	0.100
MPV	10.6 (9.9-11.7)	10.3 (9.6-11.0)	**0.001**	10.3 (9.7-11.2)	10.3 (9.7-11.1)	0.674
PLT/MPV	20.2 (13.0-26.8)	23.0 (17.1-30.1)	**0.003**	22.2 (14.8-30.7)	23.1 (17.5-29.7)	0.227
CRP	64 (34-128)	18 (4.9-62)	**<0.001**	48 (22-101)	14 (4-53)	**<0.001**

HT: Hypertension, DM: Diabetes mellitus, CAD: Coronary artery disease, CHF: Chronic heart failure, CRF: Chronic renal failure, RA: Rheumatoid arthritis, CTD: Connective tissue disease, CVE: Cerebrovascular event, COPD: Chronic obstructive pulmonary disease, CCI: Charlson Comorbidity Index, Hb: Hemoglobin, WBC: White blood cell, NLR: Neutrophil to lymphocyte ratio, PLT: Platelet, MPV: Mean platelet volume, CRP: C-reactive protein

Table 3. Independent risk factors for 30-day and 12-month mortality according to logistic regression analysis

	30-day mortality	p	12-month mortality	p
	Multivariate OR (95% CI)		Multivariate OR (95% CI)	
Infectious diseases (yes or no)	2.109 (1.241-3.584)	**0.006**	1.978 (1.174-3.332)	**0.010**
Palliative support (yes or no)	5.982 (2.229-16.050)	**<0.001**	6.506 (2.482-17.054)	**<0.001**
DM (yes or no)	0.434 (0.266-0.708)	**0.001**	0.462 (0.288-0.742)	**0.001**
CAD (yes or no)	0.496 (0.268-0.915)	**0.025**		
Malignancy (yes or no)	2.514 (1.451-4.356)	**0.001**	2.654 (1.568-4.493)	**<0.001**
CCI (1-unit increase)	1.219 (1.117-1.353)	**<0.001**	1.200 (1.093-1.317)	**<0.001**
MPV (1-unit increase)	1.525 (1.294-1.798)	**<0.001**		
CRP (1-unit increase)	1.006 (1.003-1.009)	**<0.001**	1.006 (1.004-1.009)	**<0.001**

OR: Odds ratio, CI: Confidence interval, DM: Diabetes mellitus, CAD: Coronary artery disease, CCI: Charlson Comorbidity Index, MPV: Mean platelet volume, CRP: C-reactive protein

In our study, we observed that high NLR was associated with higher mortality in hospitalized older adults at 1 and 12 months. Although the exact relationship between NLR and mortality among hospitalized patients is not clear, possible mechanisms include systemic inflammation caused by acute disease. NLR may also increase mortality due to underlying sepsis or bacteremia. Another possible cause is chronic inflammation, which naturally increases with age[16]. However, more studies are needed to elucidate the relationship between NLR and mortality in hospitalized older patients.

PLTs are involved in a wide range of pathophysiological processes, such as hemostasis, thrombosis, coagulation, vascular constriction and repair, atherosclerosis, host defense, and tumor growth and metastasis[17]. PLT size is expressed as MPV, a parameter that serves as an indicator of PLT function. Higher PLT volume is associated with PLT reactivation, reduced bleeding time, increased PLT aggregation, and higher risk of thrombosis[18]. The PLTs found in circulating blood differ in size. Large PLTs are more active and release more GPIIb-IIIa and P-selectin. In addition, the proteins on the surface of these PLTs have higher activation, aggregation, and endothelial binding capacities[19,20]. Epidemiological studies have shown that MPV is associated with obesity[21], hyperlipidemia[22], diabetes[23], hypertension[24], and arterial thickening[25]. In metabolic syndrome, adipose tissue releases cytokines such as tumor necrosis factor-alpha and interleukin-6, and adinopectins such as adiponectin and leptin. These proinflammatory cytokines cause a chronic increase in PLT number[26-28]. Low MPV can also increase the number of PLTs and eventually lead to metabolic syndrome[29]. High MPV levels have been associated with myocardial infarction[30], stroke[31], and peripheral vascular disease[32,33]. A study of 25,923 patients in Norway showed that high MPV increased the risk of venous thrombosis in the absence of surgery, trauma, immobilization, and malignancy. In addition, high MPV has been shown to increase the risk of ischemic stroke and subsequent death[34]. In a Copenhagen study involving 39,531 people, the prevalence of myocardial infarction was found to be higher in those with high MPV[35-37]. In our study, we determined that MPV was an independent risk factor for 30-day mortality in hospitalized older patients, with a one-unit increase in MPV associated with a 50% higher risk of death. In addition to thrombopoietin, inflammatory cytokines such as IL-1, IL-6, and tumor necrosis factor-alpha are among the factors that stimulate thrombopoiesis[38]. Therefore, MPV is thought to increase in severe inflammation. Although the relationship between MPV and increased mortality is also unclear, several mechanisms have been proposed. The first is that large PLTs contain larger prothrombotic material such as thromboxane A2 and alpha granules, thus leading to PLT activation, adhesion, and vascular proliferation[39,40]. At the same time, large PLTs have larger glycoprotein Ib and IIb/IIIa adhesion receptors, which may require more cleavage to achieve antiplatelet treatment response[41].

Studies demonstrating the effect of comorbidity on mortality are contradictory. Among epidemiological studies conducted in different European countries, some have shown that mortality increases with comorbidity[42,43]. However, other studies have indicated that comorbidity has no effect on mortality[44-46]. Frenkel et al.[47] reported that CCI was a good predictor of post-discharge mortality in older patients hospitalized for acute causes. Studies on patients followed up after hip surgery in China also showed that CCI was an indicator of long-term mortality[48]. A one-unit increase in CCI corresponded to 20% higher odds of both 30-day and 12-month mortality.

In our study, it was shown that the presence of anemia increased mortality. It has been shown in the literature that anemia increases mortality[49]. In addition, we determined that a one-unit increase in CRP was associated with slightly mortality risk at 30 days and 1 year. Similar results have been observed in studies with older people in the general population[50,51] and hospitalized older patients[46].

Long-term hyperglycemia is known to increase reactive oxygen release, lead to cellular damage and electrolyte imbalance, and impair immune functions[52]. However, there are publications in the literature showing that the presence of diabetes does not increase mortality[53,54]. In contrast, other reports indicate that mortality is higher in diabetic patients with pneumonia[55]. In our study, the presence of diabetes was actually associated with lower mortality. However, the diabetic patients in our study were not asked about the treatment they received and whether their diabetes was effectively managed.

Study Limitations

The strength of our study is that it was conducted with a very large patient cohort from two major university hospitals in two major cities. However, one of the limitations of our study is that biomarkers that might affect mortality, such as albumin, were not investigated. A second limitation is that the study was retrospective. In addition, we evaluated the association between mortality and NLR, but this ratio was affected by systemic diseases and the use of drugs such as steroids. We did not determine parameters such as steroid use. Finally, we did not evaluate the severity of disease in the patients.

CONCLUSION

In conclusion, the results of this study showed that CCI, CRP, and NLR were associated with higher mortality both at 30 days and 12 months. A one-unit increase in MPV was associated with 52.5% higher odds of 30-day mortality. Our study provides preliminary results that may guide further investigations on this subject.

Ethics

Ethics Committee Approval: The study was conducted after obtaining ethical approval from İzmir Tınaztepe University Ethics Committee (decision no: 13, dated: 20/04/2021).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: Ö.K., Design: S.Ş., F.Ş.A., Data Collection or Processing: P.T.T., Z.K.Ö., F.Ş.A., Analysis or Interpretation: P.T.T., S.Ş., Ö.K., M.Ü., Z.K.Ö., F.Ş.A., Literature Search: P.T.T., Ö.K., Writing: P.T.T., S.Ş., Ö.K., M.Ü., Z.K.Ö., F.Ş.A.

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REFERENCES

1. Kanasi E, Ayilavarapu S, Jones J. The aging population: demographics and the biology of aging. Periodontol 2000. 2016;72:13-8.
2. Covinsky KE, Palmer RM, Fortinsky RH, Counsell SR, Stewart AL, Kresevic D, et al. Loss of independence in activities of daily living in older adults hospitalized with medical illnesses: increased vulnerability with age. J Am Geriatr Soc. 2003;51:451-8.
3. Lamont CT, Sampson S, Matthias R, Kane R. The outcome of hospitalization for acute illness in the elderly. J Am Geriatr Soc. 1983;31:282-8.
4. Anpalahan M, Gibson SJ. Geriatric syndromes as predictors of adverse outcomes of hospitalization. Intern Med J. 2008;38:16-23.
5. Campbell SE, Seymour DG, Primrose WR, Lynch JE, Dunstan E, Espallargues M, et al. A multi-centre European study of factors affecting the discharge destination of older people admitted to hospital: analysis of in-hospital data from the ACMEplus project. Age Ageing. 2005;34:467-75.
6. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis. 1987;40:373-83.
7. Deyo RA, Cherkin DC, Ciol MA. Adapting a clinical comorbidity index for use with ICD-9-CM administrative databases. J Clin Epidemiol. 1992;45:613-9.
8. Zahorec R. Ratio of neutrophil to lymphocyte counts--rapid and simple parameter of systemic inflammation and stress in critically ill. Bratisl Lek Listy. 2001;102:5-14.
9. Sarraf KM, Belcher E, Raevsky E, Nicholson AG, Goldstraw P, Lim E. Neutrophil/lymphocyte ratio and its association with survival after complete resection in non-small cell lung cancer. J Thorac Cardiovasc Surg. 2009;137:425-8.
10. Cho H, Hur HW, Kim SW, Kim SH, Kim JH, Kim YT, et al. Pre-treatment neutrophil to lymphocyte ratio is elevated in epithelial ovarian cancer and predicts survival after treatment. Cancer Immunol Immunother. 2009;58:15-23.
11. Azab B, Bhatt VR, Phookan J, Murukutla S, Kohn N, Terjanian T, et al. Usefulness of the neutrophil-to-lymphocyte ratio in predicting short- and long-term mortality in breast cancer patients. Ann Surg Oncol. 2012;19:217-24.
12. Liu X, Shen Y, Wang H, Ge Q, Fei A, Pan S. Prognostic Significance of Neutrophil-to-Lymphocyte Ratio in Patients with Sepsis: A Prospective Observational Study. Mediators Inflamm. 2016;2016:8191254.
13. Hwang SY, Shin TG, Jo IJ, Jeon K, Suh GY, Lee TR, et al. Neutrophil-to-lymphocyte ratio as a prognostic marker in critically-ill septic patients. Am J Emerg Med. 2017;35:234-9.
14. Terradas R, Grau S, Blanch J, Riu M, Saballs P, Castells X, et al. Eosinophil count and neutrophil-lymphocyte count ratio as prognostic markers in patients with bacteremia: a retrospective cohort study. PLoS One. 2012;7:e42860.
15. Xue J, Huang W, Chen X, Li Q, Cai Z, Yu T, et al. Neutrophil-to-Lymphocyte Ratio Is a Prognostic Marker in Acute Ischemic Stroke. J Stroke Cerebrovasc Dis. 2017;26:650-7.
16. Bandeen-Roche K, Walston JD, Huang Y, Semba RD, Ferrucci L. Measuring systemic inflammatory regulation in older adults: evidence and utility. Rejuvenation Res. 2009;12:403-10.
17. Sanchez O, Planquette B, Roux A, Gosset-Woimant M, Meyer G. Triaging in pulmonary embolism. Semin Respir Crit Care Med. 2012;33:156-62.
18. Kim SC, Sun KH, Choi DH, Lee YM, Choi SW, Kang SH, et al. Prediction of Long-Term Mortality Based on Neutrophil-Lymphocyte Ratio After Percutaneous Coronary Intervention. Am J Med Sci. 2016;351:467-72.
19. Stellos K, Panagiota V, Kögel A, Leyhe T, Gawaz M, Laske C. Predictive value of platelet activation for the rate of cognitive decline in Alzheimer's disease patients. J Cereb Blood Flow Metab. 2010;30:1817-20.
20. Canan F, Dikici S, Kutlucan A, Celbek G, Coskun H, Gungor A, et al. Association of mean platelet volume with DSM-IV major depression in a large community-based population: the MELEN study. J Psychiatr Res. 2012;46:298-302.
21. Arslan N, Makay B. Mean platelet volume in obese adolescents with nonalcoholic fatty liver disease. J Pediatr Endocrinol Metab. 2010;23:807-13.
22. Pathansali R, Smith N, Bath P. Altered megakaryocyte-platelet haemostatic axis in hypercholesterolaemia. Platelets. 2001;12:292-7.
23. Papanas N, Symeonidis G, Maltezos E, Mavridis G, Karavageli E, Vosnakidis T, et al. Mean platelet volume in patients with type 2 diabetes mellitus. Platelets. 2004;15:475-8.
24. Nadar S, Blann AD, Lip GY. Platelet morphology and plasma indices of platelet activation in essential hypertension: effects of amlodipine-based antihypertensive therapy. Ann Med. 2004;36:552-7.
25. Wang RT, Li Y, Zhu XY, Zhang YN. Increased mean platelet volume is associated with arterial stiffness. Platelets. 2011;22:447-51.
26. Aypak C, Türedi O, Bircan MA, Yüce A. Could mean platelet volume among complete blood count parameters be a surrogate marker of metabolic syndrome in pre-pubertal children? Platelets. 2014;25:393-8.
27. Park BJ, Shim JY, Lee HR, Jung DH, Lee JH, Lee YJ. The relationship of platelet count, mean platelet volume with metabolic syndrome according to the criteria of the American Association of Clinical Endocrinologists: a focus on gender differences. Platelets. 2012;23:45-50.
28. Baatout S. Interleukin-6 and megakaryocytopoiesis: an update. Ann Hematol. 1996;73:157-62.
29. Jones RL, Paradise C, Peterson CM. Platelet survival in patients with diabetes mellitus. Diabetes. 1981;30:486-9.
30. Fay WP. Linking inflammation and thrombosis: Role of C-reactive protein. World J Cardiol. 2010;2:365-9.
31. Chu SG, Becker RC, Berger PB, Bhatt DL, Eikelboom JW, Konkle B, et al. Mean platelet volume as a predictor of cardiovascular risk: a systematic review and meta-analysis. J Thromb Haemost. 2010;8:148-56.
32. Muscari A, Puddu GM, Cenni A, Silvestri MG, Giuzio R, Rosati M, et al. Mean platelet volume (MPV) increase during acute non-lacunar ischemic strokes. Thromb Res. 2009;123:587-91.
33. Berger JS, Eraso LH, Xie D, Sha D, Mohler ER 3rd. Mean platelet volume and prevalence of peripheral artery disease, the National Health and Nutrition Examination Survey, 1999-2004. Atherosclerosis. 2010;213:586-91.

34. Braekkan SK, Mathiesen EB, Njølstad I, Wilsgaard T, Størmer J, Hansen JB. Mean platelet volume is a risk factor for venous thromboembolism: the Tromsø Study, Tromsø, Norway. J Thromb Haemost. 2010;8:157-62.

35. Greisenegger S, Endler G, Hsieh K, Tentschert S, Mannhalter C, Lalouschek W. Is elevated mean platelet volume associated with a worse outcome in patients with acute ischemic cerebrovascular events? Stroke. 2004;35:1688-91.

36. Pikija S, Cvetko D, Hajduk M, Trkulja V. Higher mean platelet volume determined shortly after the symptom onset in acute ischemic stroke patients is associated with a larger infarct volume on CT brain scans and with worse clinical outcome. Clin Neurol Neurosurg. 2009;111:568-73.

37. Klovaite J, Benn M, Yazdanyar S, Nordestgaard BG. High platelet volume and increased risk of myocardial infarction: 39,531 participants from the general population. J Thromb Haemost. 2011;9:49-56.

38. Kaushansky K. The molecular mechanisms that control thrombopoiesis. J Clin Invest. 2005;115:3339-47.

39. Kamath S, Blann AD, Chin BS, Lip GY. Platelet activation, haemorheology and thrombogenesis in acute atrial fibrillation: a comparison with permanent atrial fibrillation. Heart. 2003;89:1093-5.

40. Martin JF, Shaw T, Heggie J, Penington DG. Measurement of the density of human platelets and its relationship to volume. Br J Haematol. 1983;54:337-52.

41. Kamath S, Blann AD, Lip GY. Platelet activation: assessment and quantification. Eur Heart J. 2001;22:1561-71.

42. Menotti A, Mulder I, Nissinen A, Giampaoli S, Feskens EJ, Kromhout D. Prevalence of morbidity and multimorbidity in elderly male populations and their impact on 10-year all-cause mortality: The FINE study (Finland, Italy, Netherlands, Elderly). J Clin Epidemiol. 2001;54:680-6.

43. Fillenbaum GG, Pieper CF, Cohen HJ, Cornoni-Huntley JC, Guralnik JM. Comorbidity of five chronic health conditions in elderly community residents: determinants and impact on mortality. J Gerontol A Biol Sci Med Sci. 2000;55:M84-9.

44. Di Bari M, Virgillo A, Matteuzzi D, Inzitari M, Mazzaglia G, Pozzi C, et al. Predictive validity of measures of comorbidity in older community dwellers: the Insufficienza Cardiaca negli Anziani Residenti a Dicomano Study. J Am Geriatr Soc. 2006;54:210-6.

45. Ferrucci L, Guralnik JM, Baroni A, Tesi G, Antonini E, Marchionni N. Value of combined assessment of physical health and functional status in community-dwelling aged: a prospective study in Florence, Italy. J Gerontol. 1991;46:M52-6.

46. Agrawal S, Luc M, Winkowski F, Lindner K, Agrawal AK, Wozniak M, et al. Predictors of mortality in older patients admitted to a geriatric hospital. Geriatr Gerontol Int. 2019;19:70-5.

47. Frenkel WJ, Jongerius EJ, Mandjes-van Uitert MJ, van Munster BC, de Rooij SE. Validation of the Charlson Comorbidity Index in acutely hospitalized elderly adults: a prospective cohort study. J Am Geriatr Soc. 2014;62:342-6.

48. Lau TW, Fang C, Leung F. Assessment of postoperative short-term and long-term mortality risk in Chinese geriatric patients for hip fracture using the Charlson comorbidity score. Hong Kong Med J. 2016;22:16-22.

49. Zakai NA, Katz R, Hirsch C, Shlipak MG, Chaves PH, Newman AB, et al. A prospective study of anemia status, hemoglobin concentration, and mortality in an elderly cohort: the Cardiovascular Health Study. Arch Intern Med. 2005;165:2214-20.

50. Tice JA, Browner W, Tracy RP, Cummings SR. The relation of C-reactive protein levels to total and cardiovascular mortality in older U.S. women. Am J Med. 2003;114:199-205.

51. Harris TB, Ferrucci L, Tracy RP, Corti MC, Wacholder S, Ettinger WH Jr, Heimovitz H, Cohen HJ, Wallace R. Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the elderly. Am J Med. 1999;106:506-12.

52. Jafar N, Edriss H, Nugent K. The Effect of Short-Term Hyperglycemia on the Innate Immune System. Am J Med Sci. 2016;351:201-11.

53. Graham BB, Keniston A, Gajic O, Trillo Alvarez CA, Medvedev S, Douglas IS. Diabetes mellitus does not adversely affect outcomes from a critical illness. Crit Care Med. 2010;38:16-24.

54. Corrao S, Nobili A, Natoli G, Mannucci PM, Perticone F, Pietrangelo A, et al. Hyperglycemia at admission, comorbidities, and in-hospital mortality in elderly patients hospitalized in internal medicine wards: data from the RePoSI Registry. Acta Diabetol. 2021;58:1225-36.

55. Corrao S, Argano C, Natoli G, Nobili A, Corazza GR, Mannucci PM, et al. Disability, and not diabetes, is a strong predictor of mortality in oldest old patients hospitalized with pneumonia. Eur J Intern Med. 2018;54:53-9.

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Efficacy Comparison Between Weekly and Triweekly Regimens of Carboplatin-paclitaxel in Non-small Cell Lung Cancer

Küçük Hücreli Dışı Akciğer Kanserinde Haftalık ve Üç Haftalık Karboplatin Paklitaksel Tedavi Etkinliğinin Karşılaştırılması

Serdar ATA[1], Ahmet Ziya BAYHAN[1], Oğuzhan KESEN[1], Burcu ARSLAN BENLİ[1], Tolga KÖŞECİ[2], Hakan DEMİR[1], Timuçin ÇİL[1], Berna BOZKURT DUMAN[1]

[1]Adana City Training and Research Hospital, Clinic of Medical Oncology, Adana, Turkey
[2]Çukurova University Faculty of Medicine, Department of Medical Oncology, Adana, Turkey

ABSTRACT

Aim: Around 40% of non-small cell lung cancers have stage 3b or 4 disease at the time of diagnosis. In the treatment, platinum-based therapy can still be used in patients who do not carry a driver mutation or who are not suitable for immunotherapy with advanced non-small cell lung cancers (NSCLC). The objective of this study was to compare the effectiveness of weekly carboplatin-paclitaxel regimen with triweekly carboplatin-paclitaxel regimen.

Materials and Methods: This is a retrospectively structured study. Patients who were followed-up and treated for lung cancer in Adana City Training and Research Hospital's Oncology Department between January 1, 2017 and July 1, 2021 were included.

Results: Out of the 104 patients, 52 (50%) patients received weekly carboplatin-paclitaxel (C-P), and 52 (50%) received C-P every 3 weeks. The mean overall survival was 19.64±2.53 months in the weekly C-P group and 17.47±1.64 months in the triweekly C-P group (p=0.675). The mean progression-free survival (PFS) was 8.5±1.01 months in the weekly C-P group and 5.76±0.61 months in the triweekly C-P group (p=0.017).

Conclusion: We demonstrated that weekly C-P treatment, which is known to have fewer toxicity in NSCLC, provided better PFS compared to triweekly treatment.

Keywords: Non-small cell lung cancer, carboplatin-paclitaxel, triweekly, progression free survival, overall survival

ÖZ

Amaç: Küçük hücreli dışı akciğer kanser tanılı hastaların yaklaşık %40'ı tanı anında evre 3b veya 4 hastalığa sahiptir. Platin bazlı sistemik kemoterapi, şu anda ilerlemiş küçük hücreli dışı akciğer kanserlerli hastalar için yerleşik konvansiyonel tedavidir. Bu çalışmanın amacı, daha düşük toksisite beklenen haftalık karboplatin-paklitaksel (K-P) rejiminin etkinliğininin üç haftalık K-P rejimi ile karşılaştırmaktır.

Gereç ve Yöntem: Bu retrospektif yapılandırılmış bir çalışmadır; Adana Şehir Eğitim ve Araştırma Hastanesi Onkoloji Kliniği'nde 1 Ocak 2017-1 Temmuz 2021 tarihleri arasında akciğer kanseri nedeniyle takip ve tedavi edilen hastalar dahil edildi.

Bulgular: Yüz dört kişiden 52'si (%50) haftalık K-P ve 52'si (%50) 3 haftada bir K-P aldı. Ortalama genel sağkalım haftalık K-P grupda 19,64±2,53 ay ve üç haftada bir K-P alan grubunda 17,47±1,64 aydı (p=0,675). Ortalama progresyonsuz sağkalım (PFS) haftalık K-P grubunda 8,57±1,01 ay ve üç haftada bir K-P alan grupda 5,76±0,61 ay olup, anlamlı olarak daha yüksek bir değerdi (p=0,017).

Sonuç: Daha az toksisiteye sahip olduğu bilinen haftalık K-P tedavisinin benzer hasta grubunda üç haftalık tedaviye göre daha iyi PFS sağladığını gösterdik.

Anahtar Kelimeler: Küçük hücreli dışı akciğer kanseri, karboplatin-paklitaksel, haftada bir, progresyonsuz sağkalım, genel sağkalım

Address for Correspondence: Serdar ATA MD, Adana City Training and Research Hospital, Clinic of Medical Oncology, Adana, Turkey
Phone: +90 505 426 03 13 **E-mail:** drserdarata@gmail.com **ORCID ID:** orcid.org/0000-0002-8269-9884
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INTRODUCTION

Among all cancers, lung cancer is the one that most frequently leads to death, according to World Health Organization data[1]. Approximately 80% of lung cancers are non-small cell lung cancers (NSCLC)[2]. Around 40% of the patients in this group have stage 3b or 4 disease at the time of diagnosis[3]. Platinum-based systemic chemotherapy is currently the established conventional treatment for patients with advanced NSCLC[4,5]. Immunotherapies are included in current treatment guidelines as important therapeutic agents for lung cancer. Because immunotherapies are expensive and therefore, difficult to access, they have not yet become a standard for care outside developed countries. However, these agents are not routinely used in the first line due to reimbursement issues in our country, pemetrexed is also not reimbursed in the first line treatment of NSCLC in Turkey. As a result, platinum-based treatments are predominantly used in our country. One of these regimens is carboplatin-paclitaxel (C-P) treatment. Studies have compared C-P protocol with other treatment protocols and found no superiority of any of these against others[4]. In studies investigating the side effect profile, the most frequently seen toxicities in patients receiving treatment with C-P may include myelosuppression, neuropathy, nausea, weakness, and arthralgia. While no difference is seen in terms of effectiveness compared to triweekly C-P treatment, toxicity rates have been shown to decline when paclitaxel is given weekly and carboplatin is administered every 3 weeks[6]. The objective of this study was to compare the effectiveness of weekly C-P regimen with triweekly C-P regimen in NSCLC.

MATERIALS AND METHODS

This study assessed patients followed-up and treated for lung cancer in Adana City Training and Research Hospital's Oncology Clinic between January 1, 2017 and July 1, 2021. Ethical approval was obtained from Adana City Training and Research Hospital (decision no: 1913, date: 21.04.2022). Patients evaluated were those diagnosed with lung cancer in the hospital automation system. The files of 104 patients with pathologically diagnosed lung cancer were screened for suitability for the study. A total of 181 patients with neuroendocrine carcinoma or small cell lung cancer were excluded from the study. In the second step treatments given to 623 patients with NSCLC were evaluated. Four hundred forty patients who received first line therapy other than C-P were excluded. Among the remaining 176 patients, 49 who first received chemo-radiotherapy for primary lung cancer were also excluded from the study. Out of remaining 127 patients, 23 patients who received weekly C-P treatment 3 times or less, or 1 cycle of triweekly C-P treatment were also excluded. Patients with driver mutations (EGFR, ALK, ROS etc.) were not included in the study.

Patients receiving paclitaxel at a dose of 80 mg/m^2 weekly and carboplatin 2 AUC (area under the curve) for the weekly treatment protocol, or paclitaxel at a dose of 175 mg/m^2 every 3 weeks and 5 AUC doses of carboplatin every 3 weeks for the triweekly treatment protocol were included.

Data from 104 eligible patients were evaluated statistically. Response to treatment and progression were adjudicated based on the assessment of conventional imaging reports registered in the hospital automation system according to RECIST 1.1. For patients who were followed up with positron emission tomography/computed tomography, adjudication was made by the assessment of final reports.

Statistical Analysis

Statistical Package for the Social Sciences 23.0 package software was used for the statistical analysis of the data. Categorical measurements were summarized in terms of number and percentage, continuous measurements were summarized as mean, deviation and minimum-maximum. Suitability for normal distribution was examined using the Shapiro-Wilk test. The chi-square test and Fisher's exact test were used for comparison of categorical variables. The Independent Student's t-test was used in groups conforming normal distribution and the Mann-Whitney U test in those not conforming normal distribution. The Spearman correlation test was used to investigate the relationship between overall survival (OS) and progression-free survival (PFS) values and continuous measurement values. The Kaplan-Meier and log-rank tests were used in survival analyses. Statistical significance level was set to 0.05 for all tests.

RESULTS

Out of the 104 patients included in the study, 90 (86.5%) were male and 14 (13.5%) were female. Fifty-two (50%) patients received weekly C-P and 52 (50%) received C-P every 3 weeks. Fifty-seven (54.8%) patients were followed-up for the diagnosis of adenocarcinoma, 21 (20.2%) for squamous cell carcinoma, and 26 (25%) for not otherwise specified (NOS). During the course of the treatment, 48 (46.2%) patients experienced radiological progression and 66 (63.5%) patients died. Clinical and demographic findings of the patients are presented in Table 1. Patients in the weekly C-P group received treatment for a median of 7 weeks and those in triweekly C-P group received a median of 3 cycles. Size of the primary mass was the same in both groups while SUV value of the primary mass was higher in the weekly C-P group (p=0.029). No difference was noted between the groups in terms of laboratory parameters (Table 1).

The mean OS was 19.64±2.53 months in the weekly C-P group and 17.47±1.64 months in the triweekly C-P group (p=0.675) (Figure 1).

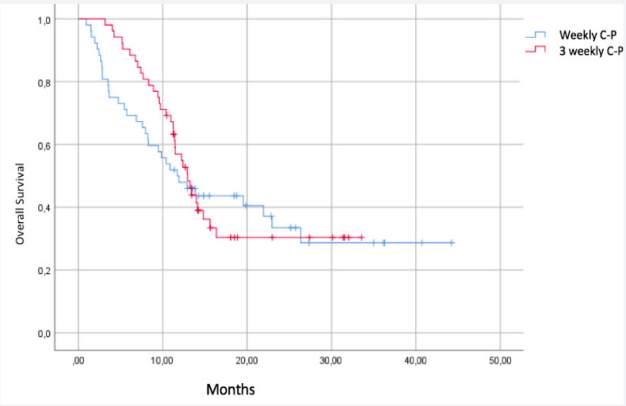

Figure 1. Overall survival according to chemotherapy regimen

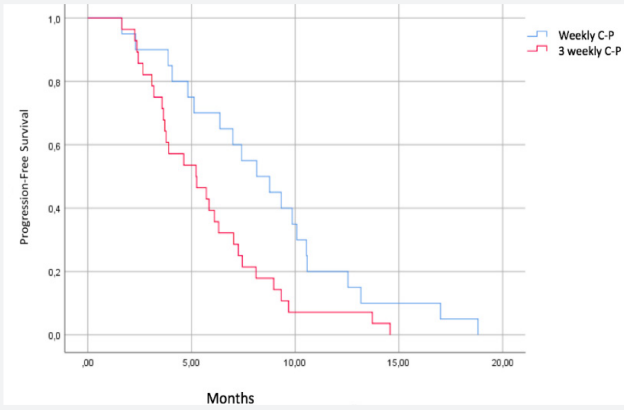

Figure 2. Progression-free survival according to chemotherapy regimen

Table 1. Demographic, clinical and laboratory characteristics of patients

	Weekly C-P[a]	Triweekly C-P[a]	Total	p
Age of diagnosis (mean, SD)	65.7 (8.9)	60.1 (8.9)	62.9 (9.3)	0.002[b,c]
Gender	**n (%)**	**n (%)**	**n (%)**	
Male	45 (86.5)	45 (86.5)	90 (86.5)	NA[d]
Female	7 (13.5)	7 (13.5)	14 (13.5)	
Diagnosis				
Adenocancer	29 (55.8)	28 (53.8)	57 (54.8)	0.896[d]
Squamous cell carcinoma	11 (21.2)	10 (19.2)	21 (20.2)	
Not otherwise specified	12 (23.1)	14 (26.9)	26 (25.0)	
Location of metastasis				
None	2 (23.1)	11 (21.2)	23 (22.1)	
Brain	7 (13.5)	9 (17.3)	16 (15.4)	0.603[d]
Other[g]	31 (59.6)	27 (51.9)	58 (55.8)	
Brain + other	2 (3.8)	5 (9.6)	7 (6.7)	
Number of metastases	2 (1-5)	2 (1-5)	2 (1-5)	0.275[e]
Number of treatments	7 (3-29)	3 (2-11)	5 (2-29)	**<0.001[e,c]**
Lactate dehydrogenase	267 (135-1736)	253 (159-10848)	260 (135-10848)	0.413[e]
Progression status				
None	32 (61,5)	24 (46.2)	56 (53.8)	0.116[f]
Yes	20 (38,5)	28 (53.8)	48 (46.2)	
Death status				
None	19 (36.5)	19 (36.5)	38 (36.5)	NA[d]
Yes	33 (63.5)	33 (63.5)	66 (63.5)	
Location of metastasis				
None	12 (23.1)	11 (21,2)	23 (22,1)	0.603[d]
Brain	7 (13.5)	9 (17,3)	16 (15.4)	
Other[g]	31 (59,6)	27 (51,9)	58 (55.8)	
Brain + other[g]	2 (3.8)	5 (9.6)	7 (6.7)	
Primary mass size in mm	48.5 (10-157)	52 (10-110)	51.5 (10-157)	0.352[b]
PET SUV value of the primary mass	13.3 (2.2-43)	11,1 (4.8-29.3)	11.8 (2.2-43)	**0.029[b,h]**
PET SUV value of the metastasis	6.8 (1.8-23)	5.6 (1.86-18.5)	6.1 (1.8-23)	0.374[b]

[a]C-P: Carboplatin-paclitaxel, [b]Independent Student's t-test (mean, SD), [c]p<0.001, [d]Chi-square test, [e]Mann-Whitney U test (median minimum-maximum), [f]Fisher's exact test, [g]Liver, bone, surrenal glands, [h]p<0.05.

PET: Positron emission tomography, LDH: Lactate dehydrogenase, SD: Standard deviation

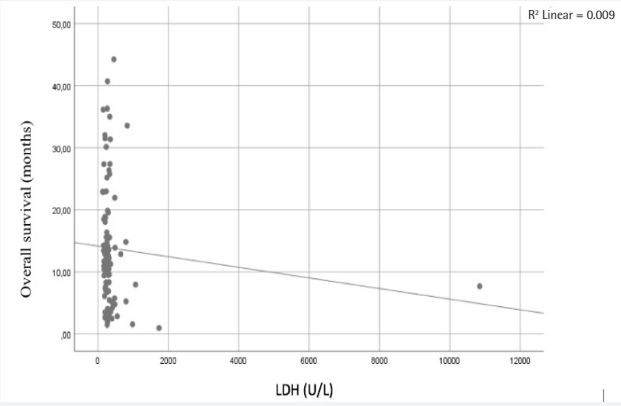

Figure 3. Overall survival according to LDH level

LDH: Lactate dehydrogenase

The mean PFS was 8.57±1.01 months in the weekly C-P group and 5.76±0.61 months in the triweekly C-P group with a significantly higher value (p=0.017) (Figure 2). There was no difference regarding lactate dehydrogenase (LDH) level between the groups (p=0.413). Considering all the patients included in the study, a correlation was found between LDH value and OS (r=-0.227; p=0.021) (Figure 3).

DISCUSSION

Many studies are available about the first-line therapy of patients with advanced NSCLC. In our study, we found that the PFS value was statistically longer with weekly C-P treatment, which is preferred because it is less toxic in advanced NSCLC patients[6]. Although it did not reach statistical significance, we found a 2.2-months prolongation in the OS value. Our study is the first to compare the effectiveness of weekly and triweekly C-P treatments. Comparative studies demonstrated that treatment with cisplatin-gemcitabine, cisplatin-vinorelbine, C-P and cisplatin-irinotecan had no superiority to each other[4,5,7,8].

OS value for triweekly C-P treatment varies between 7.3 and 11.4 months in different studies[9-12]. In the phase 2 study performed by Kallab et al.[13] in stage 3B and 4 NSCLC, paclitaxel was given at a weekly dose of 100 mg/m^2 and carboplatin was given weekly as 2 AUC. These two treatments were given during the first 3 weeks of 4-week cycle. The last week was left empty and effectiveness of the treatments was compared. In our study, paclitaxel was given at a dose of 80 mg/m^2, which is lower than they administered, and carboplatin was continuously administered weekly at 2 AUC with no breaks. Kallab et al.[13] found a PFS value of 5.4 months and OS of 10.8 months, which were longer with 8.57 and 19.64 months, respectively, in our study. Although the patients were of similar age, we attribute the difference between the spans to the fact that 97% of the patients in their study had stage 4 disease.

In the study by Sakakibara et al.[14], in which they investigated the effectiveness and toxicity in patients over the age of 70 years with stage 3B, stage 4 NSCLC and postoperative recurrence, paclitaxel was given at a weekly dose of 70 mg/m^2 and carboplatin as 3-week cycles at 6 AUC. PFS was found to be 6 months and OS 14.7 months. Despite advanced patient age, statistically fewer neutropenia and febrile neutropenia were found in weekly C-P compared to C-P every 3 weeks (p<0.0001 and p=0.018, respectively). The lower frequency of side effects makes weekly treatment favorable. Although ECOG score was not evaluated in our study, weekly treatment is preferred for patients with poorer health in our clinic owing to lower toxicity. While PFS was still longer compared to weekly treatment, our opinion is that weekly C-P treatment may be preferred to triweekly C-P in patients with advanced stage NSCLC without driver mutation.

Although the reason for not preferring weekly treatment is more frequent hospital visits and the additional financial burden on healthcare system[15], we think that lower toxicity compared to triweekly treatment may reduce hospitalizations due to side effects.

Despite the fact that the age, sex and histological subtypes of the two groups were the same, PFS was found to be statistically different between the groups. Because the groups were homogeneous, this difference was thought to be treatment-related. It may be related to the stable course of active drug blood concentration in weekly treatment.

We found a correlation between LDH value and OS (r=-0.227; p=0.021), which demonstrated that patients with higher tumor burden lived shorter as expected. Similarly, previous studies have also shown that high LDH levels are associated both with the presence of metastasis and with lower OS[16-18].

Study Limitations

The retrospective nature of the study and the inclusion of single center patients were our limitations. ECOG performance status could not be evaluated since patient data were accessed through hospital automation system. However, we consider the presence of a bias between the two groups while weekly treatment was preferred for patients with clinically poorer health and triweekly treatments were preferred in those with better health condition. Besides, drug toxicities could not be assessed in detail.

CONCLUSION

In conclusion, a considerable portion of patients with lung cancer have shorter survival because of not being suitable for local therapies at the time of diagnosis. Some of the patients who received systemic treatment die from tumor progression, and some due to drug toxicity. We demonstrated that weekly

C-P treatment, which is known to have fewer toxicity, provided better PFS in the similar patient group compared to triweekly treatment. Therefore, we assert that weekly C-P treatment may be used as the first option in patients with advanced stage NSCLC. Studies with prospective study design are needed to compare these two treatment protocols in terms of toxicity and effectiveness.

Ethics

Ethics Committee Approval: Ethical approval was obtained from Adana City Training and Research Hospital (decision no: 1913, date: 21.04.2022).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: S.A., T.K., T.Ç., Design: S.A., A.Z.B., T.K., B.B.D., Data Collection or Processing: S.A., O.K., B.A.B., H.D., Analysis or Interpretation: S.A., B.A.B., B.B.D., Literature Search: S.A., A.Z.B., Writing: S.A., T.Ç., B.B.D.

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA A Cancer J Clin. 2021;71:209-49. Available from: https://acsjournals.onlinelibrary.wiley.com/doi/full/10.3322/caac.21660
2. Govindan R, Page N, Morgensztern D, Read W, Tierney R, Vlahiotis A, et al. Changing epidemiology of small-cell lung cancer in the United States over the last 30 years: analysis of the surveillance, epidemiologic, and end results database. J Clin Oncol. 2006;24:4539-44.
3. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. CA Cancer J Clin. 2007;57:43-66.
4. Schiller JH, Harrington D, Belani CP, Langer C, Sandler A, Krook J, et al. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. N Engl J Med. 2002;346:92-8.
5. Ohe Y, Ohashi Y, Kubota K, Tamura T, Nakagawa K, Negoro S, et al. Randomized phase III study of cisplatin plus irinotecan versus carboplatin plus paclitaxel, cisplatin plus gemcitabine, and cisplatin plus vinorelbine for advanced non-small-cell lung cancer: Four-Arm Cooperative Study in Japan. Ann Oncol. 2007;18:317-23.
6. Belani CP, Ramalingam S, Perry MC, LaRocca RV, Rinaldi D, Gable PS, et al. Randomized, phase III study of weekly paclitaxel in combination with carboplatin versus standard every-3-weeks administration of carboplatin and paclitaxel for patients with previously untreated advanced non-small-cell lung cancer. J Clin Oncol. 2008;26:468-73.
7. Herbst RS, Giaccone G, de Marinis F, Reinmuth N, Vergnenegre A, Barrios CH, et al. Atezolizumab for First-Line Treatment of PD-L1-Selected Patients with NSCLC. N Engl J Med. 2020;383:1328-39.
8. Scagliotti GV, De Marinis F, Rinaldi M, Crinò L, Gridelli C, Ricci S, et al. Phase III randomized trial comparing three platinum-based doublets in advanced non-small-cell lung cancer. J Clin Oncol. 2002;20:4285-91.
9. Rosell R, Gatzemeier U, Betticher DC, Keppler U, Macha HN, Pirker R, et al. Phase III randomised trial comparing paclitaxel/carboplatin with paclitaxel/cisplatin in patients with advanced non-small-cell lung cancer: a cooperative multinational trial. Ann Oncol. 2002;13:1539-49.
10. Yoh K, Kubota K, Kakinuma R, Ohmatsu H, Goto K, Niho S, et al. Phase II trial of carboplatin and paclitaxel in non-small cell lung cancer patients previously treated with chemotherapy. Lung Cancer. 2007;58:73-9.
11. Belani CP, Lee JS, Socinski MA, Robert F, Waterhouse D, Rowland K, et al. Randomized phase III trial comparing cisplatin–etoposide to carboplatin–paclitaxel in advanced or metastatic non-small cell lung cancer. Annals of Oncology. 2005;16:1069-75.
12. Kosmidis P, Mylonakis N, Skarlos D, Samantas E, Dimopoulos M, Papadimitriou C, et al. Paclitaxel (175 mg/m2) plus carboplatin (6 AUC) versus paclitaxel (225 mg/m2) plus carboplatin (6 AUC) in advanced non-small-cell lung cancer (NSCLC): A multicenter randomized trial. Annals of Oncology. 2000;11:799-805.
13. Kallab AM, Nalamolu Y, Dainer PM, Jillella AP. A phase II study of weekly paclitaxel and carboplatin in previously untreated patients with advanced non-small-cell lung cancer. Med Oncol. 2005;22:145-51.
14. Sakakibara T, Inoue A, Sugawara S, Maemondo M, Ishida T, Usui K, Abe T, Kanbe M, Watanabe H, Saijo Y, Nukiwa T. Randomized phase II trial of weekly paclitaxel combined with carboplatin versus standard paclitaxel combined with carboplatin for elderly patients with advanced non-small-cell lung cancer. Ann Oncol. 2010;21:795-9.
15. Pignata S, Scambia G, Katsaros D, Gallo C, Pujade-Lauraine E, De Placido S, et al. Carboplatin plus paclitaxel once a week versus every 3 weeks in patients with advanced ovarian cancer (MITO-7): a randomised, multicentre, open-label, phase 3 trial. Lancet Oncol. 2014;15:396-405.
16. Liu L, He Y, Ge G, Li L, Zhou P, Zhu Y, Tang H, Huang Y, Li W, Zhang L. Lactate dehydrogenase and creatine kinase as poor prognostic factors in lung cancer: A retrospective observational study. PLoS One. 2017;12:e0182168.
17. Deng T, Zhang J, Meng Y, Zhou Y, Li W. Higher pretreatment lactate dehydrogenase concentration predicts worse overall survival in patients with lung cancer. Medicine (Baltimore). 2018;97:e12524.
18. de Jong C, Deneer VHM, Kelder JC, Ruven H, Egberts TCG, Herder GJM. Association between serum biomarkers CEA and LDH and response in advanced non-small cell lung cancer patients treated with platinum-based chemotherapy. Thorac Cancer. 2020;11:1790-800.

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Systemic Immune-inflammation Index in Patients with Migraine: Clinical, Scale and Radiological Characteristics

Migrenli Hastalarda Sistemik İmmün-enflamasyon İndeksi: Klinik, Skala ve Radyolojik Özellikler

Duygu ARSLAN MEHDIYEV, Zeynep ÖZÖZEN AYAS, Gülgün UNCU

Eskişehir City Hospital, Clinic of Neurology, Eskişehir, Turkey

ABSTRACT

Aim: Migraine is a common neurological disorder in which inflammation plays a role in its pathophysiology. Systemic immune inflammation index (SII) (platelet x neutrophil/lymphocyte) is a vital parameter that indicates inflammatory response and is used in follow-up and evaluation of prognosis for various diseases. The aim of this study is to compare the hematological parameters of patients with migraine and healthy controls and to determine the correlation between SII with the clinical features of migraine and migraine-related hyperintense lesions on brain magnetic resonance imaging (MRI).

Materials and Methods: Migraine patients over 18 years old, who were admitted to the neurology outpatient clinic in a 48-month period, were included in the study. Healthy individuals were included in the study as the control group. Age, gender, duration of migraine diagnosis, migraine attack frequency, presence of aura, smoking, family history, presence of systemic disease, visual analog scale and migraine disability scale scores, presence of migraine-related hyperintense lesions on brain MRI were recorded for all patients in the study. Hemoglobin (Hb), red cell distribution width (RDW), neutrophil, lymphocyte, thrombocyte counts and SII values of the control group and migraine patients were compared.

Results: Hb, lymphocyte, thrombocyte, and RDW levels were significantly higher in migraine patients (n=150) than in the control group (n=178) (p=0.03, p=0.05, p=0.002, p=0.000, respectively). SII was found to be significantly higher in female patients with a diagnosis of migraine compared to males (p=0.01). RDW value was significantly higher in patients with hyperintense lesions on MRI than in those without lesions (p=0.001).

Conclusion: In our study, it is thought that RDW in patients with migraine may be a marker for the presence of migraine-related hyperintense lesions on MRI. However, although SII had a difference between genders in migraine, it has been observed that it is not a parameter that will contribute to the prediction for the disease for now.

Keywords: Migraine, inflammation, red cell distribution width, magnetic resonance image

ÖZ

Amaç: Migren fizyopatolojisinde enflamasyonun rol oynadığı sık görülen nörolojik bir hastalıktır. Sistemik immün-enflamatuvar indeks (SII) (trombosit x nötrofil/lenfosit) enflamatuvar yanıtı gösteren ve pek çok hastalık için takipte ve prognozu değerlendirmede önemli bir parametredir. Çalışmamızın amacı, migren hastalarının hematolojik parametrelerinin sağlıklı kontrollerle kıyaslanması ve SII'nin migren kliniği ile ve beyin manyetik rezonans görüntülemedeki (MRG) migren-ilişkili hiperintens lezyonlarla arasındaki bağlantıyı saptamaktır.

Gereç ve Yöntem: Çalışmaya, 48 aylık süreçte nöroloji polikliniğine başvuran ve migren tanısıyla izlenen 18 yaş üzeri hastalar alındı. Çalışmaya kontrol grubu olarak sağlıklı bireyler dahil edildi. Çalışmadaki tüm hastaların yaş, cinsiyet, migren tanısının süresi, migren atak sıklığı, aura varlığı, sigara içiciliği, aile hikayesi, sistemik hastalık varlığı, görsel analog skala ve migrene bağlı dizabilite ölçeği skorları, beyin MRG'de migren-ilişkili hiperintens lezyonların varlığı kaydedildi. Kontrol grubu ve migren hastalarının hemoglobin (Hb), eritrosit dağılım hacmi (RDW), nötrofil, lenfosit, trombosit ve SII değerlerine bakılarak birbirleriyle karşılaştırıldı.

Bulgular: Hb, lenfosit, trombosit ve RDW düzeyleri migrenli hastalarda (n=150), kontrol grubuna göre (n=178) anlamlı oranda yüksekti (sırasıyla p=0,03, p=0,05, p=0,002, p=0,000). Migren tanılı kadın hastalarda erkeklere kıyasla SII anlamlı olarak daha yüksek saptandı (p=0,01). MRG'de hiperintens lezyonu olan hastalarda, olmayanlara göre RDW değeri anlamlı düzeyde daha yüksek tespit edildi (p=0,001).

Address for Correspondence: Zeynep ÖZÖZEN AYAS MD, Eskişehir City Hospital, Clinic of Neurology, Eskişehir, Turkey
Phone: +90 505 903 96 05 **E-mail:** zozozen@hotmail.com **ORCID ID:** orcid.org/0000-0002-9302-5543
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Sonuç: Çalışmamızda migrenli hastalarda RDW'nin MRG'deki migren-ilişkili hiperintens lezyon varlığı açısından bir belirteç olabileceği düşünülmektedir. Ancak SII'nin migrende cinsiyetler arası farklılığı olsa da, hastalık için şimdilik öngörüye katkıda bulunacak bir parametre olmadığı gözlenmiştir.

Anahtar Kelimeler: Migren, enflamasyon, eritrosit dağılım genişliği, manyetik rezonans görüntüleme

INTRODUCTION

Headache is a common complaint in society, which almost everyone experiences at least once and is the most common reason for admission to a neurology outpatient clinic. Among the primary headaches, migraine is the third most common disease in the world and the third most common disease that causes disability in both men and women under the age of 50 years[1]. The overall prevalence of migraine is 12%, 18% in women and 6% in men[2]. In Turkey, the prevalence of migraine in the age group of 15-55 years is 16.4%; 21.8% in women and 10.9% in men[3].

Although the pathophysiology of migraine remains unclear, it is currently accepted that it starts with cortical spreading depolarization and results in peripheral and central sensitization. With this depolarization wave stimulating the trigeminovascular system, various neuropeptides are secreted, causing vascular dilatation and sterile neurogenic inflammation resulted from the extravasation of plasma proteins. Sterile neurogenic inflammation is the mechanism responsible for the pain phase[4].

There are studies regarding the use of inflammation and related biomarkers in many diseases. As the neutrophil count increases in response to inflammation, the lymphocyte count decreases, so the neutrophil/lymphocyte ratio can be used as an inflammation marker[5]. Systemic immune-inflammation index (SII) is a parameter that shows inflammation and immune status calculated using neutrophil/lymphocyte ratio and thrombocyte value[6]. It was shown that SII could be used as a marker in different cancer types such as hepatocellular carcinoma, small cell lung cancer, pancreatic cancer, and cervical cancer and in the prognosis of many diseases such as coronary artery disease and Severe acute respiratory syndrome-Coronavirus-2[6-9].

Since there is a neurogenic inflammation in migraine, studies have been performed to evaluate the neutrophil/lymphocyte ratio[10-12]. It was also shown that platelet activation may play a role in the pathophysiology of migraine[13]. Studies in which neutrophil, lymphocyte, monocyte and thrombocyte values and their ratios to each other in migraine patients have been studied[14,15]. However, as of May 2022, there is no publication in the literature evaluating the relationship of SII with migraine. Our study, which presents these data for the first time, makes a significant contribution to the literature. We designed this study to understand the relationship between SII and migraine, as it is a marker of inflammation and sterile neurogenic inflammation plays a role in the pathogenesis of migraine.

The purpose of our study is to examine hemoglobin (Hb), red cell distribution width (RDW), neutrophil, lymphocyte and platelet values in patients diagnosed with migraine, as well as SII level, which is a crucial parameter in inflammation. Also, the clinical characteristics of migraine patients and the presence of migraine-related hyperintense lesions on magnetic resonance imaging (MRI) and their correlation with clinical and blood parameters were investigated.

MATERIALS AND METHODS

Patients over the age of 18 years, who were followed up with the diagnosis of migraine according to the International Classification of Headache Disorders (ICHD) and who were admitted to Eskişehir City Hospital Neurology Outpatient Clinic between November 1, 2018, and November 1, 2020, were included in the study. Individuals over the age of 18 years, who were referred for work-school applications and were not diagnosed with systemic diseases including migraine, were included in the study as the control group. The presence of aura, nausea, vomiting, and photo-phonophobia was recorded in patients diagnosed with migraine. The duration of the diagnosis of migraine, the frequency of pain, smoking, family history in terms of migraine, and presence of a systemic disease was questioned. The diseases classified as systemic diseases in our study included diabetes, hypertension, asthma, chronic obstructive pulmonary disease, and thyroid diseases. The severity of migraine pain was calculated using the visual analog scale (VAS) and functional loss using the Migraine Disability Scale (MIDAS)[16,17].

In the VAS evaluation, the headache was grouped as mild (1-3 points), moderate (4-6 points), severe (7-8 points), and very severe (9-10 points).

MIDAS is a practical questionnaire used to evaluate the degree of migraine, consisting of 7 parts. While the first five questions evaluate functionality, the frequency and severity of pain are scored in the last two questions. The total score is graded as grade I between 0 and 5 points, grade II between 6 and 10, grade III between 11 and 20, and grade IV between 21 and above[18].

Demographic characteristics, Hb, RDW, lymphocyte, thrombocyte, neutrophil, and SII values of the patient and control groups were recorded. These values were compared between subgroups in migraine patients and between migraine patients and healthy controls.

Patients with a diagnosis of migraine were divided into cases with periventricular white matter localization in brain MRI, hyperintense lesions smaller than 5 mm and the number of the lesions limited with 4-12 and patients without lesions in brain MRI. It was checked whether there was any difference in clinical features and hemogram parameters in patients with and without migraine-related hyperintense lesions in MRI.

According to the ICHD[1], patients with headaches other than migraine and ischemic and/or vasculitic lesions in brain MRI were not included in the study. Those with the signs of active infection were also excluded from the study.

Approval for this study was obtained from the Non-invasive Clinical Studies Ethics Committee of Eskişehir Osmangazi University Faculty of Medicine (number: E-25403353-050.99-122340, date: 15.12.2020).

Statistical Analysis

Mean, standard deviation, median, interquartile difference, ratio, and frequency values were used in the descriptive statistics of the data. The distribution of variables was determined with the Kolmogorov-Smirnov test. In quantitative data analysis, the independent sample t-test was used in groups with normal distribution and the Mann-Whitney U test in groups that did not show normal distribution. The chi-square and Fisher exact tests analyzed the qualitative data, and the Spearman correlation analysis was used for non-parametric data in correlation analysis. Statistical Package for the Social Sciences 21.0 program was used in the analyses. A p value of <0.05 was considered statistically significant in all analyses.

RESULTS

A total of 150 migraine patients, 121 (80.7%) female and 29 (19.3%) male, were included in the study. Of the healthy individuals in the control group, 129 (72.5%) were female, and 49 (27.5%) were male. The mean age of migraine patients was 33.25±8.93 years, while the mean age of the healthy control group was 30.77±9.77 years.

The mean duration of diagnosis of migraine was 7.58 years (0.8-35), the frequency of pain was 8.71 (0.08-30) month on average, and the mean VAS score was 8.37 (3-10) (Table 1). The mean monthly pain frequency was found to be 8.71 (0.08-30) and the mean VAS score was 8.37 (3-10) (Table 1). It was determined that 22.7% (34) of the migraine patients were MIDAS grade I, 24% (36) were grade II, 20.7% (31) were grade III, and 32.7% (49) were grade IV (Table 1). Eighty-four (56%) patients had a family history, 58 (38.7%) patients were found to be smoking, and 28 (18.7%) patients (9 patients with hypertension, 6 with diabetes mellitus, 6 with thyroid disorder, 4 with chronic obstructive pulmonary disease and 3 with asthma) had a comorbid systemic disease (Table 1). Considering the clinical features, photo/phonophobia was detected in 136 (90.7%) patients, aura in 69 (46%) patients, nausea in 106 (70.7%) patients, and vomiting in 50 (33.3%) patients (Table 1). All patients included in the study underwent brain MRI, and a hyperintense lesion on MRI was detected in 57 (38%) patients (Table 1).

The blood parameters of the migraine and control groups were compared. Hb level, RDW, thrombocyte, and lymphocyte counts were significantly higher in the migraine group than in the control group (p=0.03, p=0.000, p=0.002, p=0.05, respectively) (Table 2).

When the migraine group was compared by gender, it was found that platelet, RDW, and SII values were significantly

Table 1. Clinical features of migraine patients

Variables	Migraine (n=150) Mean±standard deviation (minimum-maximum) / n (%)	
MIDAS	2.63±1.16 1-4	
The duration of the diagnosis	7.58±7.47 0-35	
The frequency of pain	8.71±8.47 0-30	
VAS	8.37±1.44 3-10	
Family history	n	%
Yes	84	56
No	66	44
Smoking	n	%
Yes	58	38.7
No	92	61.3
Systemic disease	n	%
Evet	28	18.7
Hayır	122	81.3
Hyperintense lesion on MRI	n	%
Yes	57	38
No	93	62
Photophonophobia	n	%
Yes	136	90.7
No	14	9.3
Nausea	n	%
Yes	106	70.7
No	44	29.3
Vomiting	n	%
Yes	50	33.3
No	100	66.7
Aura	n	%
Yes	69	46
No	81	54

MIDAS: Migraine Disability Scale, VAS: Visual analog scale, MRI: Magnetic resonance imaging

higher in women than in men, but the Hb values were significantly lower (Table 3).

Control group data were compared according to gender. Age and Hb values were markedly lower in women than in men, and RDW values were markedly higher (p=0.001, p<0.001, p=0.01, respectively). SII value did not differ significantly between men and women in the control group (Table 4).

Considering the correlation of clinical and laboratory data with each other, it was seen that Hb level was positively correlated with smoking.

Table 2. Comparison of blood parameters of migraine-control group

	Migraine (n=150) Mean±standard deviation (minimum-maximum)	Control (n=178) Mean±standard deviation (minimum-maximum)	p value
Hb	14.02±1.47 9-18	13.7±1.38 10-17	**p=0.03***
RDW	13.75±2.97 10-39	11.87±1.39 2-19	**p<0.001****
Neutrophil	4450±1477 1750-11160	4365±1217 1760-7380	p=0.97
Thrombocyte	266.288±66.390 85000-481000	245.083±49.901 153000-441000	**p=0.002***
Lymphocyte	2340±712 1050-5300	2182±586 930-4860	**p=0.05***
SII	541.530±2.73 158673-1900100	521.021±2.29 156922-1525465	p=0.7

*p<0.05, **p<0.001.

Hb: Hemoglobin, RDW: Red cell distribution width, SII: Systemic inflammatory index

Table 3. Comparison of the clinical and laboratory values of the migraine group by gender

	Female (n=121) Mean±standard deviation (minimum-maximum)	Male (n=29) Mean±standard deviation (minimum-maximum)	p value
Age	34±9 18-62	31±8 20-47	p=0.13
The duration of the diagnosis	8±7 0-35	7±7 0-30	p=0.17
The frequency of pain	8±8 0-30	10±7 1-30	p=0.17
VAS	8±1 3-10	8±7 6-10	p=0.99
MIDAS	3±1 1-4	3±7 1-4	p=0.08
Hb	13±1 9-16	16±1 13-18	**p<0.001****
RDW	14±3 10-39	13±2 11-19	**p=0.01***
Neutrophil	4410±1477 1750-11160	4614±1491 2960-9470	p=0.69
Thrombocyte	273.464±67488 154000-481000	236.034±52580 85000-327000	**p=0.01***
Lymphocyte	2273±626 1050-3860	2618±958 1490-5300	p=0.14
SII	564.134±282010 158673-1900100	447.217±215927 165952-1118044	**p=0.01***

*p<0.05, **p<0.001.

Hb: Hemoglobin, RDW: Red cell distribution width, SII: Systemic inflammatory index, MIDAS: Migraine Disability Scale, VAS: Visual analog scale

The presence of hyperintense lesions on MRI was found to be positively associated with the RDW count. Platelet count was negatively correlated with family history and smoking (Table 5).

RDW value was positively correlated with hyperintense lesions on MRI. When the migraine group was compared by the presence of hyperintense lesions in MRI, the attack frequency and RDW values were found to be significantly higher in those with hyperintense lesions on MRI. There was no significant relationship between neutrophil, lymphocyte, thrombocyte, and SII and MRI lesions (Table 6).

When migraine patients were compared in terms of the presence of aura, the mean age and duration of illness of those with aura were significantly higher than those without aura (p=0.008, p=0.01, respectively). No significant differences were observed between patients with and without aura in Hb, RDW, and SII values.

Family history of migraine was positive in 55.3% of women and 56.6% of men.

When patients diagnosed with migraine were compared for smoking, it was found that Hb levels were significantly higher in smokers compared to non-smokers (p=0.007), and platelet count was significantly lower (p=0.01).

When the migraine group was compared for the presence of systemic disease, it was found that the mean age of those with the systemic disease was significantly higher (p=0.004) compared to those without systemic disease.

When the relationship of Hb level with the presence of smoking and systemic disease was evaluated, it was revealed that there was a predictive relationship between smoking and Hb (sig: 0.009) (Table 7).

The relationship of smoking and systemic diseases with the presence of hyperintense lesions on MRI was not found to be significantly predictive (Table 8).

Table 4. Comparison of the control group by gender

	Female (n=129) Mean±standard deviation (minimum-maximum)	Male (n=49) Mean±standard deviation (minimum-maximum)	p value
Age	33.25±8.93 18-62	36±9.22 18-66	**p=0.001****
Hb	13±1 10-16	15±0.9 13-17	**p<0.001****
RDW	12±1.57 2-19	11.5±0.66 10-14	**p=0.01***
Neutrophil	4413±1233 1930-7380	4237±1177 1760-7180	p=0.38
Thrombocyte	248.455±54.065 153000-441000	236.204±35.727 171000-339000	p=0.29
Lymphocyte	2187±611 930-4860	2187±611 930-4860	p=0.99
SII	533.520±236.374 199169-1525465	488115±211025 156992-1061219	p=0.16

*p<0.05, **p<0.001.

Hb: Hemoglobin, RDW: Red cell distribution width, SII: Systemic inflammatory index

Table 5. Correlation of clinical and laboratory data of the migraine group

Variable		Hb	RDW	SII	Lymphocyte	Neutrophil	Thrombocyte
Hyperintense lesion on MRI	R	-0.033	0.264**	0.075	-0.103	-0.006	-0.077
	P	0.691	0.001	0.362	0.211	0.945	0.348
Family history	R	0.158	-0.128	-0.061	-0.025	0.091	**-0.173***
	P	0.054	0.118	0.455	0.766	0.269	**0.035**
Smoking	R	**0.236****	0.037	-0.077	0.074	0.142	**-0.194***
	P	**0.004**	0.652	0.350	0.366	0.082	**0.017**

Hb: Hemoglobin, RDW: Red cell distribution width, SII: Systemic inflammatory index, MRI: Magnetic resonance imaging

Table 6. Comparison of the migraine group according to the presence of lesions in MRI

	Presence of lesions in MRI (n=57) Mean±standard deviation (minimum-maximum)	Absence of lesions in MRI (n=93) Mean±standard deviation (minimum-maximum)	p value
Gender **Female** **Male**	**n** **%** 47 82.5 10 17.5	**n** **%** 74 79.6 19 20.4	p=0.66
Age	34.84±8.44 20-55	32.27±9.12 18-62	p=0.08
The duration of the diagnosis	7.31±7.2 0-35	7.7±7.6 0-30	p=0.86
The frequency of pain	6.9±7.34 0-30	9.78±8.9 1-30	**p=0.03***
VAS	8.46±1.46 3-10	8.32±1.4 4-10	p=0.51
MIDAS	2.44±1.13 1-4	2.75±1.16 1-4	p=0.1
Hb	13.28±1.82 9-18	13.38±1.16 9-18	p=0.71
RDW	14.29±2.3 10-25	13.41±3.28 11-39	**p=0.001***
Neutrophil	4413±1233 1930-7380	4237±1177 1760-7180	p=0.94
Thrombocyte	263263±66444 146000-481000	268.045±66.651 85000-461000	p=0.34
Lymphocyte	2271±744 1170-5140	2382±693 1050-5300	p=0.21
SII	552479±2.68 158673-1582094	534819±2.78 165952-1900100	p=0.36

*p<0.05.

Hb: Hemoglobin, RDW: Red cell distribution width, SII: Systemic inflammatory index, MRI: Magnetic resonance imaging, MIDAS: Migraine Disability Scale, VAS: Visual analog scale

Table 7. Linear regression analysis of hemoglobin level with the presence of smoking and systemic disease

Model		Unstandardized coefficients		Standardized coefficients	T	Sig.
		B	Standard error	Beta		
1	**(Constant)**	13.143	0.186		70.780	0.000
	Smoking	0.732	0.276	0.212	2.649	0.009
	Systemic disease	-0.461	0.345	-0.107	-1.336	0.184

a. Dependent variable: hemoglobin

Table 8. Binary logistic regression analysis of the relationship between smoking and systemic consumption with the presence of hyperintense lesion on MRI

		B	S.E.	Wald	df	Sig.	Exp(B)	95% CI for EXP(B)	
								Lower	Upper
	Smoking (1)	-0.040	0.349	0.013	1	0.908	0.960	0.485	1.903
	Systemic disease (1)	-0.737	0.475	2.413	1	0.120	0.478	0.189	1.213
	Constant	-0.737	0.242	9.287	1	0.002	0.478		

a. Dependent variable: hyperintense lesion on MRI. Variable(s) entered on step 1: smoking, systemic disease.

CI: Confidence interval, MRI: Magnetic resonance imaging

DISCUSSION

Migraine is a chronic disease that affects a large part of society and causes loss of labour and economic and social impact, especially in young adults. In this study, the characteristics of headache and hematological parameters that may guide the diagnosis, prognosis, and radiological findings of migraine disease diagnosed with associated symptoms were investigated.

The annual prevalence of migraine in the general population was reported to be 12% and it was found that the prevalence was mainly between the ages of 30 and 39 years in studies[19,20]. Similarly, the mean age of migraine patients in our study was found to be 33.25±8.93 years.

While the annual and lifetime prevalence of migraine is 18% and 33%, respectively, in women, this rate is 6% and 13%, respectively, in men. Migraine, which is three times more common in women, was found to have a female/male ratio of approximately 4 in our study[21.]

Migraine is a disease in which a genetic background and environmental and lifestyle factors are combined. About 70% of migraine patients have first-degree relatives with a history of migraine, and it has been reported that the risk of migraine increases fourfold in the relatives of patients with aura[22].

Our study found that 56% of the patients had a history of migraine in their first-degree family members. Although the prevalence of migraine is higher in women, it has been reported that the genetic predisposition in men is similar or higher than in women[23]. Again, in a recent study, monthly migraine frequency was associated with genetic predisposition only in men[24]. Although the number of male patients was less in our study, family history was found in similar rates (55.3% and 56.6%) in males and females.

In patients with migraines, the presence of aura generally associated with brainstem dysfunction is seen in approximately one-third of patients[22]. In our study, 46% of patients with migraines were found to have an aura. Our study observed that the mean age and duration of disease diagnosis of migraine patients with aura were higher than those without aura. It is thought that this difference may be related to the fact that people with long-term illness better recognize the nature and auras of the disease over time. No significant difference was found between Hb, RDW, and SII values between migraine patients with and without aura in our study.

It is known that there are hyperintense lesions in the subcortical and white matter that do not cause any clinical symptoms in brain MRI examinations of migraine patients. In our study, hyperintense involvement in migraine-associated white matter was observed in 38% of brain MRI. It was reported as 43.1% in a recent study and 32% in the study of Zhang et al.[25,26]. Methodological differences in studies are thought to alter the results. Age, presence of aura, the severity of headache, and duration of migraine have been reported to be risk factors for the development of white matter hyperintensities[25]. Although there was no significant difference in these risk factors in our study, it was found that the frequency of attacks was significantly higher in those with MRI hyperintense lesions than those without. In addition, the relationship between the presence of hyperintense lesions in MRI and the elevation of RDW suggests that RDW may be a marker for the presence of lesions.

Migraine and anemia are two common diseases that can be seen in young people, and a clear relationship between them has not been defined. In a study, the Hb level measured in migraine attacks was found within normal limits, and no significant difference was found with the control group[27]. On the other hand, it was shown that Hb values measured during the non-attack period were lower in migraine patients compared to healthy control, and in a study consisting of 100 patients in which patients were evaluated during an attack, it was shown that Hb values significantly decreased during an acute migraine attack[11,14]. In our study, Hb and RDW values were found to be significantly higher in migraine patients compared to the control group ($p=0.03$ and $p<0.001$, respectively). RDW, which indicates erythrocyte distribution width and shows anisocytosis, is expected to increase in cases of decreased Hb and iron deficiency. However, we think that the expected relationship between Hb and RDW may not have been observed since iron parameters were not evaluated in our study, and multifactorial reasons may affect the results.

A population-based, large cross-sectional study showed that migraine prevalence was lower in 2385 women, especially in patients with Hb values below 11.5 g/dL[28]. In society, low Hb levels are already more common in females[29]. In our study, when women and men in the migraine and healthy control groups were compared within themselves, as expected in both groups, the Hb level was found to be low, and the RDW level was higher in women than in men. Also, in correlation analyses, no relationship was found between Hb and RDW values of migraine patients and clinical characteristics (pain frequency, migraine diagnosis time, pain severity, nausea, vomiting, photo-phonophobia).

It is thought that platelet activation may be increased in patients with migraines, and this may be a part of sterile neurogenic inflammation in migraine etiology[30]. One study revealed that the platelet level was increased in migraine patients compared to controls[11]. On the other hand, some studies do not show a statistically significant difference in platelet levels in adult and pediatric migraine patients than in controls[12,31]. In our study, when the migraine and control groups were compared, platelet

values were significantly higher in patients diagnosed with migraines than in the control group (p=0.002). In addition, in our study, when migraine patients were compared in terms of gender, platelet value was found to be significantly higher in women compared to men, but no significant difference was observed between men and women in the control group. These data suggest that platelets may play a role in the inflammatory vascular process in the pathogenesis of migraine, which may be more pronounced, especially in women.

SII is a parameter for clinical worsening and invasive ventilation support in Coronavirus disease-2019 disease[32]. In evaluating the risk of major cardiovascular events in coronary artery disease, it was found to be prognostically significant in many types of cancer[6,8]. No study evaluating the systemic inflammatory index related to headache or migraine was found.

In our study, the SII value was significantly higher in women with migraine than in men. No similar difference was found between men and women in the control group. It was found that female gender was associated with high SII value only in the presence of migraine. It suggests that high SII values may lead to a diagnosis of migraine in women although not in all migraine patients, especially in women with a more common disease. The absence of similar changes in male patients may be related to the low number of male patients in our study. Studies involving larger patient populations are needed.

Lymphocyte values were significantly higher in patients diagnosed with migraine than controls. It is expected that there is a neurogenic inflammation in migraine and neutrophils increase while lymphocytes decrease in inflammation. However, contrary to expectations, lymphocyte counts were found to be high in migraine patients in our study. This result may be related to taking blood samples during the inter-attack period, not during an attack.

Inflammatory markers were also expected to be higher in patients with MRI lesions, with the prediction that white matter lesions that can be seen in brain MRI in migraine patients are caused by inflammation. A study has shown that white matter lesions on MRI are seen more frequently in patients with a higher neutrophil/lymphocyte ratio[10]. However, no significant relationship was found between laboratory parameters such as neutrophil, lymphocyte, thrombocyte, and SII values and MRI lesions in our study.

When the neutrophil/lymphocyte ratio, which is considered an inflammation marker, is considered during the attack period of migraine patients, it was significantly higher[14,27] but the same relationship could not be demonstrated in the period between attacks[12]. Our study concluded that inflammation markers were not high because the patients were evaluated in the outpatient clinic during or outside the attack.

In the relationship between migraine and RDW, inflammation and oxidative stress play a role by changing iron metabolism. The erythrocyte half-life is shortened, and the response of the bone marrow to erythropoietin decreases. It was reported that there is a positive correlation between RDW inflammation and cytokines[33,34]. In our study, it should be considered that it can be used in diagnosis and prognosis because the RDW level was significantly higher in both migraine patients compared to controls and MRI positive patients compared to MRI negative ones.

As shown and expected in previous studies, it is found that smokers have higher Hb levels and lower platelet levels[31]. In our study, the mean age of patients with systemic diseases was higher, and the frequency of systemic diseases such as diabetes and hypertension increased with age, which supports this finding.

Study Limitations

The study's limitations are its retrospective nature, failure to obtain laboratory values during a migraine attack, and failure to look for concurrent inflammatory cytokines.

CONCLUSION

In our study, we could not find a significant relationship between the clinic and MRI lesions and SII, but we found that SII significantly increased in the control group, especially in female patients compared to men. This result has revealed that studies in which SII can provide more guidance in the diagnosis of female patients and/or with more male patients are needed. We have found that RDW and thrombocyte elevation in blood parameters may be much more significant than Hb and lymphocytes in migraine patients. The presence of RDW correlation with MRI lesions suggested that RDW could guide in diagnosis, follow-up, and prognosis.

Since our study was conducted in the inter-attack period, there is a need for comprehensive studies to be conducted by including more cases that look for similar blood parameters during the attack period and compare with each other.

Ethics

Ethics Committee Approval: Approval for this study was obtained from the Non-invasive Clinical Studies Ethics Committee of Eskişehir Osmangazi University Faculty of Medicine (number: E-25403353-050.99-122340, date: 15.12.2020).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: D.A.M., Z.Ö.A., Concept: D.A.M., G.U., Design: D.A.M., Z.Ö.A., G.U., Data Collection or Processing: D.A.M., Z.Ö.A., G.U., Analysis or Interpretation: D.A.M., Z.Ö.A., Literature Search: D.A.M., Z.Ö.A., G.U., Writing: D.A.M.

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REFERENCES

1. Headache Classification Committee of the International Headache Society (IHS) The International Classification of Headache Disorders, 3rd edition. Cephalalgia. 2018;38:1-211.
2. Robbins MS, Lipton RB. The epidemiology of primary headache disorders. Semin Neurol. 2010;30:107-19.
3. Siva A. Başağrısı Epidemiyolojisi. Turkiye Klinikleri J Neur. 2003;1:94-7.
4. Ward TN. Migraine diagnosis and pathophysiology. Continuum (Minneap Minn). 2012;18:753-63.
5. Ramachandran R. Neurogenic inflammation and its role in migraine. Semin Immunopathol. 2018;40:301-4.
6. Yang YL, Wu CH, Hsu PF, Chen SC, Huang SS, Chan WL, et al. Systemic immune-inflammation index (SII) predicted clinical outcome in patients with coronary artery disease. Eur J Clin Invest. 2020;50:e13230.
7. Jomrich G, Gruber ES, Winkler D, Hollenstein M, Gnant M, Sahora K, et al. Systemic Immune-Inflammation Index (SII) Predicts Poor Survival in Pancreatic Cancer Patients Undergoing Resection. J Gastrointest Surg. 2020;24:610-8.
8. Huang H, Liu Q, Zhu L, Zhang Y, Lu X, Wu Y, et al. Prognostic Value of Preoperative Systemic Immune-Inflammation Index in Patients with Cervical Cancer. Sci Rep. 2019;9:3284.
9. Fois AG, Paliogiannis P, Scano V, Cau S, Babudieri S, Perra R, et al. The Systemic Inflammation Index on Admission Predicts In-Hospital Mortality in COVID-19 Patients. Molecules. 2020;25:5725.
10. Karabulut KU, Egercioglu TU, Uyar M, Ucar Y. The change of neutrophils/lymphocytes ratio in migraine attacks: A case-controlled study. Ann Med Surg (Lond). 2016;10:52-6.
11. Acar E, Beydilli H, Karagöz Ü, Yıldırım B, Kirli İ, Kılınç RM, et al. "Neutrophil-lymphocyte ratio can distinguish migraine patients from other patients with nonspecific headache in the emergency department." Acta Medica. 2015;31:829.
12. Onder H, Deliktas MM. May Neutrophil to Lymphocyte Ratio Serve a Role in the Prediction of Clinical Features of Migraine? J of Neurol Res 2020;10:38-43.
13. Danese E, Montagnana M, Lippi G. Platelets and migraine. Thromb Res. 2014;134:17-22.
14. Zeller JA, Lindner V, Frahm K, Baron R, Deuschl G. Platelet activation and platelet-leucocyte interaction in patients with migraine. Subtype differences and influence of triptans. Cephalalgia. 2005;25:536-41.
15. Zeller JA, Lenz A, Eschenfelder CC, Zunker P, Deuschl G. Platelet-leukocyte interaction and platelet activation in acute stroke with and without preceding infection. Arterioscler Thromb Vasc Biol. 2005;25:1519-23.
16. Stewart WF, Lipton RB, Whyte J, Dowson A, Kolodner K, Liberman JN, et al. An international study to assess reliability of the Migraine Disability Assessment (MIDAS) score. Neurology. 1999;53:988-94.
17. Gedikoglu U, Coskun O, Inan LE, Ucler S, Tunc T, Emre U. Validity and reliability of Turkish translation of Migraine Disability Assessment (MIDAS) questionnaire in patients with migraine. Cephalalgia. 2005;25:452-6.
18. Ertaş M, Siva A, Dalkara T, Uzuner N, Dora B, Inan L, et al. Validity and reliability of the Turkish Migraine Disability Assessment (MIDAS) questionnaire. Headache. 2004;44:786-93.
19. Burch R, Rizzoli P, Loder E. The Prevalence and Impact of Migraine and Severe Headache in the United States: Figures and Trends From Government Health Studies. Headache. 2018;58:496-505.
20. Lipton RB, Bigal ME, Diamond M, Freitag F, Reed ML, Stewart WF, et al. Migraine prevalence, disease burden, and the need for preventive therapy. Neurology. 2007;68:343-9.
21. Broner SW, Bobker S, Klebanoff L. Migraine in Women. Semin Neurol. 2017;37:601-10.
22. Charles A. The Migraine Aura. Continuum (Minneap Minn). 2018;24:1009-22.
23. Nyholt DR, van den Maagdenberg AM. Genome-wide association studies in migraine: current state and route to follow. Curr Opin Neurol. 2016;29:302-8.
24. Pelzer N, Louter MA, van Zwet EW, Nyholt DR, Ferrari MD, van den Maagdenberg AM, et al. Linking migraine frequency with family history of migraine. Cephalalgia. 2019;39:229-36.
25. Negm M, Housseini AM, Abdelfatah M, Asran A. Relation between migraine pattern and white matter hyperintensities in brain magnetic resonance imaging. Egypt J Neurol Psychiatr Neurosurg. 2018;54:24.
26. Zhang Y, Parikh A, Qian S. Migraine and stroke. Stroke Vasc Neurol. 2017;2:160-7.
27. Celikbilek A, Zararsiz G, Atalay T, Tanik N. Red cell distribution width in migraine. Int J Lab Hematol. 2013;35:620-8.
28. Sarıcam G. Relationship between migraine headache and hematological parameters. Acta Neurol Belg. 2021;121:899-905.
29. Poyrazoğlu HG, Öztürk AB. Predictive value of laboratory parameters in childhood migraine. Acta Neurol Belg. 2020;120:907-14.
30. Morkavuk G, Akkaya E, Koç G, Ataç GK, Leventoğlu A. Relationship between white matter lesions and neutrophil–lymphocyte ratio in migraine patients. Neurol Sci Neurophysiol. 2020;37:129-33.
31. Kutlu R, Demirbas N. The Effects of Smoking on Platelet Count, Mean Platelet Volume and Cardiovascular Risk Factors: A Case-control Study. Medical Bulletin of Haseki. 2018;55:299-305.
32. Muhammad S, Fischer I, Naderi S, Faghih Jouibari M, Abdolreza S, Karimialavijeh E, et al. Systemic Inflammatory Index Is a Novel Predictor of Intubation Requirement and Mortality after SARS-CoV-2 Infection. Pathogens. 2021;10:58.
33. Patel KV, Ferrucci L, Ershler WB, Longo DL, Guralnik JM. Red blood cell distribution width and the risk of death in middle-aged and older adults. Arch Intern Med. 2009;169:515-23.
34. Semba RD, Patel KV, Ferrucci L, Sun K, Roy CN, Guralnik JM, et al. Serum antioxidants and inflammation predict red cell distribution width in older women: the Women's Health and Aging Study I. Clin Nutr. 2010;29:600-4.

ORIGINAL ARTICLE

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The Role of Uterine Artery Pulsatility and Resistance Index in Detection of Pre-invasive Cervical Lesions

Preinvaziv Servikal Lezyonların Saptanmasında Uterus Arter Pulsatilite ve Rezistans İndeksinin Rolü

Mine DAĞGEZ[1], Cem YENER[2]

[1]Tekirdağ City Hospital, Clinic of Gynecological Oncology, Tekirdağ, Turkey
[2]Tekirdağ City Hospital, Clinic of Perinatology, Tekirdağ, Turkey

ABSTRACT

Aim: Our aim was to evaluate the diagnostic strength of uterine arteries (UA) pulsatility index (PI) and resistance index (RI) in the detection of pre-invasive cervical lesions.

Materials and Methods: Data were prospectively collected from a total of 225 patients who were diagnosed with cervical ıntraepithelial neoplasia (CIN) 1 (Group 1) (n=75) and CIN-2/3 (Group 2) (n=75) by colposcopy-guided cervical biopsy previously and from patients with normal cytology assigned as control (Group 3) (n=75). PI and RI of UA were determined by Doppler sonography and the results were compared among the groups.

Results: The mean UA-PI values were found to be 2.52±0.9, 2.51±0.9, and 2.53±0.3 in Group 1, 2, and 3, respectively. The mean UA-RI values were observed as 0.83±0.7, 0.81±0.25, and 0.84±0.1 in Group 1, 2, and 3, respectively. However, UA-PI and UA-RI values were not significantly different among the groups (p>0.05).

Conclusion: PI and RI values are decreased with CIN lesions. Nevertheless, the difference is not large enough to implement these values in current cervical cancer screening program.

Keywords: Cervical intraepithelial neoplasia, Doppler ultrasonography, pulsatility index, uterine artery

ÖZ

Amaç: Preinvaziv servikal lezyonların saptanmasında uterus arter (UA) pulsatilite indeksi (PI) ve direnç indeksinin (RI) tanısal gücünün incelenmesidir.

Gereç ve Yöntem: Kolposkopi eşliğinde servikal biyopsi ile servikal intraepitelyal neoplazi (CIN) 1 (Grup 1) (n=75), CIN-2/3 (Grup 2) (n=75) tanısı alan toplam 225 hastadan prospektif olarak veriler toplandı. Sitolojisi normal olan hastalar kontrol (Grup 3) (n=75) olarak çalışmaya dahil edildi. UA'ların PI ve RI değerleri Doppler sonografi ile ölçüldü ve sonuçlar gruplar arasında karşılaştırıldı.

Bulgular: UA-PI ortalama değerleri Grup 1, 2 ve 3'te sırasıyla 2,52±0,9, 2,51±0,9 ve 2,53±0,3 olarak bulundu. UA-RI ortalama değerleri ise Grup 1, 2 ve 3'te sırasıyla 0,83±0,7, 0,81±0,25, 0,84±0,1 olarak gözlendi. Ancak UA-PI ve UA-RI değerleri gruplar arasında anlamlı farklılık göstermedi (p>0,05).

Sonuç: PI ve RI değerlerinin CIN lezyonlarında daha düşük olduğu gözlendi. Bununla birlikte, bu fark mevcut rahim ağzı kanseri tarama programında bu değerleri uygulayacak kadar istatistiksel açıdan büyük değildir.

Anahtar Kelimeler: Servikal intraepitelyal neoplazi, Doppler ultrason, pulsatilite indeks, uterus arter

Address for Correspondence: Cem YENER MD, Tekirdağ City Hospital, Clinic of Perinatology, Tekirdağ
Phone: +90 532 748 61 80 **E-mail:** drcemyener@hotmail.com **ORCID ID:** orcid.org/0000-0002-3976-4492
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INTRODUCTION

Cervical cancer is the fourth most common cancer in women worldwide[1]. Latterly, the exquisite steps in diminution cervical cancer mortality have been observed with the onset and implementation of screening programs. Paramount approaches have also occurred in the diagnosis and treatment of cervical cancer[2]. Screening for cervical cancer has assuredly gave rise to a drop in cervical cancer incidence and mortality in many countries[3].

Cervical intraepithelial neoplasia (CIN), also known as cervical dysplasia, is the abnormal growth of cells on the surface of the cervix which can likely advance to cervical cancer[4]. An abnormal Pap smear result can necessitate an endorsement for colposcopy of the cervix. A biopsy is employed of any ominous regions and CIN can be detected subsequently. According to patients age, human papillomavirus (HPV) positivity or lesion type, it may be followed or treated by various surgical methods such as LEEP and cold knife conization afterwards[5].

Doppler ultrasonography of the fetoplacental circulation is highly used in daily practice of obstetricians[6]. The use of Doppler ultrasound as an appliance for screening/prediction of preeclampsia is recommended[7]. It was demonstrated that Doppler ultrasound is a valuable and decent predictor of ovarian malignancies[8]. In addition, using Doppler ultrasound for the pre-treatment evaluation of cervical cancer is increasing eventually[9].

In the present study, our aim was to evaluate the discriminative role of uterine artery (UA) Doppler in the detection of cervical intraepithelial lesions.

MATERIALS AND METHODS

This prospective study was conducted at the Gynecologic Oncology Outpatient Clinic at Tekirdağ City Hospital. The study was approved by the Tekirdağ Dr. İsmail Fehmi Cumalıoğlu State Hospital of Local Institutional Ethics Committee (protocol no: 2022/002, date: 04.11.2022). A total of 225 patients were divided into 3 groups. Group 1 represented patients with CIN 1 (n=75), Group 2 included patients with CIN 2-3 (n=75) and Group 3 consisted of patients without cervical pathology as control (n=75). Our exclusion criteria were non-Caucasian ethnics, age <21 and >50 years, body mass index (BMI) <18 or >28 kg/m^2, previous preeclampsia history, hysterectomized patients, history of any vaginal medical application or oral contraceptive use, cervical conization, embolization of the UAs and previous administration of chemo-radiotherapy. Postmenopausal patients or the ones in the menstrual and gestational period were also excluded from the study. Data were prospectively collected including age, parity and BMI.

All measurements were acquired by the same sonographer (C.Y.) with experience in Doppler ultrasound to avoid inter-observer variability. In all cases, Hitachi ARIETTA 60 (Aloka Medical, Ltd. Tokyo, Japan) transvaginal probe (4-9 MHz) with Doppler capability was utilized. For the assessment of UA, patient was placed in the lithotomy position, with her bladder empty in mid-luteal phase. The probe was moved laterally until the paracervical vascular plexus was seen, and the UA was identified at the level of the internal cervical os. The pulsatility index (PI) and resistance index (RI) was calculated when at least three identical waveforms were acquired (Figure 1).

Statistical Analysis

Statistical Package for Social Sciences (SPSS) 25 (SPSS, Chicago, II, USA) Windows package program was applied for statistical analysis. Descriptive statistical methods (mean, standard deviation) were used when evaluating study data. The Mann-Whitney U test was employed to compare differences between two independent groups when the dependent variable was either ordinal or continuous, but not normally distributed. The Kolmogorov-Smirnov test and the Shapiro-Wilk test were used to investigate the conformity of the variables to the normal distribution. Differences were defined as significant for $p<0.05$.

RESULTS

In the present study, there was no statistically significant difference between Groups 1, 2 and controls in terms of age, BMI, and parity (Table 1).

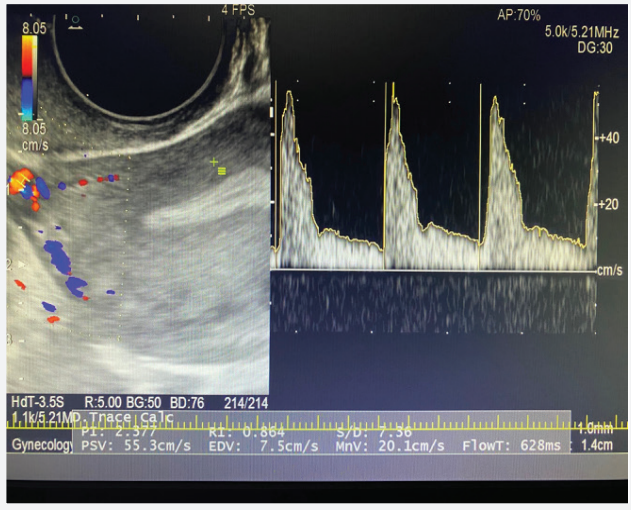

Figure 1. Transvaginal Doppler ultrasound examination of uterine artery is depicted. The uterine artery loop is located in a paracervical section, and at least three identical waveforms are recorded

Table 1. Characteristics of the patients			
	Group 1 and 2 (n=150)	Group 3 (n=75)	p
Age (year)	36.4±91	35.8±82	0.758
BMI (kg/m^2)	25.94±12	26.45±34	0.676
Parity	2 (0-6)	3 (1-7)	0.240
n: Number, BMI: Body mass index			

Table 2. Comparison of Doppler indices of Group 1 and Group 2			
	Group 1 (n=75)	Group 2 (n=75)	p
UA-PI	2.52±0.9	2.51±0.9	0.778
UA-RI	0.83±0.7	0.81±0.25	0.686
n: Number, UA-PI: Uterine artery pulsatility index, UA-RI: Uterine artery resistance index			

Table 3. Comparison of Doppler indices of Group 1 and Group 3			
	Group 1 (n=75)	Group 3 (n=75)	p
UA-PI	2.52±0.9	2.53±0.3	0.869
UA-RI	0.83±0.7	0.84±0.1	0.810
n: Number, UA-PI: Uterine artery pulsatility index, UA-RI: Uterine artery resistance index			

Table 4. Comparison of Doppler indices of Group 2 and Group 3			
	Group 2 (n=75)	Group 3 (n=75)	p
UA-PI	2.51±0.9	2.53±0.3	0.450
UA-RI	0.81±0.25	0.84±0.1	0.388
n: Number, UA-PI: Uterine artery pulsatility index, UA-RI: Uterine artery resistance index			

The mean UA-PI values were found to be 2.52±0.9, 2.51±0.9, and 2.53±0.3 in Group1, 2, and 3, respectively. The mean UA-RI values were observed as 0.83±0.7, 0.81±0.25, and 0.84±0.1 in Group 1, 2, and 3, respectively. There was no statistically significant difference among the groups in terms of UA-PI (p>0.05).

Regarding to UA-PI, we did not detect considerable differences among the groups (p>0.05) (Table 2-4).

DISCUSSION

In our study, UA-PI values were compared among the groups that were similar in terms of age, BMI and parity and no statistically significant difference was found. As the grade of CIN increased, UA-PI and UA-RI was observed to be lower due to increasing vascularization, but this was not statistically significant.

This is the second study to our knowledge investigating the use of Doppler ultrasound indices in CIN lesions. Doğan et al.[10] investigated the diagnostic strength of UA and cervical vascularity by itself or in association with HPV DNA testing and with cytology. They found that using the Doppler indices of UA and cervical arteries was indecisive in discerning CIN-I or above lesions in the early phases, which is compatible with our study.

Transvaginal and transrectal ultrasound is commonly applied to ascertain the characteristics of the cervical tumor[11]. Adding advanced techniques in ultrasound has increased the implementation of ultrasound for cervical cancer in clinical settings. If performed by well-trained gynecologists, ultrasound may give substantially precise information especially on tumor detection and local tumor extension[9]. Finally, ultrasound, besides being faster, radiation-free and non-invasive, is a broadly available imaging technique with lower cost compared to others. High-frequency ultrasound may provide comprehensive delineation of any cervical tumor, particularly when the probe is placed proximal to the tumor transvaginally and transrectally[12]. Even though, sensitivity was low when combined with other screening methods, evaluating the PI and RI of UA and cervical arteries may still stimulate experts given the fact that increased vascularization and thus lower RI and PI is attributed to cervical cancer and they may serve as a prognostic factor[13]. Lower PI and RI levels should alert the clinicians and meticulous investigation should be warranted.

Putting the vascularity index of cervical preinvasive and invasive lesions in the diagnostic algorithm to evaluate the diagnostic efficiency of a 3-step screening approach (cytology, HPV based testing and vascularity index) has recently been investigated. It is shown that to combine 3D vascular findings of a tumor with

cytology and HPV-testing essentially enhances the precision of screening for cervical cancer[14]. The characteristics of the cervical blood flow in healthy controls and in the ones with precancerous lesions or invasive cancers were studied by employing 3D power Doppler ultrasound findings, including the intensity of flow at the time of volume acquisition, the number of vessels within the volume of interest (VI), and both blood flow and vascularization[15]. All of these indices were quite higher in the group with cervical lesions, compared to the controls. The VI was also higher in advanced staged cervical cancer patients compared to less advanced ones. Cervical cancer screening could evolve by adding vascular indices obtained by ultrasound to the algorithm, especially in women of reproductive age, to refrain overdo use of LEEP or conization procedures and this approach may be apparently beneficial.

The strength of our study is that all ultrasonographic measurements were performed by the same gynecologist, a factor that may have an impact on inter-observer reproducibility and the patient number compared to the previous study.

Study Limitations

The most important limitation of our study is that it was conducted in a single center and with a relatively small number of patients.

CONCLUSION

In our research, we noticed that PI and RI values were decreased with CIN lesions. Nevertheless, the difference is not large enough to implement these values in current cervical cancer screening program. Hence, future studies with larger number of participants are needed to improve cervical cancer screening with ultrasound, and further studies are indispensable to provide reference values for Doppler ultrasound indices in cervical lesions and utilization of the tool to determine the vascularity of cervical lesions.

Ethics

Ethics Committee Approval: The study was approved by the Tekirdağ Dr. İsmail Fehmi Cumalıoğlu State Hospital of Local Institutional Ethics Committee (protocol no: 2022/002, date: 04.11.2022).

Informed Consent: Consent form was filled out by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: M.D., Concept: M.D., Design: C.Y., Data Collection or Processing: C.Y., Analysis or Interpretation: M.D., C.Y., Literature Search: M.D., C.Y., Writing: M.D.

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. Buskwofie A, David-West G, Clare CA. A Review of Cervical Cancer: Incidence and Disparities. J Natl Med Assoc. 2020;112:229-32.
2. Moore DH. Cervical cancer. Obstet Gynecol 2006;107:1152-61.
3. Beral V, Hermon C, Muñoz N, Devesa SS. Cervical cancer. Cancer Surv. 1994;19-20:265-85.
4. Mitra A, Tzafetas M, Lyons D, Fotopoulou C, Paraskevaidis E, Kyrgiou M. Cervical intraepithelial neoplasia: screening and management. Br J Hosp Med (Lond). 2016;77:C118-23.
5. Petry KU. Management options for cervical intraepithelial neoplasia. Best Pract Res Clin Obstet Gynaecol. 2011;25:641-51.
6. Bhide A, Acharya G, Baschat A, Bilardo CM, Brezinka C, Cafici D, et al. ISUOG Practice Guidelines (updated): use of Doppler velocimetry in obstetrics. Ultrasound Obstet Gynecol. 2021;58:331-9.
7. Sotiriadis A, Hernandez-Andrade E, da Silva Costa F, Ghi T, Glanc P, Khalil A, et al; ISUOG ISUOG CSC Pre-eclampsia Task Force. ISUOG Practice Guidelines: role of ultrasound in screening for and follow-up of pre-eclampsia. Ultrasound Obstet Gynecol. 2019;53:7-22.
8. Shah D, Shah S, Parikh J, Bhatt CJ, Vaishnav K, Bala DV. Doppler ultrasound: a good and reliable predictor of ovarian malignancy. J Obstet Gynaecol India. 2013;63:186-9.
9. Hsiao YH, Yang SF, Chen YH, Chen TH, Tsai HD, Chou MC, et al. Updated applications of Ultrasound in Uterine Cervical Cancer. J Cancer. 2021;12:2181-9.
10. Doğan O, Pulatoğlu Ç, Başbuğ A, Ellibeş Kaya A, Yassa M. Discriminating Performance of Early Uterine and Cervical Artery Pulsatility and Resistivity In Pre-Invasive Cervical Lesions. Sisli Etfal Hastan Tip Bul. 2018;52:206-11.
11. Fischerova D, Cibula D, Stenhova H, Vondrichova H, Calda P, Zikan M, et al. Transrectal ultrasound and magnetic resonance imaging in staging of early cervical cancer. Int J Gynecol Cancer. 2008;18:766-72.
12. Testa AC, Di Legge A, De Blasis I, Moruzzi MC, Bonatti M, Collarino A, et al. Imaging techniques for the evaluation of cervical cancer. Best Pract Res Clin Obstet Gynaecol. 2014;28:741-68.
13. Alcázar JL, Castillo G, Martínez-Monge R, Jurado M. Transvaginal color Doppler sonography for predicting response to concurrent chemoradiotherapy for locally advanced cervical carcinoma. J Clin Ultrasound. 2004;32:267-72.
14. Liang H, Fu M, Zhou J, Song L. Evaluation of 3D-CPA, HR-HPV, and TCT joint detection on cervical disease screening. Oncol Lett. 2016;12:887-92.
15. Belitsos P, Papoutsis D, Rodolakis A, Mesogitis S, Antsaklis A. Three-dimensional power Doppler ultrasound for the study of cervical cancer and precancerous lesions. Ultrasound Obstet Gynecol. 2012;40:576-81.

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Production of 3D-Printed Ribs with a Novel Material (Nylon 680 Co-Polymer) for Chest Wall Reconstruction on a Pig Model: Preliminary Results of an Experimental Study

Domuzlarda Göğüs Duvarı Rekonstrüksiyonu için 3D Printer ile Kaburga Üretimi: Yeni Bir Malzeme (Nylon 680 Co-Polymer) Deneysel Çalışmanın Preliminer Sonuçları

Nezih Onur ERMERAK[1], Mustafa YÜKSEL[2], Barış YÜKSEL[3], Tunç LAÇİN[1], İpek ERBARUT SEVEN[4], Berna KARAKOYUN[5]

[1]*Marmara University Faculty of Medicine, Department of Thoracic Surgery, İstanbul, Turkey*
[2]*Bilim Demiroğlu University Faculty of Medicine, Department of Thoracic Surgery, İstanbul, Turkey*
[3]*Google Company, Department of Computer Engineering, New York, USA*
[4]*Marmara University Faculty of Medicine, Department of Medical Pathology, İstanbul, Turkey*
[5]*University of Health Sciences Turkey, Hamidiye Faculty of Medicine, Department of Physiology, İstanbul, Turkey*

ABSTRACT

Aim: Three-dimensional (3D) printing has gained popularity among all fields of science in recent years. New research studies about the utilization of 3D printing in the medical field, in terms of medical devices and implants, have been published recently. We tried to adapt this technology into thoracic surgery by implanting 3D produced ribs following chest wall resection with a novel material called Nylon 680 Co-Polymer. We, hereby, present the preliminary results of this experimental study.

Materials and Methods: We ordered multi detector computerized tomography of the chest for 2 pigs. We measured the area that we planned to resect on chest wall and used the data for printing custom-made rib for the reconstruction of the resected area. Then, we produced ribs with a USA Food and Drug Administration approved material called Nylon 680 Co-polymer (Taulman 3D, Saint Peters, MO, USA) by using 3D printer (Afinia H480, Chanhassen, MO, USA). Pigs were operated under general anesthesia and the resected areas were reconstructed with custom-made 3D printed ribs.

Results: One of the pigs passed away due to myocardial infarction while waking up from anesthesia. We followed up the other pig for 45 days. Then, we sacrificed the animal and resected the operated part for histopathological evaluation. Histopathologic evaluation revealed moderate chronic inflammation with few giant cells containing pigmented foreign bodies.

Conclusion: Although we need more studies, it is an important step for adapting 3D-printing into thoracic surgery. Additionally, it is important to identify a potential new material (Nylon 680 Co-polymer) for the future studies. We can use this new material for 3D-printed implant and mesh production, which enables us to produce custom-made products with lower cost in shorter time.

Keywords: 3D printing, thoracic surgery, Nylon 680 Co-polymer, chest wall resection and reconstruction, novel technology

ÖZ

Amaç: Üç boyutlu (3D) baskı son yıllarda tüm bilim dalları arasında popülerlik kazanmıştır. 3D yazıcılarda kullanılabilen Nylon 680 Co-polymer adlı yeni bir malzeme ile kaburga üreterek, domuzlarda göğüs duvarı rekonstrüksiyonu için kullanmayı amaçladık. Bu yeni malzeme ile gerçekleştirilen deneysel çalışmanın ön sonuçlarını sunmaktayız.

Gereç ve Yöntem: Göğüs duvarında rezeke etmeyi planladığımız iki domuza çok kesitli bilgisayarlı toraks tomografisi çektirerek rezeke edeceğimiz alanı ölçtük. ABD Gıda ve İlaç Dairesi onaylı Nylon 680 Co-Polymer (Taulman 3D, Saint Peters, MO, ABD) ile 3D yazıcı kullanarak kaburgalar

Address for Correspondence: Nezih Onur ERMERAK MD, Marmara University Faculty of Medicine, Department of Thoracic Surgery, İstanbul, Turkey
Phone: +90 533 714 14 63 **E-mail:** onur.ermerak@marmara.edu.tr **ORCID ID:** orcid.org/0000-0003-1939-3222
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ürettik (Afinia H480, Chanhassen, MO, ABD). Denekleri opere ederek göğüs duvarı rezeksiyonu sonrası yeni üretilmiş kaburgalar ile göğüs duvarı rekonstrüksiyonu gerçekleştirdik.

Bulgular: Deneklerden bir tanesi anesteziden uyanırken miyokard enfarktüsü nedeniyle kaybedildi. Diğer domuz 45 gün boyunca, deney hayvanları için barınak olan merkezde 45 gün boyunca takip edildi. Sonrasında sakrifiye edildi ve opere edilmiş olan alan histopatolojik inceleme için rezeke edildi. Histopatolojik değerlendirme, pigmente yabancı cisimler içeren dev hücreler ve orta derecede kronik enflamasyon olarak yorumlandı.

Sonuç: Bu çalışma 3D yazıcı teknolojisinin göğüs cerrahisi alanında kullanımı açısından önemlidir. Ayrıca yeni bir malzeme olan Nylon 680 Co-polymer'in tanımlanması yeni bir çalışma alanı oluşturmaktadır. Bu yeni malzeme ile hastalara özel tasarlanmış implantlar veya meşler kısa süre içinde üretilebildiği gibi çok daha ucuza mal edilebileceği ön görülmektedir.

Anahtar Kelimeler: 3D baskı, göğüs cerrahisi, Nylon 680 Co-polymer, göğüs duvarı rezeksiyon ve rekonstrüksiyonu, yeni teknoloji

INTRODUCTION

Three-dimensional (3D) printing has gained popularity among all fields of science in recent years[1-4]. New research studies about the utilization of 3D printing in medical field in terms of medical devices and implants for the body have been published recently. 3D printing gained attraction also in pharmaceutical field, illustrated by USA Food and Drug Administration (FDA) approval of 3D-printed drug product in August 2015. Owais et al.[5] produced mitral annulus by 3D printing using echocardiographic data and experienced the potential use in cardiac surgeries. Thawani et al.[6] presented a 3D-printed model of arteriovenous malformation and demonstrated arterial and venous phases separately. Moreover, in orthopedic field, use of this technology in the aspect of surgical view is getting much more popular. Zhang et al.[7] constructed a 3D-printable, bioceramic articular spacer assembly and used it in arthroplasty surgeries. In addition, Mulford et al.[8] published a review article for defining current and future applications used in orthopedic surgery.

Surgical management of chest wall defects after oncological resection is a common situation in thoracic surgery. Management of this entity requires creativity and flexibility. The material used for the reconstruction of the chest wall can vary from mesh, methyl methacrylate to prosthetic systems like titanium plates or bars[9-11].

We tried to adapt this technology into thoracic surgery by using Nylon 680 Co-polymer, which is a novel material with lower cost.

MATERIALS AND METHODS

The research was reviewed and approved by Acıbadem University Local Ethics Committee for Animal Experiments (ACU-HADYEK), İstanbul, Turkey (decision no: 2015/17, date: 02.03.2015). This research supported by the Scientific and Technological Research Council of Turkey (TUBITAK-115S797).

All animals received care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals prepared by National Academy of Sciences and published by the National Institutes of Health, NIH publication no. 80-23, revised 1985.

The mean body weight of two male domestic pigs was 32.5 kg and the mean body surface was 1.02 m^2. Multidetector computerized tomography (MDCT) scans of the thorax were performed and 3D reconstruction images were developed for better evaluation (Figure 1). We planned to perform 5 cm incisions in the midaxillary line and 5 cm partial resection of the 5^{th} ribs (Figure 2). Measurements of the resection were made by using MDCT and all data were transferred to AutoCAD software system (Autodesk, Inc. California, USA). All mesh models were produced by AutoCAD.

We used the data for printing custom-made rib for replacing the resected part. We produced ribs in different size with Nylon 680 Co-polymer/FDA approved material (Taulman 3D, Saint Peters, MO, USA) by using 3D Printer (Afinia H480, Chanhassen, MO, USA). The 3D-printed implants were sterilized with ethylene oxide.

Pigs were operated at Acibadem University Center of Advanced Simulation and Education (CASE) Laboratories, İstanbul, Turkey. General anesthesia was induced with intravenous ketamine (1 mg/kg per body weight) and propofol (4.0 mg/kg per body weight), and was maintained by volatile anesthetics (isoflurane 1.5-2.5%). The animals were equipped with a femoral arterial catheter and rectal probe for hemodynamic, thermal monitoring and five-lead electrocardiogram.

We made a 5 cm incision in the midaxillary line and performed 5 cm rib resection for both animals. 3D-printed custom-made Nylon 680 Co-polymer rib was implanted into the resected area and stabilized with 2-0 polypropylene sutures on both margins. We checked the rib for stabilization and closed the anatomical structures according to proper anatomical manner.

Animals were wakened up from anesthesia and weaned off the mechanical ventilator. One of the animals passed away during the wakening up period due to a possible myocardial infarction. Second animal recovered uneventfully.

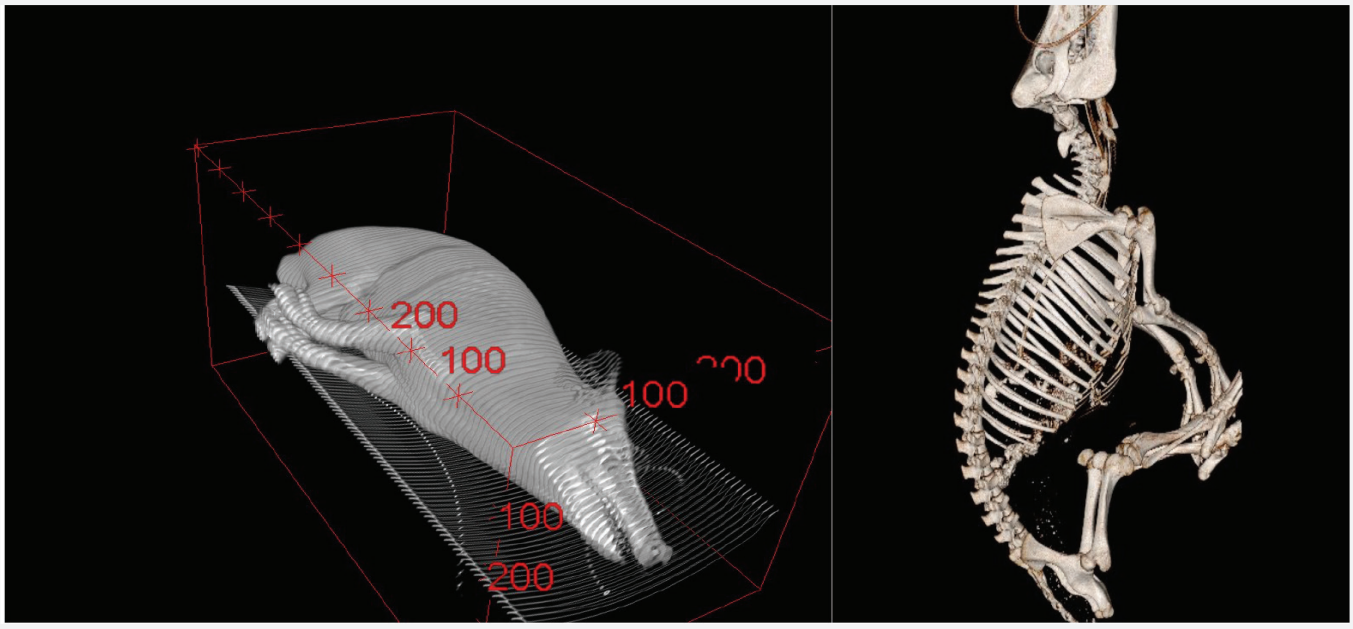

Figure 1. Multidetector computerized tomography scans and 3D reconstructed images of the animal before reconstruction
3D: Three-dimensional

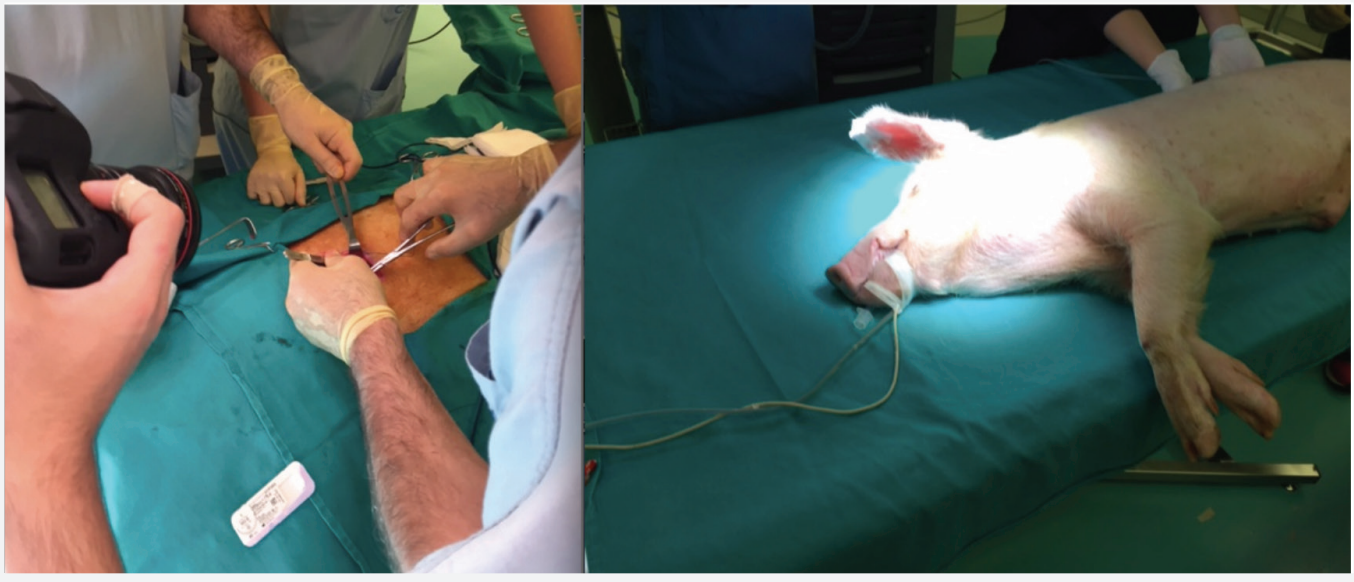

Figure 2. Procedure was performed under general anesthesia with a 5 cm incision

RESULTS

The animal received care for 45 days. He was followed up every day by one of the members from the research team. General condition, weight and appetite of the animal were recorded daily on regular basis. The pig was sacrificed on the 45th postoperative day and operated chest wall was resected totally. Specimen was delivered to the Marmara University Department of Pathology for histopathologic evaluation.

Tissue samples of implanted area were fixed in 10% formalin and processed for paraffin embedding. Sections of 4-5 µm thickness were processed for microscope slides. Slides were stained with hematoxylin and eosin. Histopathologic evaluation

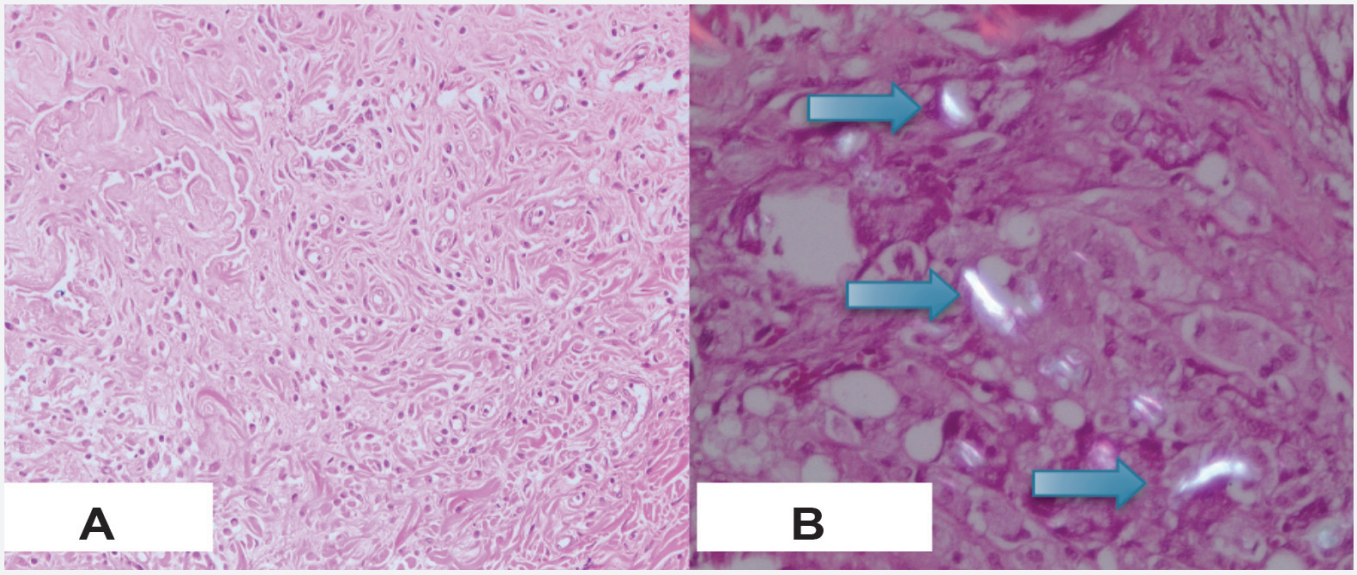

Figure 3. Histopathological evaluation showed moderate chronic inflammation with few giant cells containing pigmented foreign bodies. A) Chronic inflammation. B) Giant cells with pigmented foreign bodies in polarized light (blue arrow)

revealed moderate chronic inflammation with scattered giant cells. In polarized light, a few giant cells containing foreign bodies were observed (Figure 3).

One cartridge of Nylon-Co polymer weighs almost one kilogram, and it costs only 30 USD. Although total expense depends on the size of the implant, it is much more affordable than the regular implants produced with existing materials.

DISCUSSION

3D printing is a novel technology, and it is getting more involved in every scientific field each day. It is very important to adapt this technology in all fields of medicine. Especially in surgical branches, it is a valuable prospect in terms of medical devices, implants, and retractors. Couple of studies were published regarding the usage of 3D printing in thoracic surgery. Kurenov et al.[12] published their data about 3D-printed pulmonary artery models for patients receiving regional lung chemotherapy. They declared that 3D rapid prototyping allowed the replication of sophisticated anatomical structures that could be used to facilitate anatomic study, surgical planning, and device development. Biglino et al.[13] published an article about experimental cardiovascular modeling by 3D printing with a new rubber-like material for compliant arterial phantoms for in vitro studies and device testing. 3D-printed models save time in surgery planning as much as two thirds and help visualize complex pre-operative anatomical structures[14]. In thoracic surgery, 3D printing is being used to assess the invasion of vital structures by tumors and to assist in diagnosis and treatment of upper and lower airway diseases[15-17]. This technology provides a great opportunity to new technological developments and could be a game-changer in the surgical field[18]. By this technology, we can produce any kind of devices within the limits of our creativity. We can produce different types of custom-made surgical materials by ourselves with low cost in shorter time. Although we need more studies, this research is an important step for adapting 3D printing into thoracic surgery.

Study Limitations

Sample size is one of the limitations of the study. We need more studies with larger sample size to move into next steps. We did not compare the histopathologic results with the control group operated with the standard approved implants used in chest wall reconstruction. This is the second limitation of the study, but this study declares the preliminary results. In the light of these data, we can organize studies with larger sample size including control groups. This study is one of the pioneer data declaring the usage of implants with new material directly produced by 3D printing in thoracic surgery field. 3D printing technology is affordable and accessible, which mostly makes it possible to create accurate anatomic models, represents a new tool in thoracic surgery. These advances could aid physicians to personalize treatment approaches, improve surgical techniques, and reduce morbidity and mortality.

CONCLUSION

The application of 3D-printed materials enables precise planning for surgical procedures necessitating reconstruction or replacement. Although there has been progress in application

part of this technology, there are still limited data about the use and identification of new materials providing benefit in cost. We believe that the application of this new material in 3D-printing is going to help us for rapid prototyping with a lower cost for reconstruction in thoracic surgery in the future.

Ethics

Ethics Committee Approval: The study was approved by the Acıbadem University Animal Experiments Local Ethics Committee (ACU-HADYEK), İstanbul, Turkey (decision no: 2015/17, date: 02.03.2015).

Informed Consent: Animal experiment.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: N.O.E., M.Y., Concept: N.O.E., M.Y., B.Y., B.K., Design: N.O.E., M.Y., B.Y., İ.E.S., B.K., Data Collection or Processing: N.O.E., B.Y., İ.E.S., B.K., Analysis or Interpretation: N.O.E., M.Y., B.Y., T.L., İ.E.S., Literature Search: N.O.E., B.Y., Writing: N.O.E., B.Y.

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. Norman J, Madurawe RD, Moore CM, Khan MA, Khairuzzaman A. A new chapter in pharmaceutical manufacturing: 3D-printed drug products. Adv Drug Deliv Rev. 2017;108:39-50.
2. Olivieri L, Krieger A, Chen MY, Kim P, Kanter JP. 3D heart model guides complex stent angioplasty of pulmonary venous baffle obstruction in a Mustard repair of D-TGA. Int J Cardiol. 2014;172:e297-8.
3. Hyde J, MacNicol M, Odle A, Garcia-Rill E. The use of three-dimensional printing to produce in vitro slice chambers. J Neurosci Methods. 2014;238:82-7.
4. Huang W, Zheng Q, Sun W, Xu H, Yang X. Levofloxacin implants with predefined microstructure fabricated by three-dimensional printing technique. Int J Pharm. 2007;339:33-8.
5. Owais K, Pal A, Matyal R, Montealegre-Gallegos M, Khabbaz KR, Maslow A, et al. Three-dimensional printing of the mitral annulus using echocardiographic data: science fiction or in the operating room next door? J Cardiothorac Vasc Anesth. 2014;28:1393-6.
6. Thawani JP, Pisapia JM, Singh N, Petrov D, Schuster JM, Hurst RW, et al. Three-Dimensional Printed Modeling of an Arteriovenous Malformation Including Blood Flow. World Neurosurg. 2016;90:675-83.
7. Zhang Y, Zhu J, Wang Z, Zhou Y, Zhang X. Constructing a 3D-printable, bioceramic sheathed articular spacer for infected hip arthroplasty. J Med Hypo Idea. 2015;9:13-9.
8. Mulford JS, Babazadeh S, Mackay N. Three-dimensional printing in orthopaedic surgery: review of current and future applications. ANZ J Surg. 2016;86:648-53.
9. Ng CS. Recent and Future Developments in Chest Wall Reconstruction. Semin Thorac Cardiovasc Surg. 2015;27:234-9.
10. Czyz M, Addae-Boateng E, Boszczyk BM. Chest wall reconstruction after en bloc Pancoast tumour resection with the use of MatrixRib and SILC fixation systems: technical note. Eur Spine J. 2015;24:2220-4.
11. Chudacek J, Bohanes T, Szkorupa M, Klein J, Stasek M, Zalesak B, et al. Strategies of treatment of chest wall tumors and our experience. Rozhl Chir. 2015;94:17-23.
12. Kurenov SN, Ionita C, Sammons D, Demmy TL. Three-dimensional printing to facilitate anatomic study, device development, simulation, and planning in thoracic surgery. J Thorac Cardiovasc Surg. 2015;149:973-9.e1.
13. Biglino G, Verschueren P, Zegels R, Taylor AM, Schievano S. Rapid prototyping compliant arterial phantoms for in-vitro studies and device testing. J Cardiovasc Magn Reson. 2013;15:2.
14. Singhal AJ, Shetty V, Bhagavan KR, Ragothaman A, Shetty V, Koneru G, et al. Improved Surgery Planning Using 3-D Printing: a Case Study. Indian J Surg. 2016;78:100-4.
15. Giannopoulos AA, Steigner ML, George E, Barile M, Hunsaker AR, Rybicki FJ, et al. Cardiothoracic Applications of 3-dimensional Printing. J Thorac Imaging. 2016;31:253-72.
16. Wang L, Cao T, Li X, Huang L. Three-dimensional printing titanium ribs for complex reconstruction after extensive posterolateral chest wall resection in lung cancer. J Thorac Cardiovasc Surg. 2016;152:e5-7.
17. Cheng GZ, San Jose Estepar R, Folch E, Onieva J, Gangadharan S, Majid A. Three-dimensional Printing and 3D Slicer: Powerful Tools in Understanding and Treating Structural Lung Disease. Chest. 2016;149:1136-42.
18. Gillaspie EA, Matsumoto JS, Morris NE, Downey RJ, Shen KR, Allen MS, et al. From 3-Dimensional Printing to 5-Dimensional Printing: Enhancing Thoracic Surgical Planning and Resection of Complex Tumors. Ann Thorac Surg. 2016;101:1958-62.

ORIGINAL ARTICLE

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The Clinical Outcomes of Ultrasonography Usage in Percutaneous Dilatational Tracheostomy in the Intensive Care Unit: A Retrospective Trial

Yoğun Bakım Ünitesinde Perkütan Dilatasyonel Trakeostomide Ultrasonografi Kullanımının Klinik Sonuçları: Retrospektif Bir Çalışma

Onur BARAN, Ayhan ŞAHİN, Makbule Cavidan ARAR

Tekirdağ Namık Kemal University Faculty of Medicine, Department of Anesthesiology and Reanimation, Tekirdağ, Turkey

ABSTRACT

Aim: Preprocedural ultrasonographic examination of the upper airway anatomy is an effective method for deciding on a tracheostomy procedure, such as percutaneous or surgical tracheostomy. We aimed to compare the effects of superficial cervical plexus block (CPB) with translaryngeal block with those of local anesthesia infiltration to the incision site for percutaneous tracheostomy in terms of hemodynamic parameters, gag reflex, and anesthetic requirement. In addition, we evaluated the effect of preprocedural ultrasonography assessment compared with that of anatomical landmark examination in terms of reducing the risk of procedure-related complications.

Materials and Methods: A total of 148 patients aged at the range of 18-99 years, who were indicated for percutaneous tracheostomy in the intensive care unit, were enrolled in the study. The data intended for this study were obtained from the hospital's electronic patient database through retrospective scanning between 2018 and 2022. Patients who underwent ultrasonography for the evaluation of the related anatomical structures and superficial CPB with a translaryngeal block were assigned to the ultrasonography group (n=74), whereas those who underwent an anatomical landmark technique and local anesthetic infiltration to the procedure site were assigned to the traditional group (n=74).

Results: The patients' age and sex distributions did not differ significantly between the traditional and ultrasonography groups ($p>0.05$). In the ultrasonography group, the preprocedural, midprocedural, and postprocedural heart rates were significantly higher than in the traditional group ($p<0.05$). In the ultrasonography group, the mean preprocedural arterial pressure decreased significantly during and after the procedure ($p<0.05$). The mean arterial pressure decreased during the procedure and the postprocedural arterial pressure was significantly higher ($p<0.05$) in the ultrasonography group than in the traditional group.

Conclusion: Although ultrasonography-guided percutaneous tracheostomy takes more time to perform than traditional anatomical landmark percutaneous tracheostomy, we claim that the procedure is much safer and provides better clinical outcomes.

Keywords: Percutaneous dilatational tracheostomy, intensive care unit, superficial cervical plexus block, translaryngeal block

ÖZ

Amaç: Üst hava yolu anatomisinin işlem öncesi ultrasonografik muayenesi, perkütan veya cerrahi trakeostomi gibi bir trakeostomi prosedürüne karar vermede etkili bir yöntemdir. Perkütan trakeostomi için yüzeyel servikal pleksus bloğu (SPB) ile translaringeal bloğun; insizyon bölgesine lokal anestezi infiltrasyonu ile hemodinamik parametreler, öğürme refleksi ve anestezik gereksinimi açısından etkilerini karşılaştırmayı amaçladık. Ek olarak, işlemle ilgili komplikasyon riskini azaltma açısından işlem öncesi ultrasonografi değerlendirmesinin etkisini anatomik işaret noktası yöntemi incelemesiyle karşılaştırdık.

Gereç ve Yöntem: Yoğun bakım ünitesinde perkütan trakeostomi endikasyonu olan 18-99 yaş aralığındaki 148 hasta çalışmaya alındı. Bu çalışmaya yönelik veriler, hastanenin elektronik hasta veri tabanından 2018-2022 yılları arasında retrospektif tarama yoluyla elde edildi. İlgili anatomik

Address for Correspondence: Onur BARAN MD, Tekirdağ Namık Kemal University Faculty of Medicine, Department of Anesthesiology and Reanimation, Tekirdağ, Turkey
Phone: +90 539 342 25 82 **E-mail:** obaran@nku.edu.tr **ORCID ID:** orcid.org/0000-0003-0007-6315
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yapıların değerlendirilmesi için, ultrasonografi ve translaringeal blok ile yüzeyel SPB yapılan hastalar ultrasonografi grubuna (n=74), anatomik işaret noktası yöntemi ve prosedür bölgesine lokal anestezik infiltrasyonu uygulananlar geleneksel gruba (n=74) dahil edildi.

Bulgular: Hastaların yaş ve cinsiyet dağılımları, geleneksel ve ultrasonografi grupları arasında anlamlı farklılık göstermedi (p>0,05). Ultrasonografi grubunda, işlem öncesi, işlem ortası ve işlem sonrası kalp hızları, geleneksel gruptakinden anlamlı olarak daha yüksekti (p<0,05). Ultrasonografi grubunda işlem öncesi ortalama arter basıncı işlem sırasında ve sonrasında anlamlı olarak azaldı (p<0,05). Ortalama arter basıncı işlem sırasında azaldı ve işlem sonrası arter basıncı ultrasonografi grubunda geleneksel gruba göre anlamlı derecede yüksekti (p<0,05).

Sonuç: Perkütan trakeostominin ultrasonografi rehberliğinde uygulanması, geleneksel anatomik işaret yöntemi ile uygulanmasına göre daha uzun sürse de işlemin çok daha güvenli olduğu ve daha iyi klinik sonuçlar sağladığını düşünüyoruz.

Anahtar Kelimeler: Perkütan dilatasyonel trakeostomi, yoğun bakım ünitesi, yüzeyel servikal pleksus bloğu, translaringeal blok

INTRODUCTION

Since its invention by Ciaglia et al.[3] in 1985, percutaneous dilatation tracheostomy (PDT) has been commonly performed by intensivists in intensive care units[1,2]. Even if surgical tracheostomy is still performed for up to 50% of critically ill patients, PDT techniques are increasingly used owing to their easy application and shorter procedure times[4]. Among these techniques, multiple dilator tracheostomy, guide wire dilating forceps tracheostomy, translaryngeal tracheostomy, and newer techniques such as single-step dilation tracheostomy, rotational dilation tracheostomy, and balloon dilation tracheostomy are preferred in clinical practice[4].

A superficial cervical plexus block (CPB) is easy and efficient to apply and provides satisfying anesthesia and analgesia to the head and neck region[5,6]. As the role of ultrasonography in regional anesthesia has expanded, CPBs can now be performed more safely and accurately under ultrasonographic guidance, which facilitates the identification of various important landmarks such as muscles, the cervical vertebrae, the large vessels, nerves, and the cervical fascia[5]. Superficial CPB is performed solely or in combination with various brachial plexus blocks for carotid endarterectomies[7], humerus and clavicula fracture surgeries[8,9], orthognathic surgery[10], and ear surgeries[11].

A translaryngeal block, which aims to block the branches of the recurrent laryngeal nerve in the cricothyroid region, is useful for providing topical anesthesia to the distal airway mucosa[12]. Ultrasonographic guidance or anatomical landmarks are preferred for identifying the cricothyroid membrane[13]. A translaryngeal block is known to be useful for awake fiberoptic intubation, but it was also shown to facilitate awake tracheostomy procedures[12]. A 22- or 20-gauge needle is used to deliver the local anesthetic by inserting it perpendicular to the skin of the patient lying supine, with continuous aspiration administered simultaneously to penetrate the cricothyroid membrane. To anesthetize the distal airway mucosa, the local anesthetic is injected when air bubbles start to appear in the syringe, and the needle is immediately withdrawn[13].

Preprocedural ultrasonographic examination of the upper airway anatomy is an effective method for deciding on a tracheostomy procedure such as percutaneous or surgical tracheostomy[14]. Previous studies have demonstrated the importance of ultrasonographic examination in identifying the related major and vulnerable anatomical and vascular structures to reduce complication rates[14]. Ultrasonography-guided superficial cervical plexus with translaryngeal block for percutaneous tracheostomy has been routinely performed in our clinic for almost 2 years. Prior to the introduction of this technique to our clinic, we performed all percutaneous tracheostomy procedures using anatomical landmark techniques, with analgesia induced with infiltration anesthesia to the incision site and deep sedation to almost general anesthesia. We aimed to compare the effects of superficial CPB with translaryngeal block with those of local anesthesia infiltration to the incision site for percutaneous tracheostomy in terms of hemodynamic parameters, gag reflex, and anesthetic requirement. In addition, we evaluated the effect of preprocedural ultrasonography assessment compared with that of anatomical landmark examination in terms of reducing the risk of procedure-related complications.

MATERIALS AND METHODS

This retrospective study was conducted according to the ethical principles outlined in the Helsinki Declaration and the guidelines of good clinical practice and ethical approval was obtained from Tekirdağ Namık Kemal University Non-Interventional Research Ethics Committee (protocol no: 2022.223.12.01, date: 27.12.2022).

After obtaining the approval from the ethics committee, among 3.656 patients, 148 patients aged 18-99 years, who were indicated for percutaneous tracheostomy in the intensive care unit, were enrolled in the study. The data intended for this study were obtained from the hospital's electronic patient database through retrospective scanning between 2018 and 2022. Patients who underwent ultrasonography for the evaluation of the related anatomical structures and superficial CPB with a translaryngeal block before the percutaneous tracheostomy procedure were assigned to the ultrasonography

group (n=74), whereas those who underwent an anatomical landmark technique and local anesthetic infiltration to the procedure site before the percutaneous tracheostomy procedure were assigned to the traditional group (n=74).

In our 11-bed mixed tertiary intensive care unit, ultrasonography has been widely used for interventional procedures and patient follow-up. We have been using ultrasonography in our intensive care unit for more than 2 years. Before percutaneous tracheostomy procedures, we routinely assess the upper airway anatomy and the cartilages of the larynx and upper trachea, and perform bilateral superficial CPB with translaryngeal block to reduce the need for anesthetic drugs and to provide hemodynamic stability. In the past, our intensive care unit was not equipped with an ultrasonography device, so local anesthesia infiltration with deep sedation and general anesthesia in some cases had to be administered.

The Esaote MyLab Six (Genoa, Italy) ultrasonography device with a high-frequency linear probe was used in a midline longitudinal approach to identify the upper airway anatomical structures such as the thyroid and cricoid cartilages, cricothyroid membrane, tracheal rings, and the space between the tracheal rings for the insertion of the tracheostomy cannula. After this, the probe was oriented in a transverse position to ensure the location of the isthmus of the thyroid tissue and vascular structures at the intervention site. The probe was then reoriented in a midline longitudinal position to determine the exact point for the puncture and tracheostomy incision. The exact point was drawn with a marker pen.

A 14-G intravenous cannula with a plastic cover and the needle of a 5-mL syringe filled with serum physiologic solution was inserted in the tracheal lumen, between the first and second tracheal rings, with continuous aspiration after a 2- to 3-cm transverse incision. To prevent the puncture of the endotracheal tube cuff or tube itself, the needle was stopped immediately when the air in the syringe had aspirated. Leaving the plastic cannula in place, the needle was withdrawn, and the guide wire was inserted through the plastic cannula. After the plastic cannula was removed through the guide wire, a dilatator was used to expand the puncture point. A Griggs forceps was inserted through the guide wire after the removal of the dilator. The subcutaneous tissue and tracheostomy cannula insertion point were dilated with the Griggs forceps. The tracheostomy cannula was inserted through the orifice, and the guide wire and endotracheal tube were removed. The cuff of the tracheostomy cannula was inflated, and the location of the cannula was confirmed by auscultation. All PDT procedures were performed using a percutaneous tracheostomy kit (Portex, Hythe, Kent, England), and all tracheostomies were performed in elective conditions by experienced clinicians.

The primary outcome of this study was the evaluation of the anesthetic drug usage throughout the procedure and the hemodynamic response stability. Heart rate and arterial blood pressure were recorded from the medical records of the intensive care unit patients. The secondary outcomes were the incidence rates of major and minor complications and gag reflex. The patients' records were scanned for the following complications: minor and major hemorrhage during and after the procedure, subcutaneous emphysema, misplacement of the tracheostomy cannula, and conversion to surgical tracheostomy.

Statistical Analysis

Mean, standard deviation, median lowest, highest, frequency, and ratio values were used in the descriptive statistics of the data. The distribution of variables was measured with the Kolmogorov-Smirnov test. The Mann-Whitney U test was used in the analysis of quantitative independent data. The chi-square test was used in the analysis of qualitative independent data, and the Fisher test was employed when the chi-square test conditions were not met. Statistical Package for the Social Sciences 28.0 program was used in the analysis.

RESULTS

The mean age of the patients (n=148) was 56.6±20.3 years. Precise height and weight measurements of all patients were not evaluated because they could not be obtained from patient files. Of the patients, 38.5% were male, and 61.5% were female (Table 1). The complication rates were as follows, regardless of the technique used to perform the tracheostomy procedures: the presence of gag reflex (n=45; 30.4%), minor bleeding (n=30; 20.3%), major bleeding (n=12; 20.3%), cannula misplacement (n=9; 6.1%), and subcutaneous emphysema (n=9; 2%). Of the patients, 11 (7.4%) were converted to surgical tracheostomy after undergoing percutaneous tracheostomy (Table 1).

The patients' age and sex distributions did not differ significantly between the traditional and ultrasonography groups ($p>0.05$). In the ultrasonography group, the preprocedural, midprocedural, and postprocedural heart rates were significantly higher than those in the traditional group ($p<0.05$). In the traditional group, no significant differences ($p>0.05$) were observed in the midprocedural and postprocedural heart rates compared with the preprocedural heart rate. In the ultrasonography group, the midprocedural heart rate showed no significant difference ($p>0.05$) when compared with the preprocedural heart rate. In the ultrasonography group, the preprocedural heart rate decreased significantly ($p<0.05$) after the procedure. No significant differences ($p>0.05$) in the midprocedural and postprocedural heart rate changes were found between the traditional and ultrasonography groups (Table 2).

Table 1. Demographic, hemodynamic and procedure-specific parameters and complication rates

		Minimum	Maximum	Median	Mean±SD / n (%)
Age		18.0	90.0	61.0	56.6±20.3
Sex	Male				57 (38.5)
	Female				91 (61.5)
Traditional group					74 (50.0)
Ultrasound group					74 (50.0)
Heart rate (beats per minute)					
Pre-procedure		50.0	169.0	95.5	101.1±32.2
Mid-procedure		43.4	170.2	97.0	100.7±33.3
Post-procedure		42.7	172.9	94.2	99.1±32.1
Mean arterial pressure (mmHg)					
Pre-procedure		50.0	100.0	72.5	74.4±14.0
Mid-procedure		47.5	108.6	73.6	72.9±14.3
Post-procedure		41.7	115.0	72.0	72.8±15.1
Propofol consumption		0.0	250.0	80.0	98.2±71.2
Opioid consumption		25.0	150.0	75.0	77.5±38.7
Total procedure time (minute)		15.0	90.0	42.0	45.1±21.4
Neuromuscular blocking agents use	(-)				116 (78.4)
	(+)				32 (21.6)
Major bleeding	(-)				136 (91.9)
	(+)				12 (8.1)
Minor bleeding	(-)				118 (79.7)
	(+)				30 (20.3)
GAG reflex presence	(-)				103 (69.6)
	(+)				45 (30.4)
Subcutaneous emphysema presence	(-)				145 (98.0)
	(+)				3 (2.0)
Cannula misplacement	(-)				139 (93.9)
	(+)				9 (6.1)
Conversion to surgical tracheostomy	(-)				137 (92.6)
					11 (7.4)

SD: Standard deviation

In terms of preprocedural, midprocedural, and postprocedural mean arterial pressures, no significant differences ($p>0.05$) were revealed between the traditional and ultrasonography groups. In the traditional group, the changes in the mean preprocedural arterial pressures during and after the procedure were not significant ($p>0.05$). In the ultrasonography group, the mean preprocedural arterial pressure decreased significantly during and after the procedure ($p<0.05$). The mean arterial pressure decreased during the procedure and the postprocedural arterial pressure was significantly higher ($p<0.05$) in the ultrasonography group than in the traditional group (Table 2).

The propofol and opioid doses used were significantly lower ($p<0.05$) in the ultrasonography group than in the traditional group (Figure 1). The use rate of neuromuscular blocking agents was significantly lower ($p<0.05$) in the ultrasonography group than in the traditional group (Table 2, Figure 2).

The incidence rates of major bleeding and complications, including subcutaneous emphysema, cannula misplacement, and conversion to surgical tracheostomy, did not differ significantly between the traditional and ultrasonography groups ($p>0.05$ for all; Figure 3). The incidence rates of minor bleeding and gag reflex were significantly lower ($p<0.05$ for both) in the ultrasonography group than in the traditional group (Figure 4). However, the procedure time was significantly longer ($p<0.05$) in the ultrasonography group than in the traditional group (Table 2).

Table 2. Comparison of demographic, hemodynamic and procedure-specific parameters and complication rates between groups

		Traditional group		Ultrasound group			
		Mean±SD / n (%)	Median	Mean±SD / n (%)	Median	p	
Age		53.6±21.2	54.0	59.6±19.1	62.5	0.091	m
Sex	Male	23 (31.0)		34 (45.9)		0.063	X^2
	Female	51 (69.0)		40 (54.1)			
Heart rate (beats per minute)							
Pre-procedure		94.2±30.0	90.5	108.1±33.2	101.5	**0.011**	m
Mid-procedure		93.5±32.0	85.6	107.9±33.1	100.8	**0.008**	m
Post-procedure		93.4±31.1	92.2	104.8±32.2	98.5	**0.033**	m
Change according to pre-procedure							
Mid-procedure		-0.68±8.98	-1.50	-0.14±3.27	0.00	0.268	m
Intra-group change p		0.296[w]		0.821[w]			
Post-procedure		-0.86±8.93	-2.00	-3.28±1.05	-3.00	0.154	m
Intra-group change p		0.347[w]		**0.000[w]**			
Mean arterial pressure (mmHg)							
Pre-procedure		74.0±14.0	72.0	74.8±14.0	74.0	0.721	m
Mid-procedure		74.7±15.1	74.8	71.1±13.3	70.3	0.195	m
Post-procedure		75.0±16.2	75.2	70.7±13.7	69.8	0.116	m
Change according to pre-procedure							
Mid-procedure		-0.68±8.98	-1.50	-0.14±3.27	0.00	**0.000**	m
Intra-group change p		0.279[w]		**0.000[w]**			
Post-procedure		-0.86±8.93	-2.00	-3.28±1.05	-3.00	**0.000**	m
Intra-group change p		0.594[w]		**0.000[w]**			
Propofol consumption		151.2±60.4	150.0	45.3±29.4	50.0	**0.000**	m
Opioid consumption		106.1±30.9	100.0	49.0±20.0	50.0	**0.000**	m
Neuromuscular blocking agents use	(-)	49 (66.2)		67 (90.5)		**0.000**	X^2
	(+)	25 (33.8)		7 (9.5)			
Major bleeding	(-)	66 (89.2)		70 (94.6)		0.228	X^2
	(+)	8 (10.8)		4 (5.4)			
Minor bleeding	(-)	54 (73.0)		64 (86.5)		**0.041**	X^2
	(+)	20 (27.0)		10 (13.5)			
GAG reflex presence	(-)	35 (47.3)		68 (91.9)		**0.000**	X^2
	(+)	39 (52.7)		6 (8.1)			
Subcutaneous emphysema presence	(-)	72 (97.3)		73 (98.6)		1.000	X^2
	(+)	2 (2.7)		1 (1.4)			
Cannula misplacement	(-)	69 (9.2)		70 (94.6)		0.731	X^2
	(+)	5 (6.8)		4 (5.4)			
Conversion to surgical tracheostomy	(-)	66 (89.2)		71 (95.9)		0.117	X^2
		8 (10.8)		3 (4.1)			
Total procedure time (min.)		30.6±9.4	30.0	59.7±20.1	63.0	**0.000**	m

[m]Mann-Whitney U test, [X²]Chi-square test (Fisher test).

SD: Standard deviation

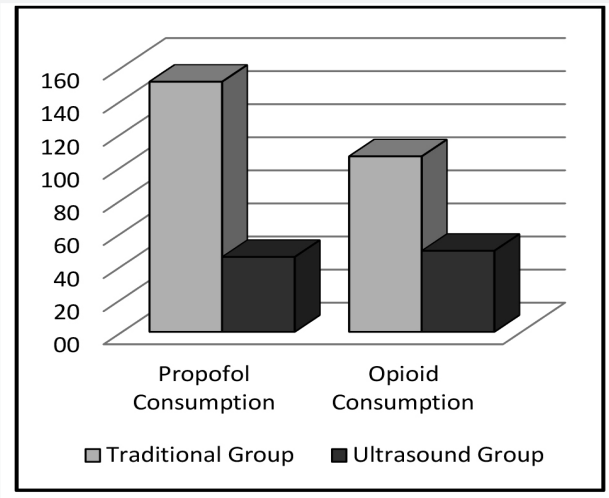

Figure 1. Propofol and opioid consumption of the patients

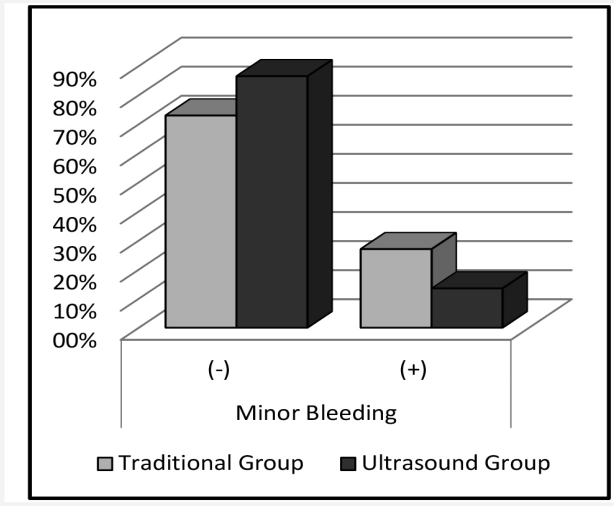

Figure 2. Neuromuscular blocking agent use of the patients

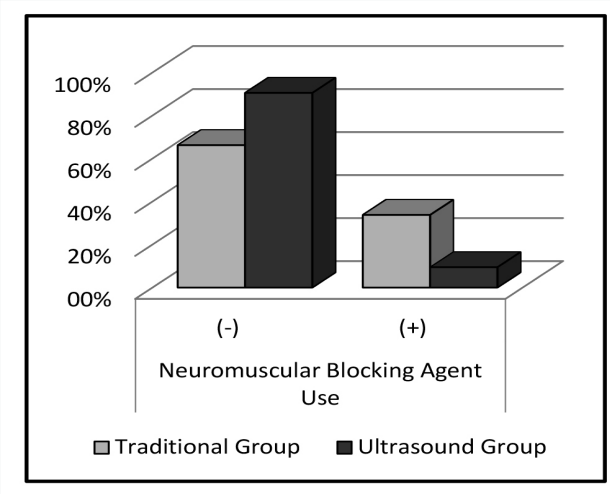

Figure 3. Minor bleeding seen between the groups

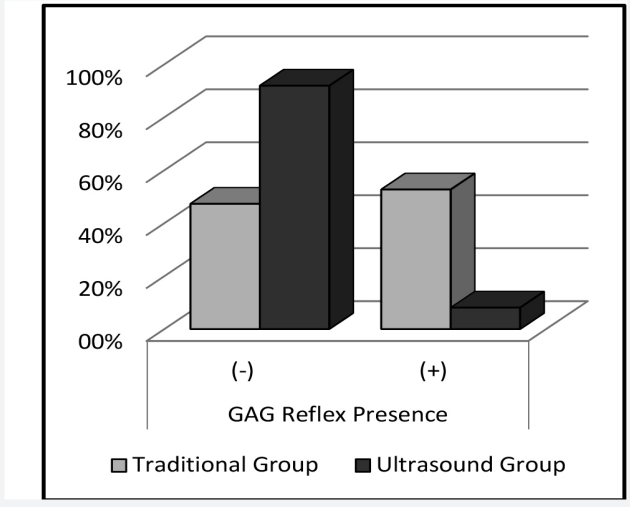

Figure 4. Presence of GAG reflex in the patients

DISCUSSION

The purpose of this study was to demonstrate that ultrasonography was an essential assessment device in percutaneous tracheostomy to facilitate the procedure and maintain effective analgesia using ultrasonography-guided regional anesthesia techniques and hemodynamic stability during the procedure. Ultrasonographic examination of the neck and puncture site in percutaneous tracheostomy is useful to avoid complications such as hemorrhages caused by puncturing the vascular structures and thyroid gland and to master the exact puncture point level to avoid damage to the cartulary structures[14-17].

As several studies have confirmed that preprocedural ultrasonographic examination of the anterior region of the neck minimizes the occurrence of complications related to percutaneous tracheostomy[17-20], we aimed to retrospectively scan for the characteristics of patients who had undergone elective tracheostomy to document the effect of ultrasonography usage in our clinic.

The traditional anatomical landmark technique for percutaneous tracheostomy consists of palpation of the underlying structures such as the cricothyroid membrane, cricoid cartilage, and tracheal rings[2,21]. Blind detection of the anatomical structures in the laryngeal region might be challenging and can have negative consequences. Placement of the tracheal tube above

the first tracheal ring may increase the risk of late subglottic stenosis. Sustić et al.[22] reported that when the tracheal tube was placed blindly, it could be mispositioned above the first tracheal ring, unlike with ultrasonography guidance.

Performing an ultrasonography-guided superficial plexus block with a translaryngeal block is essential in maintaining analgesia for percutaneous tracheostomy[12,23]. Owing to the impacts of the advantages of ultrasonography-guided regional anesthesia techniques performed in the operation theater, these techniques have also been used for pain management in critically ill patients in intensive care units[24]. To avoid the risks related to opioid and hypnotic use, we prefer ultrasonography-guided regional anesthesia techniques in intensive care. In our study, the propofol and opioid doses needed for the tracheostomy procedure were lower in the ultrasonography group. We think that this is the advantage of the preprocedural superficial CPB with a translaryngeal block. It is also related to a low hemodynamic response to pain. As patient response to the incisions during the tracheostomy procedure is heightened, higher propofol and opioid doses are needed. When the preprocedural and postprocedural heart rates and mean arterial pressures were compared within the groups, the patients in the ultrasonography group showed only decreases compared with the traditional group. We think that this was due to the regional anesthesia techniques we performed. The hemodynamic response to the tracheostomy procedure was avoided in the ultrasonography group owing to the ultrasonography-guided blocks.

Adding a translaryngeal block is also effective in preventing gag reflex, which is an involuntary defense mechanism to protect the pharynx and throat from foreign objects[12,25]. Following the studies by Şahin et al.[12] and Koshy and Thankamony[23], we experienced the advantages of adding a translaryngeal block to the superficial CPB, and related to this fact, the patients in the ultrasonography group had a lower incidence rate of gag reflex[23].

Contrary to the study of Plata and Gaszyński[17] reporting that ultrasonography guidance shortens the procedure duration, the procedure was much longer, nearly double, in the ultrasonography group than in the traditional group in our study (p=0.000)[17]. The major cause of this result was the longer time needed for the inspection of the anterior neck region followed by ultrasonography-guided superficial CPB with translaryngeal block in the ultrasonography group.

According to the study of Topcu et al.[26], which was a retrospective cohort study with 59 patients enrolled after scanning 2852 patients who were followed up in the intensive care unit, the time needed to perform ultrasonography-guided percutaneous tracheostomy was shorter than that required for Griggs percutaneous tracheostomy. By contrast, our study only required the total time for ultrasonography-guided percutaneous tracheostomy, including the time for preprocedural assessment of the anterior neck region combined with superficial CPB and translaryngeal block. As the patients' medical records contained no information on the time for the tracheostomy procedure alone apart from the preprocedural assessment and ultrasonography-guided block of the procedure region, this was considered a limitation of our study.

Several complications such as minor and major bleeding, pneumothorax, tracheal and esophageal injuries, paratracheal placement, hemodynamic instability, desaturation, and ruptured endotracheal tube cuff have been reported as immediate and early complications in the literature[2,27,28]. Rudas et al.[27] reported that they found no statistically significant difference in the mean complication rate between the two groups in their Traditional Landmark versus Ultrasound-Guided Evaluation Trial study, where in one group, the tracheal puncture site was decided using the landmark technique, whereas in the other group, ultrasonography guidance was used. On the basis of the noted complications in the patients' medical records and hospital data system, we found that only the incidence rate of minor bleeding was significantly different between the groups. The incidence rates of major bleeding, subcutaneous emphysema, and conversion to surgical tracheostomy were not significantly different between the groups. We expected the rate of conversion to surgical tracheostomy to be significantly higher in the traditional anatomical landmark group. Prospective studies are needed to improve the outcomes in terms of reducing the risk of other complications.

Neuromuscular blocking agents are not needed for some minor surgical interventions. However, they are required in the percutaneous tracheostomy procedure if the patient shows gag reflex, which indicates a direct relationship between the presence of gag reflex and the use of neuromuscular blocking agents. The results of our study corroborate this claim.

Study Limitations

In this single-center study, our case number was low compared to similar studies in the literature. The records in the hospital information system from past to present have been one of the limiting steps of our study. Prospective randomized controlled studies are needed to provide better clinical data.

CONCLUSION

Although ultrasonography-guided percutaneous tracheostomy takes more time to perform than traditional anatomical landmark percutaneous tracheostomy, we claim that the

procedure is much safer and provides better clinical outcomes. Randomized controlled trials with larger patient populations are warranted to document outcomes more accurately.

Ethics

Ethics Committee Approval: The study was approved by the Tekirdağ Namık Kemal University of Non-Interventional Research Ethics Committee (protocol no: 2022.223.12.01, date: 27.12.2022).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: O.B., Concept: O.B., M.C.A., Design: O.B., A.Ş., M.C.A., Data Collection or Processing: O.B., Analysis or Interpretation: A.Ş., M.C.A., Literature Search: A.Ş., Writing: O.B.

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. Batuwitage B, Webber S, Glossop A. Percutaneous tracheostomy. Continuing Education in Anaesthesia Critical Care & Pain 2014;14:268-72.
2. Mehta C, Mehta Y. Percutaneous tracheostomy. Ann Card Anaesth. 2017;20:S19-25.
3. Ciaglia P, Firsching R, Syniec C. Elective percutaneous dilatational tracheostomy. A new simple bedside procedure; preliminary report. Chest. 1985;87:715-9.
4. Putensen C, Theuerkauf N, Guenther U, Vargas M, Pelosi P. Percutaneous and surgical tracheostomy in critically ill adult patients: a meta-analysis. Crit Care. 2014;18:544.
5. Kim JS, Ko JS, Bang S, Kim H, Lee SY. Cervical plexus block. Korean J Anesthesiol. 2018;71:274-88.
6. Shih ML, Duh QY, Hsieh CB, Liu YC, Lu CH, Wong CS, et al. Bilateral superficial cervical plexus block combined with general anesthesia administered in thyroid operations. World J Surg. 2010;34:2338-43.
7. Pandit JJ, Satya-Krishna R, Gration P. Superficial or deep cervical plexus block for carotid endarterectomy: a systematic review of complications. Br J Anaesth. 2007;99:159-69.
8. Baran O, Kır B, Ateş İ, Şahin A, Üztürk A. Combined supraclavicular and superficial cervical plexus block for clavicle surgery. Korean J Anesthesiol. 2020;73:67-70.
9. Nakamura H, Ishitsuka S. Brachial plexus, superficial cervical plexus, and intercostobrachial nerve block under sedation in a morbidly obese patient undergoing proximal humerus fracture intramedullary nailing repair: A case report. J Clin Anesth. 2021;73:110326.
10. Ciftci B, Develi T, Münevveroglu S, Ekinci M. Superficial cervical plexus block for orthognathic surgery. Rev Esp Anestesiol Reanim (Engl Ed). 2021;68:428-9.
11. Türkyılmaz N, Aksu C, Gürkan Y. Kulak cerrahisinde yüzeyel servikal pleksus bloğu: Olgu sunumu [Superficial cervical plexus block in ear surgery: A case report]. Agri. 2020;32:106-8.
12. Şahin A, Baran O, Gültekin A, Şahin GG, Ersözlü T, Arar C. Can Ultrasound-Guided Regional Anesthesia Techniques For Tracheostomy Be An Alternative To General Anesthesia? Surg Innov. 2022;29:160-8.
13. Hadzic, A: Hadzic's textbook of regional anesthesia and acute pain management, 2nd ed. McGraw-Hill Education: 2017.
14. Sarıtaş, A, Zincircioğlu, Ç, Uzun Sarıtaş, P, Uzun, U, Köse, I, Şenoğlu, N. The efficacy of ultrasound to evaluate the neck structures before tracheostomy and to determine the methods of tracheostomy: Percutaneous or surgical? Kocaeli Med J. 2018;7:113-8.
15. Rajajee V, Fletcher JJ, Rochlen LR, Jacobs TL. Real-time ultrasound-guided percutaneous dilatational tracheostomy: a feasibility study. Crit Care. 2011;15:R67.
16. Guinot PG, Zogheib E, Petiot S, Marienne JP, Guerin AM, Monet P, et al. Ultrasound-guided percutaneous tracheostomy in critically ill obese patients. Crit Care. 2012;16:R40.
17. Plata P, Gaszyński T. Ultrasound-guided percutaneous tracheostomy. Anaesthesiol Intensive Ther. 2019;51:126-32.
18. Bonde J, Nørgaard N, Antonsen K, Faber T. Implementation of percutaneous dilation tracheotomy--value of preincisional ultrasonic examination? Acta Anaesthesiol Scand. 1999;43:163-6.
19. Sustić A, Zupan Z, Eskinja N, Dirlić A, Bajek G. Ultrasonographically guided percutaneous dilatational tracheostomy after anterior cervical spine fixation. Acta Anaesthesiol Scand. 1999;43:1078-80.
20. Singh M, Chin KJ, Chan VW, Wong DT, Prasad GA, Yu E. Use of sonography for airway assessment: an observational study. J Ultrasound Med. 2010;29:79-85.
21. Chung W, Kim BM, Park SI. Simply modified percutaneous tracheostomy using the Cook® Ciaglia Blue Rhino™: a case series. Korean J Anesthesiol. 2016;69:301-4.
22. Sustić A, Kovac D, Zgaljardić Z, Zupan Z, Krstulović B. Ultrasound-guided percutaneous dilatational tracheostomy: a safe method to avoid cranial misplacement of the tracheostomy tube. Intensive Care Med. 2000;26:1379-81.
23. Koshy RC, Thankamony H. Superficial cervical plexus block for urgent tracheostomy. Indian J Anaesth. 2019;63:65-6.
24. Capdevila M, Ramin S, Capdevila X. Regional anesthesia and analgesia after surgery in ICU. Curr Opin Crit Care. 2017;23:430-9.
25. Eachempati P, Kumbargere Nagraj S, Kiran Kumar Krishanappa S, George RP, Soe HHK, Karanth L. Management of gag reflex for patients undergoing dental treatment. Cochrane Database Syst Rev. 2019;2019:CD011116.
26. Topcu H, Ozçiftçi S, Şahiner Y. Comparative effectiveness of real-time ultrasound-guided tracheostomy and anatomic landmark percutaneous dilatational tracheostomy: a retrospective cohort study. Eur Rev Med Pharmacol Sci. 2022;26:7883-91.
27. Rudas M, Seppelt I, Herkes R, Hislop R, Rajbhandari D, Weisbrodt L. Traditional landmark versus ultrasound guided tracheal puncture during percutaneous dilatational tracheostomy in adult intensive care patients: a randomised controlled trial. Crit Care. 2014;18:514.
28. Cheung NH, Napolitano LM. Tracheostomy: epidemiology, indications, timing, technique, and outcomes. Respir Care. 2014;59:895-915.

ORIGINAL ARTICLE

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Galangin Protects AML-12 Cells Against Dactinomycin Induced Hepatotoxicity

Galangin AML-12 Hücrelerini Daktinomisine Bağlı Gelişen Hepatotoksisiteye Karşı Korur

Melek AKINCI[1], Çağatay OLTULU[2], Elvan BAKAR[3], Zatiye Ayça ÇEVİKELLİ YAKUT[4]

[1]*Trakya University Faculty of Pharmacy, Department of Pharmacology, Edirne, Turkey*
[2]*Trakya University Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Edirne, Turkey*
[3]*Trakya University Faculty of Pharmacy, Department of Basic Sciences, Edirne, Turkey*
[4]*Trakya University Faculty of Pharmacy, Department of Pharmacognosy, Edirne; Marmara University Faculty of Pharmacy, Department of Pharmacology, İstanbul, Turkey*

ABSTRACT

Aim: The purpose of this study was to evaluate the effects of galangin (Gal) on dactinomycin induced hepatotoxicity *in vitro*.

Materials and Methods: AML-12 cell line was divided into 4 groups as the control, Gal, dactinomycin, and Gal+dactinomycin groups. IC50 dose was determined by the thiazolyl blue tetrazolium bromide test. Gene expressions of glutathione (GSH), superoxide dismutase (SOD), catalase, caspase 3 (Cas-3), Cas-9, apoptotic protease activating factor-1 (Apaf-1), B cell CLL/lymphoma-2 (Bcl-2), Bcl-2 associated X protein (Bax), tumor protein p53 (p53), second mitochondria-derived activator of caspase/direct inhibitor of apoptosis-binding protein (smac/DIABLO), topoisomerase (Top) I, and Top II were determined with quantitative real-time polymerase chain reaction analysis.

Results: Dactinomycin elevated the expression of SOD, catalase, and GSH in response to oxidative effects. In the Gal+dactinomycin group, Gal administration reduced Apaf-1 expression and increased Bcl-2 expression with antiapoptotic effects. In the dactinomycin group, p53 levels increased due to the defense mechanism against DNA damage. Gal increased smac/DIABLO expression to remove damaged structures. Bcl-2 and smac/DIABLO expression levels in the groups were inversely proportional. In the Gal+dactinomycin group, Top II expression level was lower than in the dactinomycin group. This result indicated that double strand of DNA damage was diminished by Gal.

Conclusion: Gal protected against the hepatotoxicity due to dactinomycin with antioxidant and antiapoptotic effects. Further experimental studies are needed to establish the use of Gal in liver damage.

Keywords: Dactinomycin, galangin, AML-12 cell line, hepatotoxicity, oxidative stress, apoptosis

ÖZ

Amaç: Bu çalışmanın amacı galangin (Gal) daktinomisin kaynaklı hepatotoksisite üzerindeki etkilerini *in vitro* olarak incelemektir.

Gereç ve Yöntem: AML-12 hücre hattı, kontrol, Gal, daktinomisin ve Gal+daktinomisin olmak üzere 4 gruba ayrıldı. IC50 dozu tiazolil mavi tetrazolyum bromid yöntemi ile belirlendi. Glutatyon (GSH), süperoksid dismutaz (SOD), katalaz, kaspaz-3 (Cas-3), Cas-9, apoptotik proteaz aktive edici faktör 1 (Apaf-1), Bcl-2, Bax, p53, apoptoz bağlayıcı protein inhibitörü (smac/DIABLO), topoizomeraz (Top) I ve Top II gen ekspresyonları Kantitatif gerçek zamanlı polimeraz zincir reaksiyonu analizi ile incelendi.

Bulgular: Daktinomisin, oksidatif etkilere yanıt olarak SOD, katalaz ve GSH ekspresyonunu artırdı. Gal+daktinomisin grubunda Gal uygulaması, antiapoptotik etkilerle Apaf-1 ekspresyonunu azaltırken, Bcl-2 ekspresyonunu artırdı. Daktinomisin grubunda DNA hasarına karşı savunma mekanizması nedeniyle p53 seviyeleri arttı. Gal, hasarlı yapıları kaldırmak amacıyla smac/DIABLO ekspresyonunu artırdı. Gruplardaki Bcl-2 ve smac/DIABLO ekspresyon seviyeleri ters orantılı idi. Gal+daktinomisin grubunda Top II ekspresyon düzeyi, daktinomisin grubuna göre daha düşük bulundu. Bu durum, çift sarmal DNA hasarının Gal tarafından azaltıldığını göstermektedir.

Anahtar Kelimeler: Daktinomisin, galangin, AML-12 hücre hattı, hepatotoksisite, oksidatif stres, apoptoz

Address for Correspondence: Melek AKINCI MD, Trakya University Faculty of Pharmacy, Department of Pharmacology, Edirne, Turkey
Phone: +90 505 896 45 58 **E-mail:** melektamer@trakya.edu.tr **ORCID ID:** orcid.org/0000-0003-3879-4232
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INTRODUCTION

Dactinomycin is a cytotoxic drug with a polypeptide structure that has anti-cancer activity derived from *Streptomyces parvulus*. Dactinomycin is used to treat Wilms tumor in children[1]. Dactinomysin's common side effects include myelosuppression, mucositis, and hepatotoxicity[1,2]. The risk of hepatotoxicity increases when dactinomycin is combined with radiation therapy[3]. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were found to be elevated in the serum of individuals receiving dactinomycin treatment. The risk of hepatotoxicity is higher with dactinomycin therapy, especially in young children[4,5]. Obliteration of hepatic veins, severe hepatic obstruction, portal hypertension, and hepatocyte destruction are among the detrimental effects of dactinomycin that lead to liver injury[6].

In order to avoid the many adverse effects of anticancer medications on the liver and to cure hepatotoxicity if it has already occurred, phytotherapy is thought to be a useful strategy. Plants ameliorate hepatotoxicity through a variety of mechanisms. Hepatoprotective effects could be found in several phytochemicals, including flavonoids, monoterpenes, and phenols. A bioactive flavonoid called galangin (Gal) is present in honey and *Alpinia officinarum*. Gal has been demonstrated to be well tolerated and safe in rodents without adverse effects[7]. Gal has been found to have antioxidant and anti-inflammatory properties in many experimental animal studies. In addition, Gal protected against carbon tetrachloride (CCl_4)-induced hepatotoxicity and fibrosis by reducing oxidative stress and inhibiting the activation and proliferation of hepatic stellate cells. Gal was also found to ameliorate apoptosis via modulating antioxidant defense mechanisms in rats with ischemia-reperfusion (I/R)-induced liver damage. Gal has been shown to reduce oxidative stress and sustain mitochondrial activity in diabetic rats, thereby alleviating liver damage[8].

Although some pharmacological effects of Gal have been investigated, there are no studies on its ability to prevent dactinomycin-induced liver injury in the AML-12 cell line. Therefore, in our study, we investigated the potential of Gal to prevent the hepatotoxicity of dactinomycin.

MATERIALS AND METHODS

Groups

Our study was planned into 4 groups: control, Gal, dactinomycin, and Gal+dactinomycin.

Chemicals

The Gal and dactinomycin solutions were prepared in an aqueous solution containing 0.01% DMSO, and the Gal+dactinomycin mixture was prepared in a 1:1 ratio.

Cell Culture

AML-12 cells (ATCC®, CRL-2254 TM) were grown in Eagle's Minimum Essential Medium, Dulbecco's Modified Eagle's Medium, Ham's F-12 growth medium supplemented with 5% fetal bovine serum, 100 IU/mL penicillin, 10 mg/mL streptomycin and 1% L-glutamine. They were placed in the incubator that contained 95% moisture and 5% CO_2 at 37 °C. Our study started in the 5th passage and ended in the 12th passage.

Determination of Substance Concentrations to Be Administered to Cell Lines by Thiazolyl Blue Tetrazolium Bromide Method

180 µL cell culture medium was inoculated in 96 well plates with $1x10^5$ cells in each well to calculate IC_{50} values for all groups to be used in the research. After the incubation of 24 hours, substances were administered to the related groups except for the control group and doses are shown at Figure 1 (in a volume of 20 µL). Then, all groups were left in the incubator (37 °C, 5% CO_2) for 24 hours. The control group was administered an aqueous solution containing 0.01% DMSO. MTT solution (20 µL, 5 mg/mL) was added to each well. After 3 hours, DMSO (200 µL) was added to dissolve formazan crystals. The absorption value was measured using a microplate scanner at 492 nm (Thermo Scientific Multiskan Go). The control group was regarded 100% alive and the IC_{50} dose was calculated by probit analysis. MTT test was run in four replicates in all groups.

RNA Isolation and cDNA Synthesis

AML-12 cells were inoculated 3 times in culture plates to have $3x10^6$ cells in each well. After 24 hours, chemicals were administered at the dose of AML-12 cell IC_{50}. RNA was isolated (PureLink RNA Mini Kit) from the obtained cells according to the manufacturer's instructions. Concentrations and purity values of the obtained RNA samples were determined with nanodrop (NaNoQ OPTIZEN). cDNA synthesis was carried out from RNA samples (high capacity cDNA reverse transcription kit) according to the manufacturer's instructions.

Quantitative Real-Time Polymerase Chain Reaction Analysis

Quant Studio 6 Flex device of SYBR Select Master Mix was used for quantitative real-time polymerase chain reaction (qRT-PCR) analysis of gene expressions of the cells associating with SOD, CAT, GSH and gene expressions of the cells associating with caspase (Cas-3), Cas-9, Apaf-1, Bcl-2 associated X protein (Bax), B cell CLL/lymphoma-2 (Bcl-2), tumor protein p53 (p53), smac/DIABLO, Top I, Top II. PCR conditions were determined as: 1 cycle was 2 minutes at 50 °C, 10 minutes at 95 °C, afterwards 50 cycles for denaturation were 15 seconds

at 95 °C, and 1 second at 60 °C for annealing and extension. Comparative cycle threshold (2-ΔΔCt) method (User Bulletin 2, Applied Biosystems) was performed for the analysis of mRNA expression levels. To obtain a copy of the GSH gene sequences was selected "Nucleotide" from the National Center for Biotechnology Information. After that, relevant organism/gene name was entered in the search box, and FASTA was determined and the relevant genes were designed. Relative fold-changes in gene expression were calculated by comparing the experimental groups to the control group and were normalized to the expression of β-actin mRNA (Table 1).

Statistical Analysis

IC_{50} value was calculated by applying probit analysis to percent viability data obtained by MTT test. One-way ANOVA test and post hoc Tukey were administered to the relative fold-change values of gene expressions. Values at $p<0.05$ were accepted to be significant. Probit analysis and ANOVA test were done with Statistical Package for the Social Sciences 20 software (IBM).

Table 1. Primer sequences of analyzed genes for qRT-PCR analysis

Gene	Primer sequences (forward/reverse)
SOD	F: AGCTGCACCACAGCAAGCAC[8] R: TCCACCACCCTTAGGGCTCA
CAT	F: TCCGGGATCTTTTTAACGCCATTG[9] R: TCGAGCACGGTAGGGACAGTTCAC
GSH	F: ACTTGGCACTCCTCTCCTGA R: AGGCACTAGAACCTGCTGGA
Cas-3	F: GGTATTGAGACAGACAGTGG[10] R: CATGGGATCTGTTTCTTTGC
Cas-9	F: GAGTCAGGCTCTTCCTTTG[10] R: CCTCAAACTCTCAAGAGCAC
Apaf-1	F: GATATGGAATGTCTCAGATGGCC[11] R: GGTCTGTGAGGACTCCCCA
Bax	F: TTCATCCAGGATCGAGCAGA[10] R: GCAAAGTAGAAGGCAACG
Bcl-2	F: ATGTGTGTGGAGAGCGTCAA[10] R: ACAGTTCCACAAAGGCATCC
p53	F: CACGAGCGCTGCTCAGATAGC[10] R: ACAGGCACAAACACGCACAAA
Smac/DIABLO	F: CTCTGTGGCTGAGGGTTGAT[12] R: TTGTAGATGATGCCCACAGG
Top I	F: TCATACTGAACCCCAGCTCC[10] R: GTCCTGCAAGTGCTTGTTCA
Top II	F: CTTCTCTGATATGGACAAACATAAGATTCC[10] R: GGACTGTGGGACAACAGGACAATAC

SOD: Superoxide dismutase, GSH: Glutathione, Cas: Caspase, qRT-PCR: Quantitative real-time polymerase chain reaction

RESULTS

In order to investigate the effects of Gal, dactinomycin and Gal+dactinomycin on the viability of AML-12 cell lines, MTT assays were performed for 24 hours. MTT assay results detected that Gal, dactinomycin and Gal+dactinomycin reduced the cell viability on the AML-12 cell line that was dependent on the dose (Figure 1). IC_{50} doses were identified as 30.354 μM in Gal, 2.853 in dactinomycin and 3.262 μM in Gal+dactinomycin.

SOD mRNA expression levels increased in the dactinomycin (281,43-folds) and Gal+dactinomycin (15.04-folds) groups compared to the control group ($p<0.05$). SOD mRNA expression levels were elevated in the dactinomycin group (33.62-folds) compared to the Gal group ($p<0.05$) (Figure 2A).

Dactinomycin group showed an increase in CAT mRNA expression in response to the control (58.86-folds) and Gal groups (5.98-folds) ($p<0.05$) (Figure 2B).

The Gal (20,88 folds), dactinomycin (75-folds), and Gal+dactinomycin (76.92-folds) groups all showed an increase in GSH mRNA expression compared to controls ($p<0.05$). Compared to the Gal group, GSH mRNA expression levels in the dactinomycin (3.61-folds) and Gal+dactinomycin (3.7-folds) groups were increased ($p<0.05$) (Figure 2C).

When compared to the control, Cas-3 mRNA expression increased in the Gal (6.89-folds) and Gal+dactinomycin (5.68-folds) groups ($p<0.05$). Compared to the Gal group, dactinomycin group (4.82-folds) showed a decrease in Cas-3 mRNA expression ($p<0.05$) (Figure 2D).

In the Gal (12.12-folds) and Gal+dactinomycin (4.25-folds) groups, Cas-9 mRNA expression increased in comparison to control ($p<0.05$). In the dactinomycin (8.78-folds) and Gal+dactinomycin (2.85-folds) groups, Cas-9 mRNA expression decreased in comparison to the Gal group ($p<0.05$) (Figure 2E).

In comparison to the control and Gal groups, Apaf-1 mRNA expression was higher in the dactinomycin (30.34-folds and 14.81-folds) and Gal+dactinomycin (62.61-folds and 10.26-folds) groups ($p<0.05$) (Figure 2F).

When compared to the control, Bax mRNA expression increased in the Gal (11.90-folds), dactinomycin (12.19-folds), and Dactinomycin+Gal (14.04-folds) groups ($p<0.05$) (Figure 3A).

In comparison to the control group, Bcl-2 mRNA expression elevated in the Gal+dactinomycin group (2.43-folds) whereas it dropped in the Gal group (0.45-folds) ($p<0.05$). When compared to the Gal group, Bcl-2 mRNA expression was higher in the dactinomycin (2.11-folds) and Gal+dactinomycin (5.4-folds) groups (Figure 3B).

p53 mRNA expression increased in all groups when compared to the control group (54.55-folds for Gal group, 201.59-folds for dactinomycin group, 11.99-folds for Gal+dactinomycin group) ($p<0.05$). p53 mRNA expression increased in the dactinomycin group (3.64-folds), and decreased in the Gal+dactinomycin group (4.72-folds) compared to the Gal group ($p<0.05$) (Figure 3C).

In comparison to the control group, Smac/DIABLO mRNA expression increased in all groups (105.12-folds for Gal group, 59.75-folds for dactinomycin group, 56.55-folds for Gal+dactinomycin group) ($p<0.05$). In comparison to the Gal group, Smac/DIABLO mRNA expression was reduced in the dactinomycin (1.76-folds) and Gal+dactinomycin (2.26-folds) groups ($p<0.05$) (Figure 3D).

When compared to the control group and the Gal group, the expression of the top 1 mRNA was higher in the dactinomycin (3.13-folds and 3.52-folds) and Gal+dactinomycin groups (6.83-folds and 7.67-folds) ($p<0.05$) (Figure 3E).

Top II mRNA expression was higher in the dactinomycin group (1.58-folds) than in the control group ($p<0.05$). Top II mRNA expression was increased in the dactinomycin group (1.98-folds) compared to the Gal group ($p<0.05$) (Figure 3F).

DISCUSSION

Dactinomycin has serious toxic effects that could lead to hepatic injury. Depending on the dosage, combination therapy and conditions related to patients, dactinomycin elevates serum AST and ALT levels. This clinical situation could progress to liver damage[13]. One of the most significant and natural flavonoids is the polyphenolic molecule Gal[14]. In addition to Gal's beneficial effects in the treatment of cancer, it has been shown to be antigenotoxic against chemotherapy and radiotherapy[14,15]. Our study has demonstrated that Gal provides hepatoprotective effects against dactinomycin-induced injury *in vitro*.

Antioxidant enzymes present in cells include SOD and CAT. SOD converts superoxide anion to H_2O_2, which then is reduced to H_2O by CAT. Moreover, the antioxidant compound GSH

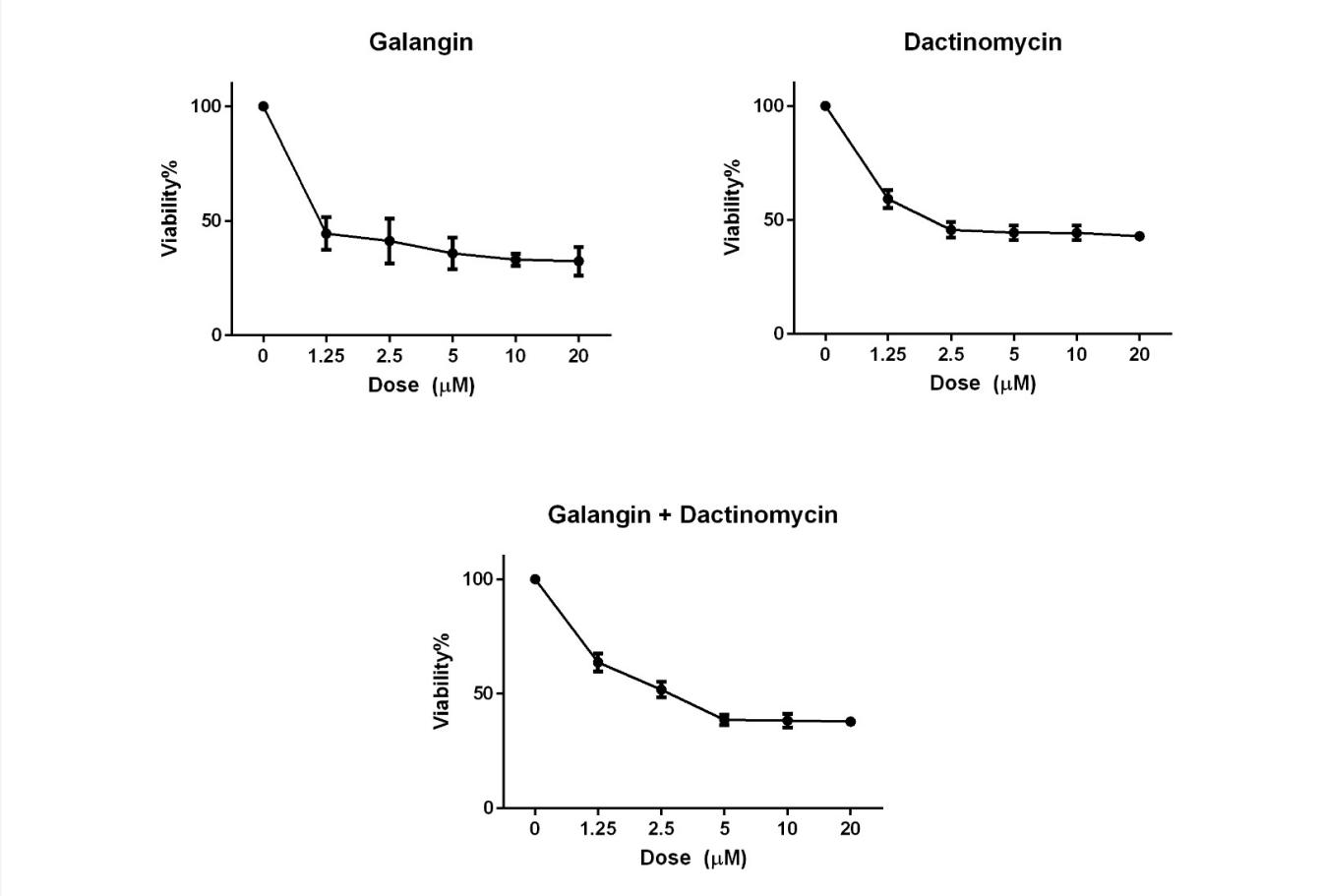

Figure 1. MTT assay results of each treatment group. Vertical bars represent standard deviation (n=4, mean±standard deviation) (viability %=Sample absorbance average / control absorbance average × 100)

reacts with free radicals, and it generates oxidized glutathione. According to the previous studies, Gal has antioxidant activity[16]. Supportively, in our study, Gal administration has elevated antioxidant enzymes SOD and CAT mRNA expression. Furthermore, it has increased GSH mRNA expression. According to our results, dactinomycin has also elevated SOD, CAT, and GSH mRNA expressions. This situation could be dependent on the response to oxidant effects of dactinomycin, since the cell

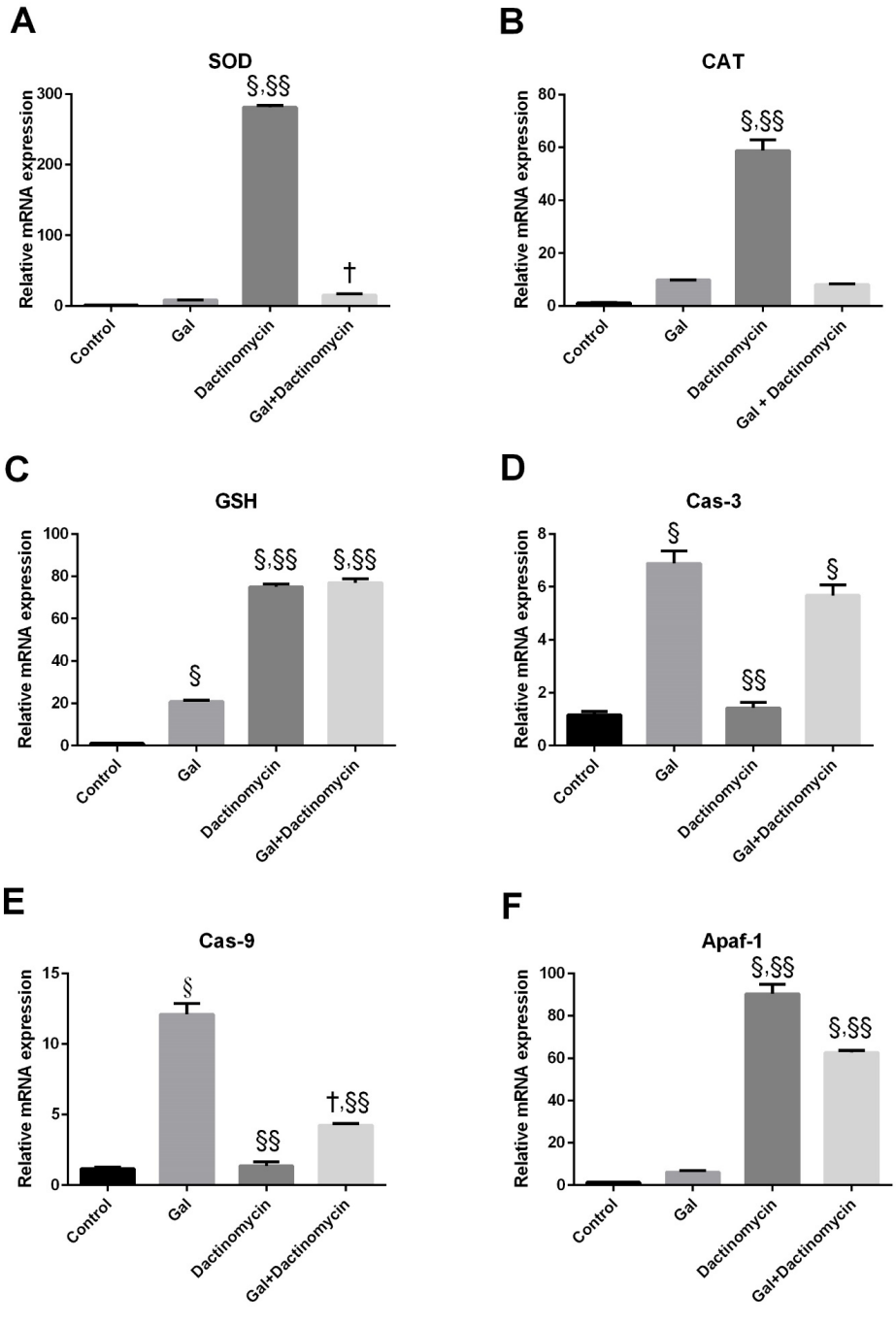

Figure 2. SOD (A), CAT (B), GSH (C), Cas-3 (D), Cas-9 (E), and Apaf-1 (F) relative mRNA expression

*p<0.05, †p<0.01, ‡p<0.001, §p<0.0001 compared to the control group; **p<0.05, ††p<0.01, ‡‡p<0.001, §§p<0.0001 compared to the Gal group

SOD: Superoxide dismutase, GSH: Glutathione, Cas: Caspase

was able to raise the antioxidant activity. Due to the protective effects of Gal, in the Gal+dactinomycin group, SOD and CAT mRNA expressions wer lower than in the dactinomycin group.

Apoptosis is the term for the controlled cell death that maintains equilibrium in living organisms. Apoptosis plays an important role in various diseases' progression. Dactinomycin triggers apoptosis in many tissues, which has positive effects in chemotherapy. However, apoptosis could also play a role in adverse effects, such as hepatic injury. During the apoptosis in cells, mitochondria releases cytochrome-c which generates a complex with Apaf-1, and ATP. Thereby in the cytosol, an

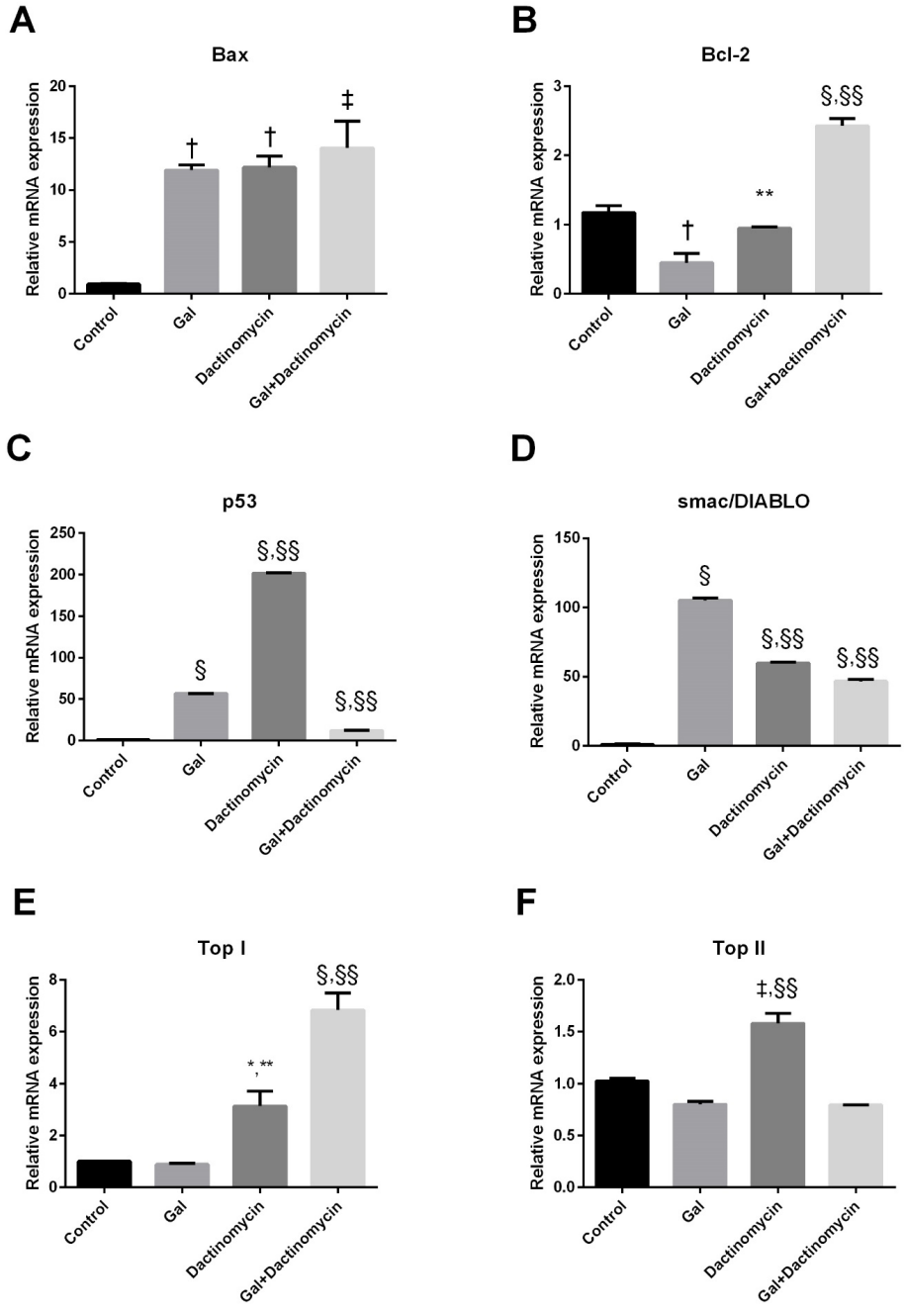

Figure 3. Bax (A), Bcl-2 (B), p53 (C), smac/DIABLO (D), Top I (E), and Top II (F) relative mRNA expression

*p<0.05, †p<0.01, ‡p<0.001, §p<0.0001 compared to the control group; **p<0.05, ††p<0.01, ‡‡p<0.001, §§p<0.0001 compared to the Gal group

Gal: Galangin

apoptosome complex is produced, which activates inhibitor Cas, and Cas-9. Moreover, Cas-9 activates Cas-3 which is an effector Cas. According to our results, in the dactinomycin group, Apaf-1 mRNA expression levels increased significantly; however, expression levels of Cas-3 and Cas-9 were not elevated. This condition indicates that liver damage-related apoptosis pathway has not progressed up to Cas-9 and Cas-3[17].

Bax is a protein that provides a proapoptotic effect. In our study, Gal and dactinomycin increased Bax gene expression with respect to the control group and induced apoptosis. Supportively, Zhang et al.[18] demonstrated that Gal triggerred apoptosis through elevation in Bax levels. Bcl-2 is one of the antiapoptotic proteins. According to our results, in the Gal+dactinomycin group, Bcl-2 expression levels were higher than in the dactinomycin group, which indicates that Gal has exerted an antiapoptotic effect. P53 is a protein that provides DNA repair. In the dactinomycin group, p53 levels increased due to the defense mechanism against DNA damage. Furthermore, it has been known that p53 could sensitize the cells to apoptosis by increasing Apaf-1 levels. Supportively, according to our results, in the dactinomycin group, both of the p53 and Apaf-1 levels were increased[19]. Moreover, Gal elevated p53 levels. According to literature, Gal increases p53 levels that prompts cell autophagy and alleviates cellular metabolic stress[20].

During the apoptosis, mitochondria releases smac/DIABLO that exacerbates apoptosis depending on decreasing IAPs inhibition on caspases[21]. Supportively, in our study, elevated smac/DIABLO expression with Gal administration provides removing damaged structures. smac/DIABLO release is attenuated by Bcl-2[22]. In this regard, results of our study have exhibited that Bcl-2 and smac/DIABLO transcript levels in the groups are inversely proportional. In a recent study, similar results have been obtained with ellagic acid, which is a phenolic compound. It has been shown that ellagic acid elevates smac/DIABLO expression in a dose-dependent manner that provides anti-cancer activity[23].

DNA-topoisomerases are essential enzymes for regulating DNA structure and metabolism, including DNA replication, transcription, and chromosomal segregation. Top I enzymes are typically monomers that break the single strand of the DNA double helix. Top II enzymes with two or more subunits are capable of breaking both strands of DNA's double helix[24]. Various situations as many diseases, oxidative stress and inflammation could evelate the topoisomerase levels[25]. According to our results, when Gal was administered with dactinomycin, Top II level reduced with respect to the dactinomycin group. This result has indicated that double strand of DNA damage is diminished by Gal. However, the amount of Gal administered along with dactinomycin was inadequate to repair the single strand of DNA damage, since Top I level in Gal+dactinomycin group was detected to be increased.

CONCLUSION

Our study has demonstrated that Gal could protect against hepatotoxicity due to dactinomycin through alleviating the oxidative stress, abnormalities in apoptotic pathways, and double strand of DNA damage in the liver tissue. Further experimental studies are needed in order to use the Gal in hepatotoxicity treatment.

Ethics

Ethics Committee Approval and Informed Consent: Since our study is a cell culture study, Ethics Committee Form and Informed Consent Form are not required.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: M.A., Ç.O., E.B., Design: M.A., Ç.O., E.B., Data Collection or Processing: M.A., Ç.O., E.B., Analysis or Interpretation: M.A., Ç.O., E.B., Z.A.Ç.Y., Literature Search: M.A., E.B., Z.A.Ç.Y., Writing: M.A., Z.A.Ç.Y.

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. Temel MK. Sitotoksik kemoterapötiklerin yirminci yüzyıldaki gelişimi. Türk Onkoloji Dergisi. 2015;30:96-108.
2. Baskin Y, Amirfallah A, Calibasi G, Olgun N. Hepatopathy-Thrombocytopenia Syndrome During Actinomycin D Treatment May Be Related to MDR1 (ABCB1) Gene Polymorphisms. Am J Ther. 2016;23:e594-6.
3. Farruggia P, Macaluso A, Tropia S, Di Marco F, Russo D, Grigoli A, et al. Hepatopathy-thrombocytopenia syndrome (HTS) after actinomycin-D therapy: report of three cases and review of the literature. Pediatr Hematol Oncol. 2011;28:237-43.
4. Raine J, Bowman A, Wallendszus K, Pritchard J. Hepatopathy-thrombocytopenia syndrome--a complication of dactinomycin therapy for Wilms' tumor: a report from the United Kingdom Childrens Cancer Study Group. J Clin Oncol. 1991;9:268-73.
5. Barnett KT, Malafa MP. Complications of hepatic artery infusion: a review of 4580 reported cases. Int J Gastrointest Cancer. 2001;30:147-60.
6. Sandrasegaran K, Alazmi WM, Tann M, Fogel EL, McHenry L, Lehman GA. Chemotherapy-induced sclerosing cholangitis. Clin Radiol. 2006;61:670-8.
7. Abbaszadeh H, Keikhaei B, Mottaghi S. A review of molecular mechanisms involved in anticancer and antiangiogenic effects of natural polyphenolic compounds. Phytother Res. 2019;33:2002-14.
8. Aladaileh SH, Abukhalil MH, Saghir SAM, Hanieh H, Alfwuaires MA, Almaiman AA, et al. Galangin Activates Nrf2 Signaling and Attenuates Oxidative Damage, Inflammation, and Apoptosis in a Rat Model of Cyclophosphamide-Induced Hepatotoxicity. Biomolecules. 2019;9:346.

9. Dkhil MA, Moneim AE, Al-Quraishy S. Indigofera oblongifolia Ameliorates Lead Acetate-Induced Testicular Oxidative Damage and Apoptosis in a Rat Model. Biol Trace Elem Res. 2016;173:354-61.

10. Oltulu C, Akıncı M, Elvan B. Antitumor Activity of Etoposide, Puerarin, Galangin and Their Combinations in Neuroblastoma Cells, International Journal of Life Sciences and Biotechnology. 2022;5:407-23.

11. Yakovlev AG, Ota K, Wang G, Movsesyan V, Bao WL, Yoshihara K, et al. Differential expression of apoptotic protease-activating factor-1 and caspase-3 genes and susceptibility to apoptosis during brain development and after traumatic brain injury. J Neurosci. 2001;21:7439-46.

12. Tokatlı C, Doğanlar O, Doğalar ZB. Meriç Delta Balıklarında Çevre Kirliliğinin Genotoksik Etkileri: Antioksidan Savunma, Isı Şok Protein Sinyali ve DNA Hasar-Onarım Mekanizmaları. Journal of Limnology and Freshwater Fisheries Research. 2020;6:14-24.

13. Dactinomycin. NCBI Bookshelf. 2017. (Accessed 10 April 2023).

14. Fang D, Xiong Z, Xu J, Yin J, Luo R. Chemopreventive mechanisms of galangin against hepatocellular carcinoma: A review. Biomed Pharmacother. 2019;109:2054-61.

15. Rampogu S, Gajula RG, Lee KW. A comprehensive review on chemotherapeutic potential of galangin. Biomed Pharmacother. 2021;141:111808.

16. Bacanlı M, Başaran AA, Başaran N. The antioxidant, cytotoxic, and antigenotoxic effects of galangin, puerarin, and ursolic acid in mammalian cells. Drug Chem Toxicol. 2017;40:256-62.

17. Ali N, Rashid S, Nafees S, Hasan SK, Sultana S. Beneficial effects of Chrysin against Methotrexate-induced hepatotoxicity via attenuation of oxidative stress and apoptosis. Mol Cell Biochem. 2014;385:215-23.

18. Zhang HT, Luo H, Wu J, Lan LB, Fan DH, Zhu KD, et al. Galangin induces apoptosis of hepatocellular carcinoma cells via the mitochondrial pathway. World J Gastroenterol. 2010;16:3377-84.

19. Shakeri R, Kheirollahi A, Davoodi J. Apaf-1: Regulation and function in cell death. Biochimie. 2017;135:111-25.

20. Wen M, Wu J, Luo H, Zhang H. Galangin induces autophagy through upregulation of p53 in HepG2 cells. Pharmacology. 2012;89:247-55.

21. Roberts DL, Merrison W, MacFarlane M, Cohen GM. The inhibitor of apoptosis protein-binding domain of Smac is not essential for its proapoptotic activity. J Cell Biol. 2001;153:221-8.

22. Adrain C, Creagh EM, Martin SJ. Apoptosis-associated release of Smac/DIABLO from mitochondria requires active caspases and is blocked by Bcl-2. EMBO J. 2001;20:6627-36.

23. Abubakar MB, Abdullah WZ, Sulaiman SA, Suen AB. A review of molecular mechanisms of the anti-leukemic effects of phenolic compounds in honey. Int J Mol Sci. 2012;13:15054-73.

24. Mastrangelo S, Attina G, Triarico S, Romano A, Maurizi P, Ruggiero A. The DNA-topoisomerase Inhibitors in Cancer Therapy. Biomedical and Pharmacology Journal. 2022;15:553-62.

25. Rialdi A, Campisi L, Zhao N, Lagda AC, Pietzsch C, Ho JSY, et al. Topoisomerase 1 inhibition suppresses inflammatory genes and protects from death by inflammation. Science. 2016;352:aad7993.

ORIGINAL ARTICLE

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In Which Conditions is Conservative Tratment Likely to Fail in Humeral Shaft Fractures? A Retrospective Analysis of 67 Patients

Humerus Cisim Kırıklarında Hangi Durumlarda Konservatif Tedavinin Başarısız Olma İhtimali Vardır? 67 Hastanın Retrospektif Analizi

Mehmet ALBAYRAK[1], Fatih UĞUR[2]

[1]*İstanbul Rumeli University Health Services Vocational School Therapy and Life Department Physiotherapy Program, İstanbul; Tekirdağ Yaşam Hospital, Clinic of Orthopedics and Traumatology, Tekirdağ, Turkey*
[2]*Kastamonu University Faculty of Medicine, Department of Orthopedics and Traumatology, Kastamonu, Turkey*

ABSTRACT

Aim: Humeral shaft fractures are one of the most common fractures of the upper extremity. Most humeral shaft fractures can be treated conservatively, but in some cases, surgical treatment is required. In this study, the characteristics of patients who needed surgical intervention due to unsuccessful conservative treatment and inability to achieve union were investigated.

Materials and Methods: Data of 67 patients [female (n=41) and male (n=26)] in whom conservative treatment was initiated due to humeral shaft fracture were evaluated retrospectively. The data of the patients in whom treatment had to be switched to surgery during the follow-up period were analyzed. Age, gender, trauma type, fracture site and vitamin D levels on the day of the fracture were noted.

Results: At the end of 54 (42-77) days, the treatment was switched to surgery due to nonunion in 14 patients. All of these patients were female, over the age of 60 years, and the humeral fractures in these patients were in the middle third of the humerus. The mean 25(OH) vitamin D [25(OH) D] level of these patients was 14 mg/dL (4-22 mg/dL).

Conclusion: It is concluded that the conservative treatment is prone to fail if humeral shaft fracture is a proximal oblique or a mid-shaft one, if the patient is over the age of 60 years and female, and if 25(OH)D level of the patient is low.

Keywords: Conservative, humerus fracture, surgery

ÖZ

Amaç: Humerus şaft kırıkları üst ekstremitenin sık görülen kırıklarından biridir. Humerus şaft kırıklarının büyük bir kısmı konservatif yöntemlerle tedavi edilebilir ancak bazı durumlarda cerrahi tedavi gereksinimleri mevcuttur. Bu çalışmada konservatif tedavinin başarısız olduğu ve kaynamanın elde edilememesi nedeniyle cerrahi girişime ihtiyaç doğan hastaların özellikleri araştırıldı.

Gereç ve Yöntem: Humerus cisim kırığı nedeniyle konservatif tedavilerine başlanan 67 hastanın [kadın (n=41) ve erkek (n=26)] verileri retrospektif olarak değerlendirildi. Takip sürecinde cerrahi tedaviye geçiş yapılmak durumunda kalınan hastaların verileri incelendi. Yaş, cinsiyet, travma tipi, kırık yeri ve kırığın olduğu gündeki D vitamini düzeyleri not edildi.

Bulgular: On dört hastada 54 (42-77) gün sonunda kaynamama nedeniyle cerrahi tedaviye geçiş yapıldığı saptandı. Bu hastaların tamamı 60 yaş üzeri kadın hastalardı ve bu hastalardaki humerus kırıkları humerusun orta üçte birlik kısmındaydı. Cerrahi tedaviye geçiş yapılan hastaların 25(OH) vitamin D [25(OH)D] seviyeleri ortalama 14 mg/dL (4-22 mg/dL) idi.

Sonuç: Bu çalışma neticesinde humeral cisim kırığının proksimal yerleşimli ve oblik olması veya orta şaft yerleşimli olması, hastanın 60 yaş üstü kadın hasta olması ve hastanın 25(OH)D seviyesinin düşük olması durumlarında konservatif tedavinin başarısız olmaya daha yatkın olduğu sonucuna varıldı.

Anahtar Kelimeler: Konservatif, humerus kırığı, cerrahi

Address for Correspondence: Mehmet ALBAYRAK MD, İstanbul Rumeli University Health Services Vocational School Therapy and Life Department Physiotherapy Program, İstanbul; Tekirdağ Yaşam Hospital, Clinic of Orthopedics and Traumatology, Tekirdağ, Turkey
Phone: +90 533 660 50 13 **E-mail:** doktorm.albayrak@gmail.com **ORCID ID:** orcid.org/0000-0002-4074-7024
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INTRODUCTION

Humerus shaft fracture (HSF) is a common upper limb injury that makes up for 1-5% of all fractures[1,2]. In terms of incidence, HSFs usually show a bimodal distribution-the first peak is seen in young males aged 21-30 years mostly due to high-energy trauma, and the second peak is in older females aged 50-80 years due to low-energy trauma (most commonly due to fall from a standing height)[3].

Although there is a lack of consensus regarding the most appropriate treatment for HSFs-conservative[4] versus surgical intervention[5], conservative methods, which include applying a coaptation splint followed by a Sarmiento functional brace[6] or directly applying the functional brace[7], are preferred more in the literature. Regarding the surgical treatment of HSFs, there is a list of absolute and relative indications that are commonly accepted by trauma surgeons (Table 1)[8-10].

Age, gender, severity of trauma, presence of an open fracture and accompanying metabolic diseases are the factors that affect the union of the fracture[2,3]. In means of fracture type, some fracture patterns like short oblique fractures of humerus have relative indication for surgery, which means conservative treatment can be initiated primarily in these cases. In case of failure, such as nonunion in the follow-up, these fracture types may need surgical intervention. Throughout the follow-up period of conservative treatment, it is well known that patient compliance gradually decreases, and in case of necessity for a further surgical intervention when conservative treatment fails, surgery becomes much more demanding and technically challenging.

In this study, the characteristics of patients who needed surgical intervention due to unsuccessful conservative treatment and inability to achieve union were investigated. It was hypothesized that conservative treatment had a tendency to fail in proximal oblique shaft fractures. The effects of sex, age and vitamin D levels on the success or failure of conservative treatment were also evaluated.

MATERIALS AND METHODS

The study was approved by the Tekirdağ Namık Kemal University of Local Ethics Committee (protocol no: 2023.77.04.13, date: 25/04/2023).

In this retrospective study, we evaluated 84 patients treated for HSFs between January 2008 and December 2018 in Tekirdağ Yaşam Hospital by reviewing archive and radiology files. Among these, 17 patients who were operated on for absolute indications were excluded from the study. The remaining 67 patients were conservatively managed at the beginning of the treatment before surgical intervention. All patient characteristics are presented in Table 2.

On the day of admission, 25(OH) vitamin D levels were measured for all patients using a blood test. After radiologic investigations [anteroposterior (AP) and lateral view radiographs of the shoulder and arm], all included patients were initially managed conservatively using a coaptation splint, which was applied in the emergency room. The next day, a custom brace was made for each patient according to the dimensions taken from the uninjured arm. After one week, the coaptation splint was removed and the custom-manufactured brace was applied. All

Table 2. Data of the patients

Data	Number of patients
Male	26
Female	41
Total	67
Location on the shaft	
Proximal third	16
Middle third	39
Distal third	12
Total	67
Trauma type	
Major	11
Minor	56
Total	67

Table 1. Absolute and relative indications for surgical treatment of humerus shaft fractures

Absolute indications	Relative indications
- Open fracture - Vascular injury requiring repair - Brachial plexus injury - Ipsilateral forearm fracture (floating elbow) - Compartment syndrome - Periprosthetic humeral shaft fractures at the tip of the stem	- Bilateral humerus fracture - Polytrauma or associated lower extremity fracture - Pathologic fractures - Burns or soft tissue injury that precludes bracing - Fracture characteristics – Distraction at fracture site – Short oblique or transverse fracture pattern – Intraarticular extension

patients were followed up for the next two weeks using serial weekly X-rays. Criteria for acceptable alignment were listed as less than 20° apex anterior or posterior angulation, less than 30° varus/valgus angulation, maximum 15° malrotation and 3 cm of shortening[2]. The data of the patients in whom treatment had to be switched to surgery during the follow-up period were analyzed. Age, gender, trauma type and fracture site were noted.

In Figure 1, the radiograph of a 67-year-old female patient at the time of first admission is seen and in Figure 2, the radiograph of the same patient is seen, showing deterioration in the fracture alignment and nonunion on the 29th day. In Figure 3, the radiograph of the patient taken at the end of 4 weeks after the operation is seen.

In Figure 4, the radiograph of another 64-year-old female patient at the time of first admission is seen. Figure 5 shows the radiograph of the same patient, demonstrating deterioration in the fracture alignment and nonunion. AP and lateral radiographs of the same patient 3 weeks after the operation are seen in Figure 6 and in Figure 7, respectively.

Statistical Analysis

A binary logistic regression model was used to examine the effects of age, sex, fracture site, and fracture type on the union status of fractures, and the Wald test was performed to evaluate the collective significance of the aforementioned variables. Furthermore, to determine the differences in fracture healing according to sex, fracture type, and age, the Fisher's exact test was employed. A p value of <0.05 was used to determine statistical significance.

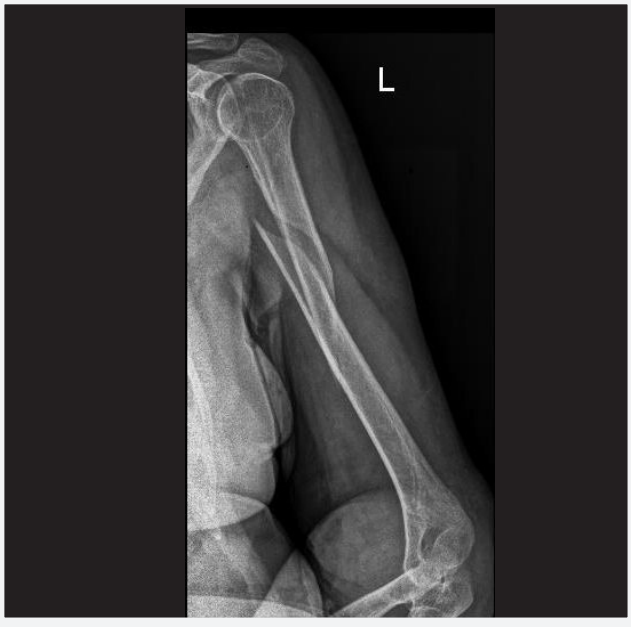

Figure 1. The radiograph of a 67-year-old female patient at the time of first admission

RESULTS

Sixteen of the fractures were located in the proximal third of the humerus, 39 in the middle third and 12 in the distal third. Eleven patients had fractures after major trauma like falling from height and the rest had fractures due to simple trauma or falls from a standing height. Out of the 67 patients, 53 healed without any complications and attained fracture union by the

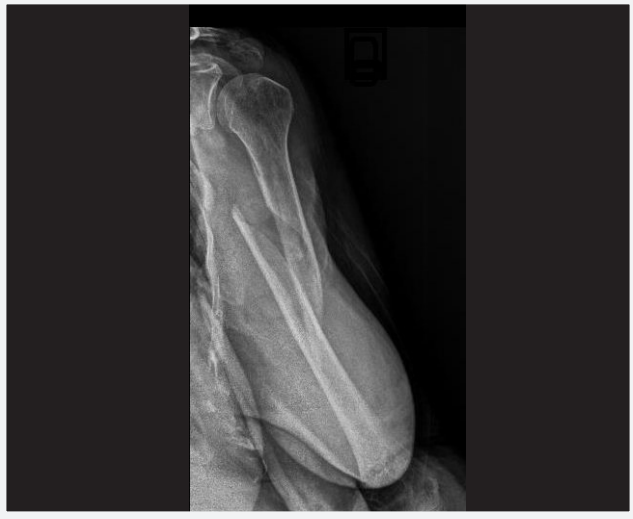

Figure 2. The radiograph of the same patient, taken on the 29th day, showing deterioration in the fracture alignment and nonunion

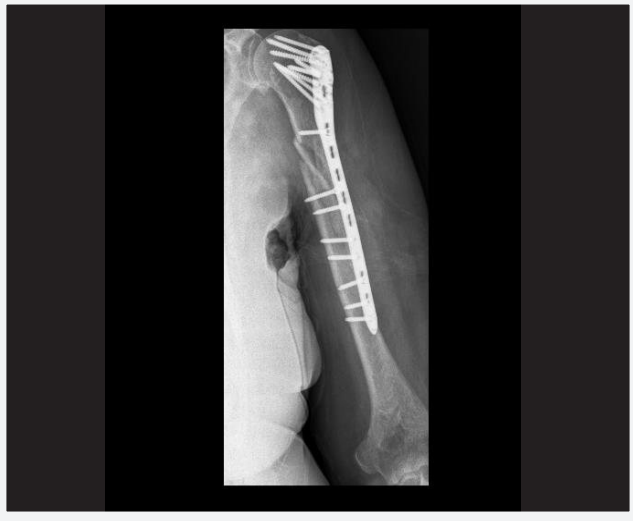

Figure 3. Follow-up radiograph of the patient at the 4th week postoperatively

end of the 8-10-week period conservatively. Radiographically, callus formation was visible between 4 and 6 weeks for all 53 patients, and bony alignment was within the acceptable range in both AP and lateral radiographs.

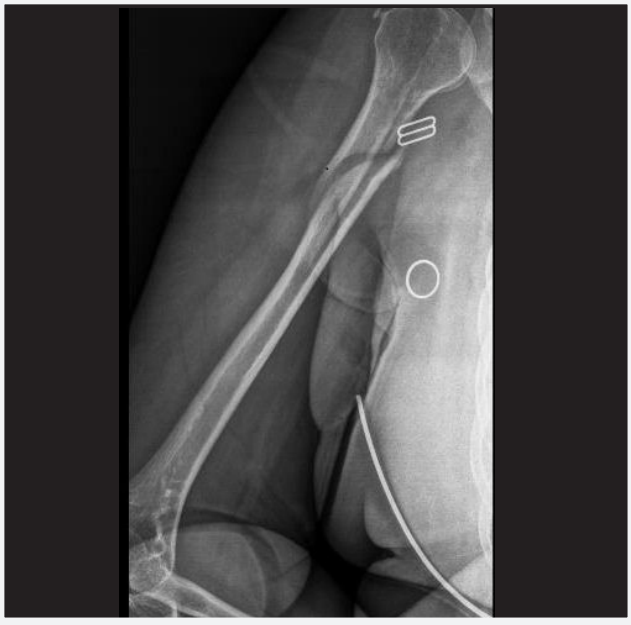

Figure 4. The radiograph of a 64-year-old female patient at the time of first admission

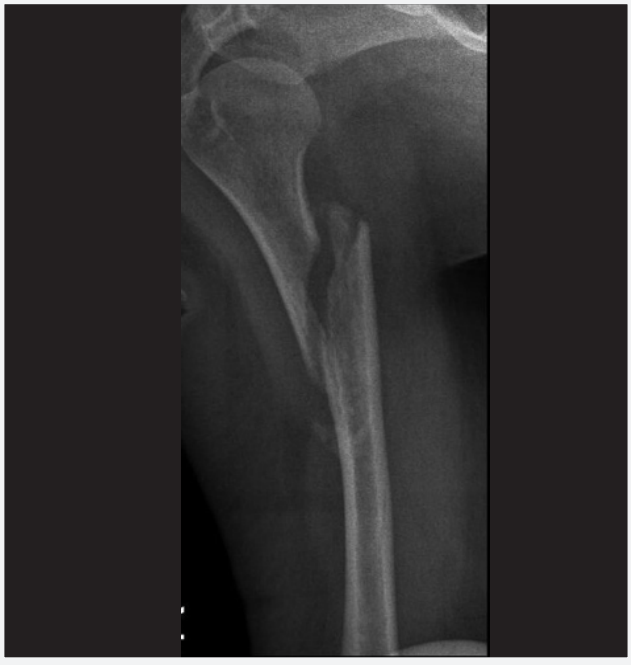

Figure 5. The radiograph of the same patient, taken on the 32nd day, showing deterioration in the fracture alignment and nonunion

The remaining 14 patients (20.89%), in whom conservative treatment had failed, were subsequently treated surgically. The average duration for determining the failure of conservative management and opting for surgery was 41 days (38±14 days).

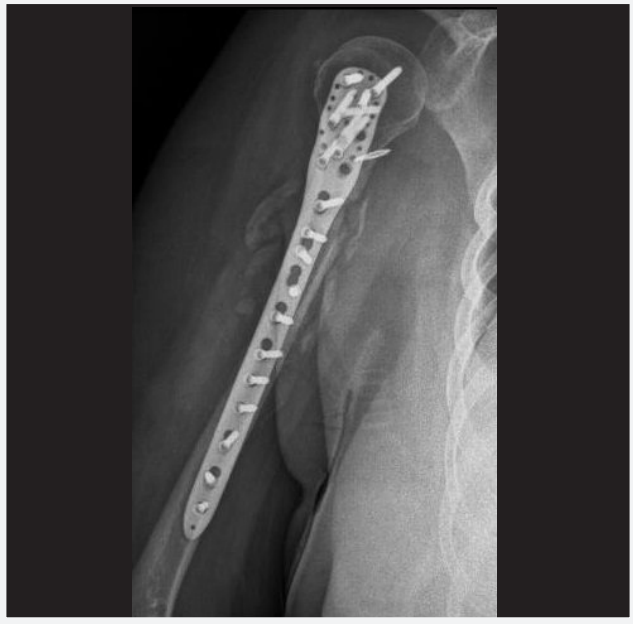

Figure 6. Follow-up AP radiograph of the patient at the 3rd week postoperatively

AP: Anteroposterior

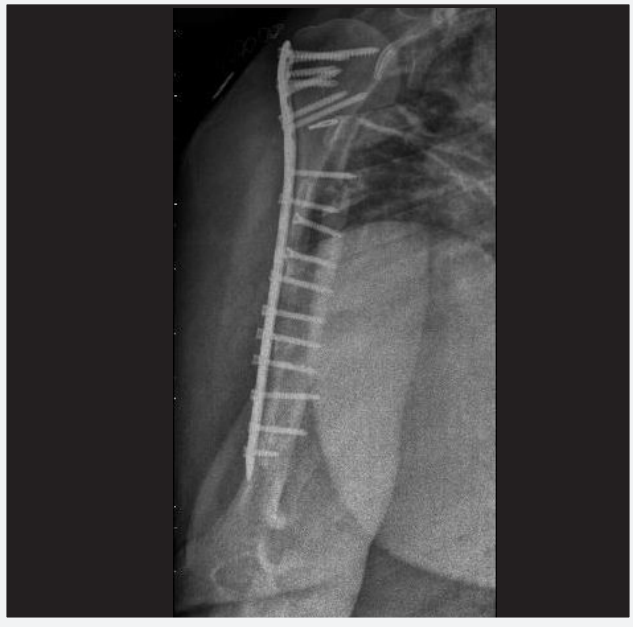

Figure 7. Follow-up lateral radiograph of the patient at the 3rd week postoperatively

The reasons for failure were malalignment and lack of adequate callus formation in the follow-up radiographs. Eventually, all 14 patients were treated with open reduction and internal fixation using plate and screws. Postoperatively, the patients were followed for 2 weeks, all fractures healed and showed radiographic union at the end of 10 weeks postoperatively. As for the complications, superficial wound infections, which were healed with a 15-day antibiotic treatment and dressings, were detected in two patients. In three patients, 30° of decrease in elbow total arc range of motion, which recovered with a 20-day physical therapy conducted at home and in the hospital, developed.

In terms of fracture location, 25% of the proximal one-third (n=4/16), 89.74% of the middle third (n=35/39), and 100% of the distal third (n=12/12) fractures were united using a functional brace. There was a statistically significant difference between the healing rates in the proximal and middle third fractures (p=0.039) and the proximal and distal third fractures (p=0.043) but the difference in the healing rates for the middle and distal third fractures was statistically not significant (p=0.051).

When comparing the conservatively treated (n=53) and surgically treated (n=14) groups, we found that the former had relatively younger patients (44±12 years), while the mean age for the surgically treated ones was 61±11 years, which was statistically significant (p=0.040). On the other hand, the mean vitamin D3 levels at the time of admission were significantly low in the surgically treated group compared to the conservatively treated group [14 mg/dL (16±8 mg/dL) versus 29 mg/dL (44±14 mg/dL); p=0.036].

When stratified according to sex, all male patients had bony unions while 33.3% of women had nonunion. On comparing different age groups, we found that all patients in age group of 16-59 years had union while 35% of the patients aged >60 years had no union. Additionally, there was no significant relationship between union status and trauma type, such as major or minor (p=0.061).

The Wald test performed to determine the effects of age, sex, and vitamin D levels revealed that a one-year increase in the age variable reduced the probability of fracture union by 0.836 times (β=-0.18, odds ratio=0.836, p=0.048). Furthermore, we found a significant relationship between fracture union status and sex (p=0.011) and age (p=0.044).

DISCUSSION

Functional treatment (in the form of splinting/bracing) is an integral part of the conservative management of HSFs, which usually yields satisfactory results by allowing appropriate stabilization to ensure fracture healing and patient compliance[11]. Although obesity and nonconformity of the patient to brace therapy are the primary impediments to conservative treatment[10,11], functional bracing is the first choice of treatment for HSFs[10-12]. In our study sample, all patients were first started with conservative treatment with splints followed by functional bracing, unless absolutely indicated for surgery. However, Denard et al.[5] stated that closed treatment of humerus fractures had a significantly higher rate of nonunion and malunion, and surgical treatment was better than conservative treatment without significant complication. In contrast, only 20.89% of our patients had nonunion or malunion with conservative management, which corroborates the choice of conservative treatment as the first-line treatment for HSF.

However, despite the success of conservative treatment for HSFs, many studies have pointed out that oblique fractures of the humerus, especially in the proximal one-third, require surgical intervention[4,12,13]. In our study, all of our patients who underwent surgery due to malunion or nonunion had oblique fractures in the proximal humerus. Furthermore, Koch et al.[14] stated that transverse fractures of the humerus are more prone to nonunion and often require surgery. However, in our study, transverse fractures healed adequately with conservative treatment, whereas the oblique fracture group required surgery.

Notably, previous studies with concurring evidence about proximal oblique HSFs being more prone to nonunion with conservative methods have also highlighted the role of the patient's age as important for nonunion[15,16]. In contrast, Ali et al.[4] found that nonunion could occur in this particular fracture type, irrespective of the patient's age. In our study, patients over the age of 60 years were particularly prone to nonunion after sustaining a proximal oblique HSF.

It has also been reported that the only criterion to ensure union in an HSF, regardless of its type according to the AO classification[16], is the fracture location within the shaft-proximal or not[11]. We also observed that patients with fractures in the proximal third of the humerus were likely to go into nonunion. It is uncertain why proximal third humerus fractures, especially the oblique type, do not unite conservatively and require surgery. Walker et al.[10] reasoned that this area was between the insertion points of two strong muscles, deltoid and pectoralis major, which tend to distract the fracture site by pulling in different directions. Therefore, if rotational alignment is maintained but there is a gap between the fracture ends, muscle interposition must be taken into consideration. It is well established that if there is a significant gap between fracture ends, the fracture tends to go into atrophic nonunion.

Similar to our study, Decomas and Kaye[17] also performed a multifactorial analysis of nonunion in HSF patients and confirmed that obesity, a history of cigarette smoking, metabolic bone disease, cardiovascular disease, short oblique fractures, open fractures, and fractures of the proximal third of the shaft were potential risk factors. Accordingly, they claimed that handling these patients by conservative treatment increased the risk of nonunion; hence, they should be managed surgically and directly[17]. Our results also corroborate these findings that HSFs in the proximal one-third tend to go into nonunion when managed conservatively, especially in older patients.

Study Limitations

We did not account for the patient's medical history, such as accompanying illnesses that can impair fracture healing, namely diabetes mellitus, hypothyroidism, obesity, and smoking habits. A lack of information about these information limits the applicability of our results. Furthermore, the fractures were not classified according to the AO classification. Larger studies comparing the nonunion cases by their fracture classifications and incorporating more factors related to the patient's personal characteristics are required to verify these results.

CONCLUSION

Our results confirm that oblique HSFs (short or long) in the proximal or middle one-third regions tend to become absolute indications for surgical management, especially if the patient is female and above the age of 60 years. In such patients, clinicians can promptly opt for surgery without using conservative methods to ensure bony union and recovery.

Ethics

Ethics Committee Approval: The study was approved by the Tekirdağ Namık Kemal University of Local Ethics Committee (protocol no: 2023.77.04.13, date: 25/04/2023).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: M.A., Concept: M.A., Design: M.A., Data Collection or Processing: M.A., Analysis or Interpretation: F.U., Literature Search: F.U., Writing: M.A.

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. Cole PA, Wijdicks CA. The operative treatment of diaphyseal humeral shaft fractures. Hand Clin. 2007;23:437-48.
2. Spiguel AR, Steffner RJ. Humeral shaft fractures. Curr Rev Musculoskelet Med. 2012;5:177-83.
3. Tytherleigh-Strong G, Walls N, McQueen MM. The epidemiology of humeral shaft fractures. J Bone Joint Surg Br. 1998;80:249-53.
4. Ali E, Griffiths D, Obi N, Tytherleigh-Strong G, Van Rensburg L. Nonoperative treatment of humeral shaft fractures revisited. J Shoulder Elbow Surg. 2015;24:210-4.
5. Denard A Jr, Richards JE, Obremskey WT, Tucker MC, Floyd M, Herzog GA. Outcome of nonoperative vs operative treatment of humeral shaft fractures: a retrospective study of 213 patients. Orthopedics. 2010;33.
6. Sarmiento A, Kinman PB, Galvin EG, Schmitt RH, Phillips JG. Functional bracing of fractures of the shaft of the humerus. J Bone Joint Surg Am. 1977;59:596-601.
7. Zagorski JB, Latta LL, Zych GA, Finnieston AR. Diaphyseal fractures of the humerus. Treatment with prefabricated braces. J Bone Joint Surg Am. 1988;70:607-10.
8. Latta LL, Sarmiento A, Tarr RR. The rationale of functional bracing of fractures. Clin Orthop Relat Res. 1980;28-36.
9. Ekholm R, Tidermark J, Törnkvist H, Adami J, Ponzer S. Outcome after closed functional treatment of humeral shaft fractures. J Orthop Trauma. 2006;20:591-6.
10. Walker M, Palumbo B, Badman B, Brooks J, Van Gelderen J, Mighell M. Humeral shaft fractures: a review. J Shoulder Elbow Surg. 2011;20:833-44.
11. Toivanen JA, Nieminen J, Laine HJ, Honkonen SE, Järvinen MJ. Functional treatment of closed humeral shaft fractures. Int Orthop. 2005;29:10-3.
12. Rutgers M, Ring D. Treatment of diaphyseal fractures of the humerus using a functional brace. J Orthop Trauma. 2006;20:597-601.
13. Ring D, Chin K, Taghinia AH, Jupiter JB. Nonunion after functional brace treatment of diaphyseal humerus fractures. J Trauma. 2007;62:1157-8.
14. Koch PP, Gross DF, Gerber C. The results of functional (Sarmiento) bracing of humeral shaft fractures. J Shoulder Elbow Surg. 2002;11:143-50.
15. Gallusser N, Barimani B, Vauclair F. Humeral shaft fractures. EFORT Open Rev. 2021;6:24-34.
16. Kehtari S, Gallusser N, Vauclair F. Mise au point sur les fractures diaphysaires de l'humérus [Update on humeral shaft fractures]. Rev Med Suisse. 2020;16:2421-5.
17. Decomas A, Kaye J. Risk factors associated with failure of treatment of humeral diaphyseal fractures after functional bracing. J La State Med Soc. 2010;162:33-5.

ORIGINAL ARTICLE

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Can the Side Effects of Mydriatics Be Reduced with the ROP-bundle Protocol?

ROP-bundle Protokolü ile Midriyatiklerin Yan Etkileri Azaltılabilir mi?

Özlem ŞAHİN[1], Özmert M.A. ÖZDEMİR[2], Ebru Nevin ÇETİN[3], Kazım KÜÇÜKTAŞÇI[4], Hacer ERGİN[2]

[1]*University of Health Sciences Turkey, Ümraniye Training and Research Hospital, Clinic of Neonatal Intensive Care, İstanbul, Turkey*
[2]*Pamukkale University Faculty of Medicine, Department of Neonatal Intensive Care, Denizli, Turkey*
[3]*Pamukkale University Faculty of Medicine, Department of Ophthalmology, Denizli, Turkey*
[4]*Denizli Private Hospital, Clinic of Neonatal Intensive Care, Denizli, Turkey*

ABSTRACT

Aim: The mydriatics that used for pupil-dilation in the examination of retinopathy-of-prematurity (ROP) may cause some side-effects in the neurological, gastrointestinal, cardiovascular systems by absorption from the nasal mucosa, cornea, conjunctiva, skin. In order to minimize these side-effects, it's recommended to prepare mydriatics in appropriate concentrations, combinations, and to apply pressure on the naso-lacrimal canal by closing the eyes after the application. In this study, we aimed to evaluate the systemic side-effects in the early period of using 0.5% cyclopentolate-1% phenylephrine combination for pupil-dilatation before the ROP-examination and with the protocol we applied after drip in our unit.
Materials and Methods: Thirty-three ROP examinations of 17 cases were included in the study, which was planned retrospectively. After instillation of eye drops containing 0.5% cyclopentolate-1% phenylephrine combination in accordance with our ROP-bundle-protocol, the eye was closed, pressure was applied to the naso-lacrimal canal for 1-2 minutes, and the excess part that had leaked into the skin was wiped off. Oxygen saturation (SaO_2), the amount of oxygen given, blood pressure arterial (TA), heart rate were recorded before and after the drop at 10., 30., and 60. minutes. In addition, patients were followed up for 24 hours in dimensions of gastric-residue, distention, apnea and other side-effects.
Results: The mean-week of gestation, body weight of 17 newborns, 35.3% (6) of whom were boys, 64.7% (11) of girls, were found to be 27.6±3 weeks, 1025±389 g, respectively. In 33 evaluations made before and after mydriatic in 17 cases; distension developed in two cases, apnea in one, and pallor of the skin in one patient. Although there was a statistically significant difference only in SaO_2 and 60th minute systolic TA-measurements between pre- and post-treatment measurements, hemodynamic changes were not evaluated as clinically significant.
Conclusion: In-order-to reduce the side-effects that may develop due to mydriatics, it's necessary to standardize the practices before and after the ROP-examination, close follow-up of the cases after the ROP-examination in terms of early intervention.

Keywords: Retinopathy of prematurity, newborn, cyclopentolate, phenylephrine

ÖZ

Amaç: Prematüre retinopatisi (ROP) muayenesinde pupil dilatasyonu için kullanılan midriyatikler; nazal mukoza, kornea, konjonktiva ve deriden emilerek nörolojik, gastrointestinal ve kardiyovasküler sistemde bazı yan etkilere neden olabilmektedir. Bu yan etkilerin en aza indirilebilmesi için midriyatiklerin uygun konsantrasyon ve kombinasyonlarda hazırlanması, uygulama sonrasında gözlerin kapatılıp nazo-lakrimal kanal üzerine bası uygulanması önerilmektedir. Bu çalışmada, ünitemizde ROP muayenesi öncesinde pupil dilatasyonu amacıyla kullanılan %0,5 siklopentolat-%1 fenilefrin kombinasyonu ve damla sonrası ünitemizde uyguladığımız protokol ile erken dönemdeki sistemik yan etkileri değerlendirmeyi amaçladık.
Gereç ve Yöntem: Retrospektif olarak planlanan çalışmaya 17 olguya ait 33 ROP muayenesi dahil edildi. ROP-bundle protokolümüze uygun olarak %0,5 siklopentolat-%1 fenilefrin kombinasyonunu içeren göz damlalarının damlatılmasından sonra göz kapatılıp, nazo-lakrimal kanala 1-2 dakika süreyle bası uygulandı ve deriye sızan fazla kısmı silindi. Damla öncesinde ve sonrası 10., 30., 60. dakikalarda oksijen satürasyonu (SaO_2), verilen oksijen miktarı, tansiyon arteriyel (TA), kalp tepe atımı kaydedildi. Ayrıca hastalar 24 saat süreyle gastrik rezidü, distansiyon, apne ve diğer yan etkiler açısından takip edildi.

Address for Correspondence: Özlem ŞAHİN MD, University of Health Sciences Turkey, Ümraniye Training and Research Hospital, Clinic of Neonatal Intensive Care, İstanbul, Turkey
Phone: +90 505 373 77 65 **E-mail:** colkozlem@yahoo.com **ORCID ID:** orcid.org/0000-0001-9951-8624
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Bulgular: Çalışma kapsamında %35,3'ü (6) erkek ve %64,7'si (11) kız olmak üzere değerlendirilen 17 yenidoğanın ortalama gestasyon haftası ve vücut ağırlıkları sırasıyla 27,6±3 hafta ve 1025±389 gr saptandı. On yedi olguda midriyatik öncesi ve sonrası yapılan 33 değerlendirmede; iki olguda distansiyon, birinde apne ve bir olguda da deride solukluk gelişti. Tedavi öncesi ile sonrası ölçümler arasında sadece SaO_2 ve 60. dakika sistolik TA ölçümünde istatistiksel açıdan anlamlı bir farklılık saptansa da hemodinamik değişiklik bakımından klinik olarak anlamlı değerlendirilmedi. **Sonuç:** Midriyatiklere bağlı gelişebilecek yan etkilerin azaltılması amacıyla ROP muayenesi öncesi ve sonrasındaki uygulamaların standardize edilmesi, erken müdahale açısından ROP muayenesi sonrası olguların yakın izlemi gereklidir.

Anahtar Kelimeler: Prematüre retinopatisi, yenidoğan, siklopentolat, fenilefrin

INTRODUCTION

Premature births are an important public health problem all over the world. In recent years, developments related to neonatal intensive care in developing countries such as China, India and Turkey have caused some morbidities such as retinopathy of prematurity (ROP) to be seen more frequently in this group of premature babies, together with the increase in survival rates of premature babies. ROP, which is a common complication of preterm birth, is a pathology in which immature blood vessels in the retina are affected and which can cause severe visual impairment and even blindness. ROP, which affects approximately 80% of premature babies with a birth weight of less than 1000 g, is the most important cause of visual impairment in children under 5 years of age in developed countries. Its incidence is tried to be reduced with optimal oxygen use strategy, early diagnosis with ROP screening protocols, and appropriate treatments[1,2]. In order not to delay the treatment, screening examinations should be done on time, and adequate pupil dilation should be ensured before the ROP examination. Due to the possible side effects of eye drops used for mydriasis, different concentrations and combinations of phenylephrine, cyclopentolate, and tropicamide have been tried to provide both safe and adequate pupil dilation. The main reason for concern in the use of mydriatics is that 80% of the drops enter the nasolacrimal duct after ocular administration and pass into the systemic circulation via the nasal mucosa and their systemic effects occur[2-5]. Hypertension, hypotension, tachycardia, bradycardia, apnea, cardiopulmonary arrest, seizure, necrotizing enterocolitis (NEC), sepsis and even death may develop in patients in relation to cardiovascular, respiratory, central nervous system and gastrointestinal side effects due to the systemic effects of mydriatics[5-8]. Phenylephrine makes vasoconstriction and causes increase in blood pressure and tachycardia; anticholinergics cause temporary bradycardia followed by tachycardia, palpitations and arrhythmias[3].

In this retrospective study, we aimed to evaluate how the ROP bundle protocol, which we routinely applied in our unit, affected the short-term results after drop.

MATERIALS AND METHODS

The files of premature infants who underwent ROP examination in our 18-bed tertiary neonatal intensive care unit between May 2011 and January 2012 were retrospectively reviewed. Approval for the retrospective study was obtained from the Non-Interventional Clinical Research Ethics Committee of Pamukkale University (decision no: E.349665, date: 28.03.2023).

Selection and Definition of Cases

Considering the recommendations of the American Academy of Pediatrics and the American Academy of Ophthalmology, ROP screening was performed for all infants with a birth weight of ≤1500 g and/or a gestational week of ≤32 weeks and a birth weight of 1500-2000 g or a gestational age greater than 32 weeks, taking cardiopulmonary support, who were clinically unstable and who were thought to be at risk for ROP by the clinician[9].

Patients with congenital anomaly, cardiovascular instability and need for inotropes were not included in the study.

Technical Information

ROP examinations are carried out by the specialist ophthalmologist in our clinic regularly on the same days, more frequently when necessary, at the bedside.

One hour before the ROP examination, 1% phenylephrine-0.5% cyclopentolate combination for pupil dilation was applied three times in each eye at 0^{th}, 5^{th} and 10^{th} minutes. In order to minimize the passage of eye drops into the systemic circulation, the eye was closed after the procedure, pressure was applied to the lacrimal canal for 1-2 minutes, and the excess part of the drop that leaked into the skin was wiped with a sterile sponge. In patients with adequate pupil dilation with 1% phenylephrine-0.5% cyclopentolate, eye examinations were performed 60 minutes after the start of mydriasis by the same experienced ophthalmologist, independent of the study.

Before and after mydriatic administration, at the 10^{th}, 30^{th}, 60^{th} minutes, oxygen saturation (SaO_2), amount of oxygen delivered, arterial blood pressure, heart apex beat and gastric residue for 24 hours, abdominal distension, apnea, and skin pallor were evaluated. Hemodynamic changes were also evaluated before the ROP examination was performed.

Statistical Analysis

Patient data collected within the scope of the study were analyzed with the IBM Statistical Package for the Social

Sciences for Windows 23.0 (IBM Corp., Armonk, NY) package program. Frequency and percentage for categorical data and mean and standard deviation for continuous data were given as descriptive values. ANOVA test was used for repeated measurements and paired samples test was used for paired comparisons to evaluate the difference between pre-treatment and post-treatment measurement values. Cases with a p value less than 0.05 were considered statistically significant.

RESULTS

A total of 17 patients, including 6 boys (35.3%) and 11 girls (64.7%), were included in the evaluation within the scope of the study. The distribution of demographic and clinical data of infants is given in Table 1.

In 33 ROP examinations of 17 patients included in the study, the pre- and post-mydriatic evaluation revealed distension in two cases, apnea in one patient, and skin pallor in one patient after the drip (Table 2). Complications developed in the patients were not resistant and additional medical treatment was not required.

Table 1. Distribution of demographic and clinical findings of patients

n=17 case	n (%) or Mean±SD
Gender	
Male	6 (35.3)
Female	11 (64.7)
Gestational week	27.6±3
Body weight (gr)	1025.9±389.4
Adjusted age (week)	34.4±3.4
Type of birth	
NSVD	2 (11.8)
C/S	15 (88.2)
Apgar score (1st minute)	6.2±1.1
Apgar score (5th minute)	7.9±0.7
Time of examination (day)	49±17.8

SD: Standard deviation, NSVD: Normal spontaneous vaginal delivery, C/S: Cesarean section

Table 3 shows the distribution of clinical data measured at the pre-treatment and post-treatment 10th, 30th and 60th minutes of the babies included in the evaluation. In the table, considering the analyses of repeated measurements before and after the treatment, although there was a statistically significant difference only in the measurement of SaO_2, SaO_2 was within the target saturation range and there was no need for additional oxygen. When we made a double comparison between the pre-treatment and the 60th minute of the treatment, a significant increase in systolic blood pressure was found (p=0.019), but it was not at the border of hypertension and did not persist.

DISCUSSION

In our study, a low concentration of 1% phenylephrine-0.5% cyclopentolate combination was used for mydriatic effect before the ROP examination. In order to reduce the systemic absorption of the drug after the drop, the patient's eyelid was closed, the excess part that had leaked into the skin was wiped off, and pressure was applied to the nasolacrimal duct for 1-2 minutes. After this application, which we called the ROP-bundle protocol, it was determined that there were no serious systemic side effects and no significant deterioration in hemodynamic parameters that would affect the clinical condition of the baby.

After absorption of topically applied mydriatics through nasal mucosa, cornea or conjunctiva, they pass into the systemic circulation, and may cause some side effects such as increased blood pressure, bradycardia or tachycardia in the cardiovascular system; desaturation and apnea in the respiratory system; feeding intolerance, abdominal distention, ileus, NEC in the

Table 2. Distribution of complications after drops in patients

ROP examination (n=17)	n (%)
Apnea	1 (5.9)
Skin pallor	1 (5.9)
Distention (4th hour)	1 (5.9)
Distention (12th hour)	1 (5.9)

ROP: Retinopathy of prematurity

Table 3. Distribution of clinical findings of patients before and after treatment

Variables (n=33 measurements)	Pre-treatment	Post-treatment 10th minute	Post-treatment 30th minute	Post-treatment 60th minute	p value
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
SaO_2	**94.4±3.1**	**94.8±2.6**	**93.8±3.2**	**95.3±3.2**	**0.049**
FiO_2	31.5±16.1	31.5±16.2	31.6±16.8	31.7±14.3	0.936
HPB	147.1±12.7	149.4±15.1	148.8±14.4	149.5±13.4	0.743
Systolic blood pressure	66.8±12.5	69.6±12.8	68.3±13.5	71.7±14.3	0.051
Diastolic blood pressure	38±11	38.7±13	37.8±11.1	39.8±11.1	0.694

SD: Standard deviation, SaO_2: Arterial oxygen saturation, FiO_2: Fractionated oxygen concentration, HPB: Heart peak beat

gastrointestinal tract; and apnea and convulsions in the central nervous system. In order to prevent the passage of ocularly applied drugs into the systemic circulation, applications such as applying pressure to the nasolacrimal canal, wiping the excess part of the drop that leak into the skin, using the microdrop form, reducing the number and the frequency of drops are recommended[4,5,8,10].

In the meta-analysis published by Kremer et al.[3] in 2019, in which mydriatics were evaluated, it was stated that different concentrations and combinations were used, the lowest dose providing adequate pupil dilation in terms of efficacy and the most appropriate content was the combination of 1% phenylephrine-0.2% cyclopentolate (1-2 drops), and the safety profile would increase even more with low dose microdrop application, but more studies were needed for the evaluation of efficacy. In addition, the demonstration of that low-dose mydriatics have comparable efficacy with higher doses has led clinicians to use low doses for pupil dilation[3]. We were able to provide adequate pupil dilation for ROP examination in all our patients with 1% phenylephrine-0.5% cyclopentolate, which we applied in low concentration for mydriasis before the ROP examination.

Due to the fact that mydriatics available in the market are produced for adults, their higher concentrations and larger volume drop forms pose a risk in terms of side effects. In order to reduce complications, mydriatics are administered in neonatal units by diluting them to the targeted concentration, and measures are taken for drop size[3,8]. Elibol et al.[5], in their prospective study, compared clinical efficacy and systemic side effects of mydriatics by using microdrop (mean drop volume 5.6 µL) and standard drop (mean drop volume 35.4 µL) forms. Adequate pupil dilation in all patients and the mean blood pressure, which was significantly higher in the group in which standard drops were applied, suggested that reducing the droplet volume might reduce possible side effects. It was concluded that mydriatics should be dropped with a small diameter intravenous cannula until the use of microdrops in premature infants is standardized in well-designed studies. In our study, no planning could be made regarding the drop size of mydriatics.

Although two or three drops of mydriatics are generally recommended for adequate pupil dilation in ROP screening, a single drop is considered sufficient in outpatient examinations. In a study using 1% phenylephrine-0.2% cyclopentolate combination for ROP screening and comparing the effectiveness of different numbers of drops, 64 eye examinations were performed on 15 babies, and no significant difference was found between pupil sizes. It was stated that pupil dilation could be achieved with less than three drops, and even a single drop might be sufficient for most infants[11]. The Turkish Neonatology Society recommended that 2.5% phenylephrine-0.5% tropicamide should be administered 2-3 times with 5-minute intervals before the ROP examination[6]. In our study, we dropped mydriatics three times with a 5-minute interval and provided sufficient pupil dilation. Considering the studies conducted, we think that the number of drops can be reduced in practice, since adequate pupil dilation can be achieved, considering the side-effect profile.

Kremer et al.[3] reported a statistically a significant increase in mean blood pressure of 3.4-22.8% in eight studies, and a significant decrease in mean blood pressure of 1-17.1% in four studies. In our study, although there was a statistically significant increase in systolic blood pressure at the 60th minute after the drop, it was observed that it did not persist and it improved.

Vasoconstriction, which occurs with the effect of phenylephrine, disrupts the perfusion of the intestines, and anticholinergics reduce peristalsis and may cause nutritional intolerance, abdominal distention, ileus and NEC in the gastrointestinal tract[1,4,12]. In the study of Jiang et al.[1] published in 2016, it was reported that after phenylephrine and tropicamide, approximately 10% of NEC developed and there was an increase in upper gastrointestinal system bleeding. Bonthala et al.[11] showed that with 1% phenylephrine and 0.2% cyclopentolate, duodenal motor contractions were reduced approximately four times and gastric emptying was significantly delayed. In a randomized controlled study that compared three different drug regimens to evaluate gastrointestinal side effects, it was concluded that the combination of 1% phenylephrine-0.2% cyclopentolate provided adequate pupil dilation with minimal systemic side effects[13]. In the study conducted by Mitchel et al.[2] blood levels of mydriatics were evaluated in patients with GI involvement after 1% phenylephrine-0.2% cyclopentolate combination, and a positive correlation was found between cyclopentolate level and residue. In our study, after -1% phenylephrine-0.5% cyclopentolate, GIS involvement was not detected in two cases, except for abdominal distension.

Many case reports about the side effects of mydriatics have been reported: Apnea with 1% phenylephrine-0.2% cyclopentolate in two patients who underwent outpatient ROP examination[14]; periorbital pallor, which regressed spontaneously in both eyes after 2.5% phenylephrine-0.5% cyclopentolate drops within 20 minutes and was thought to occur due to the cutaneous absorption of vasoconstricting phenylephrine[10]; transient paralytic ileus with abdominal distention and feeding intolerance within hours in two cases after drop of 1% phenylephrine-0.2% cyclopentolate[15]. In our study, pallor in the periorbital region, which resolved spontaneously, developed in one patient, and apnea developed in one patient, which resolved with tactile stimulation. We

think that ROP examinations should be done in a place with intensive care conditions or resuscitation materials should be available in the examination room for the safety of the patients and for emergency response when necessary.

Study Limitations

The inability to use the microdrop form, the small number of patients, and the inability to compare the side effects of different concentrations of mydriatics are important limitations of our study.

CONCLUSION

Despite the widespread use of mydriatic eye drops used for targeted pupil dilation before ROP screening in neonatal units, there are great differences in practice and the optimal dose, number of drops, time between drops, time between examination and drops are not standardized. In this study, we observed that there may be an increase in systolic blood pressure, abdominal distension, apnea and pale skin in premature and very low birth weight babies despite the ROP-bundle protocol we applied to minimize side effects. We planned to add mydriatic drop volume reduction, which we could not do in our study, to our ROP-bundle protocol. In conclusion, further randomized, controlled, multicenter studies are needed to develop approaches to reduce the transmission of mydriatic drugs into the systemic circulation in premature infants, to establish and standardize appropriate protocols such as ROP-bundle.

Ethics

Ethics Committee Approval: Approval for the retrospective study was obtained from the Non-Interventional Clinical Research Ethics Committee of Pamukkale University (decision no: E.349665, date: 28.03.2023).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: Ö.Ş., Ö.M.A.Ö., E.N.Ç., K.K., H.E., Design: Ö.Ş., Ö.M.A.Ö., E.N.Ç., K.K., H.E., Data Collection or Processing: Ö.Ş., Ö.M.A.Ö., Analysis or Interpretation: Ö.Ş., Ö.M.A.Ö., H.E., Literature Search: Ö.Ş., Ö.M.A.Ö., E.N.Ç., K.K., H.E., Writing: Ö.Ş., Ö.M.A.Ö.

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REFERENCES

1. Jiang JB, Zhang ZW, Zhang JW, Wang YL, Nie C, Luo XQ. Systemic changes and adverse effects induced by retinopathy of prematurity screening. Int J Ophthalmol. 2016;9:1148-55.
2. Mitchell A, Hall RW, Erickson SW, Yates C, Lowery S, Hendrickson H. Systemic Absorption of Cyclopentolate and Adverse Events After Retinopathy of Prematurity Exams. Curr Eye Res. 2016;41:1601-7.
3. Kremer LJ, Reith DM, Medlicott N, Broadbent R. Systematic review of mydriatics used for screening of retinopathy in premature infants. BMJ Paediatr Open. 2019;3:e000448.
4. Dincer E, Gonen I, Bornaun HA, Yasa B, Babayigit A, Kurum O, et al. Early Hemodynamic Effects of Mydriatic Eye Drops in Preterm Infants. Am J Perinatol. 2022.
5. Elibol O, Alçelik T, Yüksel N, Caglar Y. The influence of drop size of cyclopentolate, phenylephrine and tropicamide on pupil dilatation and systemic side effects in infants. Acta Ophthalmol Scand. 1997;75:178-80.
6. Koç E, Baş AY, Özdek Ş, Ovalı F, Başmak H. Turkish Neonatal and Turkish Ophthalmology Societies consensus guideline on the retinopathy of prematurity. Turk Pediatri Ars. 2018;53:S151-60.
7. Akın O, Erdeve Ö, Bolat A, Sarıcı SÜ. Prematüre Bir Bebekte Midriyatik Göz Damlası Kullanımına Bağlı Gelişen Akut Gastrik Dilatasyon ve İleus Tablosu. Turkiye Klinikleri J Pediatr. 2009;18:240-3.
8. Seliniotaki AK, Prousali E, Lithoxopoulou M, Kokkali S, Ziakas N, Soubasi V, et al. Alternative mydriasis techniques for retinopathy of prematurity screening. Int Ophthalmol. 2020;40:3613-9.
9. Section on Ophthalmology American Academy of Pediatrics; American Academy of Ophthalmology; American Association for Pediatric Ophthalmology and Strabismus. Screening examination of premature infants for retinopathy of prematurity. Pediatrics. 2006;117:572-6.
10. Sesham R, Puvanachandra N, Muthukumar P. Phenylephrine eye drops: a rare complication in preterm neonate. Arch Dis Child Fetal Neonatal Ed. 2014;99:F180.
11. Bonthala S, Sparks JW, Musgrove KH, Berseth CL. Mydriatics slow gastric emptying in preterm infants. J Pediatr. 2000;137:327-30.
12. Chew C, Rahman RA, Shafie SM, Mohamad Z. Comparison of mydriatic regimens used in screening for retinopathy of prematurity in preterm infants with dark irides. J Pediatr Ophthalmol Strabismus. 2005;42:166-73.
13. Vicente GV, Bahri M, Palafoutas JJ, Wang H, Mehta N. A randomized controlled trial to determine the lowest effective dose for adequate mydriasis in premature infants. J AAPOS. 2012;16:365-9.
14. Wood MG, Kaufman LM. Apnea and bradycardia in two premature infants during routine outpatient retinopathy of prematurity screening. J AAPOS. 2009;13:501-3.
15. Lim DL, Batilando M, Rajadurai VS. Transient paralytic ileus following the use of cyclopentolate-phenylephrine eye drops during screening for retinopathy of prematurity. J Paediatr Child Health. 2003;39:318-20.

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Burnout in Turkish Adult Neurology Specialists

Türk Erişkin Nöroloji Uzmanlarında Tükenmişlik

Hasan Armağan UYSAL[1], Ahmet Onur KESKİN[2], Halil GÜLLÜOĞLU[1], Fatma Gökçem YILDIZ SARIKAYA[3]

[1]İzmir University of Economics Faculty of Medicine, Medical Point Hospital, Clinic of Neurology, İzmir, Turkey
[2]Başkent University Faculty of Medicine, Turgut Noyan Application and Research Center, Department of Neurology, Adana, Turkey
[3]Hacettepe University Faculty of Medicine, Department of Neurology, Ankara, Turkey

ABSTRACT

Aim: Burnout in medical doctors may worse affect patient care or physical performance of clinician. We aimed to investigate the burnout ratio and the factors associated with burnout in Turkish neurology specialists.

Materials and Methods: The neurology specialists in Turkey were included in the study. The participants were asked to fill a questionnaire comprised of 33 questions regarding various thoughts and experiences. The participants gave a response to the questions as follows: strongly disagree, slightly agree, moderately agree, strongly agree, completely agree. According to the meaning value of the question (negative or positive meaning), the answer was given a point in a range of 1-5. Sum of points divided by the maximum point (165) gave a burnout ratio.

Results: The mean age was 38.78 (±8.42) years, and the female/male ratio was 461/255. The mean burnout ratio was found to be 46.73% (±8.95). Male sex, academic membership, higher academic degree, working in medical faculty hospital, lower salary, being single or nonparent, nightshift, absence of on call work, or working in the intensive care unit were detected to be associated with a higher burnout ratio. Burnout ratio was in positive correlation with age, number of patients examined, and duration of working hours, but in negative correlation with number of auxiliary staff or neurologists in hospital.

Conclusion: Our study is the first study to demonstrate a high burnout ratio in a large sample of Turkish adult neurology specialists. Being male, older, academician, professor, single or nonparent, working in medical faculty hospital or in intensive care, low salary, nightshift, and high patient number or working hours seem to be associated with burnout.

Keywords: Burnout, neurology, Turkish, neurologist

ÖZ

Amaç: Tıp doktorlarında tükenmişlik hasta bakımını veya klinisyenin fiziksel performansını kötü etkileyebilir. Çalışmamızda Türk nöroloji uzmanlarındaki tükenmişlik oranını ve tükenmişlikle ilişkili faktörleri araştırmayı amaçladık.

Gereç ve Yöntem: Çalışmaya Türkiye'deki nöroloji uzmanları dahil edildi. Katılımcılardan çeşitli düşünce ve deneyimler ile ilişkili 33 sorudan oluşan bir anketi doldurmaları istendi. Katılımcılar sorulara şu şekilde cevap verdiler: Kesinlikle katılmıyorum, kısmen katılıyorum, katılıyorum, kuvvetle katılıyorum, kesinlikle katılıyorum. Sorunun anlamına göre (negatif veya pozitif), verilen cevap 1-5 arasında puanlandırıldı. Puanların toplamının maksimum (165) puana bölünmesiyle tükenmişlik oranı elde edildi.

Bulgular: Ortalama yaş 38,78 (±8,42) yıl, kadın/erkek oranı 461/255 olarak bulundu. Ortalama tükenmişlik oranı %46,73 (±8,95) idi. Erkek cinsiyet, akademisyenlik, akademik derecenin yüksek olması, tıp fakültesi hastanesinde çalışmak, düşük maaş, bekar olmak, ebeveyn olmamak, nöbet tutmak, icapçı olmamak veya yoğun bakım ünitesinde çalışmak daha yüksek tükenmişlik oranı ile ilişkili bulundu. Tükenmişlik oranı, yaş, muayene edilen hasta sayısı ve çalışma saatleri ile pozitif, yardımcı sağlık personeli veya nörolog sayısı ile negatif korelasyon içindeydi.

Sonuç: Bizim çalışmamız, Türk erişkin nöroloji uzmanlarından oluşan büyük bir örneklemde yüksek tükenmişlik oranını gösteren ilk çalışmadır. Erkek olmak, ileri yaş, akademisyen, profesör veya bekar olmak, ebeveyn olmamak, tıp fakültesi hastanesinde veya yoğun bakım ünitesinde çalışmak, düşük maaş, nöbet tutmak, yüksek hasta sayısı veya çalışma saati tükenmişlikle ilişkili görünmektedir.

Anahtar Kelimeler: Tükenmişlik, nöroloji, Türk, nörolog

Address for Correspondence: Hasan Armağan UYSAL MD, İzmir University of Economics Faculty of Medicine, Medical Point Hospital, Clinic of Neurology, İzmir, Turkey
Phone: +90 555 570 30 45 **E-mail:** druysalarmagan@yahoo.com **ORCID ID:** orcid.org/0000-0002-4867-304X
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INTRODUCTION

Burnout in medical doctors may worse affect personal mental health, patient care, and may decrease physical performance of the clinician. Burnout might lead physicians to tend depressive episode and maybe suicidal behavior[1,2]. Previous studies showed that burnout might affect more than a half of the physicians in the United States (US) or China[3,4]. Medical neurologists were shown to have higher burnout rates and lower satisfaction rates, compared to many other medical specialties[3,5,6].

Burnout among neurologists was shown to be associated with low salary, low satisfaction with work-life balance and meaning in work, long clinical documentation, and violence[3,7,8].

In previous studies, burnout was observed at a high frequency among Turkish doctors working in emergency department[9]. Coronavirus disease-2019 (COVID-19) pandemics aggravated the burnout among Turkish medical doctors, especially working in emergency department, inpatient or outpatient clinics, or intensive care units[10,11]. Besides difficulty in assessing personal protective equipment, working in COVID-19 care units, alone, was shown to be associated with burnout among physicians in Turkey[10,11]. In a study, the prevalence of burnout during the pandemic was shown to be very high among neurology residents in Philippines[12].

In one study conducted before COVID-19 pandemic, violence was experienced by over 80% of the medical physicians in Turkey[13]. Violence in the health care units was suggested to be associated with burnout in that study[13]. However, burnout studies conducted among Turkish neurologists either before or during the pandemic are limited.

We hypothesized that workload, working hours, type of hospital, duration passed as specialist, working as an academic member, or salary problems might affect the job satisfaction or emergence of burnout in Turkish neurologists. We aimed to evaluate the burnout rate and the factors associated with burnout in Turkish adult neurology specialists.

MATERIALS AND METHODS

Study Design

This prospective observational study was conducted in Hacettepe University Hospital, and approved by the Local Ethics Committee of Hacettepe University with approval number, GO 18/898-05 (date: 25.09.2018). The study was conducted in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Written informed consent was obtained from all of participants.

Study Population

The neurology specialists, so-called neurologists, participated in the study. In Turkey, to become a neurologist, neurology specialty training for at least 5-years is to be completed after graduation from medical faculty. The participants were asked to fill a questionnaire, which was formed by the authors to evaluate burnout. The questionnaire was distributed physically to the neurology specialists working in state hospitals, training and research hospitals, and medical faculty hospitals throughout Turkey. We included most of these hospitals in our country, so that we might present a representative sample for Turkey. We performed this survey between November 2018 and January 2020.

The neurologists who were not willing to fill the questionnaire were excluded from the study. Moreover, incompletely filled questionnaire forms were also not included in the study.

Data Collection

Demographic parameters (age and sex), academic status (academic member or not), academic degree (specialist, associate professor, full professor), childbearing (parent or nonparent) of the participants were recorded. Type of hospital (state hospital, private hospital, training and research hospital, or medical faculty hospital) in which the participant was working, the duration of working in that institution as a neurology specialist, and features of job such as nightshift, off of work after nightshift, on call work, or working in intensive care unit were recorded. The mean daily number of patients that the participant examined, the number of auxiliary staff, the number of neurologists in the hospital, and the mean duration of working hours were also recorded.

Evaluation of Burnout

We performed a questionnaire to evaluate the degree of burnout in the participants. We prepared the questionnaire by 33 questions regarding various thoughts and experiences. The participants gave a response to the questions as follows: strongly disagree, slightly agree, moderately agree, strongly agree, completely agree. The answers were evaluated with a range of 1-5 points. According to the meaning value of the question (negative or positive meaning), the answer was given a point. Sum of points divided by the maximum point (165) gave a burnout ratio.

Statistical Analysis

Data obtained in the study were analyzed statistically using Statistical Package for the Social Sciences 27.0 software (IBM Corporation, Armonk, New York, US). The conformity of the data to normal distribution was evaluated using the Shapiro-Wilk Francia test. When comparing more than two independent groups according to quantitative variables, the Kruskal-Wallis H test with Monte Carlo simulation was used with Dunn's test for post hoc analysis. When comparing two independent

groups according to quantitative variables, the Mann-Whitney U test with Monte Carlo simulation was used. To analyze the correlations among the variables, the Spearman's Rho test was employed. To reveal the causality between dependent and independent variables as a mathematical model, one of Ensemble Machine Learning methods, Boosting/Bagging linear regression analyses were used. To increase the predictive value, Automatic Data Preparation steps (include: Adjustment of measurement level, Outlier and missing value handling, Supervised merging, Outlier and missing value handling and supervised merging) were performed. As a model selection method, information criterion (AICC) method in Best subsets/Forward stepwise method was used. Because high-accuracy results could not be achieved, we did not report it. Quantitative variables were stated as mean (standard deviation), median (minimum-maximum), and median (1st quartile-3rd quartile) values, and categorical variables as number (n) and percentage (%) on the tables. Variables were evaluated at a 95% confidence level, and a value of $p<0.05$ was accepted as statistically significant.

RESULTS

The mean age of the participants was 38.78 (±8.42) years. The female/male ratio was 461/255. 80% of whole participants were not academic member, and monthly salary was higher than 50000 Turkish Liras in 30.3%. The mean burnout ratio was 46.73% (±8.95), the mean working hour was 9.02 (±3.30) hours, and the mean daily number of patients examined was 40 (5-120) (Table 1).

Number of responses to each item in the questionnaire was demonstrated on Table 2.

Male sex, academic membership, higher academic degree, work in medical faculty hospital, lower salary, being single or nonparent, nightshift, absence of on call work, or intensive care unit work were associated with a higher burnout ratio. Burnout ratio was in positive correlation with age, number of patients examined, and duration of working hours, but in negative correlation with number of auxiliary staff or number of neurologists in hospital (Table 3).

DISCUSSION

We found that being male, older, academician, professor, single or nonparent, working in medical faculty hospital or in intensive care, low salary, nightshift, high patient number or working hours were associated with higher burnout ratio.

In one study conducted among US neurology residents and fellows, greater satisfaction with work-life balance, meaning in work, or older age were associated with less burnout[3]. To evaluate the impact of age on burnout, it is more accurate to evaluate it in a wide range of age, and in a group of clinicians both with low and high experience. We found that older age was associated with more burnout, but we included neurology specialists comprising of both new specialists and professors. A large study conducted in US including both specialists and residents revealed that working hours, number of patients, on call work and clerical work were associated with high risk for burnout[14]. However, they showed that age was associated with lower risk of burnout. Discrepancies in the association of age with burnout risk might be explained by discrepancies in job conditions and satisfaction which might be affected by national policies. We found that the mean duration passed as neurology specialist was not correlated with burnout ratio. Being academic member or full professor, salary, marital status, or on call work might affect burnout. We suggest that age might be a complex factor interacting with all these factors which have an impact on burnout ratio.

Gender discrepancies have been investigated in a number of studies analyzing burnout in neurologists[15,16]. In a large study analyzed in Chinese neurologists, female neurologists were found as younger, less married or to have children, less to hold senior role, but working hours and on call work did not differ according to sex[15]. Multivariate analysis showed that risk factors for burnout were similar in men and women. A previous survey of US neurologists revealed that female neurologists made more negative comments regarding workload, although working hours, on call work, or patient volume were independent of sex[16]. In our study, female preponderance was observed (female/male ratio: 1.8) in contrast to the previous studies[14,16]. We showed that male sex was associated with higher burnout ratio but we did not examine the sex differences in academic or job properties. Discrepancies in salary or representation as an academic member were found to be an important factor for attrition of women from neurology in US[7]. We found that being a parent decreased the burnout ratio. An online survey analyzing women neurologists in US showed that self-reported gender discrimination or having more children was associated with burnout and dissatisfaction[17]. Among Japanese neurologists, it was found that working hours was higher in men, but housework load was higher among women neurologists[18]. We observed that career dissatisfaction was high among Turkish neurologists according to the statements in the questionnaire.

We found that working hours might increase burnout ratio. We did not specifically examine the actual cause of working hours, such as clerical work, but high patient number probably might increase it. In previous studies, clerical workload and patient number were found to be associated with higher working hours, poor work-life balance, and higher burnout risk[14,16,19]. We also revealed that low number of auxiliary staff or neurologists might increase burnout ratio. The absence of adequate co-existent staff may increase both clerical work,

Table 1. Demographic and job properties of the participants		
		n (%)
Sex		
	Male	255 (35.6)
	Female	461 (64.4)
Academic status		
	Academic member	143 (20.0)
	Not academic member	573 (80.0)
Academic degree		
	Specialist	622 (86.9)
	Associate professor	60 (8.4)
	Full professor	34 (4.7)
Hospital		
	State hospital	262 (36.6)
	Private hospital	159 (22.2)
	Training and research hospital	140 (19.6)
	Medical faculty hospital	155 (21.6)
Monthly salary*		
	<7500 TL	46 (6.4)
	7500-12500 TL	453 (63.3)
	12500-20000 TL	185 (25.8)
	>20000 TL	32 (4.5)
Marital status (married)		521 (72.8)
Childbearing (parent)		461 (64.4)
Nightshift (present)		328 (45.8)
Off of work after nightshift (present)		100 (14.0)
On call work (present)		478 (66.8)
Intensive care unit work (present)		539 (75.3)
	Mean (SD)	**Median (min-max)**
Age (year)	38.78 (8.42)	38 (25-65)
Burnout ratio	46.73 (8.95)	46.67 (27.88-76.36)
Mean daily number of patients examined	47.99 (29.78)	40 (5-120)
Duration passed as specialist (year)	10.45 (7.04)	9 (1-35)
Number of auxiliary staff	2.61 (1.22)	2 (1-5)
Number of neurologists in hospital	6.17 (6.31)	4 (1-30)
Duration of working hours	9.02 (3.30)	8 (5-36)

*Refers to salaries between the years of 2018 and 2020.

TL: Turkish Liras, SD: Standard deviation, min-max: Minimum-maximum

patient number, working hours, or frequency of nightshift, which might contribute to increased burnout risk. Inadequate staff was also demonstrated to be associated with burnout in previous studies[3,16]. The factors associated with burnout, which have been investigated both in the present and the previous studies, seem not to be independent of each other[14,16,19]. We thought that increased working hours might also be associated with documentation work but we did not analyze it. Documentation work or time requirement for documentation was shown as an important problem for burnout in a previous study[17].

The daily number of patients was ranged in 5-120 in the present study. This broad range might be resulted from the inclusion of various types of hospitals, and from the inclusion of both academic members and specialists. Upper limit of the range was extremely high for a neurologist to examine such many patients in one day. The annual number of patients examined was also shown to be high in a burnout study investigated in

Table 2. Response of the participants to the questionnaire

	Strongly disagree	Slightly agree	Moderately agree	Strongly agree	Completely agree
The neurologists' workload is heavy.	5 (0.7)	0 (0.0)	23 (3.2)	122 (17.0)	566 (79.1)
Salary payments are delayed.	269 (37.6)	158 (22.1)	171 (23.9)	66 (9.2)	52 (7.3)
Interpersonal disharmony affects the decision to collaborate.	8 (1.1)	8 (1.1)	98 (13.7)	256 (35.8)	346 (48.3)
Healthcare workers are exposed to violence in hospitals.	0 (0.0)	5 (0.7)	42 (5.9)	224 (31.3)	445 (62.2)
The healthcare workers are verbally insulted.	0 (0.0)	5 (0.7)	58 (8.1)	168 (23.5)	485 (67.7)
I often worry about being reported.	21 (2.9)	45 (6.3)	236 (33.0)	143 (20.0)	271 (37.8)
I believe that professional organizations adequately represent neurologists.	293 (40.9)	156 (21.8)	120 (16.8)	59 (8.2)	88 (12.3)
Not doing extra work is a problem for me.	106 (14.8)	99 (13.8)	197 (27.5)	142 (19.8)	172 (24.0)
My patients' knowledge of neurology is insufficient.	0 (0.0)	10 (1.4)	120 (16.8)	323 (45.1)	263 (36.7)
I believe that the news in the media has negatively affected my relationships with my patients.	0 (0.0)	12 (1.7)	82 (11.5)	280 (39.1)	342 (47.8)
I am disturbed because of having to take care of the patient who creates trouble for me.	6 (0.8)	18 (2.5)	44 (6.1)	104 (14.5)	544 (76.0)
Bureaucratic work prevents me from sparing enough time for patients.	0 (0.0)	20 (2.8)	120 (16.8)	198 (27.7)	378 (52.8)
Off-label report and medication requests bother me.	6 (0.8)	12 (1.7)	52 (7.3)	110 (15.4)	536 (74.9)
There are problems regarding taking leave.	40 (5.6)	94 (13.1)	214 (29.9)	152 (21.2)	216 (30.2)
I believe that the clinical practice training I have received is adequate.	36 (5.0)	36 (5.0)	209 (29.2)	358 (50.0)	77 (10.8)
Neurologists' job satisfaction is taken into account.	400 (55.9)	175 (24.4)	50 (7.0)	4 (0.6)	87 (12.2)
Neurologists' patients respect them.	81 (11.3)	159 (22.2)	303 (42.3)	169 (23.6)	4 (0.6)
The duties and authorities of the director neurologists are determined.	201 (28.1)	197 (27.5)	245 (34.2)	57 (8.0)	16 (2.2)
I should be able to electronically review the tests performed in other hospitals.	8 (1.1)	4 (0.6)	20 (2.8)	72 (10.1)	612 (85.5)
I can spare enough time for my registered patients.	207 (28.9)	172 (24.0)	196 (27.4)	127 (17.7)	14 (2.0)
I take extra time at the outpatient clinic and take care of patients who are waiting.	42 (5.9)	62 (8.7)	155 (21.6)	266 (37.2)	191 (26.7)
I can spare enough time for my vocational training.	272 (38.0)	201 (28.1)	162 (22.6)	73 (10.2)	8 (1.1)
I believe that my patients trust me.	28 (3.9)	44 (6.1)	121 (16.9)	415 (58.0)	108 (15.1)
In terms of my neurology career, I anticipate a promising future.	293 (40.9)	134 (18.7)	137 (19.1)	118 (16.5)	34 (4.7)
Neurologists work in harmony with the Turkish Neurological Society.	154 (21.5)	241 (33.7)	210 (29.3)	82 (11.5)	29 (4.1)
I am satisfied with the performance system.	602 (84.1)	72 (10.1)	34 (4.7)	8 (1.1)	0 (0.0)
If I was given another chance, I would like to be a neurologist again.	270 (37.7)	72 (10.1)	161 (22.5)	102 (14.2)	111 (15.5)
I would like my child to become a doctor.	449 (62.7)	124 (17.3)	62 (8.7)	38 (5.3)	43 (6.0)
I would like my child to become a neurologist.	517 (72.2)	82 (11.5)	78 (10.9)	20 (2.8)	19 (2.7)
I can spare the time for myself.	341 (47.6)	218 (30.4)	127 (17.7)	16 (2.2)	14 (2.0)
At work, I have the opportunity to express myself and am assigned duties that match my abilities.	215 (30.0)	169 (23.6)	169 (23.6)	128 (17.9)	35 (4.9)
My superiors do not expose me to physical, verbal or implied mistreatment of my rights in the workplace.	104 (14.5)	146 (20.4)	235 (32.8)	154 (21.5)	77 (10.8)
The institution for which I work pays me the income I deserve as a result of my overtime work (extra payment/ progress payment) without any deductions.	241 (33.7)	190 (26.5)	139 (19.4)	104 (14.5)	42 (5.9)

Table 3. Association of burnout ratio with demographic and job properties of the participants

		n		Burnout ratio Median (q1-q3)	p
Sex					**0.002ᵘ**
	Male	255		47.27 (41.21-55.76)	
	Female	461		46.06 (39.39-52.73)	
Academic status					**<0.001ᵘ**
	Academic member	143		51.52 (47.27-56.36)	
	Not academic member	573		45.45 (38.79-52.12)	
Academic degree					**<0.001ᵏ**
	Specialist	622	A	45.45 (39.39-52.12)[A, B]	
	Associate professor	60	B	52.12 (47.27-56.36)	
	Full professor	34	C	53.94 (51.52-56.36)	
Hospital					**<0.001**
	State hospital	262	A	44.85 (39.39-47.88)	
	Private hospital	159	B	46.67 (38.18-54.55)	
	Training and research hospital	140	C	46.36 (40.61-52.12)	
	Medical faculty hospital	155	D	53.33 (49.09-56.36)[A, B, C]	
Monthly salary					**<0.001ᵏ**
	<30000 TL	46	A	50.91 (37.58-61.82)	
	30000-50000 TL	453	B	45.45 (39.39-52.73)[C]	
	50000-80000 TL	185	C	49.09 (46.06-55.15)	
	>80000 TL	32	D	39.39 (37.58-43.94)[A, B, C]	
Marital status					**<0.001ᵘ**
	Married	521		45.45 (39.39-52.73)	
	Single	195		50.3 (44.85-54.55)	
Childbearing					**<0.001ᵘ**
	Parent	461		44.24 (38.18-52.12)	
	Nonparent	255		49.7 (45.45-55.15)	
Nightshift					**0.001ᵘ**
	Present	328		47.27 (41.21-55.15)	
	Absent	388		45.45 (38.79-52.73)	
Off of work after nightshift					0.402ᵘ
	Present	100		46.66 (40-52.73)	
	Absent	616		46.67 (39.39-53.94)	
On call work					**0.004ᵘ**
	Present	478		46.06 (39.39-52.12)	
	Absent	238		48.785 (39.39-55.15)	
Intensive care unit work					**0.002ᵘ**
	Present	539		49.09 (43.64-56.36)	
	Absent	177		46.06 (39.39-52.73)	
				r	
Mean daily number of patients examined		716		**0.272**	**<0.001ˢ**
Duration passed as specialist (year)		716		**-0.062**	0.097ˢ
Number of auxiliary staff		716		**-0.170**	**<0.001ˢ**
Number of neurologists in hospital		716		**-0.233**	**<0.001ˢ**
Age (year)		716		**0.074**	**0.048ˢ**
Duration of working hours		716		**0.182**	**<0.001ˢ**
Monthly salary		716		**0.064**	0.085ˢ

ᵘMann-Whitney U test (Monte Carlo), ᵏKruskal Wallis Test (Monte Carlo); post-hoc test: Dunn's test, ˢSpearman's rho test, r: Correlation coefficient, q1: 1st Quartile, q3: 3rd Quartile, [A, B, C, D]Expresses significance according to relevant subgroups

China[4]. The relationship of patient number with burnout among neurologists was also suggested in that study. Working in public hospital was also found as a risk for burnout[4]. According to our findings, working in a medical faculty hospital seems to be associated with a higher burnout ratio than working in a state hospital or training and research hospital. This discrepancy might be related to that salary, working hours, or patient numbers might differ among these hospitals in Turkey. Our study is the first study to analyze burnout ratio in a large sample of Turkish adult neurology specialists. We revealed that nightshift, rather than on call work, was associated with higher burnout ratio. However, on call work seems to be a risk factor for burnout among neurologists in another country[4]. This also might be resulted from different policies applied for health care workers. In our study, off of work after nightshift did not affect the burnout ratio. To our knowledge, no previous studies include such a parameter. The explanation might be that off of work after nightshift might be a routine procedure in other countries.

We showed that monthly salary of >80000 Turkish Liras was associated with lower burnout ratios. However, minority of Turkish neurologists have earned such a salary. Lower income was shown as an important factor for burnout among neurologists also in other countries, such as China or US[4,7]. Neurologists were shown to earn one of the least salaries among medical specialties in US[7]. Actually, we thought that evaluation of purchase power for a neurologist might have a higher importance to compare Turkish neurologists with those in other countries. However, it necessitates a different design of study.

In one study investigating burnout among neurology residents during the COVID-19 pandemic, burnout frequency was found as extremely high (94%)[12]. We analyzed burnout among neurology specialists independent of COVID-19 pandemic.

Study Limitations

We analyzed only neurology specialists including both academic members and non-members, and excluded residents. To our knowledge, off of work after nightshift is the first time analyzed as a parameter that may affect burnout ratio among neurologists. Our analysis was performed independent of COVID-19 pandemic.

CONCLUSION

We found a high burnout ratio among women and men neurology specialists, and that being male, older, academician, professor, single or nonparent, working in medical faculty hospital or in intensive care, low salary, nightshift, high patient number or working hours were associated with higher burnout ratio. Burnout ratio is affected by a number of factors interacting each other, and considering the impact of burnout both on the neurologist and the patient health, national policies become important to prevent or decrease these factors in this population.

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Ethics

Ethics Committee Approval: This prospective observational study was conducted in Hacettepe University Hospital, and approved by the Local Ethics Committee of Hacettepe University with approval number, GO 18/898-05 (date: 25.09.2018).

Informed Consent: Written informed consent was obtained from all of participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: H.A.U., F.G.Y.S., Design: A.O.K., H.G., H.A.U., F.G.Y.S., Data Collection or Processing: A.O.K., H.G., Analysis or Interpretation: A.O.K., H.G., H.A.U., F.G.Y.S., Literature Search: A.O.K., H.G., H.A.U., F.G.Y.S., Writing: A.O.K., H.G., H.A.U., F.G.Y.S.

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REFERENCES

1. Shanafelt TD, Balch CM, Dyrbye L, Bechamps G, Russell T, Satele D, et al. Special report: suicidal ideation among American surgeons. Arch Surg. 2011;146:54-62.
2. Center C, Davis M, Detre T, Ford DE, Hansbrough W, Hendin H, et al. Confronting depression and suicide in physicians: a consensus statement. JAMA. 2003;289:3161-6.
3. Levin KH, Shanafelt TD, Keran CM, Busis NA, Foster LA, Molano JRV, et al. Burnout, career satisfaction, and well-being among US neurology residents and fellows in 2016. Neurology. 2017;89:492-501.
4. Zhou X, Pu J, Zhong X, Zhu D, Yin D, Yang L, et al. Burnout, psychological morbidity, job stress, and job satisfaction in Chinese neurologists. Neurology. 2017;88:1727-35.
5. Shanafelt TD, Boone S, Tan L, Dyrbye LN, Sotile W, Satele D, et al. Burnout and satisfaction with work-life balance among US physicians relative to the general US population. Arch Intern Med. 2012;172:1377-85.
6. Shanafelt TD, Hasan O, Dyrbye LN, Sinsky C, Satele D, Sloan J, et al. Changes in Burnout and Satisfaction With Work-Life Balance in Physicians and the General US Working Population Between 2011 and 2014. Mayo Clin Proc. 2015;90:1600-13.
7. Hasan TF, Turnbull MT, Vatz KA, Robinson MT, Mauricio EA, Freeman WD. Burnout and attrition: Expanding the gender gap in neurology? Neurology. 2019;93:1002-8.

8. Rodríguez-Fernández JM, Loeb JA, Hier DB. It's time to change our documentation philosophy: writing better neurology notes without the burnout. Front Digit Health. 2022;4:1063141.

9. Erdur B, Ergin A, Yüksel A, Türkçüer İ, Ayrık C, Boz B. Assessment of the relation of violence and burnout among physicians working in the emergency departments in Turkey. Ulus Travma Acil Cerrahi Derg. 2015;21:175-81.

10. Tarhan Ş, Ömeroğlu Şimşek G, Direk Tecirli N, Uçan ES, Atik M, İtil BO, et al. Facing the Pandemic: Burnout in Physicians in Turkey. Turk Thorac J. 2021;22:439-45.

11. Uz B, Savaşan E, Soğancı D. Anxiety, Depression and Burnout Levels of Turkish Healthcare Workers at the End of the First Period of COVID-19 Pandemic in Turkey. Clin Psychopharmacol Neurosci. 2022;20:97-108.

12. Turalde CWR, Espiritu AI, Macinas IDN, Jamora RDG. Burnout among neurology residents during the COVID-19 pandemic: a national cross-sectional study. Neurol Sci. 2022;43:1503-11.

13. Çevik M, Gümüştakim RŞ, Bilgili P, Ayhan Başer D, Doğaner A, Saper SHK. Violence in healthcare at a glance: The example of the Turkish physician. Int J Health Plann Manage. 2020;35:1559-70.

14. Busis NA, Shanafelt TD, Keran CM, Levin KH, Schwarz HB, Molano JR, et al. Burnout, career satisfaction, and well-being among US neurologists in 2016. Neurology. 2017;88:797-808.

15. Pu J, Zhou X, Zhu D, Zhong X, Yang L, Wang H, et al. Gender differences in psychological morbidity, burnout, job stress and job satisfaction among Chinese neurologists: a national cross-sectional study. Psychol Health Med. 2017;22:680-92.

16. LaFaver K, Miyasaki JM, Keran CM, Rheaume C, Gulya L, Levin KH, et al. Age and sex differences in burnout, career satisfaction, and well-being in US neurologists. Neurology. 2018;91:e1928-41.

17. Moore LR, Ziegler C, Hessler A, Singhal D, LaFaver K. Burnout and Career Satisfaction in Women Neurologists in the United States. J Womens Health (Larchmt). 2019;28:515-25.

18. Burnout in Japanese neurologists: comparison of male and female physicians]. Rinsho Shinkeigaku. 2021;61:219-27.

19. Miyasaki JM, Rheaume C, Gulya L, Ellenstein A, Schwarz HB, Vidic TR, et al. Qualitative study of burnout, career satisfaction, and well-being among US neurologists in 2016. Neurology. 2017;89:1730-8.

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Effect of PDE 5 Inhibitor-Avanafil on Renal Ischemia/Reperfusion Injury in Rats

Sıçanlarda Renal İskemi/Reperfüzyon Hasarı Üzerine PDE 5 İnhibitörü-Avanafilin Etkisi

Tuğba Nurcan YÜKSEL[1], Zekai HALICI[2], Cihangir KAYA[3], Ayşe BOZKURT[4], Taha TAVACI[5], Maide Sena CİVELEK[6], Bengül ÖZDEMİR[7]

[1]*Tekirdağ Namık Kemal University Faculty of Medicine, Department of Pharmacology, Tekirdağ, Turkey*
[2]*Atatürk University Faculty of Medicine, Department of Pharmacology-Clinical Research, Development and Design Application and Research Center, Erzurum, Turkey*
[3]*İstanbul Pendik Veterinary Control Institute, Diagnostic Department, İstanbul, Turkey*
[4]*Van Yüzüncü Yıl University Faculty of Medicine, Department of Pharmacology, Van, Turkey*
[5]*Sakarya University Faculty of Medicine, Department of Pharmacology, Sakarya, Turkey*
[6]*Atatürk University Faculty of Medicine, Department of Pharmacology, Erzurum, Turkey*
[7]*Kafkas University Faculty of Medicine, Department of Histology and Embryology, Kars, Turkey*

ABSTRACT

Aim: Renal ischemia-reperfusion injury (RI/RI) damages many organs, especially the kidney. Phosphodiesterase (PDE) 5 inhibitors has antioxidant and anti-inflammatory effects. Avanafil (AVA) is a second-generation PDE 5 inhibitor with greater PDE isoform selectivity. The aim of this study is to investigate the effects of AVA on RI/RI in rats.

Materials and Methods: Forty rats were randomly divided into five groups (n=8): Sham; AVA 10; RI/RI; RI/RI + 5 mg/kg AVA, and RI/RI + 10 mg/kg AVA. RI/RI in rats was established by clamping renal artery. An acute surgical experiment was performed for the induction of renal ischemia for 45 min by renal artery clamping followed by reperfusion for 24 h. Kidney tissues were investigated biochemically [malondialdehyde (MDA) and glutathione (GSH) with ELISA], molecularly [relative quantification of IL-1β, nuclear factor-kappa B (NF-κB), and tumor necrosis factor-alpha (TNF-α) mRNA gene expression with qRT-PCR], and histopathologically (staining with Harris hematoxylin and eosin Y).

Results: AVA administration ameliorated disturbances in MDA and GSH levels caused by RI/RI. AVA treatment improved the increase in the mRNA expressions of IL-1β, NF-κB, and TNF-α in kidney tissues induced ischemia/reperfusion injury. AVA administration ameliorated histopathologic injury in kidney tissues caused by renal ischemia reperfusion. Moreover, the values closest to those of the sham group were obtained by administering 10 mg/kg AVA to rats with RI/RI.

Conclusion: AVA administration improved renal ischemia/reperfusion-induced tissue injury by alleviating oxidative stress and inflammatory cascades that could be important in ischemia-reperfusion injury. These findings may provide a mechanistic basis for using AVA to treat RI/RI.

Keywords: Anti-inflammatory, antioxidant, avanafil, phosphodiesterase 5 inhibitor, renal ischemia/reperfusion injury

ÖZ

Amaç: Renal iskemi-reperfüzyon hasarı (RI/RI) başta böbrek olmak üzere birçok organa zarar verir. Fosfodiesteraz (PDE) 5 inhibitörleri, antioksidan ve anti-enflamatuvar etkilere sahiptir. Avanafil (AVA), daha yüksek PDE izoform seçiciliğine sahip ikinci nesil bir PDE 5 inhibitörüdür. Bu çalışmanın amacı sıçanlarda RI/RI üzerine AVA'nın etkilerini incelemektir.

Gereç ve Yöntem: Kırk sıçan rastgele beş gruba (n=8) ayrıldı: Kontrol; AVA 10 mg/kg; RI/RI; RI/RI + 5 mg/kg AVA ve RI/RI + 10 mg/kg AVA. RI/RI sıçan modeli, renal arter klemplenerek oluşturuldu. Renal arter klempleme ile 45 dakika renal iskemi indüksiyonu ve ardından 24 saat reperfüzyon

Address for Correspondence: Tuğba Nurcan YÜKSEL MD, Tekirdağ Namık Kemal University Faculty of Medicine, Department of Pharmacology, Tekirdağ, Turkey
Phone: +90 546 573 81 41 **E-mail:** tnyuksel@nku.edu.tr **ORCID ID:** orcid.org/0000-0001-5092-1674
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için akut bir cerrahi deney yapıldı. AVA, iskemiden 6 ve 1 saat önce oral olarak sonda ile uygulandı. Yirmi dört saatlik reperfüzyondan sonra moleküler ve biyokimyasal inceleme için böbrek dokuları çıkarıldı. Böbrek dokuları biyokimyasal [ELISA ile malondialdehit (MDA) ve glutatyon (GSH)], moleküler [qRT-PCR ile IL-1β, nükleer faktör-kappa B (NF-κB), and tümör nekroz faktörü-alfa (TNF-α) mRNA gen ekspresyonları] ve histopatolojik (Harris hematoksilen ve eosin Y ile boyama) olarak incelendi.

Bulgular: AVA uygulaması, RI/RI'nin sebep olduğu MDA ve GSH düzeylerindeki değişiklikleri iyileştirdi. AVA tedavisi iskemi/reperfüzyon hasarından kaynaklanan böbrek dokularındaki IL-1β, NF-κB ve TNF-α mRNA gen ekspresyonlarındaki artışı düzeltti. AVA uygulaması renal iskemi reperfüzyonun neden olduğu böbrek dokularındaki histopatolojik hasarı iyileştirdi. Ayrıca kontrol grubuna en yakın değerler RI/RI'li sıçanlara 10 mg/kg AVA uygulanması ile elde edildi.

Sonuç: AVA uygulaması, iskemi/reperfüzyon hasarında önemli olabilecek oksidatif stresi ve enflamatuvar kaskadları hafifleterek RI/RI kaynaklı doku hasarını iyileştirmiştir. Bu bulgular, RI/RI'yi tedavi etmek için AVA kullanımına ilişkin mekanik bir temel sağlayabilir.

Anahtar Kelimeler: Anti-enflamatuvar, antioksidan, avanafil, fosfodiesteraz 5 inhibitörü, renal iskemi/reperfüzyon hasarı

INTRODUCTION

Kidneys are one of the most perfused organs in the body, making them extremely vulnerable to changes in blood perfusion[1]. They play a pivotal role in physiological functions[2]. In the situation of ischemia/reperfusion, both molecular and cellular events set off an inflammatory cascade that disrupts the normal function of tissues by releasing reactive oxygen species (ROS), amplifying the actions of cytokines, and recruiting leukocytes. Reperfusion is the critical process of providing substrate and oxygen for adenosine triphosphate synthesis to the ischemic tissue. Nevertheless, it exacerbates tissue damage by producing additional ROS[3]. Exaggerated ROS generation causes renal ischemia-reperfusion injury (RI/RI) via ROS-induced anomalous signal pathways, inflammatory infiltration, cellular disorder, and renal cell death[4]. Moreover, reperfusion duration and the re-oxygenation stage are the key causes of negative impacts on the kidney, according to a growing body of evidence[5]. RI/RI can occur following an infarction, sepsis, or organ transplantation, and it magnifies tissue injury by activating an inflammatory cascade that involves ROS, chemokines, proinflammatory cytokines, and leukocytes[6]. The generated ROS triggers lipid peroxidation [with malondialdehyde (MDA) generated as the end product][7]. Increased MDA levels with lipid oxidation damage cell membrane functions and cellular integrity[8,9]. Increasing the concentrations of nonenzymatic compounds such as glutathione (GSH) stimulates the cellular defense system against oxidative damage[10]. In addition, I/R-induced ROS production causes the activation of nuclear factor-kappa B (NF-κB)[11]. NF-κB activation increases the transcription of pro-inflammatory cytokines including tumor necrosis factor-alpha (TNF-α)[12]. TNF-α can exacerbate the harm to tissues and organs by promoting the production of IL-1β and other inflammatory markers[13]. Thus, many different inflammatory mediators take part in RI/RI. Consequently, pharmacological agents with multiple effects including anti-oxidative and anti-inflammatory, and inhibiting tubular necrosis features may be a hopeful approach for avoiding renal tissue injury.

Phosphodiesterase (PDE) 5 inhibitors are a widely used primary treatment of erectile dysfunction as well as many other illnesses, such as prostatic hyperplasia, hypertension, and coronary heart disease[14]. Preclinical studies have shown that PDE 5 inhibition may mitigate oxidative and inflammatory damage to the kidneys, resulting in decreased albuminuria, glomerular hyperfiltration and hypertrophy, and decreased glomerulosclerosis overall[15]. These renoprotective impacts and enhancements in renal tissue injury could be attributed to PDE 5 inhibitor activity through both hemodynamic and intrarenal antioxidant, anti-inflammatory, and anti-proliferative mechanisms[16]. Avanafil (AVA) is a second-generation PDE 5 inhibitor. The Food and Drug Administration and the European Medicines Agency introduced AVA in 2013 as PDE 5 inhibitor compounds[17]. AVA is more PDE isoform selective and has different physical and chemical characteristics from first-generation PDE 5 inhibitors[18]. In experimental studies, the defensive impacts of various PDE 5 inhibitors on ischemia/reperfusion-induced tissue injury have been individually proven in diverse tissues, including the myocardium[19], spinal cord[20], brain[21], ovary[22], and even kidney[14]. AVA's effects in various renal situations such as nephropathy have also been studied[23,24]. However, the effect of AVA on renal tissue injury ischemia/reperfusion-induced is not known. In light of this knowledge, we designed to investigate the possible protective effects of AVA on RI/RI in rats. Furthermore, the underlying mechanisms were investigated molecularly, biochemically, and histopathologically.

MATERIALS AND METHODS

Animals

In this study, 40 Wistar rats aged 4-5 months (weight: 250-290 gr) were purchased from Atatürk University Medical Experimental Application and Research Center. All the animals were kept in standard plastic cages under standard conditions (temperature: 22±1 °C, relative humidity: 40-80%, 12 h light-dark cycle). Throughout the experiment, the animals had

unlimited access to the usual rat water and food (ad libitum). All experimental procedures were carried out in accordance with national guidelines for the use and care of laboratory animals.

This animal study and all its protocols were approved by Atatürk University Medical Experimental Application and Research Center of Ethics Committee on 04-04-2022 with document number E-75296309-050.01.04-2200103613.

Chemicals

The PDE 5 inhibitor, AVA (Qty: 1g) was purchased from the BLDpharm Company (Cat. No: BD289977, MW: 483.95). Xylazine (Basilazin 2%) was obtained from BioTek, Turkey. Ketamine (Ketalar 500 mg/10 mL) was obtained from Pfizer, Turkey. The lab experiments required additional chemicals, all of which were bought from Sigma and Merck (Germany).

Experimental Strategy

The 40 rats were randomly separated into 5 groups (n=8 per group, Table 1). AVA (5 and 10 mg/kg) was orally administered by gavage 6 and 1 h before the operation, as the half-life of the AVA is 6 hours, in related groups[25,26].

Surgical Procedure for Inducing Renal Ischemia/Reperfusion Injury

All animals were anesthetized with an injection of 80 mg/kg ketamine + 8 mg/kg xylazine). After disinfecting the dorsal wall, a 2.5 cm longitudinal incision was made along the posterior dorsal midline area and a right nephrectomy was performed. To achieve RI/RI, a clamp was applied to the vessels for 45 minutes, occluding the renal artery in the kidney. The organ itself was protected from harm with extra care. Color changes throughout the entire kidney provided evidence for the occlusion's effectiveness. The rats were then carefully reperfused for 24 hours to provide blood flowed into the kidneys after the clamp was removed (Table 1)[27,28]. All the animals were kept warm after surgery to protect them from hypothermia. After the completion of the 24 h reperfusion phase, all rats were anesthetized with a high dose of ketamine-xylazine. All kidney tissues were collected and kept at -80 °C for biochemical and molecular investigations and at 10% formalin solution for histopathological investigations.

Biochemical Investigations

100 mg of all specimens reserved for biochemical investigations were treated with 1 mL of PBS, ground in liquid nitrogen with a Tissue Lyser II (Qiagen), and centrifuged. Supernatants obtained by centrifugation were used as sample. MDA levels[29] and GSH[30] levels were determined with an enzyme-linked immunosorbent assay (ELISA) reader[31]. MDA and GSH levels of the kidney tissues were expressed respectively as nmol/mg protein. The mean and standard deviation for each set of data was displayed per mg of protein.

Protein Determination

Utilizing commercial protein standards (Sigma Aldrich, total protein kit-TP0300-1KT-(USA), the Lowry technique was employed to calculate the protein concentrations[32].

Molecular Investigations

Gene Expressions Analyses

A qRT-PCR was designed to assess interleukin 1 beta (IL-1β), NF-κB, and tumor necro factor-α (TNF-α) mRNA expression levels. In order to do this, kidney tissues were homogenized, RNA was isolated, cDNA was created, and the expression levels of various mRNAs were quantitatively assessed.

RNA Extraction from Kidney Tissues

Kidney tissue specimens were measured separately at 20 mg. Specimens were stabilized in RNAlater RNA Stabilization Reagent (Qiagen) and homogenized with the Tissue LyserII (Qiagen). Using the RNeasy Mini Kit Qiagen and following the manufacturer's instructions in Qiaqube (Qiagen, Hilden, Germany), total RNA was purified. The total amount of mRNA was determined utilizing nanodrop spectrophotometry (All Sheng) at 260 nm[33].

Table 1. Experimental groups and designs to investigate the effects of AVA in the renal tissue on RI/RI

Groups	6 and 1 h before the operation	0th hour ischemia-induced	45 min after ischemia	24 h after reperfusion-induced
1 Sham		Sham operation		Sacrification
2 AVA 10 mg/kg	AVA	Sham operation		Sacrification
3 RI/RI		Ischemia	Reperfusion	Sacrification
4 RI/RI + AVA 5 mg/kg	AVA	Ischemia	Reperfusion	Sacrification
5 RI/RI + AVA 10 mg/kg	AVA	Ischemia	Reperfusion	Sacrification

AVA: Avanafil, AVA 5: 5 mg/kg AVA, AVA 10: 10 mg/kg AVA, RI/RI: Renal ischemia/reperfusion injury

Reversed Transcriptase Reaction and cDNA Synthesis

cDNA production from total RNA was performed with a high capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA). 10 µL RNA was used for each reaction. cDNA synthesis was achieved with T100 Thermal Cycler (BIO-RAD) according to temperature measurements. By using nanodrop spectrophotometry (All Sheng), the quantity of cDNA was determined, and the obtained cDNA was kept at -20 °C. For the cDNA synthesis reaction, the following ingredients were used: total RNA (10 µL), 25X dNTP mix (0.8 µL), 10X RT random primers (2 µL), reverse transcription 10X buffer (2 µL), diethylpyrocarbonate H_2O (4.2 µL) and MultiScribe reverse transcriptase (1 µL). The cDNA concentrations were assessed and quantified using the Epoch Spectrophotometer System and Take3 Plate (Biotek)[34,35].

Quantitative Determination of IL-1β, NF-κB, and TNF-α mRNA Gene Expression by qRT-PCR

Utilizing the StepOnePlus qRT-PCR system technology (Applied Biosystems, USA) and cDNA produced from RNA of rats, analyses of relative and IL-1β, NF-κB, and TNF-α expression analyses were carried out, as previously described[36]. TaqMan Gene Expression Assays: Rat IL-1β (Rn00580432_m1), rat NFKβ (Rn01399583_m1), and rat TNF-α (Rn00562055_m1) primers were used for the real-time polymerase chain reaction. β-actin (housekeeping gen) (Rn00667869_m1) expression results in each tissue were used as the reference gene. Amplification and quantification processes were performed on Corbett Rotor-Gene (Thermo Fisher Scientific) device. The following TaqMan® Gene Expression Assays for 100ng cDNA were pipetted for 40 cycles with 100 ng cDNA, 10 µL TaqMan Master Mix, and 1 µL Assay and completed to 20 µL with RNase-free H_2O. The cycle threshold (Ct) is the cycle count at which the quantity of fluorescent signal observed in qRT-PCR experiments exceeds the lowest value. Ct values were transformed automatically into delta delta Ct ($2^{-\Delta\Delta Ct}$)[37] and the findings were statistically analyzed.

Histopathological Analysis

The preparation phase of solutions, dehydration and clearing of tissue samples, section planning, and staining with Harris Hematoxylin and Eosin Y were all done by previous studies for histopathological assessment[38]. The kidney tissue sections obtained from rats for histopathological analysis were quickly fixed in a 3.7% formaldehyde (10% formalin) solution for 48 h. For each series of increasing alcohol concentrations of 50%, 70%, 80%, 96%, and 99%, all tissues were preserved for 1 hour to test for dehydration. The tissues were cleared for three to fifteen minutes in a solution of xylene. For infiltration, it was preserved in liquid paraffin that was molten. Tissues were meticulously blocked in paraffin after being processed. Each paraffin block of tissue was cut to a thickness of 5 micrometers for histopathological analysis after blocking. On the slide covered in adhesive, paraffin sections were cut. All slides were then stained using Harris hematoxylin and eosin Y. For histopathological examinations, tubular necrosis, inflammation, and hemorrhage areas in kidney tissues were evaluated by light microscope.

Statistical and Semi-quantitative Analysis

For the statistical analysis of molecular results (IL-1β, NF-kB, and TNF-α) and biochemical results (MDA and GSH), we used GraphPad Prism, version 5.0, and the results are presented as the means±standard deviation. Comparisons between the groups were performed using the One-Way ANOVA and Tukey's multiple comparison tests; significance was accepted at $p<0.05$. P value less than 0.05 was considered statistically significant. Significant differences were detected among all groups, compared to the sham group (#$p<0.05$, ##$p<0.01$, ###$p<0.001$) and compared to the RI/RI group (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

For comparison of histopathological data among all groups and to better show the extent of tissue damage, the presence of tubular necrosis, inflammation, and hemorrhage findings were scored semiquantitatively as none: 0, few: 1, moderate: 2, and severe: 3. For each tissue slide, at least five areas were assessed at ×100 magnification, and the median staining concentration score was taken into consideration. The One-Way ANOVA and Tukey's multiple comparison tests were used to compare the groups (*$p<0.0$)[39].

RESULTS

Impacts of AVA on Oxidant and Antioxidant Parameters in Renal Tissue

MDA (Figure 1A) and GSH (Figure 1B) levels were investigated to research the antioxidant impact of AVA. MDA levels, a sign of the oxidant situation, increased in the RI/RI group ($p<0.001$). AVA administration reduced MDA levels in a dose-dependent manner, in comparison to the RI/RI group. GSH levels, a sign of the antioxidant situation, were dramatically lower in the RI/RI group in comparison to the sham group ($p<0.001$). In a dose-dependent manner, the AVA therapy reduced the drop in GSH levels brought on by ischemia/reperfusion.

Impacts of AVA on Anti-inflammatory Parameters in Renal Tissue

To investigate the anti-inflammatory effect of AVA, IL-1β (Figure 2A), NF-kB (Figure 2B), and TNF-α (Figure 2C) mRNA expression levels in the renal tissue of rats were analyzed. IL-1β, NF-kB, and TNF-α mRNA expression, signs of the anti-inflammatory situation, were significantly increased in the RI/RI group, compared to those in the sham group ($p<0.001$). The

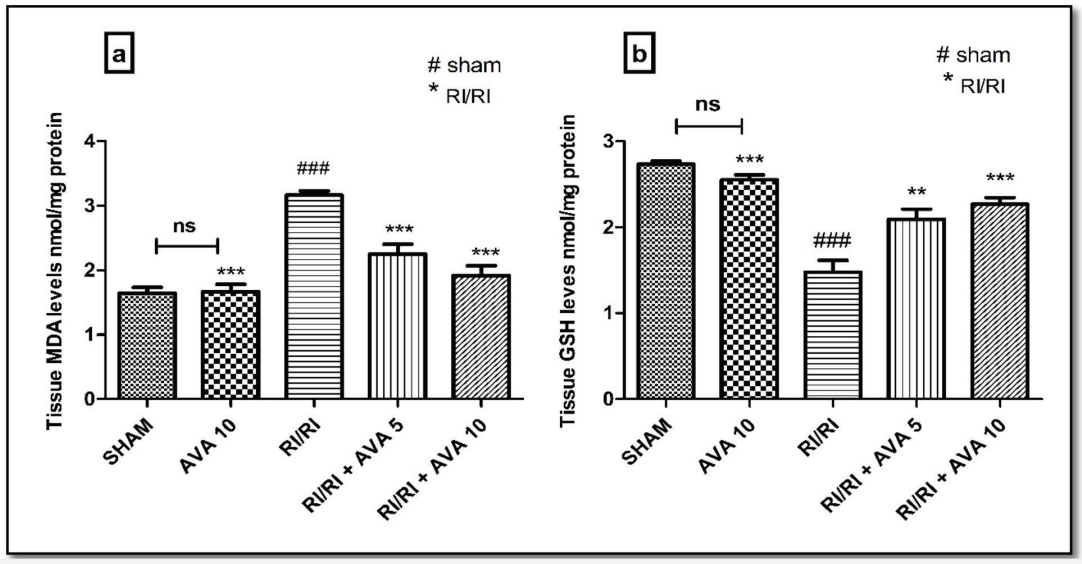

Figure 1. The biochemical results of the effects of AVA in the renal tissue on RI/RI. A) MDA levels (nmol/mg protein). B) GSH levels (nmol/mg protein). AVA 5: 5 mg/kg AVA; AVA 10: 10 mg/kg AVA. The levels of MDA and GSH were measured according to the modified methods with an ELISA reader. GraphPad Prism, version 5.0 was used for the statistical analysis, and the results are presented as the means±standard deviation. Comparisons between the groups were performed using the One-Way ANOVA and Tukey's multiple comparison tests; p value less than 0.05 was considered statistically significant. Significant differences were detected among all groups, compared to the sham group (#p<0.05, ##p<0.01, ###p<0.001) and compared to the RI/RI group (*p<0.05, **p<0.01, ***p<0.001)

AVA: Avanafil, RI/RI: Renal ischemia/reperfusion injury, MDA: Malondialdehyde, GSH: Glutathione

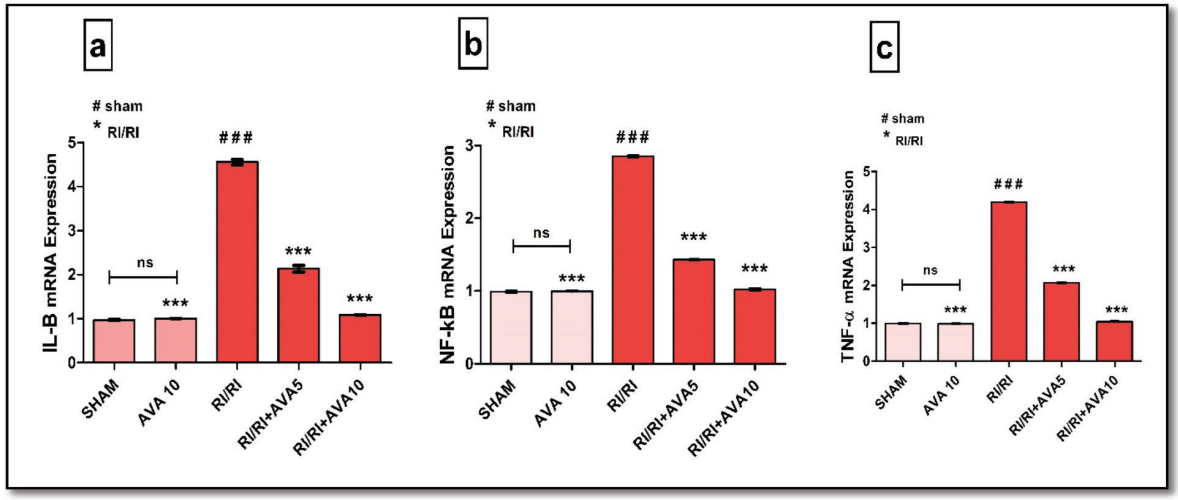

Figure 2. The molecular results of the effects of AVA in the renal tissue on RI/RI. A) IL-1β mRNA expression levels. B) NF-κB expression levels. C) TNF-α mRNA expression level. AVA 5: 5 mg/kg AVA; AVA 10: 10 mg/kg AVA. The expression of mRNAs was detected using qRT-PCR analysis. β-actin was used as the reference gene. GraphPad Prism, version 5.0 was used for the statistical analysis, and the results are presented as the means±standard deviation. Comparisons between the groups were performed using the One-Way ANOVA and Tukey's multiple comparison tests; p value less than 0.05 was considered statistically significant. Significant differences were detected among all groups, compared to the sham group (#p<0.05, ##p<0.01, ###p<0.001) and compared to the RI/RI group (*p<0.05, **p<0.01, ***p<0.001)

AVA: Avanafil, RI/RI: Renal ischemia/reperfusion injury, MDA: Malondialdehyde, GSH: Glutathione, NF-κB: Nuclear factor-kappa B, TNF-α: Tumor necrosis factor-alpha

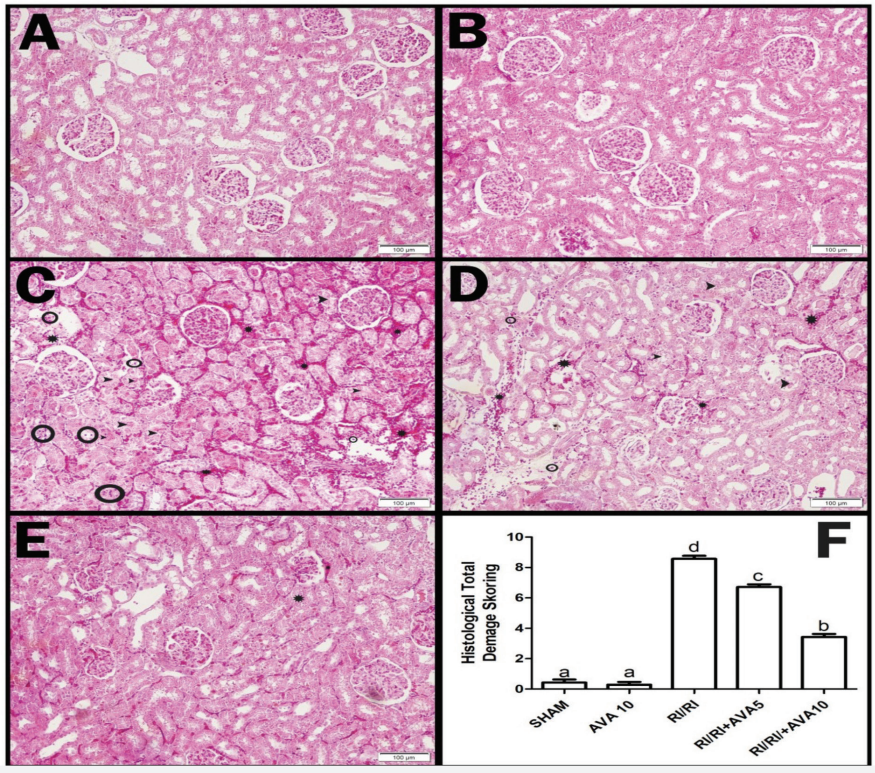

Figure 3. Pathologic changes: Hematoxylin-eosin staining findings and histological total tissue damage scoring results of the effects of AVA in the renal tissue on RI/RI. A) Sham group, B) AVA-10 group, C) RI/RI group, D) RI/RI + AVA 5 group, E) RI/RI + AVA 10 group, F) Histopathology scoring graph (star: Hemorrhage, ring: Inflammatory cells, arrowhead: Tubular necrosis) (*p<0.0)

AVA: Avanafil, RI/RI: Renal ischemia/reperfusion injury

AVA treatment improved the increase in IL-1β, NF-kB, and TNF-α mRNA expression induced by ischemia/reperfusion in a dose-dependent manner (p<0.001).

Impacts of AVA on Histopathological Changes in Renal Tissue

Light Microscopy Results

To investigate the histopathological effect of AVA in RI/RI, kidney tissue samples were stained with Harris Hematoxylin and Eosin Y staining and evaluated by a light microscope. (Figure 3A-E). Also, semi-quantitative scoring of histopathologic findings was shown in Figure 3F.

Under light microscopy, normal-looking glomerular structures, and distal and proximal tubule structures were observed in the kidney tissues of the sham group (Figure 3A). In the kidney tissues of the AVA10 group, no pathological damage was observed. The light microscopic findings of the histopathological appearance of this group's glomerular, distal, and proximal tubule structures were similar to those of the sham group (Figure 3B).

Signs of severe tissue injury caused by ischemia/reperfusion were observed in the kidney tissue samples of the RI/RI group. In the kidney tissues of this group, hemorrhage areas in which erythrocyte cells formed piles were observed around the glomerulus, distal and proximal tubules (star). Inflammatory cells were found in some areas (ring). Along with dilatation in glomerular structures, deteriorations due to necrotic cell death were observed in proximal and distal tubules (Figure 3C). The histopathological injury resulting from ischemia/reperfusion improved in AVA-administered groups, depending on the dose (Figure 3D, 3E). Furthermore, the histopathological appearance of the 10 mg/kg AVA-administered group was similar to the sham group (Figure 3E).

DISCUSSION

RI/RI is a pathological condition that causes additional organ damage in ischemic tissues by causing low blood supply, followed by the restoration of blood flow[40]. RI/RI damages many organs, especially the kidney, which raises the mortality rate. RI/RI is regarded as a significant cause of end-stage renal failure and chronic renal failure. Kidney transplantation, embolism trauma, vascular and cardiac surgery, atherosclerosis,

and chronic renal artery stenosis are a few of the conditions that can expose the kidneys to RI/RI[41]. Additionally, RI/RI is the cause of acute kidney injury in more than 60% of patients[42]. Reperfusion of the ischemic kidney leads to necrosis or apoptosis, which exacerbates the inflammatory and oxidative condition and harms cellular honesty. The primary pathophysiological mechanisms of this situation involve the release of ROS and the generation of pro-inflammatory mediators[43]. With this information in mind, In the present study, we aimed to determine the effects of AVA, which is a PDE 5 inhibitor on RI/RI in rats by biochemical, molecular, and histopathological analyses.

ROS and oxidative stress are crucial factors in the development of renal tissue injury[44]. Exaggerated ROS production throughout RI/RI may result in endothelial dysfunction, tubular injury, and interstitial inflammation. Thus, oxidative stress is important in the development of RI/RI[45,46]. The MDA level is a sign of lipid peroxidation, free oxygen radical content, and the extent of renal tissue injury caused by these radicals[47,48]. GSH is essential in endogenous protection against oxygen-free radicals[49]. We investigated oxidative stress factors related to RI/RI; GSH, and the lipid peroxidation product MDA. Previous studies have shown that after RI/RI, MDA level is increased and GSH level is decreased[50,51]. Also, Prem and Kurian[52] demonstrated that markers of oxidative stress were significantly raised in a rat model of RI/RI. Our research detected the levels of GSH and MDA in kidney tissue and we found that compared to the sham group, the MDA level in the kidney tissue of rats in the RI/RI group was significantly increased while GSH levels were significantly decreased, suggesting that after RI/RI, oxidative stress reaction was aggravated in the kidney tissues. Conversely, in the RI/RI plus AVA groups, GSH levels importantly raised and MDA levels importantly reduced depending on the AVA doses. These effects of AVA seemed to be associated with the inhibition of oxidative stress, to a lesser extent, inflammatory responses. These findings demonstrated that AVA enhanced the oxidative situation. As a result, it reduced oxidative stress and alleviated RI/RI. Chowdari Gurram et al.[53] reported that AVA significantly reduced the markers of oxidative stress in a mice model. In addition, some researchers also reported that AVA significantly improved oxidative parameters[54]. Similarly, it has previously been established that PDE 5 inhibitors have a protective impact by regulating oxidative stress in kidney tissue[55,56]. Consistent with other findings reported in the literature, our result suggests that AVA treatment reduces oxidant parameter generation while increasing anti-oxidant parameter generation.

Inflammation is important in tissue repair, which is a response to damage[57]. ROS induces tissue injury in a range of manners involving inflammatory response, mitochondrial dysfunction, ER, and oxidative stress[58]. As a result, blocking ROS generation is an effective method for RI/RI protection. RI/RI is a significant pathological condition and an inflammatory reaction in which the NF-kB family is crucial. ROS generation activates NF-kB, which then triggers ICAM-1 expression. Nevertheless, ROS and inflammatory reactions play important parts in the development of ischemia/reperfusion injury via NF-kB and the ICAM-1 progression[59]. By secreting TNF-α, which controls a spectrum of immune, inflammatory, and hematopoietic responses, immune cells play a crucial role in the inflammatory process[60]. TNF-α can stimulate the production of other inflammatory markers, including IL-1β, and aggravate the damage of tissues and organs[13]. Furthermore, the importance of the inflammatory response in RI/RI, which leads to the activation of IL-1, makes IL-1 a valuable target in RI/RI by modulating its effect. Hence, it is crucial for RI/RI treatment to reduce proinflammatory cytokines including IL-1β, NF-kB, and TNF-α[61]. In light of this information, in this study, the IL-1β, NF-κβ, and TNF-α mRNA expression in kidney tissues after RI/RI and treatment with AVA were evaluated to appraise the possible medical significance of AVA in RI/RI. We found that compared to the sham group, the IL-1β, NF-κβ, and TNF-α mRNA expression levels in the RI/RI group were significantly increased, suggesting that after RI/RI, the inflammatory reaction was aggravated in the kidney tissues. These results are consistent with previous research that found RI/RI-induced increases in cytokine levels[62,63]. Embaby et al.[64] demonstrated that renal TNF-α and NF-κB mRNA expressions increased in acute RI/RI in rats. Çakır et al.[65] showed that levels of IL-1β, NF-κβ, and TNF-α mRNA expressions increased in rats with RI/RI. In addition, several recent studies have shown that the levels of anti-inflammatory cytokines, including IL-1β, NF-κβ, and TNF-α, increase after RI/RI[66,67]. The current findings demonstrated that AVA fixed modifications to anti-inflammatory parameters were caused by RI/RI, indicating the strong anti-inflammatory feature of AVA. These findings indicated that AVA reduced tissue injury by avoiding RI/R-induced increases in cytokine levels. As a result, the anti-inflammatory effect of AVA appears to be related to the reduction of oxidative damage. AVA's anti-inflammatory effects, including suppression of pro-inflammatory cytokines, have earlier been shown in various animal models. Aydin et al.[68] demonstrated that AVA modulated NF-kB and IL-1 β levels in the LPS-induced acute lung injury model in rats. Chowdari Gurram et al.[53] demonstrated that AVA improved neuroinflammatory cytokines such as Tnf R1 and NF-kB in LPS-induced neuroinflammation in mice. Likewise, it has earlier been established that PDE 5 inhibitors have a protective role via inhibition of IL-1, NF-kB, and TNF-α in prostate ischemia[69] and acute kidney toxicity[70] models. Consistent with previous research, our findings show that AVA decreases tissue injury by avoiding the increase in IL-1β, NF-kB, and TNF-α levels due to ischemia/reperfusion.

Regarding the histopathological scores in the current investigation, the histological total tissue damage score was quite high in the RI/RI group, while the score was reduced in the groups treated with 5 and 10 mg/kg of AVA. In the RI/RI group, kidney tissues also showed severe pathological alterations. The RI/RI group displayed hemorrhage, extensive inflammatory cell infiltration, and necrosis. However, in the groups that received 5 and 10 mg/kg of AVA, the histological aspect of the kidney tissues was nearly normal. Our biochemical and molecular findings were corroborated by our histology findings.

Study Limitations

The limitations of this study include the fact that biochemical markers such as SOD data and renal function markers such as BUN could not be investigated. We think that further more detailed studies are now needed for a clearer understanding of the effect mechanism revealed in this study. However, this study is particularly valuable and original as the first to reveal the effect of AVA on RI/RI. We hope that the study will lead to further studies.

CONCLUSION

Based on the biochemical, molecular, and histopathological findings, we have shown that AVA significantly improved ischemia/reperfusion-induced renal injury. AVA may intervene with both anti-inflammatory and antioxidant processes that may be essential in renal ischemia/reperfusion damage. The effects of AVA on RI/RI may be related to the treatment (1) reducing immunopositivity of inflammatory cytokines including IL-1β, NF-kB, and TNF-α, (2) alleviating adverse changes in oxidative stress-related components including MDA and GSH, (3) reducing severe histopathological modifications. AVA should be considered as a potential therapeutic agent in addition to surgery in the clinical treatment of RI/RI. These findings may provide a mechanistic basis for using AVA to treat ischemia/reperfusion-induced renal injury.

Ethics

Ethics Committee Approval: This animal study and all its protocols were approved by Atatürk University Medical Experimental Application and Research Center of Ethics Committee on 04-04-2022 with document number E-75296309-050.01.04-2200103613.

Informed Consent: Animal experiment.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: T.N.Y., Z.H., C.K., A.B., T.T., M.S.C., B.Ö., Concept: T.N.Y., Z.H., C.K., A.B., T.T., M.S.C., B.Ö., Design: T.N.Y., Z.H., C.K., A.B., T.T., M.S.C., B.Ö., Data Collection or Processing: T.N.Y., Z.H., C.K., A.B., T.T., M.S.C., B.Ö., Analysis or Interpretation: T.N.Y., Z.H., C.K., A.B., T.T., M.S.C., B.Ö., Literature Search: T.N.Y., Z.H., C.K., Writing: T.N.Y., Z.H., C.K., A.B., T.T., M.S.C., B.Ö.

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REFERENCES

1. Ray SC, Mason J, O'Connor PM. Ischemic Renal Injury: Can Renal Anatomy and Associated Vascular Congestion Explain Why the Medulla and Not the Cortex Is Where the Trouble Starts? Semin Nephrol. 2019;39:520-9.
2. Quadri SS, Culver S, Siragy HM. Prorenin receptor mediates inflammation in renal ischemia. Clin Exp Pharmacol Physiol. 2018;45:133-9.
3. Wu MY, Yiang GT, Liao WT, Tsai AP, Cheng YL, Cheng PW, et al. Current Mechanistic Concepts in Ischemia and Reperfusion Injury. Cell Physiol Biochem. 2018;46:1650-67.
4. Inhibition of Brd4 alleviates renal ischemia/reperfusion injury-induced apoptosis and endoplasmic reticulum stress by blocking FoxO4-mediated oxidative stress. Redox Biol. 2019;24:101195.
5. Martin JL, Gruszczyk AV, Beach TE, Murphy MP, Saeb-Parsy K. Mitochondrial mechanisms and therapeutics in ischaemia reperfusion injury. Pediatr Nephrol. 2019;34:1167-74.
6. Malek M, Nematbakhsh M. Renal ischemia/reperfusion injury; from pathophysiology to treatment. J Renal Inj Prev. 2015;4:20-7.
7. Akhigbe R, Ajayi A. The impact of reactive oxygen species in the development of cardiometabolic disorders: a review. Lipids Health Dis. 2021;20:23.
8. Sengul O, Ferah I, Polat B, Halici Z, Bayir Y, Yilmaz M, Kilic N, Keles ON. Blockade of endothelin receptors with bosentan limits ischaemia/reperfusion-induced injury in rat ovaries. Eur J Obstet Gynecol Reprod Biol. 2013;170:458-63.
9. Aslan M, Erkanli Senturk G, Akkaya H, Sahin S, Yılmaz B. The effect of oxytocin and Kisspeptin-10 in ovary and uterus of ischemia-reperfusion injured rats. Taiwan J Obstet Gynecol. 2017;56:456-62.
10. Nayki C, Nayki U, Keskin Cimen F, Kulhan M, Yapca OE, Kurt N, et al. The effect of rutin on ovarian ischemia-reperfusion injury in a rat model. Gynecol Endocrinol. 2018;34:809-14.
11. Hashmi SF, Rathore HA, Sattar MA, Johns EJ, Gan CY, Chia TY, et al. Hydrogen Sulphide Treatment Prevents Renal Ischemia-Reperfusion Injury by Inhibiting the Expression of ICAM-1 and NF-kB Concentration in Normotensive and Hypertensive Rats. Biomolecules. 2021;11:1549.
12. Prince PD, Rodríguez Lanzi C, Fraga CG, Galleano M. Dietary (-)-epicatechin affects NF-κB activation and NADPH oxidases in the kidney cortex of high-fructose-fed rats. Food Funct. 2019;10:26-32.
13. Zhang X, Jin C, Li Y, Guan S, Han F, Zhang S. Catalpol improves cholinergic function and reduces inflammatory cytokines in the senescent mice induced by D-galactose. Food Chem Toxicol. 2013;58:50-5.
14. Kucuk A, Yucel M, Erkasap N, Tosun M, Koken T, Ozkurt M, et al. The effects of PDE5 inhibitory drugs on renal ischemia/reperfusion injury in rats. Mol Biol Rep. 2012;39:9775-82.
15. Swiecicka A. The efficacy of PDE5 inhibitors in diabetic patients. Andrology. 2023;11:245-56.
16. Brown KE, Dhaun N, Goddard J, Webb DJ. Potential therapeutic role of phosphodiesterase type 5 inhibition in hypertension and chronic kidney disease. Hypertension. 2014;63:5-11.
17. Zucchi A, Costantini E, Scroppo FI, Silvani M, Kopa Z, Illiano E, et al. The first-generation phosphodiesterase 5 inhibitors and their pharmacokinetic issue. Andrology. 2019;7:804-17.

18. Ahmed WS, Geethakumari AM, Biswas KH. Phosphodiesterase 5 (PDE5): Structure-function regulation and therapeutic applications of inhibitors. Biomed Pharmacother. 2021;134:111128.

19. Ockaili R, Salloum F, Hawkins J, Kukreja RC. Sildenafil (Viagra) induces powerful cardioprotective effect via opening of mitochondrial K(ATP) channels in rabbits. Am J Physiol Heart Circ Physiol. 2002;283:H1263-9.

20. Serarslan Y, Yonden Z, Ozgiray E, Oktar S, Guven EO, Sogut S, et al. Protective effects of tadalafil on experimental spinal cord injury in rats. J Clin Neurosci. 2010;17:349-52.

21. Ko IG, Shin MS, Kim BK, Kim SE, Sung YH, Kim TS, et al. Tadalafil improves short-term memory by suppressing ischemia-induced apoptosis of hippocampal neuronal cells in gerbils. Pharmacol Biochem Behav. 2009;91:629-35.

22. Celik M, Aksoy AN, Aksoy H, Aksoy Y, Halici Z. Sildenafil reduces ischemia-reperfusion injury in rat ovary: biochemical and histopathological evaluation. Gynecol Obstet Invest. 2014;78:162-7.

23. Zisis IE, Georgiadis G, Docea AO, Calina D, Cercelaru L, Renoprotective Effect of Vardenafil and Avanafil in Contrast-Induced Nephropathy: Emerging Evidence from an Animal Model. J Pers Med. 2022;12:670.

24. Huyut Z, Bakan N, Yildirim S, Akbay HI, Huyut MT, Ahlatci A, et al. Can zaprinast and avanafil induce the levels of angiogenesis, bone morphogenic protein 2, 4 and 7 in kidney of ovariectomised rats? Arch Physiol Biochem. 2022;128:945-50.

25. Huyut Z, Bakan N, Yıldırım S, Alp HH. Effects of the Phosphodiesterase-5 (PDE-5) Inhibitors, Avanafil and Zaprinast, on Bone Remodeling and Oxidative Damage in a Rat Model of Glucocorticoid-Induced Osteoporosis. Med Sci Monit Basic Res. 2018;24:47-58.

26. Huyut Z, Bakan N, Akbay Hİ, Yıldırım S, Şekeroğlu MR. Zaprinast and avanafil increase the vascular endothelial growth factor, vitamin D3, bone morphogenic proteins 4 and 7 levels in the kidney tissue of male rats applied the glucocorticoid. Arch Physiol Biochem. 2022;128:1290-6.

27. Ziypak T, Halici Z, Alkan E, Akpinar E, Polat B, Adanur S, et al. Renoprotective effect of aliskiren on renal ischemia/reperfusion injury in rats: electron microscopy and molecular study. Ren Fail. 2015;37:343-54.

28. Nezamoleslami S, Sheibani M, Jahanshahi F, Mumtaz F, Abbasi A, Dehpour AR. Protective effect of dapsone against renal ischemia-reperfusion injury in rat. Immunopharmacol Immunotoxicol. 2020;42:272-9.

29. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95:351-8.

30. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem. 1968;25:192-205.

31. Bayir Y, Un H, Cadirci E, Akpinar E, Diyarbakir B, Calik I, et al. Effects of Aliskiren, an RAAS inhibitor, on a carrageenan-induced pleurisy model of rats. An Acad Bras Cienc. 2019;91:e20180106.

32. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193:265-75.

33. Kose D, Kose A, Halici Z, Cadirci E, Tavaci T, Gurbuz MA, et al. Bosentan, a drug used in the treatment of pulmonary hypertension, can prevent development of osteoporosis. Iran J Basic Med Sci. 2021;24:922-7.

34. Cadirci E, Ugan RA, Dincer B, Gundogdu B, Cinar I, Akpinar E, et al. Urotensin receptors as a new target for CLP induced septic lung injury in mice. Naunyn Schmiedebergs Arch Pharmacol. 2019;392:135-45.

35. Sipahi H, Orak D, Reis R, Yalman K, Senol O, Palabiyik-Yucelik SS, et al. A comprehensive study to evaluate the wound healing potential of okra (Abelmoschus esculentus) fruit. J Ethnopharmacol. 2022;287:114843.

36. Köse D, Yüksel TN, Halıcı Z, Çadırcı E, Gürbüz MA. The Effects of Agomelatine Treatment on Lipopolysaccharide-Induced Septic Lung Injuries in Rats. Eurasian J Med. 2021;53:127-31.

37. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25:402-8.

38. Yüksel TN, Yayla M, Duygu K, Uğan RA, Toktay E, Kılıçle PA, et al. Investigation of the Protective Effects of Pomegranate (Punica Granatum L.) Peel Extract on Lipopolysaccharide-induced Uveitis in Rats. Trakya University Journal of Natural Sciences. 2023;24:11-20.

39. Toktay E, Tastan TB, Gurbuz MA, Erbas E, Demir O, Ugan RA, et al. Potential protective effect of astaxanthin on ovary ischemia-reperfusion injury. Iran J Basic Med Sci. 2022;25:173-8.

40. Pefanis A, Ierino FL, Murphy JM, Cowan PJ. Regulated necrosis in kidney ischemia-reperfusion injury. Kidney Int. 2019;96:291-301.

41. Abbas W, Altemimi M, Qassam H, Hameed AA, Zigam Q, Abbas L, et al. Fimasartan ameliorates renal ischemia reperfusion injury via modulation of oxidative stress, inflammatory and apoptotic cascades in a rat model. J Med Life. 2022;15:241-51.

42. Park M, Kwon CH, Ha HK, Han M, Song SH. RNA-Seq identifies condition-specific biological signatures of ischemia-reperfusion injury in the human kidney. BMC Nephrol. 2020;21:398.

43. Shan Y, Chen D, Hu B, Xu G, Li W, Jin Y, et al. Allicin ameliorates renal ischemia/reperfusion injury via inhibition of oxidative stress and inflammation in rats. Biomed Pharmacother. 2021;142:112077.

44. Tian Y, Shu J, Huang R, Chu X, Mei X. Protective effect of renal ischemic postconditioning in renal ischemic-reperfusion injury. Transl Androl Urol. 2020;9:1356-65.

45. Zhao M, Wang Y, Li L, Liu S, Wang C, Yuan Y, et al. Mitochondrial ROS promote mitochondrial dysfunction and inflammation in ischemic acute kidney injury by disrupting TFAM-mediated mtDNA maintenance. Theranostics. 2021;11:1845-63.

46. Li F, Bahnson EM, Wilder J, Siletzky R, Hagaman J, Nickekeit V, et al. Oral high dose vitamin B12 decreases renal superoxide and post-ischemia/reperfusion injury in mice. Redox Biol. 2020;32:101504.

47. Zhu MM, Wang L, Yang D, Li C, Pang ST, Li XH, et al. Wedelolactone alleviates doxorubicin-induced inflammation and oxidative stress damage of podocytes by IκK/IκB/NF-κB pathway. Biomed Pharmacother. 2019;117:109088.

48. Hu B, Tang J, Zhang Y, Ma ZY, Shan YG, Liu J, et al. Glycogen Synthase Kinase-3β Inhibitor Attenuates Renal Damage Through Regulating Antioxidant and Anti-inflammation in Rat Kidney Transplant With Cold Ischemia Reperfusion. Transplant Proc. 2019;51:2066-70.

49. Aboutaleb N, Jamali H, Abolhasani M, Pazoki Toroudi H. Lavender oil (Lavandula angustifolia) attenuates renal ischemia/reperfusion injury in rats through suppression of inflammation, oxidative stress and apoptosis. Biomed Pharmacother. 2019;110:9-19.

50. Bayir Y, Cadirci E, Polat B, Baygutalp NK, Albayrak A, Karakus E, et al. Aliskiren - a promising strategy for ovarian ischemia/reperfusion injury protection in rats via RAAS. Gynecol Endocrinol. 2016;32:675-83.

51. Huang D, Chen D, Hu T, Liang H. GATA2 promotes oxidative stress to aggravate renal ischemia-reperfusion injury by up-regulating Redd1. Mol Immunol. 2023;153:75-84.

52. Prem PN, Kurian GA. Fisetin attenuates renal ischemia/reperfusion injury by improving mitochondrial quality, reducing apoptosis and oxidative stress. N-S Arch Pharmacol. 2022;395:547-61.

53. Chowdari Gurram P, Satarker S, Kumar G, Begum F, Mehta C, Nayak U, et al. Avanafil mediated dual inhibition of IKKβ and TNFR1 in an experimental paradigm of Alzheimer's disease: in silico and in vivo approach. J Biomol Struct Dyn. 2022:1-19.

54. Kurakula M, Naveen N R, Patel B, Manne R, Patel DB. Preparation, Optimization and Evaluation of Chitosan-Based Avanafil Nanocomplex Utilizing Antioxidants for Enhanced Neuroprotective Effect on PC12 Cells. Gels. 2021;7:96.

55. Jorge ARC, Marinho AD, Silveira JAM, Nogueira Junior FA, de Aquino PEA, Alves APNN, et al. Phosphodiesterase-5 inhibitor sildenafil attenuates kidney injury induced by Bothrops alternatus snake venom. Toxicon. 2021;202:46-52.

56. Mehanna OM, El Askary A, Al-Shehri S, El-Esawy B. Effect of phosphodiesterase inhibitors on renal functions and oxidant/antioxidant

parameters in streptozocin-induced diabetic rats. Arch Physiol Biochem. 2018;124:424-9.

57. Lamkanfi M, Dixit VM. Inflammasomes and their roles in health and disease. Annu Rev Cell Dev Biol. 2012;28:137-61.

58. Malek M, Nematbakhsh M. Renal ischemia/reperfusion injury; from pathophysiology to treatment. J Renal Inj Prev. 2015;4:20-7.

59. Hashmi SF, Rathore HA, Sattar MA, Johns EJ, Gan CY, Chia TY, et al. Correction: Hashmi et al. Hydrogen Sulphide Treatment Prevents Renal Ischemia-Reperfusion Injury by Inhibiting the Expression of ICAM-1 and NF-kB Concentration in Normotensive and Hypertensive Rats. Biomolecules 2021, 11, 1549. Biomolecules. 2022;12:593.

60. Kong DH, Kim YK, Kim MR, Jang JH, Lee S. Emerging Roles of Vascular Cell Adhesion Molecule-1 (VCAM-1) in Immunological Disorders and Cancer. Int J Mol Sci. 2018;19:1057.

61. Vannay A, Fekete A, Langer R, Toth T, Sziksz E, Vasarhelyi B, et al. Dehydroepiandrosterone pretreatment alters the ischaemia/reperfusion-induced VEGF, IL-1 and IL-6 gene expression in acute renal failure. Kidney Blood Press Res. 2009;32:175-84.

62. Li Y, Xu B, Yang J, Wang L, Tan X, Hu X, et al. Liraglutide protects against lethal renal ischemia-reperfusion injury by inhibiting high-mobility group box 1 nuclear-cytoplasmic translocation and release. Pharmacol Res. 2021;173:105867.

63. Bai T, Wang X, Qin C, Yang K, Duan Z, Cao Z, et al. Deficiency of mindin reduces renal injury after ischemia reperfusion. Mol Med. 2022;28:152.

64. Embaby EM, Saleh RM, Marghani BH, Barakat N, Awadin W, Elshal MF, et al. The combined effect of zinc oxide nanoparticles and milrinone on acute renal ischemia/reperfusion injury in rats: Potential underlying mechanisms. Life Sci. 2023;323:121435.

65. Çakır M, Aydın A, Tekin S. Protective effect of fatty acid amide hydrolase inhibitor URB597 and monoacylglycerol lipase inhibitor KML29 on renal ischemia-reperfusion injury via toll-like receptor 4/nuclear factor-kappa B pathway. Int Immunopharmacol. 2023;114:109586.

66. Younis NS. Myrrh Essential Oil Mitigates Renal Ischemia/Reperfusion-Induced Injury. Curr Issues Mol Biol. 2023;45:1183-96.

67. Abdel-Razek HA, Rizk MS, Amer GS, Kora MA, Afifi AM, Donia SS. Impact of combined ischemic preconditioning and melatonin on renal ischemia-reperfusion injury in rats. Iran J Basic Med Sci. 2023;26:235-40.

68. Aydin P, Magden ZBA, Uzuncakmak SK, Halici H, Akgun N, Mendil AS, et al. Avanafil as a Novel Therapeutic Agent Against LPS-Induced Acute Lung Injury via Increasing CGMP to Downregulate the TLR4-NF-κB-NLRP3 Inflammasome Signaling Pathway. Lung. 2022;200:561-72.

69. Kobayashi H, Zha X, Nagase K, Inamura S, Taga M, Aoki Y, et al. Phosphodiesterase 5 inhibitor suppresses prostate weight increase in type 2 diabetic rats. Life Sci. 2022;298:120504.

70. Wadie W, Abdel-Razek NS, Salem HA. Phosphodiesterase (1, 3 & 5) inhibitors attenuate diclofenac-induced acute kidney toxicity in rats. Life Sci. 2021;277:119506.

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Suicidal Attempt in Adolescents with Major Depressive Disorder

Majör Depresif Bozukluğu Olan Ergenlerde İntihar Girişimi

Esen YILDIRIM DEMİRDÖĞEN, Mehmet Akif AKINCI, Abdullah BOZKURT, Halime DAĞCI

Atatürk University Faculty of Medicine, Department of Child and Adolescent Psychiatry, Erzurum, Turkey

ABSTRACT

Aim: In this study, we aimed to assess the sociodemographic and clinical characteristics of adolescents diagnosed with major depressive disorder (MDD), with and without suicide attempts, as well as to investigate the factors predicting suicide attempts.

Materials and Methods: This study included 151 adolescents aged between 12 and 18 years, who were diagnosed with MDD between January 2021 and June 2022. This study has a retrospective design, and data including family sociodemographic characteristics, suicide attempts and characteristics, non-suicidal self-injury (NSSI) attempts, history of abuse, comorbid psychiatric disorders, and scores on depression and anxiety scales were extracted from the cases' polyclinic records. The participants were divided into two groups as those with suicide attempts (n=40) and those without suicide attempts (NSSI n=111), and the sociodemographic and clinical data were compared between the groups. In addition, a binary logistic regression analysis was performed to identify the predictors for suicide attempts.

Results: The results of the study revealed that the suicide attempt group had a higher age (p=0.023), less maternal years of education (p=0.026), higher rates of self-injurious behavior (p<0.001), more severe depression (p=0.021) and anxiety (p=0.018) symptoms, and higher rates of history of childhood abuse (p=0.001). The binary logistic regression analysis performed to predict suicide attempts in those with an MDD diagnosis determined NSSI and history of abuse to be predictors.

Conclusion: A better understanding of predictive factors of suicide attempts in adolescents with depression may help establish targets for early intervention and inform more effective prevention strategies. Particularly, the presence of self-injurious behaviors and history of childhood abuse should be warning for suicide attempts.

Keywords: Major depressive disorder, adolescents, suicide attempt, non-suicidal self-injury, childhood abuse

ÖZ

Amaç: Çalışmamızda intihar girişimi olan ve olmayan majör depresif bozukluk (MDB) tanılı ergenlerin sosyodemografik ve klinik özelliklerini değerlendirmeyi ve intihar girişimini yordayan faktörleri araştırmayı amaçladık.

Gereç ve Yöntem: Çalışmaya Ocak 2021-Haziran 2022 tarihleri arasında MDB tanısı alan 12-18 yaş aralığında 151 ergen dahil edildi. Çalışmamız retrospektif dizaynda olup olgu ve ailesine ait sosyodemografik özellikler, intihar girişimi ve niteliği, intihar dışı kendine zarar verme (NSSI) girişimi, istismar öyküsü, eşlik eden komorbid psikiyatrik bozukluklar, depresyon ve anksiyete ölçek skorları gibi tüm veriler olguların poliklinik dosyalarından kaydedildi. Katılımcılar intihar girişimi olan (n=40) ve olmayan (n=111) olarak iki gruba ayrılarak gruplar arası sosyodemografik ve klinik özellikler karşılaştırıldı. Ayrıca intihar girişimini yordayan faktörleri belirlemek için iki boyutlu lojistik regresyon analizi yapıldı.

Bulgular: Çalışmanın sonuçları suisidal girişimi olan grubun daha büyük yaşta (p=0,023), anne eğitim süresinin daha düşük (p=0,026), kendine zarar verme davranışının daha fazla (p<0,001), depresyon (p=0,021) ve anksiyete semptom şiddetinin (p=0,018) daha yüksek, çocukluk çağı istismar öyküsünün daha fazla (p=0,001) olduğunu gösterdi. MDB tanısında intihar girişimini yordamak için yapılan iki boyutlu regresyon analizinde ise NSSI ve istismar öyküsünün yordayıcı olduğu tespit edildi.

Sonuç: Depresyonu olan ergenlerde intihar girişimini yordayan faktörlerin daha iyi anlaşılması, erken müdahale için hedeflerin belirlenmesine yardımcı olabilir ve daha etkili önleme stratejileri konusunda bilgi sağlayabilir. Özellikle kendine zarar verme davranışının olması ve çocukluk çağı istismar öyküsü, intihar girişimleri için uyarıcı olmalıdır.

Anahtar Kelimeler: Majör depresif bozukluk, ergenler, intihar girişimi, intihar dışı kendine zarar verme davranışı, çocukluk çağı istismar öyküsü

Address for Correspondence: Esen YILDIRIM DEMİRDÖĞEN MD, Atatürk University Faculty of Medicine, Department of Child and Adolescent Psychiatry, Erzurum, Turkey
Phone: +90 553 552 50 62 **E-mail:** esenyildirim08@hotmail.com **ORCID ID:** orcid.org/0000-0002-2457-5832
Received: 13.04.2023 **Accepted**: 05.07.2023

INTRODUCTION

Major depressive disorder (MDD) is a psychiatric disorder affecting the social adaptation, cognitive and emotional development of children and adolescents and resulting in a significant loss of functioning in their daily lives. It is characterized by a persistent and repetitive depressed or irritable mood along with diminished interest in pleasurable activities as well as symptoms in domains including attention, sleep, and appetite[1]. The lifetime prevalence of adolescent depression is 11%[2]. Depressive episodes during this period are associated with poor psychosocial functioning (e.g., more conflict with parents, poorer academic performance) and more frequent risk-taking behaviors (e.g., substance abuse, early onset sexual behaviors, suicide attempts)[3,4].

Adolescent depression and suicide are serious public health problems. Previous studies have indicated a strong relationship between adolescent depression and suicide attempts. Patients experiencing depression are under a higher risk for suicidal behaviors including ideation, planning and attempts compared to those without depression[5]. Parallel to the literature on adults, MDD is a well-known risk factor for suicide attempts in adolescents, and adolescents with depression have a six-times higher risk of attempting suicide compared to adolescents without depression[6]. Nearly 30% of adolescents diagnosed with depression were shown to attempt suicide[7].

The high risk of suicide attempts in depressed adolescents emphasizes the importance of identifying the other associated risk factors in this population. Factors such as a low socioeconomic level, history of parental divorce, parental psychiatric illness, and problematic familial relationships were reported to have significant influence on suicide attempts in depressed adolescents[8,9]. Adolescents and young adults who experienced sexual abuse are under a higher risk for suicide[10]. In a review of the factors associated with suicide attempts in young individuals with depression, depression characteristics (type and severity), psychiatric comorbidities (particularly, anxiety and substance abuse disorders), and neurological characteristics (structural and functional changes in prefrontal, subcortical and cerebellar regions) were shown to be associated with suicide outcomes[11]. A history of non-suicidal self-injury (NSSI) was also described to be a clinical indicator of future suicide attempts[12,13].

A better understanding of factors increasing the related factors associated with suicide attempts in adolescents with depression may help establish targets for early intervention and inform more effective prevention strategies. Thus, we aimed to assess the sociodemographic and clinical characteristics of adolescents diagnosed with MDD with and without suicide attempts, and to investigate the relationship between these characteristics and suicide attempts.

MATERIALS AND METHODS

This study included 151 adolescents aged between 12 and 18 years, who were diagnosed with MDD in the Child and Adolescent Psychiatry Clinic of Atatürk University, Faculty of Medicine between January 2021 and June 2022. This study has a retrospective design and data including family sociodemographic characteristics, suicide attempts and characteristics, NSSI attempts, history of abuse, comorbid psychiatric disorders, scores on depression and anxiety scales were extracted from the cases' polyclinic records. The authors, who are child and adolescent psychiatrists, and who were responsible for data collection, evaluated the patients' files and reached consensus on all cases. The inclusion criteria were as follows: (I) patients aged between 12 and 18 years; (II) patients meeting the diagnostic criteria for depression in the Diagnostic and Statistic Manual of Mental Disorders-5th edition (DSM-5). Meanwhile, the exclusion criteria were as follows: (I) patients with other mental disorders such as schizophrenia, bipolar disorder, intellectual disability, autism spectrum disorder, etc.; (II) patients with serious organic disorders; (III) patients with files that were missing the data to be recorded for the present study.

This study was approved by Atatürk University, Faculty of Medicine, Clinical Research Ethics Committee (approval number: B.30.2.ATA.0.01.00/506, date: 30/06/2022). After receiving approval, files of the cases from the specified dates were inspected and a data set was constructed based on the inclusion and exclusion criteria. The participants were divided into two groups as those with and without previous suicide attempts, and statistical analyses were performed.

Data Collection Tools

Children's Depression Inventory (CDI): The scale developed by Kovacs[14] to assess depression in children aged between 6 and 17 years consists of 27 items[14]. Each item receives a score of 0, 1, or 2 according to symptom severity. High scores indicate a high level of depression. The Turkish validity and reliability study established the scale's Cronbach's α internal consistency coefficient as 0.80[15].

Beck Anxiety Inventory (BAI): The scale adapted to Turkish by Ulusoy et al.[16] assesses certain attitudes and symptoms related to anxiety. The scale is composed of 21 items and one of four statements is chosen for each item. The highest possible score is 84, while the lowest possible score is 21. The scale's internal consistency coefficient is between 0.92 and 0.94.

Sociodemographic and Clinical Characteristics Data Form: This is a form constructed by the authors based on the data from the polyclinic files. It includes data such as the child's age and gender, parental age, level of education, status of

employment, history of mental illness, history of NSSI/suicide, family economic status, problems in familial relationships, problems in peer relationships, psychiatric and medical comorbidities, history of abuse, NSSI/suicide patterns.

Statistical Analysis

All statistical analyses were performed using the Statistical Package for Social Sciences) version 22.0. The Kolmogorov-Smirnov test was used to assess data for normal distribution. Descriptive analyses were used to summarize sociodemographic and clinical data. Descriptive statistics were presented as mean, standard deviation values and percentages. Categorical variables were compared using the chi-square test. Numeric variables were compared using the independent samples t-test or Mann-Whitney U test based on whether the groups had a normal distribution. A binary logistic regression analysis was conducted to identify the predictors of suicide attempts. P<0.05 was considered statistically significant.

RESULTS

This study included 151 adolescents aged between 12 and 18 years. Forty individuals (33 girls, 7 boys) had previous suicide attempts, while 111 (77 girls, 34 boys) did not have a history of suicide attempts. The groups were not significantly different with regard to gender (p=0.079). The mean age of the suicide attempt group was (15.05±1.7), which was significantly higher than the mean age of the non-suicide attempt group (14.23±2.3) (p=0.023). The suicide attempt group had significantly less maternal years of education (suicide attempt=5.6±3.5, non-suicide attempt=7.62±4.8; p=0.026). The groups were not significantly different in terms of the other sociodemographic characteristics. Sociodemographic characteristics of the cases are shown in Table 1.

Upon the examination of suicidal patterns in the suicide attempt group, it was found that 69.2% of the cases attempted suicide by taking drugs or chemicals, 12.7% by jumping from a height, 10.3% by cutting, and 7.7% by hanging. When the

Table 1. Sociodemographic characteristics of adolescents and their parents

	SA (n=40) Mean±SD/%	Non-SA (n=111) Mean±SD/%	t/z/χ^2	p
Age	15.05±1.7	14.23±2.3	2.307	0.023
Gender				0.079
Female	82.5%	69.4%	2.563	
Male	26.5%	30.6%		
Chronic physical illness	2.6%	9.9%	2.025	0.297
Mothers' age (years)	42.00±5.71	41.65±6.25	0.286	0.776
Fathers' age (years)	46.34±6.04	45.92±6.159	0.338	0.737
Mother's education (years)	5.6±3.5	7.62±4.8	-2.289	0.026
Father's education (%) (years)	8.65±4.64	9.43±4.48	-0.741	0.463
Mother employed	14.7%	25.3%	1.612	0.204
Father employed	88.2%	90.8%	0.189	0.663
Mother's psychiatric disorder	17.6%	19.8%	0.076	0.783
Father's psychiatric disorder	11.4%	9.3%	0.134	0.714
Mother's physical illness	23.5%	24.8%	0.021	0.886
Father's physical illness	27.8%	19.0%	0.134	0.714
Family type Nuclear family Extended family Fragmented family	 79.5% 12.8% 7.7%	 74.3% 16.5% 9.2%	1.406	0.495
Family income				0.563
Low	44.7%	35.8%		
Middle	47.4%	52.3%	1.148	
High	7.9%	11.9%		
Family history of NSSI/suicide attempt	7.7%	4.6%	0.542	0.435

SA: Suicidal attempt, Non-SA: Non-suicidal attempt, non-NSSI: Non-suicidal self injury, SD: Standard deviation

Table 2. Clinical characteristics of adolescents

	SA (n=40) Mean±SD/%	Non-SA (n=111) Mean±SD/%	t/z/χ2	p
Problems in familial relationships	72.5%	68.2%	0.257	0.612
Problems in peer relationships	68.4%	62.4%	0.445	0.505
NSSI	67.5%	22.3%	26.729	**<0.001**
CDI total score	29.67±10.03	23.44±9.7	2.405	**0.021**
BAI total score	36.1±13	21.89±13.29	2.730	**0.018**
Psychiatric comorbidity	50%	43.6%	4.108	0.579
Childhood abuse	37.5%	13.5%	10.626	**0.001**

SA: Suicidal attempt, Non-SA: Non-suicidal attempt, Non-NSSI: Non-suicidal self-injury, CDI: Child Depression Inventory, BAI: Beck Anxiety Inventory, SD: Standard deviation

Table 3. Predictors of suicide attempts in adolescents diagnosed with major depressive disorder

	β	S.E.	p	Exp(B)	95% CI for EXP(B)	
					Lower	Upper
Constant	-1.988	1.971	0.313	0.137		
Age	0.117	0.134	0.381	1.124	0.865	1.461
Gender	-0.579	0.601	0.336	0.561	0.173	1.821
Problems in familial relationships	0.797	0.556	0.151	2.219	0.747	6.596
Problems in peer relationships	0.183	0.487	0.708	1.200	0.462	3.119
Psychiatric Comorbidity	0.017	0.456	0.971	0.983	0.402	2.404
Childhood abuse	1.159	0.533	**0.030**	0.314	0.110	0.892
Family History of NSSI/Suicide Attempt	0.790	1.065	0.458	0.454	0.056	3.661
NSSI	2.085	0.501	**<0.001**	0.124	0.047	0.332

SA: Suicidal attempt, Non-SA: Non-suicidal attempt, Non-NSSI: Non-suicidal self-injury, CI: Confidence interval

groups were compared with regard to NSSI, the rate of NSSI was found to be 67.5% in the suicide attempt group compared to 22.3% in the non-suicide attempt group (p<0.001). The suicide attempt group had significantly higher scores on CDI (suicide attempt CDI total score=29.67±10.03; non-suicide attempt CDI total score=23.44±9.7 p=0.021) and the BAI (suicide attempt total score=36.1±13.32 non-suicide attempt total score=21.89±13.29 p=0.018). Regarding psychiatric comorbidities, a rate of 50% was found in the suicide attempt group and a rate of 43.6% was found in the non-suicide attempt group, with no statistically significant difference between the groups in this regard (p=0.579). The comorbidities of the suicide attempt group included anxiety disorder (17.5%), post-traumatic stress disorder (PTSD) (12.5%), attention deficit hyperactivity disorder (7.5%), obsessive compulsive disorder (7.5%), and conversion disorder (5%). For the non-suicide attempt group, the comorbidities were anxiety disorder (12.7%), attention deficit hyperactivity disorder (12.7%), PTSD (10.9%), obsessive compulsive disorder (3.7%), and conversion disorder (3.6%). History of childhood abuse was positive at a rate of 37.5% in the suicide attempt group compared to 13.5% in the non-suicide attempt group (p=0.001). Clinical data of the groups are summarized in Table 2.

The binary logistic regression analysis performed to predict suicide attempts in the presence of a depression diagnosis revealed NSSI and history of abuse to be predictors. The results are summarized in Table 3.

DISCUSSION

In this study, we aimed to compare the sociodemographic and clinical characteristics of adolescents diagnosed with MDD with and without suicide attempts, and to investigate the factors predicting suicide attempts. The results of our study showed that the suicide attempt group had a higher age, less maternal years of education, higher rates of self-injurious behavior, more severe depression and anxiety symptoms, and higher rates of history of childhood abuse. In addition, the presence of NSSI and history of childhood trauma were found to be predictors of suicide attempts in adolescents diagnosed with MDD.

The investigation of the factors associated with suicidal behavior, a risk factor for completed suicide, which is among the leading causes of death among youth, the identification of individuals under risk in order to prevent suicidal behavior; and the development of appropriate intervention programs for these individuals are of major importance[17]. The reduction

of suicide attempts, especially among individuals with psychiatric disorders, is an important public health goal in various countries[18]. Considering that MDD is the most common psychiatric disorder that has a relationship with suicide attempts, the investigation of the factors associated with suicide attempts in adolescents diagnosed with MDD should be a focus of studies concerning suicide prevention[19].

The comparison of the sociodemographic characteristics of adolescents diagnosed with MDD with and without suicide attempts revealed differences regarding age and maternal years of education. The suicide attempt group had a higher mean age. Studies have shown that suicide attempts are more common in older adolescents due to the increase in stressors related to academic and interpersonal relationships that increase with age in both MDD and community samples, increase in comorbid psychiatric disorders and more severe depressive symptoms[20-22]. This result is consistent with evidence from the literature suggesting that suicide attempts are more common among older adolescents. A low parental education level was shown to be associated with the suicide attempts in adolescents both in a population sample and in a clinical sample, and it was suggested that the effects of parental education on the mental states and suicide risk in adolescents were considered seriously. Moreover, it was described that this relationship could vary across different geographical and economic contexts depending on cultural, psychosocial and/or biological factors and the importance of considering cultural and familial contexts in the clinical management of adolescent suicidal behaviors was stressed[23,24]. In a study conducted in Turkey to evaluate the severity of suicidal behavior in depressed female adolescents, maternal perception of social gender inequality, which was the only factor that predicted suicide severity, was found to be related to the mother's level of education[25]. Our result that the level of parental education is lower in the suicide attempt group corroborates the results reported in the literature. The fact that only the maternal educational status differed between the groups may be attributed to the effects of cultural and familial contexts.

An important finding of our study was that the suicide attempt group had higher rates of NSSI behavior. Previous studies have also shown that a significant suicide risk followed self-injury in adolescents[26]. NSSI was shown to be associated with suicide attempts in depressed adolescents. In the Treatment of SSRI-resistant Depression in Adolescents (TORDIA) study, a history of NSSI (but not suicide attempt) was found to be an important indicator of suicide attempt over a period of 28 weeks[27]. In the Adolescent Depression, Antidepressant and Psychotherapy (ADAPT) study, NSSI predicted suicide attempt over a follow-up period of 28 weeks[13]. In a longitudinal study that monitored depressed adolescents for 8 years, NSSI was determined to be a strong predictor of suicidal behavior[12]. Our result, which is consistent with the literature, emphasizes the need for comprehensive assessment and treatment of NSSI in depressed adolescents. Improved assessment and intervention strategies for NSSI may facilitate the prevention of suicidal behavior.

Regarding the severity of depression, symptom severity was determined to be significantly higher in the suicide attempt group. The results on depression severity and suicidality vary in the literature. Although some studies show a relationship between depression severity and suicidality[12,28], others suggest that these are not related[29]. These different results from the studies are attributed to the fact that the relationship between depression severity and suicidal tendencies in young individuals is affected by a multitude of psychological and social factors. The higher depression severity in the suicide attempt group highlights the critical importance of understanding the severity of depressive disorder symptoms from the perspectives of adolescents in recognizing the risk of suicide attempt. These results may help guide the interventions that will target these clinical risk factors.

The suicide attempt group had significantly more severe anxiety symptoms. However, the two groups were not significantly different in terms of comorbid anxiety disorder. In young individuals with depression, psychiatric comorbidities, particularly comorbid anxiety disorder, have been associated with suicidality. However, it has not been clarified whether the higher suicidality associated with comorbid anxiety disorder is related to the specific characteristics of anxiety or to a general increase in psychiatric illness burden. Some studies have even suggested that this relationship could arise from the specific symptoms of anxiety rather than categorical anxiety disorder diagnoses[30]. In a study that investigated the relationship between suicide attempts, anxiety and poor treatment in childhood in adolescents and young adults experiencing their first depressive episodes, anxiety symptoms were shown to predict suicide attempts as well as serve as a mediator in the relationship between poor treatment in childhood and suicide attempts[10]. This result suggests that anxiety symptoms should be a therapeutic target in suicide prevention strategies even when comorbid anxiety disorder is not present.

Another important result of our study is that the suicide attempt group had higher rates of history of childhood abuse. Results from previous studies are generally in line with this result. These studies have reported that those with a history of childhood abuse could have a greater suicide risk due to factors such as a heightened susceptibility to psychosocial stress resulting from the negative effect of trauma on the HPA axis, as well as the presence of negative familial and environmental conditions that hamper the development of appropriate emotional regulation and coping abilities[10,31-33]. In addition to these causes, PTSD may also have considerable effects. In our

study, the groups were not significantly different with regard to comorbid PTSD. However, as is the case in anxiety disorder, an effect through specific symptoms may be possible despite the absence of a diagnosis. Therefore, further studies that will include PTSD symptoms along with a history of abuse are needed.

Finally, we investigated the predictors of suicide attempts in adolescents with MDD. We found that NSSI and childhood abuse history predicted suicide attempt. In a follow-up study examining the predictors of suicide attempt in adolescents with MDD, similar to our results, a history of NSSI and a history of physical and/or sexual abuse were found as important predictors[27]. In addition, previous studies have shown that these two predictive factors (NSSI and history of childhood abuse) often coexist, and a history of abuse poses a risk for NSSI[34]. Childhood abuse may adversely affect the development of emotion regulation strategies, followed by poor emotion regulation strategies may increase the risk of using the NSSI as a form of emotion coping behavior[35]. This result should warn that NSSI and history of abuse may require urgent evaluation and treatment in adolescents with MDD. In addition, the consideration of common risk and protective factors for these closely related conditions points to the necessity of including interventions for common underlying mechanisms (such as difficulties in emotion regulation) into the treatment plan.

Study Limitations

The results of our study should be interpreted in consideration of certain limitations. Due to the cross-sectional design of our study, it could not be determined whether a longitudinal relationship between sociodemographic-clinical characteristics and suicide attempts exists. Our study did not use structured interview tools, and DSM-5-based clinical interviews were employed. The assessment of suicide did not use a scale and relied on information from patient files. Therefore, suicidal ideation could not be clearly isolated and no specific data in this regard could be presented. Lastly, the absence of a PTSD scale is a major limitation. For this reason, we could not evaluate the relationship between suicide attempts in MDD and PTSD symptoms, which are closely related to a history of childhood abuse.

CONCLUSION

Suicide attempts constitute an important problem for adolescents diagnosed with MDD. A better understanding of the factors associated with the suicide attempts in these young individuals may help identify targets for early intervention and inform more effective prevention strategies. Especially, the presence of self-injurious behaviors and a history of childhood abuse should be a warning sign for suicide attempts and need to be addressed in order to prevent suicide attempts. Longitudinal studies are needed to increase our understanding of the causation of suicidal attempts and actionable strategies for clinical prediction and prevention of these behaviors in adolescents with MDD. Future research, especially involving analysis of specific psychiatric symptoms or symptom networks, may help us better understand suicidality among adolescents with MDD.

Ethics

Ethics Committee Approval: The study approval was obtained from the Atatürk University Faculty of Medicine of Clinical Research Ethics Committee and was conducted in accordance with the Declaration of Helsinki and Good Clinical Practices (decision number: B.30.2.ATA.0.01.00/506, date: 30.06.2022).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.Y.D., M.A.A., A.B., H.D., Concept: E.Y.D., M.A.A., H.D., Design: E.Y.D., M.A.A., H.D., Data Collection or Processing: E.Y.D., M.A.A., A.B., H.D., Analysis or Interpretation: E.Y.D., M.A.A., A.B., H.D., Literature Search: E.Y.D., M.A.A., A.B., Writing: E.Y.D., M.A.A., A.B.

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REFERENCES

1. American Psychiatric Association D, Association AP. Diagnostic and statistical manual of mental disorders: DSM-5. vol 5. American psychiatric association Washington, DC; 2013.
2. Avenevoli S, Swendsen J, He JP, Burstein M, Merikangas KR. Major depression in the national comorbidity survey-adolescent supplement: prevalence, correlates, and treatment. J Am Acad Child Adolesc Psychiatry. 2015;54:37-44.
3. Auerbach RP, Tsai B, Abela JR. Temporal relationships among depressive symptoms, risky behavior engagement, perceived control, and gender in a sample of adolescents. Journal of research on adolescence. 2010;20:726-47.
4. Fröjd SA, Nissinen ES, Pelkonen MU, Marttunen MJ, Koivisto AM, Kaltiala-Heino R. Depression and school performance in middle adolescent boys and girls. J Adolesc. 2008;31:485-98.
5. Ribeiro JD, Huang X, Fox KR, Franklin JC. Depression and hopelessness as risk factors for suicide ideation, attempts and death: meta-analysis of longitudinal studies. Br J Psychiatry. 2018;212:279-86.
6. Nock MK, Green JG, Hwang I, McLaughlin KA, Sampson NA, Zaslavsky AM, et al. Prevalence, correlates, and treatment of lifetime suicidal behavior among adolescents: results from the National Comorbidity Survey Replication Adolescent Supplement. JAMA Psychiatry. 2013;70:300-10.
7. Rey JM, Bella-Awusah TT, Liu J. Dépression de l'enfant et de l'adolescent. Rey, JM, IACAPAP e-Textbook of Child and Adolescent Mental Health. 2012. Available from: https://iacapap.org/_Resources/Persistent/7ab800bfe028ae766e96c066fd2bf998aeaa2081/E.1-Depression-FRENCH-2015.pdf

8. van Velzen LS, Toenders YJ, Kottaram A, Youzchalveen B, Pilkington V, Cotton SM, et al. Risk Factors for Suicide Attempt During Outpatient Care in Adolescents With Severe and Complex Depression. Crisis. 2023;44:232-9.

9. Consoli A, Peyre H, Speranza M, Hassler C, Falissard B, Touchette E, et al. Suicidal behaviors in depressed adolescents: role of perceived relationships in the family. Child Adolesc Psychiatry Ment Health. 2013;7:8.

10. Chen H, Li W, Cao X, Liu P, Liu J, Chen X, et al. The Association Between Suicide Attempts, Anxiety, and Childhood Maltreatment Among Adolescents and Young Adults With First Depressive Episodes. Front Psychiatry. 2021;12:745470.

11. Moller CI, Davey CG, Badcock PB, Wrobel AL, Cao A, Murrihy S, et al. Correlates of suicidality in young people with depressive disorders: A systematic review. Aust N Z J Psychiatry. 2022;56:910-48.

12. Tuisku V, Kiviruusu O, Pelkonen M, Karlsson L, Strandholm T, Marttunen M. Depressed adolescents as young adults - predictors of suicide attempt and non-suicidal self-injury during an 8-year follow-up. J Affect Disord. 2014;152-154:313-9.

13. Wilkinson P, Kelvin R, Roberts C, Dubicka B, Goodyer I. Clinical and psychosocial predictors of suicide attempts and nonsuicidal self-injury in the Adolescent Depression Antidepressants and Psychotherapy Trial (ADAPT). Am J Psychiatry. 2011;168:495-501.

14. Kovacs M. Rating scales to assess depression in school-aged children. Acta Paedopsychiatr. 1981;46:305-15.

15. Oy B. Cocuklar icin Depresyon Olcegi gecerlik ve guvenirlik calismasi. Turk. Psikiyatri Dergisi. 1991;2:137-40.

16. Ulusoy M, Sahin NH, Erkmen H. Turkish version of the Beck Anxiety Inventory: psychometric properties. Journal of cognitive psychotherapy. 1998;12:163.

17. Shain BN; American Academy of Pediatrics Committee on Adolescence. Suicide and suicide attempts in adolescents. Pediatrics. 2007;120:669-76.

18. Service UPH. The Surgeon General's call to action to prevent suicide. Washington, DC; 1999.

19. Li XY, Tabarak S, Su XR, Qin Z, Chai Y, Zhang S, et al. Identifying clinical risk factors correlate with suicide attempts in patients with first episode major depressive disorder. J Affect Disord. 2021;295:264-70.

20. Cho S-J, Jeon H-J, Kim J-K, Suh T-W, Kim S-U, Hahm B-J, et al. Prevalence of suicide behaviors (suicidal ideation and suicide attempt) and risk factors of suicide attempts in junior and high school adolescents. Journal of Korean Neuropsychiatric Association. 2002:1142-55.

21. Barbe RP, Williamson DE, Bridge JA, Birmaher B, Dahl RE, Axelson DA, et al. Clinical differences between suicidal and nonsuicidal depressed children and adolescents. J Clin Psychiatry. 2005;66:492-8.

22. Brent DA, Baugher M, Bridge J, Chen T, Chiappetta L. Age- and sex-related risk factors for adolescent suicide. J Am Acad Child Adolesc Psychiatry. 1999;38:1497-505.

23. Chen PJ, Mackes N, Sacchi C, Lawrence AJ, Ma X, Pollard R, et al. Parental education and youth suicidal behaviours: a systematic review and meta-analysis. Epidemiol Psychiatr Sci. 2022;31:e19.

24. Assari S, Boyce S, Bazargan M, Caldwell CH. African Americans' Diminished Returns of Parental Education on Adolescents' Depression and Suicide in the Adolescent Brain Cognitive Development (ABCD) Study. Eur J Investig Health Psychol Educ. 2020;10:656-68.

25. Kafali HY, Işık A, Ocakoglu BK, Kardaş B, Kardaş Ö, Müjdecioğlu Demir G, et al. Depresif kız ergenlerde intihar davranışının şiddeti, algılanan cinsiyet eşitliği ve çocukluk çağı travmalarının ilişkisi: Türkiye'nin bölgelerine göre farklılıklar. Klinik Psikiyatri Dergisi. 2022;25.

26. Hawton K, Saunders KE, O'Connor RC. Self-harm and suicide in adolescents. Lancet. 2012;379:2373-82.

27. Asarnow JR, Porta G, Spirito A, Emslie G, Clarke G, Wagner KD, et al. Suicide attempts and nonsuicidal self-injury in the treatment of resistant depression in adolescents: findings from the TORDIA study. J Am Acad Child Adolesc Psychiatry. 2011;50:772-81.

28. Soylu N, Taneli Y, Taneli S. Depresyonu Olan Ergenlerde İntihar Davranışını Etkileyen Sosyal, Emosyonel ve Kognitif Faktörlerin Araştırılması. Archives of Neuropsychiatry/Noropsikiatri Arsivi. 2013;50:352-9.

29. Zhang S, Chen JM, Kuang L, Cao J, Zhang H, Ai M, et al. Association between abnormal default mode network activity and suicidality in depressed adolescents. BMC Psychiatry. 2016;16:337.

30. Tuisku V, Pelkonen M, Karlsson L, Kiviruusu O, Holi M, Ruuttu T, et al. Suicidal ideation, deliberate self-harm behaviour and suicide attempts among adolescent outpatients with depressive mood disorders and comorbid axis I disorders. Eur Child Adolesc Psychiatry. 2006;15:199-206.

31. Dias de Mattos Souza L, Lopez Molina M, Azevedo da Silva R, Jansen K. History of childhood trauma as risk factors to suicide risk in major depression. Psychiatry Res. 2016;246:612-6.

32. Kuhlman KR, Geiss EG, Vargas I, Lopez-Duran NL. Differential associations between childhood trauma subtypes and adolescent HPA-axis functioning. Psychoneuroendocrinology. 2015;54:103-14.

33. Kessler RC, McLaughlin KA, Green JG, Gruber MJ, Sampson NA, Zaslavsky AM, et al. Childhood adversities and adult psychopathology in the WHO World Mental Health Surveys. Br J Psychiatry. 2010;197:378-85.

34. Thomassin K, Shaffer A, Madden A, Londino DL. Specificity of childhood maltreatment and emotion deficit in nonsuicidal self-injury in an inpatient sample of youth. Psychiatry Res. 2016;244:103-8.

35. Qian H, Shu C, Feng L, Xiang J, Guo Y, Wang G. Childhood Maltreatment, Stressful Life Events, Cognitive Emotion Regulation Strategies, and Non-suicidal Self-Injury in Adolescents and Young Adults With First-Episode Depressive Disorder: Direct and Indirect Pathways. Front Psychiatry. 2022;13:838693.