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Editorial

Dear collagues,

We are very pleased to announce the launch of the September issue of the Medical Journal of Bakırköy.

The Medical Journal of Bakırköy will continue to be an international journal mainly devoted to the publication of original studies and research. In this September issue, we have put together a group of original articles.

I hope you will enjoy this new issue of BMJ and consider submitting your future work to this promising academic venue.

With my best regards,

Prof. Dr. Esra Şevketoğlu
Chief Editor

Original Article

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Medical Journal of Bakirkoy

Association of Myeloperoxidase Gene Functional Variant with Schizophrenia and Smoking in a Turkish Population

Türk Toplumunda Myeloperoksidaz Geni Fonksiyonel Varyantının Şizofreni ve Sigara ile İlişkisi

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ABSTRACT

Objective: *Etiopathogenesis of schizophrenia (SCZ) involves several risk genes that induce inflammation, environmental stress factors and changes in the innate immune system. Patients with SCZ have the highest rate of cigarette smoking and severe nicotine dependence. Myeloperoxidase (MPO), a member of subfamily of peroxidases, is most abundantly expressed in immune cells. The aim of this study was to investigate the relationship between the MPO rs2333227 variant and SCZ/smoking etiopathogenesis.*
Method: *The study included 54 patients with SCZ, 94 smokers and 92 healthy controls. MPO rs2333227 variant was genotyped by polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) method. Odds ratio (OR) and 95% confidence interval (95%CI) were calculated using the χ^2 test.*
Results: *G/G and G/A genotypes of MPO rs2333227 were detected in our study samples. The frequencies of the G/G and G/A genotypes were 53.7%, 46.3%; 56.3%; 43.7%; 68.9%, 31.1% in SCZ patients, smokers, and the control group, respectively. The allele frequencies were G: 76.9% (SCZ patients), 77.4% (smokers) 83.7% (controls); A: 23.1% (SCZ patients), 22.6% (smokers), and 16.3% (controls). There was no significant difference between the SCZ patients, smokers and controls regarding MPO rs2333227 variant either in terms of allele frequency or genotype frequency. Then we genotyped the groups as women and men. MPO rs2333227 variant genotype distribution did not differ between men and women (p>0.05).*
Conclusion: *This study does not support the role of MPO rs2333227 variant in increasing genetic risk for SCZ/smoking in Turkish population.*

Keywords: *schizophrenia, smoking, myeloperoxidase, variant*

ÖZ

Amaç: *Şizofreni (SCZ) etyopatogenezi, enflamasyon, çevresel stres faktörleri ve doğal bağışıklık sistemindeki değişiklikleri indükleyen çeşitli risk genlerini içerir. SCZ'li hastalar yüksek sigara içme oranına ve şiddetli nikotin bağımlılığına sahiptir. Peroksidaz alt familyasının bir üyesi olan miyeloperoksidaz (MPO), en çok bağışıklık hücrelerinde eksprese edilir. Bu çalışmanın amacı MPO rs2333227 varyantı ile SCZ/sigara içimi etyopatogenezi arasındaki ilişkiyi araştırmaktır.*
Yöntem: *Çalışmaya 54 SCZ hastası, 94 sigara içen ve 92 sağlıklı kontrol dahil edildi. MPO rs2333227 varyantı polimeraz zincir reaksiyonu-sınırlayıcı enzim parça uzunluk polimorfizmi (PZR-RFLP) yöntemi ile genotiplendi. OR ve %95Cl güven aralığı X2 testi kullanılarak hesaplandı.*
Bulgular: *Çalışmada hemşirelerin %84.25'i "ERAS protokolünü bilmediklerini, %88,97'si çalıştıkları klinikte ERAS protokolü uygulamalarına yer verilmediğini, %99,21'i ise "ERAS protokolüne yönelik herhangi bir yayını takip etmediğini, %99,21'i ERAS protokolünü içeren herhangi bir eğitim almadığını belirtti.*
Sonuç: *Çalışma örneklerimizde MPO rs22333227 G/G ve G/A genotipleri saptandı. G/G ve G/A genotiplerinin sıklığı SCZ hastalarında, sigara içenlerde ve kontrol grubunda sırasıyla, %53,7, %46,3; %56,3, %43,7; %68,9, %31,1 idi. Alel sıklıkları G: %76,9 (SCZ hastaları), %77,4 (sigara içenler), %83,7 (kontroller); A %23,1 (SCZ hastaları), %22,6 (sigara içenler), %16,3 (kontroller) idi. SCZ hastaları, sigara içenler ve kontroller arasında MPO rs2333227 varyantı alele frekansı ve genotip sıklığı açısından anlamlı bir fark yoktu. Sonra grupları kadın ve erkek olarak genotipledik. MPO rs22333227 varyant genotip dağılımı kadınlar ve erkekler arasında farklı değildi (p>0.05).*

Anahtar kelimeler: *şizofreni, sigara içimi, myeloperoksidaz, varyant*

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INTRODUCTION

Schizophrenia (SCZ) is a common mental disease of unknown etiology that generally has onset in young adulthood and manifests several combinations of positive, and negative psychoticsymptoms, and cognitive impairment in attention and information processing [1]. The cause and pathogenesis of SCZ remain unclear. Many studies have reported that SCZ is caused by several risk factors that can be roughly classified into genetic factors and environmental factors. Hence, SCZ occurs as a result of an interaction between genes and the environment [2]. Relationships between the immune genes, such as those affecting the inflammatory response regulation and SCZ risk, have been revealed thanks to the advancements in molecular genetics [3]. Evidence supports that there are abnormalities of inflammatory markers in the blood, cerebrospinal fluid (CSF), and central nervous system (CNS), such as cytokines, leukocytes, acute-phase reactants among the cases with SCZ [3]. It is well known that smoking leads to various physical health problems. It is the major cause of preventable death, since it enhances the risk of lung cancer and other malignancies [4]. Recently, there has been an increasing interest in tobacco smoking as a risk factor for SCZ spectrum disorders [5]. Some studies have reported that patients who had mental diseases were more than three times as likely to smoke compared to those without psychiatric disorders [6]. The studies have shown chronic inflammatory changes in smokers as compared to nonsmokers such as higher levels of leukotriene B4 and interleukin (IL)-6 and IL-8 [7].

Myeloperoxidase (MPO) belongs to a subfamily of peroxidases. It is most commonly expressed in immune cells, including neutrophilic polymorphonuclear leukocytes (neutrophils) and lymphocytes monocytes, and macrophages and is also synthesized in other body cells [8].

MPO has potent pro-inflammatory characteristics that facilitate the injury of tissue via inflammatory site oxidative damage. The MPO gene found in 17q23.1 encodes human MPO protein. The expression level or structure of the relevant protein may be changed with polymorphisms in the MPO gene [9]. At the promoter region of the MPO gene, polymorphism of rs2333227 (-463A/G) is found, binding with the site of SP1. The gene can be transcribed due to changes in the locus, hence affecting the MPO level [10].

Therefore, this study aimed to investigate the genetic association between MPO rs2333227 variant and risk of SCZ and smoking in a Turkish cohort.

MATERIALS and METHODS

Study population

A total of 54 patients with SCZ+smoker, 94 smokers, and 92 gender and age-matched healthy individuals as controls were included in the study. The subjects referring to Istanbul, Turkey, Yedikule Hospital for Chest Diseases and Thoracic Surgery Training and Research Hospital and Bakirkoy Research and Training Psychiatry Hospital, Istanbul Turkey were included in the study. SCZ was clinically diagnosed by psychiatrists strictly based on DSM-IV criteria (Diagnostic and Statistical Manual of Mental Disorders, the fourth edition) [11]. The scores obtained from the Fagerström Test for Nicotine Dependence (FTND) and Heaviness of Smoking Index (HSI) were determined to evaluate the severity of smoking [12]. There was random recruitment of the healthy controls from the same hospital. The healthy subjects who had a psychiatric problem in their life, any serious endocrine or neurological disorder, those who received any treatment or underwent medical condition affecting the brain, and mental retardation determined based on the patient version of the Structured Clinical Interview for DSM-IV-TR Axis I Disorders were excluded from the study. Also, the control group of patients did not smoke. All participants belonged to the Turkish population in Turkey. The patients submitted the informed, written consent. There was anonymous information about the patient before submission. Local Ethics Committee approved the work. All the procedures which were performed for the study followed the Declaration of Helsinki.

Genotyping

Samples of peripheral blood were taken from all participants.Tthe genomic DNA was extracted from peripheral venous blood sample treated by EDTA (ethylenediamine tetraacetate) with the salting-out method [13], and it was stored at -20 °C until the time of analysis. MPO rs2333227 variant was genotyped

using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method described in a previous study [14]. G/G and G/A genotypes of MPO rs2333227 were detected in our study samples.

Statistical analysis

Software SPSS version 20.0 (SPSS Inc., Chicago, IL, USA) for Windows was used to analyze all data. The statistically significant differences between the controls and cases were estimated by logistic regression analysis. Odds ratio (OR) and 95% confidence interval (CI) were also calculated. Chi-square test was used to compare the differences in MPO rs2333227 variant genotype and allele frequencies between cases and controls, and if necessary, the Fisher's exact test was used. Two-tailed analyses were done, and $p<0.05$ showed statistically significant differences.

RESULTS

For MPO rs2333227 variant, 54 SCZ+smoker patients (41 females, 13 males), 94 smokers (43 females, 51 males), and 92 controls (55 females, 37 males) were evaluated. The groups had the allele and genotype distributions of MPO rs2333227, as shown in Table 1. G/G and G/A genotypes of MPO rs2333227 were detected in our study samples. The frequencies of the G/G and G/A genotypes were 53.7%, 46.3%; 56.3%; 43.7%; 68.9%, 31.1% in SCZ patients, smokers, and the control group, respectively. The allele frequencies were G: 76.9%, 77.4% 83.7%; A: 23.1%, 22.6%, and 16.3%. There was no significant difference between the SCZ patients, smokers, and controls regarding the MPO rs2333227 variant either in terms of allele or genotype frequencies.

Then we genotyped the groups into women and men. The distribution of MPO rs2333227 variant genotype did not differ between male and male patients ($p>0.05$) (Data not shown).

DISCUSSION

Cells and mediators which have evolved mainly to defend humans against malignancy and infection have been complexly organized in the immune system. Complex interactions between the immune system and brain influencing neural function, survival, and development may be etiologically and therapeutically associated for numerous CNS diseases such as psychiatric diseases [15]. SCZ is seen in about 1% of the population during their lives and mainly starts when the brain develops following puberty [16].

There was a suggestion of a possible relationship between the immune system and SCZ about one century ago, which was confirmed by epidemiological studies finding relations with systemic inflammation and infection. There is a relationship between autoimmune conditions during childhood and subclinical psychotic episodes in adolescents and SCZ in adults [17]. Also, there is a linear increase in the risk of SCZ by increasing the number of severe infections among the individuals who previously had a history of autoimmune disease [17]. An immune-mediated etiology SCZ was supported by the studies on genome association, suggesting significant links between markers near the region of major histocompatibility complex (MHC) and the SCZ on chromosome 6 [18]. Besides, this disease is significantly associated with enhancers having a potent role in the immune functions, even after excluding the MHC region genes [19]. Moreover, at least two ILs seem to be involved in the

Table 1. Genotype distribution and allele frequencies of MPO rs2333227 variant in groups.

MPO rs2333227	SCZ group	Smoker group	Control group	OR*	%95 CI*	P
Genotypes	**n=54 (%)**	**n=94 (%)**	**n=92 (%)**			
G/G	29 (53.7)	53 (56.3)	62 (68.9)	0.561 [a]	0.282-1.119 [a]	0.113 [a]
G/A	25 (46.3)	41 (43.7)	30 (31.1)	0.625 [b]	0.344-1.136 [b]	0.133 [b]
				0.897 [c]	0.458-1.758 [c]	0.864 [c]
Alleles				0.647 [a]	0.357-1.172 [a]	0.164 [a]
G	83 (76.9)	147 (77.4)	154 (83.7)	0.698 [b]	0.414-1.178 [b]	0.189 [b]
A	25 (23.1)	41 (22.6)	30 (16.3)	0.926 [c]	0.526-1.630 [c]	0.885 [c]

**Fisher's Exact Test, [a]: Sch group versus control group, [b]: Smoker group versus control group, [c]: Sch group versus smoker group.*

impacts on systems of neurotransmitter in SCZ: IL-1β, which promotes mesencephalic progenitor cells of rat converted into a dopaminergic phenotype, and IL-6, which shortens survival of serotonergic neurons in the fetus [20]. This association in humans can be supported by a cohort study of bacterial infections and studies on some infections. A higher risk for SCZ may be due to higher levels of C-reactive protein and cytokines during childhood [20].

The inflammatory markers in a vascular wall may be activated by the endothelial dysfunction induced by smoking [21]. Some studies have demonstrated that C-reactive protein with high sensitivity, plasma viscosity, several inflammatory markers, their serum concentrations, and fibrinogen are affected by smoking, and elevated slightly in ex-smokers. There is a positive correlation between duration of smoking and inflammation markers. On the contrary, there is also an inverse relationship between inflammation markers and the time elapsed after quitting smoking [22].

The lysosomal enzyme and peroxidase activity were analyzed in the MPO [23], which plays a role in the regulation of inflammatory responses, lysosomal pathway, tissue damage, lipid metabolism, and oxidative damage [24]. Not only is MPO activity engaged in the host defense physiology against microorganisms, but also it is involved in the pathophysiology of atherosclerosis, respiratory tract and CNS diseases, and cardiac dysfunction [25]. High levels of MPO have been detected in brain tissue, in some neurodegenerative disorders. There have been significantly higher MPO protein levels in Huntington's disease and Parkinson's disease in the samples of caudate and nucleus midbrain, respectively while there were no differences MPO protein contents in motor cortex samples in amyotrophic lateral sclerosis as compared to controls [26].

In a twin study, twins with a history of major depressive disorder had 32% higher blood levels of MPO [27]. Another study revealed that those suffering from comorbidity of hyperactivity disorder /attention deficit and bipolar disorder had significantly higher mean MPO levels than a bipolar disorder [28]. But Kartalci et al. found no significant association between the SCZ patient and control groups as for MPO levels [29]. It has been also revealed that the levels of MPO are elevated significantly in smokers compared with nonsmokers [30,31].

The MPO gene encodes for a single translational product that, after glycosylation and proteolytic processing, is released as mature MPO in the azurophilic granules [32]. It was reported that MPO transcriptional activity could be positively enhanced by binding reversibly to the SP1 transcription factor due to a noticeable functional polymorphism in which there was an exchange of G to A base at -463 (rs2333227) in the promoter region [33]. There is an SP1 binding the MPO -463G variant, activating 25-fold transcription in transient transfection assays, whereas the MPO -463A allele leads to notably less transcriptional activity [33]. The variant of MPO rs2333227 variant has been described as a protective genetic factor against development of several malignancies. It seems that the genotypes of A/A and MPO -463G/A have a diminished risk of colorectal adenomas, acute leukemia, gastric, lung, and ovarian carcinoma [32]. Ji et al. found MPO rs2333227 variant was positively associated with the risk of Alzheimer's disease among the population in China [24]. Galecki et al. declared a statistically significant difference in genotype distribution and allele frequency of MPO rs2333227 variants between depressive patients and healthy controls [34].

This study was conducted to elucidate the genetic role of the MPO rs2333227 variant in SCZ and smoking status. No association was observed in the analysis of SCZ/smoking and this variant in our population. There are some limitations in this study. There is no large sample size in the study, and only one Turkish cohort was included in the study group. Furthermore, the study did not consider the relationship between MPO polymorphisms and other factors, such as environmental factors.

CONCLUSIONS

In summary, as far as we know, the present study is the first one that examines the association between the MPO rs2333227 variant with SCZ and smoking among the population in Turkey. No evidence of an association between MPO rs2333227 variant with SCZ and smoking was observed in this sample of the Turkish population. Due to these limitations, the role

of MPO promoter variant in SCZ/smoking status in the future should be clarified by conducing further larger-scale studies which are well designed on multiple populations.

Ethics Committee Approval: Approval was obtained from the Istanbul Medical Faculty Clinical Research Ethics Committee (09,10,2015 / 17).
Conflict of interest: The authors have declared no conflict of interest, financial or otherwise.
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Informed Consent: Informed consent was obtained from all individual participants included in the study.

REFERENCES

1. Hennah W, Thomson P, Peltonen L, Porteous D. Genes and schizophrenia: beyond schizophrenia: the role of DISC1 in major mental illness. Schizophr Bull. 2006;32(3):409-16. https://doi.org/10.1093/schbul/sbj079
2. Lichtenstein P, Yip BH, Bjork C, Pawitan Y, Cannon TD, Sullivan PF, et al. Common genetic determinants of schizophrenia and bipolar disorder in Swedish families: a population-based study. Lancet. 2009;373(9659): 234-9. https://doi.org/10.1016/S0140-6736(09)60072-6
3. Miller BJ, Goldsmith DR. Inflammatory biomarkers in schizophrenia: Implications for heterogeneity and neurobiology. Biomarkers in Neuropsychiatry. 2019;1: 100006. https://doi.org/10.1016/j.bionps.2019.100006
4. Collaborators GT. Smoking prevalence and attributable disease burden in 195 countries and territories, 1990-2015: a systematic analysis from the Global Burden of Disease Study 2015. Lancet. 2017;389(10082):1885-906. https://doi.org/10.1016/S0140-6736(17)30819-X
5. Hunter A, Murray R, Asher L, Leonardi-Bee J. The Effects of Tobacco Smoking, and Prenatal Tobacco Smoke Exposure, on Risk of Schizophrenia: A Systematic Review and Meta-Analysis. Nicotine Tob Res. 2020;22(1):3-10. https://doi.org/10.1093/ntr/nty160
6. Dickerson F, Schroeder J, Katsafanas E, Khushalani S, Origoni AE, Savage C, et al. Cigarette smoking by patients with serious mental illness, 1999-2016: an increasing disparity. Psychiatr Serv. 2018;69(2):147-53. https://doi.org/10.1176/appi.ps.201700118
7. Mazur W, Stark H, Sovijarvi A, Myllarniemi M, Kinnula VL. Comparison of 8-isoprostane and interleukin-8 in induced sputum and exhaled breath condensate from asymptomatic and symptomatic smokers. Respiration. 2009;78(2):209-16. https://doi.org/10.1159/000206010
8. Khan AA, Alsahli MA, Rahmani AH. Myeloperoxidase as an Active Disease Biomarker: Recent Biochemical and Pathological Perspectives. Med Sci (Basel). 2018;6(2): 33. https://doi.org/10.3390/medsci6020033
9. Rudolph V, Rudolph TK, Kubala L, Clauberg N, Maas R, Pekarova M, et al. A myeloperoxidase promoter polymorphism is independently associated with mortality in patients with impaired left ventricular function. Free Radic Biol Med. 2009;47(11):1584-90. https://doi.org/10.1016/j.freeradbiomed.2009.09.001
10. Hansson M, Olsson I, Nauseef WM. Biosynthesis, processing, and sorting of human myeloperoxidase. Arch Biochem Biophys. 2006;445(2):214-24. https://doi.org/10.1016/j.abb.2005.08.009
11. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. Text Revision. 4th. Washington (DC): American Psychiatric Association; 2000.
12. Heatherton TF, Kozlowski LT, Frecker RC, Fagerström K. The Fagerström test for nicotine dependence: a revision of the Fagerström tolerance questionnaire. Br J Addict. 1991;86(9):1119-27. https://doi.org/10.1111/j.1360-0443.1991.tb01879.x
13. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988;16(3):1215. https://doi.org/10.1093/nar/16.3.1215
14. Erciyas K, Pehlivan S, Sever T, Orbak R. Genetic Variation of Myeloperoxidase Gene Contributes to Aggressive Periodontitis: A Preliminary Association Study in Turkish Population. Dis Markers. 2010;28(2):95-9. https://doi.org/10.1155/2010/734619
15. Khandaker GM, Pearson RM, Zammit S, Lewis G, Jones PB. Association of serum interleukin-6 and c-reactive protein in childhood with depression and psychosis in young adult life: a population-based longitdunal study. JAMA Psychiatry. 2014;71(10):1121-8. https://doi.org/10.1001/jamapsychiatry.2014.1332
16. Kirkbride JB, Errazuriz A, Croudace TJ, Morgan C, Jackson D, Boydell J, et al. Incidence of schizophrenia and other psychoses in England, 1950-2009: a systematic review and meta-analyses. PLoS One. 2012;7(3):e31660. https://doi.org/10.1371/journal.pone.0031660
17. Benros ME, Nielsen PR, Nordentoft M, Eaton WW, Dalton SO, Mortensen PB. Autoimmune diseases and severe infections as risk factors for schizophrenia: a 30-year population-based register study. Am J Psychiatry. 2011;168(12):1303-10. https://doi.org/10.1176/appi.ajp.2011.11030516
18. Shi J, Levinson DF, Duan J, Sanders AR, Zheng Y, Pe'er I, et al. Common variants on chromosome 6p22.1 are associated with schizophrenia. Nature. 2009; 460(7256):753-7. https://doi.org/10.1038/nature08192
19. Schizophrenia Working Group of the Psychiatric Genomics Consortium. Biological insights from 108 schizophrenia-associated genetic loci. Nature. 2014;511(7510):421-7. https://doi.org/10.1038/nature13595
20. Müller N. Inflammation in Schizophrenia: Pathogenetic Aspects and Therapeutic Considerations. Schizophr Bull. 2018;44(5):973-82. https://doi.org/10.1093/schbul/sby024

21. Mizia-Stec K, Zahorska-Markiewicz B, Gasior Z. Cigarette smoking and inflammatory indices in coronary artery disease. Int J Cardiol. 2004;93(2-3):169-74.
https://doi.org/10.1016/S0167-5273(03)00198-0
22. Daloee MH, Avan A, Mirhafez SR, Kavousi E, Hasanian-Mehr M, Darroudi S, et al. Impact of Cigarette Smoking on Serum Pro- and Anti-Inflammatory Cytokines and Growth Factors. Am J Mens Health. 2017;11(4):1169-73.
https://doi.org/10.1177/1557988315601724
23. Perianayagam MC, Tighiouart H, Liangos O, Kouznetsov D, Wald R, Rao F, et al. Polymorphisms in the myeloperoxidase gene locus are associated with acute kidney injury-related outcomes. Kidney Int. 2012;82(8):909-19.
https://doi.org/10.1038/ki.2012.235
24. Ji W, Zhang Y. The association of MPO gene promoter polymorphisms with Alzheimer's disease risk in Chinese Han population. Oncotarget. 2017;8(64):107870-6.
https://doi.org/10.18632/oncotarget.22330
25. Malle E, Furtmuller PG, Sattler W, Obinger C. Myeloperoxidase: a target for new drug development? Br J Pharmacol. 2007;152(6):838-54.
https://doi.org/10.1038/sj.bjp.0707358
26. Gellhaar S, Sunnemark D, Eriksson H, Olson L, Galter D. Myeloperoxidase-immunoreactive cells are significantly increased in brain areas affected by neurodegeneration in Parkinson's and Alzheimer's disease. Cell Tissue Res. 2017;369(3):445-54.
https://doi.org/10.1007/s00441-017-2626-8
27. Vaccarino V, Brennan ML, Miller AH, Bremner JD, Ritchie JC, Lindau F, et al. Association of Major Depressive Disorder with Serum Myeloperoxidase and other Markers of Inflammation: A Twin Study. Biol Psychiatry. 2008;64(6):476-83.
https://doi.org/10.1016/j.biopsych.2008.04.023
28. Aksoy N, Saygili EI, Bulbul F, Bahar A, Savas H, Virit O, et al. Myeloperoxidase enzyme levels and oxidative stress in bipolar disorders. African Journal of Biotechnology. 2010;9 (22):3318-23. Available from: https://www.ajol.info/index.php/ajb/article/view/80663
29. Kartalci S, Erbay LG, Zayman EP, Otlu O, Karabulut AB, Kartalci G. IL-4, TGF-®, NF-|B and MPO Levels in Patients With Treatment Resistant Schizophrenia. Turk Psikiyatri Derg. 2016;27(3):170-5. Available from: http://www.turkpsikiyatri.com/Data/UnpublishedArticles/4f9fo7.pdf
https://doi.org/10.5080/u13642
30. Lavi S, Prasad A, Yang EH, Mathew V, Simari RD, Rihal CS, et al. Smoking is associated with epicardial coronary endothelial dysfunction and elevated white blood cell count in patients with chest pain and early coronary artery disease. Circulation. 2007;115(20):2621-7. doi: 10.1161/CIRCULATIONAHA.106.641654.
https://doi.org/10.1161/CIRCULATIONAHA.106.641654
31. Andelid K, Bake B, Rak S, Lindén A, Rosengren A, Ekberg-Jansson A. Myeloperoxidase as a Marker of Increasing Systemic Inflammation in Smokers Without Severe Airway Symptoms. Respir Med. 2007;101(5):888-95.
https://doi.org/10.1016/j.rmed.2006.09.023
32. Klebanoff SJ. Myeloperoxidase. Proc Assoc Am Physicians. 1999;111(5):383-9.
https://doi.org/10.1111/paa.1999.111.5.383
33. Piedrafita FJ, Molander, RB Vansant, G Orlova, EA Pfahl, M Reynolds, WF. An Alu element in the myeloperoxidase promoter contains a composite SP1-thyroid hormone-retinoic acid response element. J Biol Chem. 1996;271(24):14412-20.
https://doi.org/10.1074/jbc.271.24.14412
34. Galecki P, Florkowski A, Bobińska K, Śmigielski J, Bieńkiewicz M, Szemraj J. Functional polymorphism of the myeloperoxidase gene (G-463A) in depressive patients. Acta Neuropsychiatr. 2010;22(5):218-22.
https://doi.org/10.1111/j.1601-5215.2010.00483.x

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Meme Kitlelerinin Malign Benign Ayırımında Sonoelastografi ve ADC Değerinin Etkinliği

Effectiveness of Sonoelastography and Diffusion MRI ADC Value In Discriminating Between Malignant and Benign Lesions of the Breast

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ABSTRACT

Objective: *We aimed to evaluate the diagnostic value and comparison of sonoelastography and diffusion-weighted magnetic resonance imaging in differentiation of benign and malignant breast masses.*
Method: *Forty-five patients who were referred to our Radiology Department for the biopsy of a known breast mass following a breast MRI were evaluated by sonoelastography using a 5-scaled Tsukuba scoring system and measurements of ADC values on diffusion weighted MRIs. Contribution of the Tsukuba scores and ADC values of the masses to the conventional methods were evaluated.*
Results: *Histopathological results of all masses with Tsukuba scores 1 and 2 were evaluated as benign. Histopathological results of 37.5% of patients with a Tsukuba score of 3 were found to be benign and 62.5% of the patients were found to be malignant. Histopathologically 80% of the patients with a Tsukuba score of 4 were evaluated to have malignant, while all (100 %) of the patients with a Tsukuba score of 5 were considered to have malignant disease. Statistically significant correlation was found between the histopathological results and Tsukuba scoring system ($p<0.05$). Sonoelastographic sensitivity, specificity, positive, and negative predictive values were 83.3%, 96.3%, 93.7% and 89.6%, respectively in the patients with Tsukuba scores of 4 and 5. The mean ADC values of histopathologically proven malignant, and benign masses were $0.95\pm0.17x10^{-3}$ mm^2/sec and $1.37\pm0.16x10^{-3}$ mm^2/sec, respectively. The mean ADC value of histopathologically proven malignant masses was significantly lower than histopathologically proven benign masses ($p<0.01$). At sonoelastographic evaluation, one false-positive and 5 false-negative results were found. Three out of 4 false-negative results were diagnosed correctly using ADC values. False-negativity was detected in one lesion diagnosed based on both sonoelastographic results, and ADC values.*
Conclusion: *We think solely sonoelastography or ADC evaluations are inadequate, however, can be used in differentiation of benign and malignant breast masses.*

Keywords: *breast neoplasms, sonoelastography, magnetik resonance imaging, diffusion*

ÖZ

Amaç: *Çalışmamızda meme kitlelerinin malign-benign ayırımında sonoelastografi ve difüzyon manyetik rezonans görüntüleme (MRG) tekniklerinin tanısal değerinin araştırılması ve karşılaştırılması amaçlanmıştır.*
Yöntem: *Meme kitlesi nedeniyle Hastanemiz Radyoloji Kliniği'ne histopatolojik inceleme için başvuran hastalardan MRG tetkiki yapılmış olan 45 hastaya işlem öncesi beş puanlı 'Tsukuba' skorlama yöntemi kullanılarak sonoelastografik inceleme ve difüzyon MRG incelemelerinden "apparent diffusion coefficient" (ADC) ölçümleri yapıldı. Tsukuba skorlaması ve kitle ADC değerlerinin konvansiyonel yöntemlere katkıları değerlendirildi.*
Bulgular: *Tsukuba skoru 1 ve 2 olan olguların tamamının histopatolojik inceleme sonucu benign değerlendirilmiştir. Tsukuba skoru 3 olan olguların %37,5'inin histopatoloji sonucu malign, %62,5'inin benign olarak saptanmıştır. Tsukuba skoru 4 olan olguların %80'inin patoloji sonucu malign iken, Tsukuba skoru 5 olan olguların %100'ü malign değerlendirilmiştir. Histopatoloji sonucu ile Tsukuba skorlaması arasında istatistiksel olarak anlamlı bir uyum bulunmaktadır ($p<0.05$). Tsukuba skor 4 ve skor 5'te duyarlılık %83,3, özgüllük %96,3, pozitif kestirim değeri %93,7 ve negatif kestirim değeri %89,6 olarak bulunmuştur. Histopatolojik olarak kanıtlanmış malign kitlelerin ortalama ADC değeri $0.95\pm0.17x10^{-3}$ mm^2/sn iken benign kitlelerin ADC değeri $1.37\pm0.16x10^{-3}$ mm^2/sn idi. Histopatolojik olarak kanıtlanmış malign kitlelerin ortalama ADC değeri, histopatolojik olarak kanıtlanmış benign kitlelerden anlamlı olarak daha düşüktü ($p<0.01$). Sonoelastografik değerlendirmede 1 yanlış pozitif ve 5 yanlış negatif sonuç saptandı. Yanlış negatif saptanan 4 lezyonun 3'üne ADC ölçümleri ile doğru tanı koyuldu. Bir lezyon hem sonoelastografik olarak, hem de ADC değerlerinde yanlış negatif saptandı.*
Sonuç: *Yalnızca sonoelastografi ve ADC ölçümlerinin tek başına malign-benign ayrımında yetersiz olduğunu ancak birbirlerini tamamlayıcı alternatif yöntemler olarak kullanılabileceğini düşünmekteyiz.*

Anahtar kelimeler: *meme kitlesi, sonoelastografi, magnetik rezonans görüntüleme, difüzyon*

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INTRODUCTION

Breast cancer is the most common malignancy in women and among the leading causes of cancer-related deaths. Early diagnosis is the most important factor determining prognosis in breast cancer. Detection of the disease at an early stage increases treatment success and survival [1-3].

Diagnostic breast USG is an inexpensive, convenient, and non-invasive method without any radiation exposure. Recently, there have been significant improvements in characterization of breast mass lesions using B-mode sonography, which can detect malignant masses with high sensitivity. However, a high false positive rate is an important problem [4,5].

Sonoelastography is based on the fact that softer sites in the tissues are more easily deformed to a greater extent than the harder parts when left under compression. The method semiquantitatively measures the degree of deformation in the tissue using B-mode USG devices [6-9]. Important advantages of sonoelastography are similar to other USG methods, as being inexpensive, noninvasive, convenient, and commonly available, as well as allowing real-time visualization and not requiring ionizing radiation [9,10].

Sonoelastography is an imaging modality that measures the tissue response to compression, and thus, measures elasticity and stiffness of the tissue. Malignant lesions have higher scores than benign lesions, as malignant lesions are usually more rigid due to desmoplastic reactions [11]. Two main sonoelastography methods are being used to evaluate breast lesions. These are five-point scoring system and strain index method. The five-point scoring system shows the degree of stiffness in the lesion and its surrounding parenchyma with different color codes in real time, and the qualitative scoring can be made visually between 1 to 5 points [12,13]. Strain index measurement determines the strain index of the lesion by proportioning the strain values of the lesion and the adjacent structures using the obtained elasticity maps. In this way, the degree of the stiffness in the lesion can be assessed quantitatively [11-14]. In addition, the shear wave elastography, the quantitative technique shows the elasticity of tissues in kPa. The advantage of the technique is the minimal interobserver difference [10].

Large-scale studies evaluating contrast-enhanced MRI showed that it is highly sensitive in detecting primary or recurrent breast cancer [15-19]. Many studies report rates of sensitivity over 90%, reaching 100% particularly in invasive breast cancer [20]. Breast MRI has been used for the purpose of preoperative staging in patients with breast cancer for the last two decades. Breast MRI can provide information about the morphological and dynamic properties of the lesion.

There are many studies using ADC values to discriminate malignant and benign lesions of the breast, to characterize malignant masses, and to evaluate peritumoral spread, tumoral cellularity and response to treatment [21,22]. In terms of ADC values, there is no consensus on which maximum b value will be used to evaluate breast lesions. ADC value of benign breast lesions is generally high. ADC value is affected by tissue features that have low cellularity such as fibrosis or necrosis. Therefore ADC values decrease in fibrotic lesions, such as fibrous fibroadenomas or invasive ductal carcinoma. ADC values of cysts are higher, because of their liquid content. In general, serous content causes a low restriction in diffusion, and mucinous content causes a slightly higher diffusion restriction. Invasive ductal carcinoma shows lower ADC values than other malignant tumors, possibly due to dense tumor cells preventing the effective movements of molecules and restricting diffusion. Noninvasive ductal carcinoma shows high ADC values than ductal carcinoma due to bleeding in the necrotic center and lower cellularity [15-17].

The present study aims to investigate the contributions of five-point scoring system in sonoelastography and ADC values measured with MRI to the diagnosis and their additive value in discriminating between malignant and benign lesions of the breast that are detected with USG.

MATERIAL and METHODS

Forty-five patients who were referred to the Radiology Clinic of Umraniye Educaton and Research Hospital for radiological examination and had previous breast MRI scans were examined with sonoelas-

tography prior to the biopsy. MRI examinations of these patients were evaluated, and measurements were made from the ADC maps.

Cases with lesions that were larger than 3 cm -as these exceed the area of visualization in elastography- or lesions that could not be localized in the ADC map of breast MRI, cases for whom a histopathological diagnosis was not made, and cases who previously underwent surgical treatment were excluded from the study.

Ümraniye Training and Research Hospital The Clinical Research Ethics Committee of the hospital (Issue: 256) approved the study protocol, and all cases included in the study provided written informed consent.

Sonoelastography technique and evaluation of images

While the patient was lying in the normal ultrasonography position, a 12 MHz linear transducer probe was centered over the lesion and positioned perpendicular to the skin, lesion, and anterior chest wall. The examination was performed using digital USG devices (Toshiba Aplio MX and Toshiba Aplio 500) that have real-time elastography software. For each lesion, evaluation at B-mode was followed by real-time elastography mode using the same probe, and images were obtained. During real-time examination, both B-mode and elastography images of the examined area could be visualized on the monitor side by side, in two separate windows. In B-mode and elastography images, the imaging area was adjusted so that the entire mass lesion was visualized together with subcutaneous fat tissue and superficial layer of pectoral muscle. While obtaining elastography images, a slight pressure was applied perpendicular to the lesion. In our study, for every pixel of the elasticity image, color codes were determined according to the degree of strain. The color scale varied from red (the highest degree of strain (softest) to blue (complete absence of strain (hardest), with green showing the average strain.

Two radiologists who were experienced in breast sonography and sonoelastography and blinded to the histopathological diagnoses of the cases evaluated the B-mode sonography and sonoelastography images that were recorded digitally during the imaging. After evaluation, an elastography score was determined for each case.

During evaluation of the sonography images, a five-point scoring system developed by Itoh et al. [13], which is known as 'Tsukuba Elasticity Score,' was employed (Figure 1). The scores were assigned according to the following classification:
Scores 1-3 were considered to indicate benign, and scores 4-5 malignant lesions.

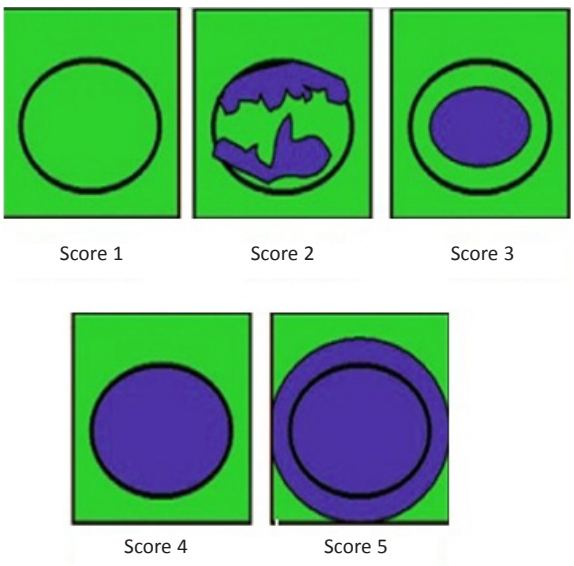

Figure 1. Schematic representation of Tsukuba scoring system.

MRI technique and evaluation of images

Breast MRI scans were performed bilaterally in each patient so as to encompass the entire breast, using 8-channel double surface breast coil, 1.5 T MRI device (Siemens Avantom). Contrast agent was administered manually as a bolus dosage of 0.1-0.2 mmol/kg. In each case, fat- suppressed T2 STIR axial images (TE:76, TR:4200, FOV=320 mm, matrix=512x512, section thickness=5 mm); turbo-spin echo T1 axial images (TE:8.7, TR:510, FOV=320 mm, matrix=512x512, section thickness=5 mm), and FLASH 3D T1A (TE:1.44, TR:4.68, FOV=320 mm, matrix=512x512, section thickness=1.7 mm) non-contrast and axial dynamic images at 1st, 2nd, 3rd, 4th, and 5th minutes were obtained. Using the subtraction program that is present as standard in the Siemens MRI console, each of the non-contrast FLASH 3D images were subtracted from the dynamic images in order

to obtain the subtracted images. Before administration of the contrast agent, echo-planar diffusion and ADC images (TR=8500, TE=109, FOV=320 mm, matrix= 256x256, section thickness=5 mm) were obtained with b=1000 values. ADC measurements were made by calculating pixel values. The measurements were evaluated by manual placement of the ROI on the mass lesion and the normal fibroglandular tissue of the same breast. Measurements were repeated several times, and the lowest value was accepted.

Statistical Analysis

For evaluation of the study data, statistical analyses were made with IBM SPSS Statistics 22 program. In addition to the descriptive statistics (mean, standard deviation, frequency), comparison of quantitative data was made using one-way ANOVA for comparing more than two groups with normally distributed parameters, and the group that caused the difference was determined with Tukey HDS test. Comparison of two groups for normally distributed parameters was made with Student's t-test. Qualitative data were compared using chi-square test, continuity (Yates) correction, and McNemar test. Sensitivity and specificity calculations were made with diagnostic screening tests. P<0.05 was accepted as statistically significant.

RESULTS

The study was conducted with a total of 45 female cases aged between 19 and 70 years. Mean age was 44.69±10.63 years. Sizes of mass lesions varied from 7 mm to 30 mm, with a mean size of 16.41±6.37 mm.

For histopathological examination, fine needle aspiration biopsy (FNAB) was performed in 19 (42.2%), and Tru-Cut biopsy in 26 (57.8%) cases.

Detected benign lesions included fibroadenomas (n=9), fibroadenomatoid changes (n=3), fibrocystic changes (n=12), and papillomas (n=3). Detected malignant lesions, included invasive ductal carcinomas (n=17), and invasive lobular carcinoma (n=1).

Tsukuba scores of 1 (n=4; 8.9%), 2 (n=17: 37.8%), 3 (n=8: 17.8%), 4 (n=5: 11.1%), and 5 (n=11: 24.4%) were detected in respective number of cases. Based on the Tsukuba scores, 16 lesions (35.6%) were diagnosed as malignant, while 29 lesions (64.4%) as benign.

Pathological examination results were benign in all cases with Tsukuba scores 1 and 2. Among cases with Tsukuba score 3, 37.5% were malignant and 62.5% were benign. Eighty percent of the cases with Tsukuba score 4 were malignant, while all (100%) of the cases with score 5 had malignant pathology.

Table 1. Distribution of pathology results according to Tsukuba scores.

	Pathology	
Tskuba	**Malignant n**	**Benign %**
1	0 (0%)	4 (100%)
2	0 (0%)	17 (100%)
3	3 (37.5%)	5 (62.5%)
4	4 (80%)	1 (20%)
5	11 (100%)	0 (0%)

There was a statistically significant concordance between pathology results and Tsukuba scores (p<0.05). The rate of accurate diagnosis of malignancy was 40% based on the pathology results, and 35.6% based on Tsukuba scores. Compared to pathology results, Tsukuba scores had diagnostic sensitivity of 83.3%, specificity of 96.3%, positive predictive value of 93.75% and negative predictive value of 89.66%.

Table 2. Concordance between Tsukuba score and pathology result.

	Pathology			
Tsukuba	**Malignant n (%)**	**Benign n (%)**	**Total n (%)**	**p**
Malignant	15 (33.3%)	1 (2.2%)	16 (35.6%)	
Benign	3 (6.7%)	26 (57.8%)	29 (64.4%)	**0.001****
Total	18 (40%)	27 (60%)	45 (100%)	

*McNemar Test ** p<0.01*

ADC values of the mass lesions of cases varied between $0.74x10^{-3}$ mm²/sec and $1.8x10^{-3}$ mm²/sec, with a mean lesion ADC value of $1.2x10^{-3}$ mm²/sec. ADC values of the normal breast tissue varied between $1.02x10^{-3}$ mm²/sec and $2.91x10^{-3}$ mm²/sec, with a mean ADC value of $1.6x10^{-3}$ mm²/sec.

After categorizing the lesions as benign and malig-

nant, the mean ADC value of the malignant lesions was 0.95±0.17x10^{-3} mm²/sec, while the mean ADC value of the benign lesions was 1.37±0.16x10^{-3} mm²/sec. Mean ADC value of the lesions was significantly lower in cases with malignant pathology results compared to the cases with benign pathology results (p<0.01).

Table 3. Concordance of mass lesion ADC and normal breast ADC values with the pathology results.

	Pathology		
	Malignant Mean±SS (Min-Max)x10^{-3}	Benign Mean±SS (Min-Max)x10^{-3}	p
Mass ADC	0.95±0.17 (0.74-1.51)	1.37±0.16 (1.1-1.8)	0.001**
Breast ADC	1.58±0.45 (1.02-2.91)	1.61±0.30 (1.17-2.25)	0.771

*Student t Test, ** p<0.01*

Mean normal breast tissue ADC values did not show a statistically significant difference according to the pathology results of the cases (p>0.05).

Table 4. Evaluation of lesion ADC values according to Tsukuba score.

		Mass ADC x10^{-3}		
Tskuba	n	Min-Max	Mean±SD	p
1	4	1.39-1.49	1.45±0.04	
2	17	1.11-1.8	1.34±0.16	
3	8	0.85-1.66	1.23±0.32	[1]0.001**
4	5	0.75-1.33	0.96±0.22	
5	11	0.74-1.51	0.99±0.21	
Malignant	16	0.74-1.51	0.99±0.21	[2]0.001**
Benign	29	0.85-1.8	1.33±0.21	

*[1] One-way ANOVA test, [2] Student t test,** p<0.01*

In comparison of Tsukuba scores of the lesions and ADC values, mean ADC values of lesions showed a statistically significant difference according to Tsukuba scores (p<0.01). Mean ADC value of the cases with Tsukuba score 1 was significantly higher than mean lesion ADC value of cases with Tsukuba scores of 4 (p=0.011) or 5 (p=0.006). Mean ADC value of the cases with Tsukuba score of 2 was significantly higher than mean ADC value of the cases with Tsukuba scores of 4 (p=0.008) or 5 (p=0.001). There was no statistically significant difference in comparison of other Tsukuba scores regarding mean ADC values of the lesions (p>0.05).

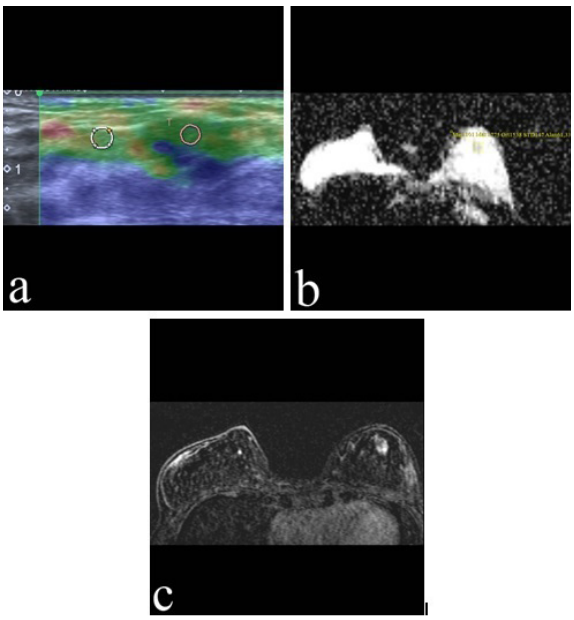

Figure 2. A 25 year-old female case. (a) In sonoelastographic examination, the lesion is coded predominantly as green, showing equal elasticity with the surrounding breast parenchyma, and was evaluated as Tsukuba elasticity score 1. (b) Post-contrast administration axial T1A FLASH 3D subtraction image. (c) ADC value in DWI was calculated as 1.538x10^{-3} mm²/sec. Histopathological diagnosis of the case was fibrocystic changes.

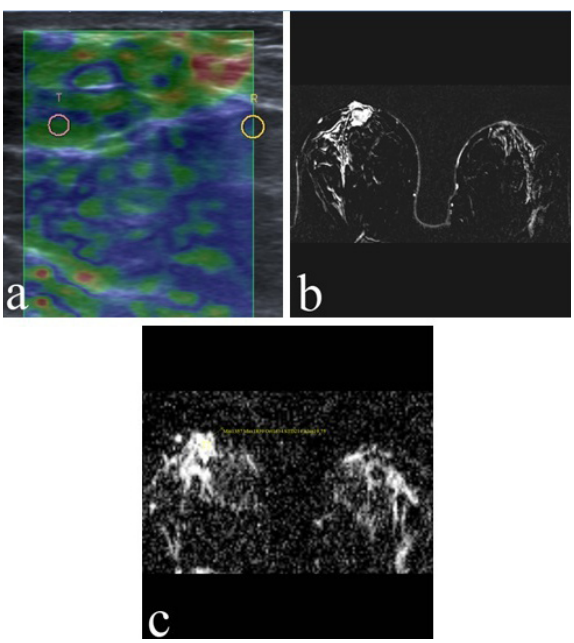

Figure 3. A 29 year-old female case. (a) In sonoelastographic examination, the lesion included blue and green areas, showing inhomogeneous elasticity, and was evaluated as Tsukuba elasticity score 2. (b) Post-contrast axial T1A FLASH 3D subtraction image. (c) ADC value in DWI was calculated as 1.634x10^{-3} mm²/sec. Histopathological diagnosis of the case was fibroadenomatoid changes.

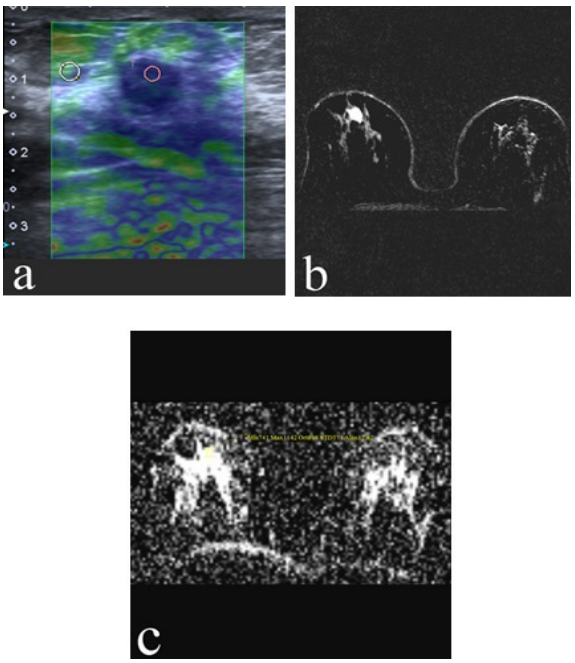

Figure 4. A 57 year-old female case. (a) In sonoelastographic examination, the surrounding tissue observed has not lost its elasticity, and the lesion coded as blue was evaluated as Tsukuba elasticity score 4. (b) Post-contrast axial T1A FLASH 3D subtraction image. (c) ADC value in DWI was calculated as 0.898x10^{-3} mm^2/sec. Histopathological diagnosis of the case was intraductal carcinoma.

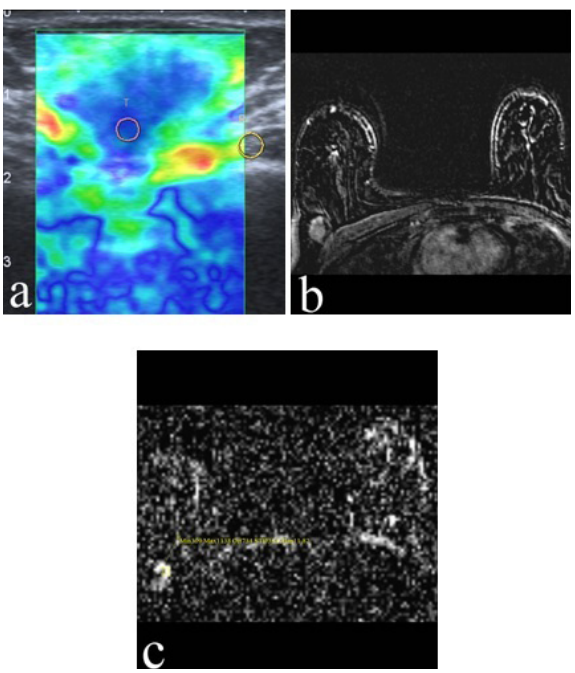

Figure 5. A 55 year-old female case. (a) In sonoelastographic examination, the surrounding tissue was observed to have lost its elasticity, and the lesion coded as blue was evaluated as Tsukuba elasticity score 5. (b) Post-contrast axial T1A FLASH 3D subtraction image. (c) ADC value in DWI was calculated as 0.74x10^{-3} mm^2/sec. Histopathological diagnosis of the case was invasive breast carcinoma.

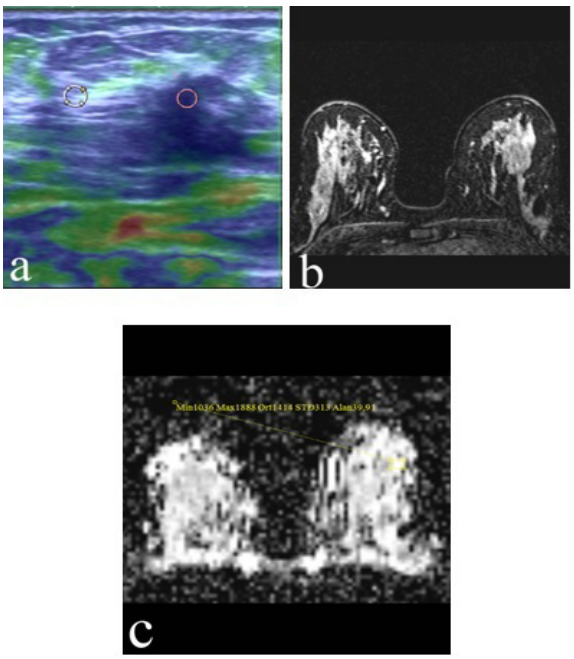

Figure 6. A 42 year-old female case. The malignant lesion was evaluated as false negative based on ADC measurement, while Tsukuba score identified it correctly. (a) In sonoelastographic examination, the lesion was evaluated as Tsukuba elasticity score 5. (b) Post-contrast axial T1A FLASH 3D subtraction image. (c) ADC value in DWI was calculated as 1.414x10^{-3} mm^2/sec. Histopathological diagnosis of the case was invasive lobular carcinoma..

Malignant lesions detected based on Tsukuba scores had significantly lower mean ADC values compared to benign lesions ($p<0.01$).

DISCUSSION

Sonoelastography may show the degree of tissue stiffness in real time with color codes, and a qualitative elasticity score between 1 to 5 points can be assigned. The five-point scoring system developed by Itoh et al. [13,14], known as "Tsukuba elasticity score," is commonly used in sonoelastographic evaluation of breast lesions. In this scoring system, the color pattern of the lesion and the surrounding breast tissue are evaluated and assigned a score on a scale of five points. We used this Tsukuba elasticity score in our study. In comparison to histopathological examination results, Tsukuba elasticity score was found to have a sensitivity of 83.3%, specificity of 96.3%, positive predictive value of 93.75%, and negative predictive value of 89.66%. Itoh et al. [13] evaluated 111 lesions and found the sensitivity and specificity of this five-point scoring method as 86.5% and 89.8%, respectively. Zhu et al. [23] evaluated 139

lesions and they found its sensitivity and specificity as 85.5% and 86.6%, respectively. Yıldız et al. evaluated 80 patients and they found sensitivity and specificity as 85.71% and 86.44%, respectively [10]. Our results are consistent with the results of previous studies using scoring methods. These findings support the utilization of this scoring system as a complementary diagnostic method to increase specificity.

In our study, we used 1.5 T magnet power device with EPI-DWI sequence and a b value of 1000 to generate ADC values. Mean ADC values of 18 malignant ($0.95\pm0.17x10^{-3}$ mm²/sec), 27 benign lesions ($1.37\pm0.16x10^{-3}$ mm²/sec), and normal tissue ($1.6x10^{-3}$ mm²/sec) were as stated. Mean ADC value of lesions that were histopathologically reported as malignant was significantly lower compared to mean ADC value of lesions that were histopathologically benign ($p<0.01$).

In their study, Guo et al. [23] used EPI sequences and took b values as 0 and 1000 mm²/sec, and they found mean ADC values of 31 malignant ($0.97x10^{-3}$ mm²/sec), and 24 benign lesions ($1.57x10^{-3}$ mm²/sec) as indicated. Using similar sequence (EPI), we obtained similar results to those of Guo et al.

Woodhams et al. used b value as 0 and 700 mm²/sec to calculate ADC values in 191 mass lesions. They found mean ADC values for malignant ($1.22\pm0.31x10^{-3}$ mm²/sec), and benign lesions ($1.67\pm0.54x10^{-3}$ mm²/sec), and normal tissue ($2.09\pm0.27x10^{-3}$ mm²/sec) as indicated [24]. Yılmaz et al. used two different b values (b=400, 800 s mm^{-2}) and found highly significant differences between the mean ADC values for normal parenchyma and malignancy ($p<0.001$)[25].

Our mean ADC value for malignant lesions was slightly lower than that found by Woodhams et al. The reason for this is that 17 of the 18 malignant lesions in our study were invasive ductal carcinomas. Woodhams et al. showed that invasive ductal carcinoma had lower ADC values compared to noninvasive ductal carcinoma. They found mean ADC values in invasive ductal, and noninvasive ductal carcinomas as $1.20x10^{-3}$ mm²/sec, and $1.35x10^{-3}$ mm²/sec, respectively Park et al. reported mean ADC value in invasive ductal carcinoma as $0.89x10^{-3}$ mm²/sec, and their result was consistent with ours [24].

There are limited number of studies investigating sonoelastography and diffusion ADC value in discriminating breast lesions. Satake et al. [26] investigated ultrasound elastography and MRI diffusion ADC values in 115 patients with only BI-RADS Category 4 and 5 lesions and they found mean elasticity score for malignant masses (4.1±0.8) was significantly higher than that for benign masses (2.7±1.1) and also mean ADC value for malignant masses ($0.89\times10^{-3}\pm0.28\times10^{-3}$ mm²/s) was significantly lower than that of benign masses ($1.1\times10^{-3}\pm0.34\times10^{-3}$ mm²/s). For BI-RADS category 4 masses, in the univariate analysis, the elasticity score ($p=0.002$) was a statistically significant predictor for malignancy, whereas the ADC value ($p=0.054$) was not significant. Using multivariate analysis, the elasticity score was also statistically significant ($p=0.005$) for BIRADS category 4 masses. In the univariate analysis, neither the elasticity score ($p=0.993$) nor the ADC value ($p=0.998$) was a statistically significant predictor of malignancy in BI-RADS category 5 masses. BI-RADS category 1-3 masses were not included in their study. In our study, in comparison of Tsukuba scores of the lesions and ADC values, mean ADC values of the lesions showed a statistically significant difference according to Tsukuba scores ($p<0.01$). Mean ADC values of the lesions in cases with Tsukuba score 1 and 2 were significantly higher than mean ADC values of the lesions in cases with Tsukuba score 4 or 5. Malignant lesions diagnosed based on Tsukuba scores had significantly lower mean ADC values compared to benign lesions. In addition in our study, 3 of the 4 lesions that had false negative results according to five-point scoring system were correctly identified as malignant with ADC measurements, while 1 lesion had false negative result with both sonoelastography and ADC. Two lesions that were evaluated as benign based on ADC values were diagnosed as malignant in histopathological examination; while both lesions were identified accurately with sonoelastography.

There are some limitations of this study. The sample size was relatively low. Sonoelastographic evaluation was performed using color- coded maps overlaying B-mode sonographic images and therefore, could not be performed independent of the B-mode sonographic examination which created a potential for bias. Furthermore, elastographic images were assigned a score on a scale of 5, but this process involved

the observer's interpretation and was not completely objective. Regarding ADC measurement, currently there is no standard b value in diffusion MRI, and different b values yield different results. Also, small cystic, necrotic components within the lesion can lead to overestimation of ADC.

CONCLUSION

Sonoelastography opens a new dimension in imaging by providing information regarding the mechanical properties of the examined tissue, and therefore it is a valuable imaging method. Rather than being used alone in discriminating between benign and malignant breast lesions, the sonoelastographic five-point Tsukuba scoring system can be used as an ancillary method in order to increase diagnostic specificity and prevent unnecessary biopsies and interventions.

Diffusion- weighted MRI is a rapid, sensitive, alternative imaging modality for characterization of breast lesions through calculation of ADC values. Additionally, since DWI is a noninvasive diagnostic method, it can prevent unnecessary biopsies.

Sonoelastography and ADC may be insufficient on their own to make a discrimination between benign and malignant breast lesions. However, these two can be used as complementary alternative methods to increase diagnostic sensitivity and specificity.

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REFERENCES

1. Denise RA, Caroline C, Bruce JH, et al. Imaging and cancer: Research strategy of the American College of Radiology Imaging Network. Radiology. 2005;235(3):741-51. https://doi.org/10.1148/radiol.2353041760
2. Leung JWT. Screening mammography reduced morbidity of breast cancer treatment. AJR Am J Roentgenol. 2005;184(5): 1508-9. https://doi.org/10.2214/ajr.184.5.01841508
3. Mahesh M. Digital mammography: An overview. Radiographics. 2004;24(6):1747-60. https://doi.org/10.1148/rg.246045102
4. Zhi H, Xiao XY, Yang HY, et al. Semi-quantitating stiffness of breast solid lesions in ultrasonic elastography. Acad Radiol. 2008;15(11):1347-53. https://doi.org/10.1016/j.acra.2008.08.003
5. Thomas A, Degenhardt F, Farrokh A, Wojcinski S, Slowinski T, Fischer T. Significant differentiation of focal breast lesions: calculation of strain ratio in breast sonoelastography. Acad Radiol. 2010;17(5):558-63. https://doi.org/10.1016/j.acra.2009.12.006
6. Lyshchik A, Higashi T, Asato R, Tanaka S, Ito J, Mai JJ, et al. Thyroid gland tumor diagnosis at US elastography. Radiology. 2005;237(1):202-11. https://doi.org/10.1148/radiol.2363041248
7. Vorländer C, Wolff J, Saalabian S, Lienenlüke RH, Wahl RA. Real-time ultrasound elastography-a noninvasive diagnostic procedure for evaluating dominant thyroid nodules. Langenbecks Arch Surg. 2010;395(7):865-71. https://doi.org/10.1007/s00423-010-0685-3
8. Ning CP, Jiang SQ, Zhang T, Sun LT, Liu YJ, Tian JW. The value of strain ratio in differential diagnosis of solid nodules. Eur J Radiol. 2012;81(2):286-91. https://doi.org/10.1016/j.ejrad.2010.12.010
9. Rago T, Santini F, Scutari M, Pinchera A, Vitti P. Elastography: New developments in ultrasound for predicting malignancy in thyroid nodules. J Clin Endocrinol Metab. 2007;92(8):2917-22. https://doi.org/10.1210/jc.2007-0641
10. Yıldız MS, Goya C, Adin ME. Contribution of sonoelastography to diagnosis in distinguishing benign and malignant breast masses. J Ultrasound Med. 2020;39(7):1395-403. https://doi.org/10.1002/jum.15236
11. Cochlin DL, Ganatra RH, Griffiths DF. Elastography in the detection of prostatic cancer. Clin Radiol. 2002;57(11):1014-20. https://doi.org/10.1053/crad.2002.0989
12. Yerli H, Yılmaz T, Ural B, Gülay H. Solid meme kitlelerinin sonoelastografi ile değerlendirilmesinin tanısal önemi. Ulus Cerrahi Derg. 2013;29(2):67-71. https://doi.org/10.5152/UCD.2013.40
13. Itoh A, Ueno E, Tohno E, et al. Breast disease: clinical application of US elastography for diagnosis. Radiology. 2006; 239(2):341-50. https://doi.org/10.1148/radiol.2391041676
14. Tardivon A, El Khoury C, Thibault F, et al. Elastography of the breast: a prospective study of 122 lesions [in French]. J Radiol. 2007;88(5 Pt 1):657-62. https://doi.org/10.1016/S0221-0363(07)89872-6
15. Tan SLL, Rahmat K, Rozalli FI, et al. Differentiation between benign and malignant breast lesions using quantitative diffusion-weighted sequence on 3 T MRI. Clin Radiol. 2014;69(1):63-71. https://doi.org/10.1016/j.crad.2013.08.007
16. Chen X, Li WL, Zhang YL, Wu Q, Guo YM, Bai ZL. Meta-analysis of quantitative diffusion-weighted MR imaging in the differential diagnosis of breast lesions. BMC Cancer. 2010;10:693. https://doi.org/10.1186/1471-2407-10-693
17. Pereira FPA, Martins G, Oliveira RVC. Diffusion magnetic resonance imaging of the breast. Magn Reson Imaging Clin N Am. 2011;19(1):95-110. https://doi.org/10.1016/j.mric.2010.09.001
18. Kolb TM, Lichy J, Newhouse JH. Comparison of the performance of screening mammography, physical examination and breast US and evaluation of factors that influences them: an analysis of 27,825 patient evaluations. Radiology. 2002;225(1): 165-75. https://doi.org/10.1148/radiol.2251011667
19. Kaiser WA. Dynamic spiral MR mammography. Radiology. 2000;215(3):919-20. https://doi.org/10.1148/radiology.215.3.r00ap45919

20. Esen G. Meme MRG, gövde manyetik rezonans. Manyetik Rezonans Derneği Yayınları; 2005.
21. Sinha S, Lucas-Quesada FA, Sinha U, DeBruhl N, Bassett LW. In vivo diffusion-weighted MRI of the breast: potential for lesion characterization. J Magn Reson Imaging. 2002;15(6):693-704. https://doi.org/10.1002/jmri.10116
22. Abdel Razek AAK, Gaballa G, Denewer A, et al. Diffusion weighted MR imaging of the breast. Acad Radiol. 2010; 17(3):382-6. https://doi.org/10.1016/j.acra.2009.10.014
23. Zhu QL, Jiang YX, Liu JB, et al. Real-time ultrasound elastography: its potential role in assessment of breast lesions. Ultrasound Med Biol. 2008;34(8):1232-8. https://doi.org/10.1016/j.ultrasmedbio.2008.01.004
24. Woodhams R, Matsunaga K, Kan S, Hata H, Ozaki M, Iwabuchi K, et al. ADC mapping of benign and malignant breast tumors. Magn Reson Med Sci. 2005;4(1):35-42. https://doi.org/10.2463/mrms.4.35
25. Yılmaz R, Bayramoglu Z, Kartal MG, Çalışkan E, Salmaslıoğlu A, Dursun M, et al. Stromal fibrosis: imaging features with diagnostic contribution of diffusion-weighted MRI. Br J Radiol. 2018;91(1085):20170706. https://doi.org/10.1259/bjr.20170706
26. Satake H, Nishio A, Ikeda M, Ishigaki S, Shimamoto K, Hirano M, et al. Predictive value for malignancy of suspicious breast masses of BI-RADS categories 4 and 5 using ultrasound elastography and MR diffusion-weighted imaging. AJR Am J Roentgenol. 2011;196(1):202-9. https://doi.org/10.2214/AJR.09.4108

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Comparison of Serum and Salivary Alpha-Fetoprotein Levels in Pregnancies Complicated with Neural Tube Defects

Nöral Tüp Defekti ile Komplike Gebeliklerde Serum ve Tükürük Alfa-fetoprotein Düzeylerinin Karşılaştırılması

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ABSTRACT

Objective: *To compare the serum and salivary values of alpha-fetoprotein (AFP) used in screening neural tube defects (NTD) during pregnancy. To investigate whether saliva can be used instead of serum in NTD screening.*
Method: *The study was conducted between May 2018 - November 2019 at Adıyaman University. The study included 41 pregnant women complicated by NTD and 44 healthy pregnant women. Three ml venous blood and 2 ml saliva samples were taken from both groups. Serum and saliva samples were not diluted. AFP concentration was measured at 450 nm by using commercially available enzyme-linked immunoassay. The intra- , and inter- test assay coefficients (CVs) of the kit were <8% and <10%, respectively. SPSS 21 program was used for data analysis. Mann-Whitney Test was used for the analysis of continuously changing parameters. The correlation coefficient was calculated by Spearman test. A p value of less than 0.05 was accepted for statistical significance.*
Results: *When serum and salivary AFP values were compared between the two groups, the AFP values were found to be higher in both NTD group samples (p<0.001, p<0.001). In both groups, correlation analysis for serum and salivary AFP values showed a strong positive correlation (r=0.730, p<0.001). When the cut-off value for serum AFP is taken as 0.26, NTD can be determined with 100% sensitivity and 90% specificity (AUC: 0.932, p<0.001). When the cut-off value for salivary AFP is taken as 0.034, it can detect NTD with 95% sensitivity and 92% specificity (AUC: 1.00, p<0.001).*
Conclusion: *Salivary and serum AFP values showed strong positive correlation between themselves. We believe that saliva can be used in NTD screening performed by AFP measurement.*

Keywords: *neural tube defect, alpha-fetoprotein, saliva, anomaly, screening test*

ÖZ

Amaç: *Gebelikte nöral tüp defekti (NTD) taramasında kullanılan alfa-fetoproteinin (AFP) serum ve tükürük değerlerini karşılaştırmak. NTD taramasında serum yerine tükürük örneğinin kullanıp kullanılamayacağını araştırmak.*
Yöntem: *Çalışma Mayıs 2018 - Kasım 2019 tarihleri arasında Adıyaman Üniversitesi'nde yapıldı. Çalışmaya gebeliği NTD ile komplike olmuş 41 gebe ve sağlıklı gebeliği olan 44 gebe dâhil edildi. Her iki grup gebelerden 3 ml venöz kan ve 2 ml tükürük örneği alındı. Serum ve tükürük numuneleri seyreltilmedi. AFP konsantrasyonu, ticari olarak temin edilebilen enzim bağlı immünolojik test kullanılarak 450 nm'de ölçüldü. Kitin intra- ve inter- test tahlil katsayısı (CV) sırasıyla <% 8 ve <% 10 idi. Verilerin analizi için SPSS 21 programı kullanıldı. Sürekli değişen parametrelerin analizi için Mann-Whitney Test kullanıldı. Korelasyon katsayısı Spearman testi ile hesaplandı. İstatistiksel anlamlılık için 0,05'den küçük p değeri kabul edildi.*
Bulgular: *İki grup arasında serum ve tükürük AFP değerleri karşılaştırıldığında, AFP değeri her iki örnekte de NTD grubunda istatistiki olarak daha yüksek saptandı (p<0,001, p<0,001). Her iki grupta Serum ve tükürük AFP değerleri için yapılan korelasyon analizinde pozitif yönde güçlü korelasyon elde edildi (r=0,730, p<0,001). Serum AFP için cut-off değer 0,26 alındığında %100 sensitivite ve %90 spesifisite ile NTD belirlenebilir (AUC: 0,932, p<0,001). Tükürük AFP için cut-off değeri 0,034 alındığında, %95 sensitivite ve %92 spesifisite ile NTD'ni belirleyebilir (AUC: 1,00, p<0,001).*
Sonuç: *Tükürük ve serum AFP değerleri güçlü pozitif korelasyon göstermiştir. AFP ölçümü ile yapılan NTD taramasında tükürüğün kullanılabileceği kanaatindeyiz.*

Anahtar kelimeler: *NTD taramasında tükürük AFP düzeyleri*

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INTRODUCTION

Neural tube defect (NTD) is the closure defect that occurs during the early embryonic development of the central nervous system. The defect can occur anywhere in the cranium and the medulla spinalis. Its incidence varies geographically over a wide range of 0.2-3% [1]. These common serious anomalies are the leading cause of death and serious disability in the neonatal period [2]. For this reason, all pregnant women are subjected to screening program for NTD in the early stages of pregnancy. Biomarkers and imaging methods are used in screening programs. The most commonly used biomarker for NTD is alpha-fetoprotein (AFP) [3]. AFP is a plasma protein produced in yolk sac and fetal liver during pregnancy. AFP passes into amniotic fluid and maternal blood at high concentrations [4]. It starts to increase in the first week of pregnancy and reaches the peak level in the first trimester. Then it decreases a little and remains at this level until the 32nd week. It is removed from the blood in a short time after birth. AFP also increases in other pregnancy pathologies such as omphalocele, gastroschisis, and yolk sac tumor [5]. It also increases in hepatocellular cancer (HCC), hepatoblastoma and germ cell tumors during adulthood [6]. The measurement of AFP is done by ELİSA methods and serum is usually used for measurement [7]. Today, medicine is shifting to noninvasive diagnostic and treatment methods day by day. Therefore, especially noninvasive screening tests will increase applicability and patient compliance. In addition to serum, screening tests are also performed on urine and saliva samples where sampling is more painless and noninvasive [8].

While urine is used frequently in these screening tests, saliva is used very rarely. However, saliva is the easiest way for ease of sampling [9].

In our study, we wanted to investigate whether AFP, which is detected in high levels in serum in second trimester pregnants complicated with NTD, can also be detected in saliva samples. Therefore, we aimed to compare the AFP levels in the serum and saliva samples of second trimester healthy pregnants and second trimester pregnants whose pregnancy was complicated by NTD.

MATERIAL and METHOD

Study design

This randomized prospective study was conducted at the Adıyaman University Obstetrics and Gynecology Clinic between May 2018 and November 2019. Before starting the study, we got approval from Adıyaman University local ethics committee on 05.22.2018 with registration number 2018/4-19. NTD screening is performed in all pregnant women between the 12th and 20th gestational weeks in our clinic. Ultrasonographic (US) examination and measurement of maternal AFP serum values were used in NTD screening. We selected pregnant women in the NTD group included in our study randomly from pregnant women who were diagnosed with NTD in our outpatient clinic or diagnosed with NTD at the maternity clinic and referred to our outpatient clinic. A total of 41 pregnant women whose pregnancy was complicated by NTD were included in the study. Pregnancies with fetal anomalies other than NTD, multiple pregnancies and pregnant women who were reluctant to participate were excluded from the study. In addition, pregnant women with chronic diseases such as diabetes mellitus, hypertension, hepatitis and those using medications regularly were also excluded.

As a control group, 44 randomly selected pregnant women in the same gestational week with the study group of patients admitted to the obstetric outpatient clinic for routine control were compared with the study group. Pregnant women with any fetal anomaly, multiple pregnancies, pregnant women with chronic disease and using medications regularly were not included in the control group of pregnant women. Cases in the NTD and control groups were informed about the study. Written consent was obtained from all pregnant women who volunteered to participate in the study.

Collection of samples

Approximately 3 ml of venous blood samples were pipetted into the anticoagulant tubes after info obtaining informed consent of the NTD and control group of pregnant women. The samples were centrifuged at 3500 rpm/min for 10 minutes without waiting. Separated plasma portions were placed in the Eppendorf tubes with automatic pipettes and stored

at -80 degrees until the day of analysis. About 2 ml of saliva samples were taken from the same patients and pipetted into the Eppendorf tubes without any treatment and stored at -80 degrees Celsius until the day of study. Patients were advised not to eat anything for at least 4-6 hours before giving a saliva sample.

Measurements

The alpha-fetoprotein concentration in serum and saliva samples was measured at 450 nm using a commercially available enzyme-bound immunoassay (Catalog no: EH0359, Wuhan Fine Biological Technologies Co. Ltd. China). The test was carried out as directed by the manufacturer. Samples were not diluted. The intra-, and inter- test assay coefficients (CVs) of the kit were <8% and <10%, respectively.

Statistics

SPSS 21 (IBM Statistical Package for Social Sciences Statistics version 21.0, Chicago, USA) program was used for data analysis. The demographic data, serum and saliva AFP values of the patients were compared between the groups with the Mann-Whitney Test. Correlation coefficient was calculated with Spearman test. Data were presented as mean±SD. P value less than 0.05 was accepted for statistical significance. ROC analysis was performed on serum and saliva AFP values to determine the cut-off value that predicts NTD for AFP.

RESULTS

All pregnant women were between 12 w + 0 d and 20 w + 0 d. There was no statistical difference between the two groups in terms of demographic data such as age, gravida, parity and BMI. Gestational weeks and demographic data of the pregnant women constituting the two groups are summarized in Table 1.

When comparing AFP values between the two groups, mean serum AFP level was statistically higher in NTD group (1.393±0.310 vs 0.357±0.097 ng/mL, p<0.001). Again, when the saliva AFP values were compared between the two groups, the AFP values were found statistically higher in the NTD group (0.518±0.259 vs 0.106±0.054 ng/mL, p<0.001) (Figure 1). The AFP values of both groups are summarized in Table 2.

Table 1. Demographic characteristics of pregnant women in both groups.

	NTD Group n=41	Control Group n=44	p
Age (year) (mean±SD)	26,6±3,3	27,8±4,4	0,176
BMI (kg/m^2) (mean±SD)	21,8±2,1	21,5±2,0	0,525
Gw (hafta) (mean±SD)	15,8±0,9	15,3±1,6	0,126
G (mean±SD)	2,7±1,2	3,2±1,4	0,092
A (mean±SD)	1,6±1,1	1,9±1,1	0,258

BMI: Body mass index, Gw: Gestational week, G: Gravidity, A: Alive SD: Standart Deviasyon

Table 2. Serum and saliva AFP levels of pregnant women in both groups.

	NTD Group n=41	Control Group n=44	p
AFP serum (ng/mL) (mean±SD)	1,393±0,310	0,357±0,097	<0,001*
AFP saliva (ng/mL) (mean±SD)	0,518±0,259	0,106±0,054	<0,001*

NTD: Neural tube defect, AFP: Alpha-fetoprotein, SD: Standard Deviation

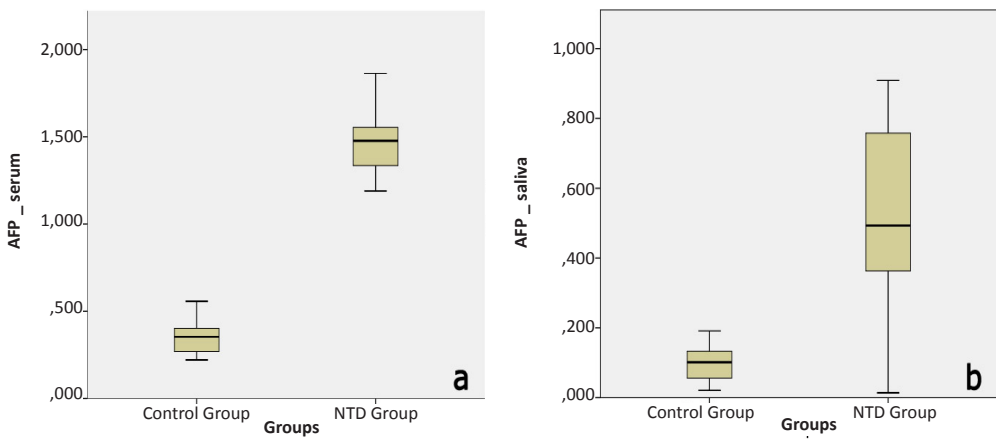

Figure 1. Average of serum (a) and saliva (b) AFP in both groups.
AFP: Alfa-fetoprotein

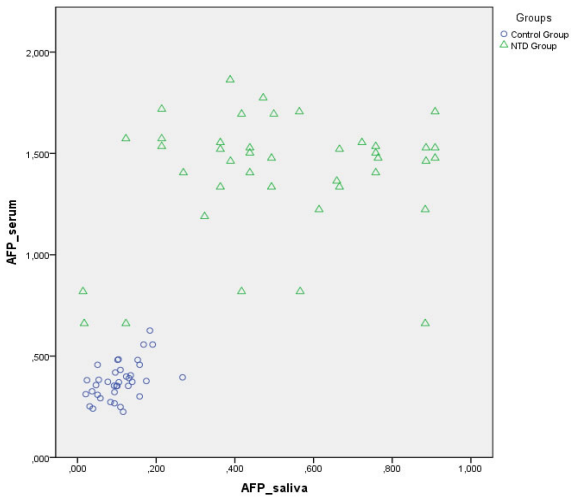

Figure 2. AFP correlation relationship between serum and saliva in both groups (r=0,730, p<0,001).
AFP: Alfa-fetoprotein

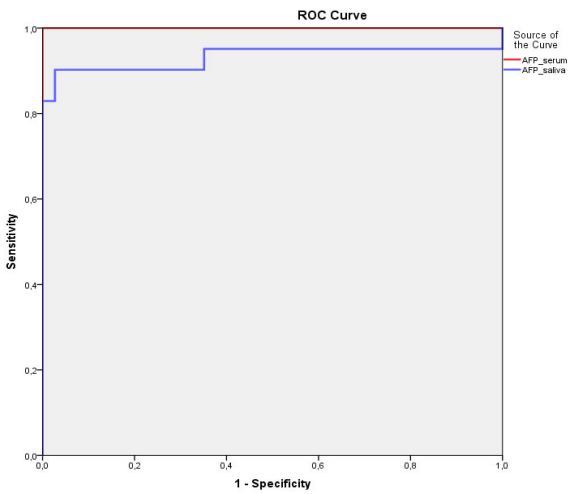

Figure 3. ROC analysis of serum and saliva AFP values in both groups (AUC: 0,932, p<0,001), (AUC: 1,00, p<0,001).
AUC: Area Under Curve, ROC: Receiver Operating Characteristic.

In the correlation analysis for serum and saliva AFP values in both groups, a positive correlation was detected (r=0.730, p<0.001) (Figure 2).

When cut-off value of 0.26 is taken for serum AFP, NTD can be determined with 100% sensitivity, 90% specificity (AUC: 0.932, p<0.001). When cut-off value of 0.034 is taken for saliva AFP, it can determine NTD with 95% sensitivity, 92% specificity (AUC: 1.00, p<0.001) (Figure 3).

DISCUSSION

A fetal anomaly such as NTD, akrania, or a defect at the level of the medulla spinalis may cause the baby to experience serious disabilities throughout its life [10]. For this reason, it is an appropriate approach to screen all pregnant women without discrimination. AFP measurements, and obstetric ultrasonography are used as screening methods all over the world [3]. Of these screening methods, USG should be performed by a gynecologist with a good obstetric experience or by a radiologist with an obstetric evaluation experience [11]. In this respect, it is difficult to use USG as a screening test, but high AFP detection in saliva or blood must be confirmed with USG. Screening tests should be a method in which all pregnant women can benefit equally in terms of cost-effectiveness, ease of application and accessibility. The measurement of AFP is quite practical compared to the ultrasonographic method in terms of applicability as a screening test [12].

AFP measurement is usually done from serum. Venous blood collection is a procedure that hurts the patient. Although it is considered noninvasive, an attempt is made to the patient's body integrity. But taking the saliva sample is quite practical. In addition, contrary to what is required when taking venous blood samples, local antiseptic, syringe, laboratory tube and most importantly, qualified medical personnel are not needed. It can even be taken at home. For this reason, investigating the presence and density of AFP in saliva will increase the ease of application of the screening test. In the literature, saliva has been used in many studies to screen biomarkers [13,14]. These studies mostly included oral and head and neck tumors [15]. In our study, pregnancies complicated by NTD were evaluated. In addition, there are very few studies in the literature investigating AFP in saliva [16]. In our study, serum AFP values were significantly higher in NTD group compared to the control group, as expected. In addition, in the NTD Group, the AFP value in saliva was statistically higher than the control group (p<0.001). When we looked at whether there was a correlation between serum and saliva AFP values, a strongly positive correlation was detected between the samples in both groups (r=0.730, p<0.001). In our study, the detection of a biomarker in saliva, which is not related to the mouth and salivary gland, indicates that plasma proteins and other molecules pass from plasma into saliva. The passage of these proteins and molecules occurs by passive or active diffusion [13].

In studies with a very small sample size in the literature, the presence of AFP in saliva was evaluated in fibrotic diseases of the liver such as HCC and cirrhosis [16,17]. You et al. compared HCC cases and control group in their study, and the serum and saliva AFP values of patients with HCC were statistically higher than values of the control group patients. In their study, a positive correlation was found between saliva and serum AFP levels in patients with HCC, as in our study [16]. As a result of studies with HCC, currently AFP is used in the follow-up of diagnosis and treatment of the patients , and provides strong relevant evidence. In this study, it has been reported that 1-10% of serum AFP enter into saliva [16]. In our current study, this rate was 35.1% on average. In our study, we attributed the higher rate of transition of AFP into saliva to higher AFP levels in NTD complicated pregnancies when compared to HCC and other GIS malignancies [18]. Because the density of the substances in passive or active diffusion is an important determinant on the extent of transition [19]. In addition, increased variations in hormonal balance and volume during pregnancy lead to relaxation in vascular smooth muscles and consequently enhance permeability. The escape of intravascular fluid into the interstitial space also increases during pregnancy [20]. These physiological changes in pregnancy helped to explain the higher rate of transition of AFP into saliva in pregnant women complicated with NTD. There are proportional differences in the transition of biomarkers in serum into saliva. The density of these biomarkers in saliva is less than that in serum. Therefore, evaluation of biomarkers in saliva with more sensitive analytical methods will help to obtain clearer data.

As a result, saliva is one of the easiest samples to store and process. Because of these advantages, using the saliva sample in the laboratory will provide convenience and advantages in many aspects. In our current study, we found that AFP measurements in screening tests for NTD can be performed in saliva samples instead of serum. We think that NTD screening test during pregnancy can be performed with saliva samples in more comprehensive studies on this subject to be performed in the future.

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Informed Consent: Informed consent was taken from all the participants.

REFERENCES

1. Greene ND, Copp AJ. Neural tube defects. Annu Rev Neurosci. 2014;37:221-42. https://doi.org/10.1146/annurev-neuro-062012-170354
2. Desai VR, Gadgil N, Saad S, Raskin JS, Lam SK. Measures of health-related quality of life outcomes in pediatric neurosurgery: literature review. World Neurosurg. 2019;122:252-65. https://doi.org/10.1016/j.wneu.2018.10.194
3. Cameron M, Moran P. Prenatal screening and diagnosis of neural tube defects. Prenat Diagn. 2009;29:402-11. https://doi.org/10.1002/pd.2250
4. Brock DJH, Sutcliffe RG. Early prenatal diagnosis of anencephaly. Lancet. 1972;9:1252-3. https://doi.org/10.1016/S0140-6736(72)92306-9
5. Seller MJ, Coltart TM, Campbell S, Singer JD. Early termination of anencephalic pregnancy after detection by raised alpha-fetoprotein levels. Lancet. 1973;14:73. https://doi.org/10.1016/S0140-6736(73)93264-9
6. Mehta N, Dodge JL, Roberts JP, Hirose R, Yao FY. Alpha-fetoprotein decrease from > 1,000 to < 500 ng/ml in patients with hepatocellular carcinoma leads to ımproved posttransplant outcomes. Hepatology. 2019;69:1193-205. https://doi.org/10.1002/hep.30413
7. Mancal P, Srámek M, Malbohan I, Simek L. The first clinical trial for determination of alpha 1 fetoprotein by means of Sevatest-ELISA AFP Kit (micro I). J Hyg Epidemiol Microbiol Immunol. 1988;32(2):209-17. PMID: 2457612.
8. Zhan Z, Guan Y, Mew K, Zeng W, Peng M, Hu P, et al. Urine alpha-fetoprotein and orosomucoid 1 as biomarkers of hepatitis B virus-associated hepatocellular carcinoma. Am J Physiol Gastrointest Liver Physiol. 2020;318:305-12. https://doi.org/10.1152/ajpgi.00267.2019
9. Kaczor-Urbanowicz KE, Carreras-Presas CM, Aro K, Tu M, Garcia-Godoy F, Wong DT. Saliva diagnostics current views and directions. Exp Biol Med. 2016;242:459-72. https://doi.org/10.1177/1535370216681550
10. Szabó N, Gergev G, Valek A, Eller J, Kaizer L, Sztriha L. Birth prevalence of neural tube defects: a population-based study in South-Eastern Hungary. Childs Nerv Syst. 2013;29:621-7. https://doi.org/10.1007/s00381-012-1951-1
11. Robinson AJ, Blaser S, Toi A, Chitayat D, Halliday W, Pantazi S, et al. The fetal cerebellar vermis: assessment for abnormal development by ultrasonography and magnetic resonance imaging. Ultrasound Q. 2007;23:211-23. https://doi.org/10.1097/RUQ.0b013e31814b162c
12. Fuchs KM, Peipert JF. First trimester down syndrome screening: public health implications. Semin Perinatol.

2005;29:267-71.
https://doi.org/10.1053/j.semperi.2005.05.003
13. Xiao H, Wong DT. Proteomics and its applications for biomarker discovery in Human Saliva. Bioinformation. 2011;5:294-6.
https://doi.org/10.6026/97320630005294
14. Nagler R, Bahar G, Shpitzer T, Feinmesser R. Concomitant analysis of salivary tumour markers-a new diagnostic tool for oral cancer. Clin Cancer Res. 2006;12:3979-84.
https://doi.org/10.1158/1078-0432.CCR-05-2412
15. Recker EN, Brogden KA, Avila-Ortiz G, Fischer CL, Pagan-Rivera K. Novel biomarkers of periodontitis and/or obesity in saliva-An exploratory analysis. Arch Oral Biol. 2015;60:1503-9.
https://doi.org/10.1016/j.archoralbio.2015.07.006
16. You XY, Jiang J, Yin FZ. Preliminary observation on human saliva alpha-fetoprotein in patients with hepatocellular carcinoma. Chin Med J (Engl). 1993;106(3):179-82. PMID: 7686840.
17. Chandra RK. Indian childhood cirrhosis: genealogic data, alpha-foetoprotein, hepatitis antigen and circulating immune complexes. Trans R Soc Trop Med Hyg. 1976;70:296-301.
https://doi.org/10.1016/0035-9203(76)90079-1
18. Johnson PJ. The role of serum alpha-fetoprotein estimation in the diagnosis and management of hepatocellular carcinoma. Clin Liver Dis. 2001;5:145-59.
https://doi.org/10.1016/S1089-3261(05)70158-6
19. Aps JK, Martens LC. Review: The physiology of saliva and transfer of drugs into saliva. Forensic Sci Int. 2005;150(2-3):119-31.
https://doi.org/10.1016/j.forsciint.2004.10.026
20. Sızlan A, Kurt E. Physiological changes in pregnancy. Turkiye Klinikleri J Surg Med Sci. 2007;3(32):1-7. Available from: http://www.turkiyeklinikleri.com/article/en-gebelik-fizyolojisi-48093.html

Original Article

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Evaluation of Systemic Steroid Response Via Digital Infrared Thermal Imaging (DITI) in Patients with Idiopathic Granulomatous Mastitis

İdiyopatik Granülomatöz Mastitli Hastalarda Dijital Kizilötesi Termal Görüntüleme (DİTİ) ile Sistemik Steroid Yanitinin Değerlendirilmesi

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ABSTRACT

Objective: *The aim of this study is to evaluate the pre-treatment and post-treatment thermography images of patients who received steroid treatment with a diagnosis of granulomatous mastitis, and to determine whether the thermography device is an effective imaging method in the follow-up.*
Method: *This retrospective study was conducted by the Breast Surgery Working Group of Cerrahpaşa Medical Faculty, Department of General Surgery. Fifteen patients who applied to the center between January 1, 2013 and May 31, 2015, and received steroid treatment due to granulomatous mastitis were included in the study. Digital Infrared Thermal Imaging (DITI) results at the beginning and end of the treatment were compared.*
Results: *The mean age of the patients was 34.6±8.0 years with a range of 22-50 years. Mean values of DITI before steroid treatment of the inflamed area was 34.3±0.7°C which was significantly higher than the post-treatment mean DITI (31.1±1.1°C). The mean pre-treatment, and post-treatment DITI values recorded in healthy breasts were 30.1±1.3°C and 29.6±1.1°C, respectively. The DITI values of patients with granulomatous mastitis were significantly higher than the results of the healthy group, both before and after treatment. In the pre-treatment DITI measurements, the thermographic image demonstrated a discerning septal image which was considered to show the region where inflammation was severe. The appearance of this 'serpentine' image, which was detected in 8 patients before the treatment, disappeared as a result of treatment.*
Conclusion: *Systemic steroid treatment is an effective option in the treatment of patients with granulomatous mastitis, and our results have shown that DITI can be used in the follow-up of response to treatment.*

Keywords: *Granulomatous mastitis, systemic steroid treatment, response to treatment, digital infrared tranformation inductor*

ÖZ

Amaç: *Bu çalışmanın amacı, granülomatöz mastit tanısı ile steroid tedavisi alan hastaların tedavi öncesi ve tedavi sonrası infrared termografi görüntülerinin değerlendirilmesi ve infrared termografi cihazının takipte etkili bir görüntüleme yöntemi olup olmadığını belirlemektir.*
Yöntem: *Bu retrospektif kohort çalışması Cerrahpaşa Tıp Fakültesi Meme Cerrahisi Çalışma Grubu tarafından gerçekleştirildi. 1 Ocak 2013 ile 31 Mayıs 2015 tarihleri arasında merkeze başvuran ve granülomatöz mastit nedeniyle steroid tedavisi alan 15 hasta çalışmaya dahil edildi. Tedavinin başlangıcında ve sonunda dijital Infrared Termal Görüntüleme (DITI) sonuçları karşılaştırıldı.*
Bulgular: *Hastaların ortalama yaşı 34,6±8,0 olup 22-50 yaş aralığındaydı. İnflamasyonlu bölgenin steroid tedavisinden önceki DITI değerleri (34,3±0,7°C), tedavi sonrası ortalamadan (31,1±1,1°C) istatistiksel olarak anlamlı ölçüde yüksekti. Sağlıklı memelerde kaydedilen ortalama tedavi öncesi DITI ölçümü tekrar ölçümde 30,1±1,3°C ve 29,6±1,1°C idi. Granülomatöz mastitli hastaların DITI sonuçları, tedaviden önce ve sonra sağlıklı grubun sonuçlarından anlamlı derecede yüksekti. Tedavi öncesi DITI ölçümlerinde, termografik görüntü, inflamasyonun şiddetli olduğu bölgeyi gösterdiği düşünülen ayırt edici bir septal görüntü gösterdi. Tedaviden önce 8 hastada saptanan bu "serpantin" görüntüsünün görünümü, tedavi sonucunda ortadan kayboldu.*
Sonuç: *Granülomatöz mastitli hastaların tedavisinde sistemik steroid tedavisi etkili bir seçenektir, sonuçlarımız DITI'nin tedaviye yanıt takibinde kullanılabileceğini göstermektedir.*

Anahtar kelimeler: *Granülomatöz mastit, sistemik steroid tedavisi, tedaviye yanıt, dijital kızılötesi transformasyon indüktörü*

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INTRODUCTION

Idiopathic Granulomatous Mastitis (IGM), a disease that is easily confused with breast cancer both clinically and radiologically, is classified as a non-infectious condition with unknown etiology without any specific treatment. Granulomatous inflammation is a special chronic inflammation that occurs when activated macrophages aggregate. After excluding other factors that cause granuloma in the breast, the diagnosis of IGM can be made. However, definitive diagnosis can be made by histopathological examination of affected breast tissue [1].

Infrared radiation detected by more advanced systems can be converted into formats that can be visualized –and more importantly– quantified and monitored. Digital Infrared Thermal Imaging (DITI) is an imaging method that has been developed for this purpose. It has found clinical use as a modality that can measure the increase in temperature caused by inflammation on the body surface [2]. DITI is a prime example of a noninvasive, simple and safe diagnostic technique. Lack of contact, pain (such as in mammography) or radiation exposure (such as in X-ray or tomography), are also considered to be important components of comfortable diagnostic methods for patients [3,4]. Despite the use of various successful methods for diagnosis and their discerning advantages over each other, several studies have suggested that thermographic imaging may be utilized to determine the size and aggressiveness of tumors [5-7]. Studies investigating mastitis with thermal images were carried out only in veterinary studies on animals and this method was shown to be effective [8].

The optimal option in the treatment of IGM is not clear. Depending on the condition of the symptoms, use of steroids up to 3 to 6 months and surgery may be required in cases not responding to initial treatments [9]. To our knowledge, there is no study in which DITI was used in the diagnosis or treatment follow-up of IGM. For this reason, the aim of this study was to investigate the pre-treatment and post-treatment DITI results of patients who were recipients of steroid treatment with a diagnosis IGM, and to determine whether the DITI could prove to be an effective imaging method in the evaluation of treatment response.

MATERIAL AND METHOD

This study was carried out between January 1, 2013 and May 31, 2015 by a commission formed by infectious disease specialists and members of the Breast Surgery Working Group of Cerrahpaşa Medical Faculty. Patients who received steroid treatment due to IGM were prospectively included in the study. Evaluation of patients were performed with DITI imaging before and after treatment at the Radiology Department of Cerrahpaşa Medical Faculty. Ethics committee approval was obtained from the Ethics Committee of Cerrahpaşa Medical Faculty at the beginning of the study (Date: 04/10/2015, Approval number: 83045809/604.01/02-109/20). Informed consent was obtained from all individual participants included in the study. All examination, diagnosis, treatment and follow-up stages of the patients were carried out by the Cerrahpaşa Medical Faculty General Surgery Department Breast Surgery Working Group.

Patient selection

The files of patients who applied to the departments Cerrahpaşa Medical Faculty of General Surgery and Infectious Diseases with mastitis findings and were determined to not have simple mastitis were examined and screened. Fifteen patients who were followed with a preliminary diagnosis of IGM and diagnosed with unilateral IGM after biopsy were included in the study.

Treatment and follow-up

Methyl-prednisolone treatment was prescribed at a dose of 0.5 mg / kg / day and thermography images were taken with the DITI device in the Cerrahpaşa Medical Faculty Radiology Department Breast Imaging Unit before starting treatment. The patients were followed at the weekly Mastitis Meeting organized jointly by Infectious Diseases and General Surgery Departments. Methylprednisolone treatment of patients who showed clinical improvement was tapered and discontinued and thermographic imaging was performed with DITI device approximately 1 month later.

DITI procedure

In our study, Meditherm med2000© device was used for digital infrared imaging. During the shooting, the temperature of the room was kept at an average of

18-22°C. Patients were kept in the pre-cooled room for 5 minutes, with the waist up naked. The patient's hands were joined at the nape, and 8 images were taken for each patient from anterior, posterior, right axilla, left axilla, right oblique and left oblique views (Figure 1). The whole procedure took an average of 12 minutes.

Using the Wintes Thermal Evaluation Software (version 1.05.0005), measurements were made by scanning the areas affected by granulomatous mastitis and healthy breasts.

Statistical Analysis

Mean, standard deviation, median, lowest, highest, frequency and ratio values were used in the descriptive statistics of the data. The distribution of continuous variables was checked by the Kolmogorov-Smirnov test. The Wilcoxon Signed-Rank Test and the McNemar test were used in the analysis of repeated measurements. The SPSS v22.0 program was used for the conduct of all statistical analyses.

RESULTS

The mean age of the patients was 34.6±8.0 with a range of 22-50 years. The mean DITI measurements of the diseased breast before methyl-prednisolone treatment was 34.3±0.7°C. One month after the treatment (post-treatment measurement) this value was 31.1±1.1°C. Compared with pretreatment values, there was a statistically significant decrease in temperature after treatment ($p<0.05$). Measurements were also made on the healthy contralateral breast for comparison . While the mean DITI measurements in healthy breasts before, and after treatment were 30.1±1.3°C, and 29.6±1.1°C, respectively. There was no significant difference between DITI measurements in healthy breasts before and after treatment ($p>0.05$). In both the pre-treatment and post-treatment period, DITI results of healthy breasts were significantly lower compared to breasts with IGM ($p<0.05$, Table 1).

Table 1. DITI results before and after steroid treatment.

	Breast with IGM (n=15)	Healthy breast (n=15)	p
Before treatment (°C)	34.3 (33-36)	30.1 (28-33)	<0.001
After treatment (°C)	31.2 (30-33)	29.9 (28-31)	<0.001
P	<0.001	0.207	

Data are given as median (minimum - maximum)

We detected that patients who had active IGM showed a septal image in the thermography shots of the abscess area. This image, which was defined as a serpentine by radiologists, was detected more prominently in patients who were in the active stage of the disease. This finding was detected in 8 of the 15 patients, but it regressed completely in 7 patients after treatment ($p<0.05$, Figure 2, Table 2).

Table 2. Serpentine detection in DITI before and after steroid treatment.

	Serpentine before treatment (n = 8)	No Serpentine before treatment (n = 8)	p
Serpentine after treatment	1 (12.5%)	-	0.016
No Serpentine after treatment	7 (87.5%)	7 (100.0%)	

Data are given as frequency (percentage of column)

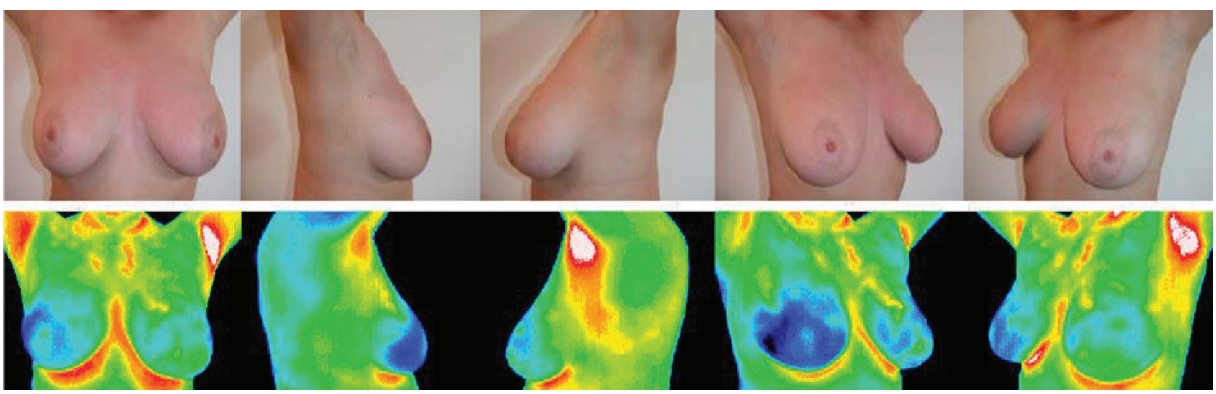

Figure 1. Shooting positions and thermographic provisions.

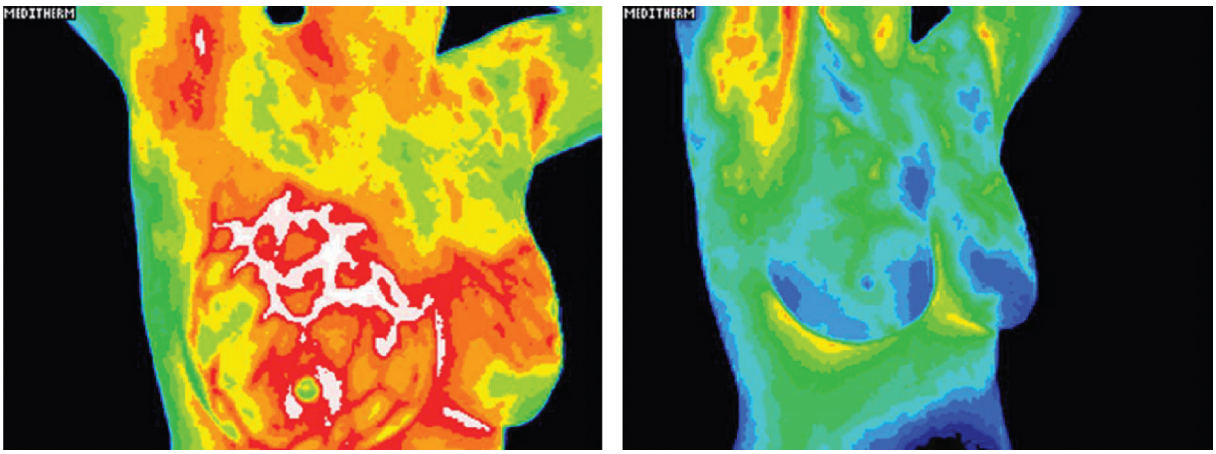

Figure 2. DITI image of the septal image we call serpentine in granulomatous mastitis (left) and healed after treatment (right).

DISCUSSION

In our study, it was determined that the increase in temperature due to inflammation in IGM can be detected with DITI. According to our results, it can be said that steroid is an effective option in IGM treatment and DITI is a successful imaging method in the treatment follow-up. However, it is also important to mention that other treatment approaches do exist. For instance, in their examination of 81 IGM patients, Aydın et al., reported that those who received antibiotics had shorter recovery time compared to people undergoing the standard immunosuppressive or surgical treatment –given that lesion size was 2 cm or smaller [10]. In our study, other treatment modalities were not examined. In future studies, better interpretations can be made by examining these features and comparing their results, especially with regard to DITI evaluation.

In the literature, no study has been encountered that performed the diagnosis and follow-up of IGM with DITI. In their reviews evaluating the imaging methods used in IGM, Hasbahceci and Kadioglu, reported that 30 studies published on this subject used ultrasonography (USG), mammography and magnetic resonance imaging (MRI) [11]. In a similar study, Fazzio et al. reported that the most ideal method for clinical follow-up of patients diagnosed with IGM was MRI, and that any infrared imaging method was not preferred in any patient [12]. Kocaoğlu et al. [13] reported that MRI was superior in comparison to USG in the diagnosis of IGM and biopsy is still required for the definitive diagnosis, but they did not mention the DITI method. Different studies have also examined the methods used in the diagnosis of IGM, but DITI has not been mentioned [14]. As far as our detailed literature review has revealed, this study is the first study in which steroid treatment follow-up in IGM was evaluated with DITI results. Although we could not compare with other methods and other lesions in our study, it was determined that DITI was an effective method in the follow-up of IGM treatment with systemic steroids. Although thermal images are not preferred in mastitis cases in humans, they are especially useful in terms of breast health of animals that are important in the dairy industry; Thermal images are widely used in screening, diagnosis, follow-up and treatment response. Many animal studies have evaluated the results of diagnosis and follow-up of the cases with mastitis with infrared thermal images. As a result of these studies, thermal imaging method has been shown to be effective [15-17].

Studies conducted in other parts of the body have reported that the infection / inflammation in any region can be detected by thermography, and the patient's well-being can be confirmed by the improvement of this abnormality. Among these studies, Kawali reported that the inflammatory or non-inflammatory state of ocular lesions can be detected by thermal imaging [18]. Denoable et al. [19] found that patellar thermal imaging results reflect the severity of osteoarthritis and that the regression or progression of the disease can be monitored with this method. In a study conducted by Saxena and Willital, the importance of thermography in determining skin

and extremity infections was demonstrated [20]. In addition, there are different studies in which inflammation and the results of treatment in different diseases were monitored by thermal imaging [21-23]. In our study, it was shown that DITI results provide information about progression and recovery in IGM, where the inflammation process is quite active. Considering the results of other studies, it seems that DITI is an advantageous option in the evaluation of inflammatory processes that are not only found in the breast area, but also in other parts of the body, especially when they are difficult to diagnose and treat with other methods.

IGM is a rare condition in which it is difficult to differentiate between infectious mastitis and breast carcinoma. The diagnosis is made by exclusion of other possible diagnoses and imaging methods facilitate this process [12]. It has been observed that studies examining the thermographic imaging of breast tissue are mostly performed for the diagnosis, treatment and prognosis of breast cancer [24]. IGM symptoms and imaging results are similar to those of malignant lesions. Therefore, this differential diagnosis is very important [25,26]. There are also studies in other diseases that have reported that distinction with DITI is inadequate [27]. Sarıgöz et al. examined the diagnostic reliability of DITI in 54 patients with palpable breast masses. They found that DITI had 95% sensitivity and 73% specificity in the distinction of malign-benign lesions. They reported that the highest temperatures were detected in IGM cases (36±1°C) which were comparable to those seen in cases of invasive ductal carcinoma (35±1). Therefore it was difficult to differentiate these two lesions with DITI. They also stated that both lesions have higher temperature than fibroadenoma, due to the presence of inflammation.

Breast temperature can be influenced by various features such as metabolic status of the body, blood supply of the breast, size and volume of the breast, breast adipose tissue and activity of the glands [28]. It has been reported that breast temperature may also change according to race, because several skin and adipose tissue-related characteristics are different among races [29]. In our study, these features that may affect DITI results have not been evaluated. Since our study is a single-center study, its generalizability is also limited. It is also evident that the reliability of the presented results were as much as those reported by healthcare professionals who performed and interpreted imaging results. It is also important to remember that while other methods have years and years of experience in imaging studies and their limitations are well known, thermography does not have these advantages. Finally, in our study, other treatment modalities could not be examined. In future studies, better interpretations can be made by examining these features.

CONCLUSION

To our knowledge, this is the first study to examine the effectiveness of DITI in IGM treatment follow-up. We have also determined that response to treatment can be monitored by temperature changes in the inflamed area with DITI. In future, researches with greater number of patients, which comparatrively evaluated other imaging methods, and thermography results of other breast diseases will contribute to more effective management of IGM diagnosis and treatment.

Ethics Committee Approval: Approval was obtained from the Istanbul University Cerrahpaşa Medical Faculty Ethics Committee (10.04.2015 - 109120).
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REFERENCES

1. Altıntoprak F, Baytekin HF, Altınay AE, Eren T. Meme kanserini taklit eden idiyopatik granülomatöz mastit. Meme Sağlığı Dergisi. 2009;5(1):40-3. Available from: https://www.eurjbreasthealth.com/content/files/sayilar/14/buyuk/1491.pdf
2. Jones BF. A reappraisal of the use of infrared thermal image analysis in medicine. IEEE Trans Med Imaging. 1998;17(6): 1019-27. https://doi.org/10.1109/42.746635
3. Parisky YR, Sardi A, Hamm R, Hughes K, Esserman L, Rust S, et al. Efficacy of computerized infrared imaging analysis to evaluate mammographically suspicious lesions. AJR Am J Roentgenol. 2003;180(1):263-9. https://doi.org/10.2214/ajr.180.1.1800263
4. Jones BF, Plassmann P. Digital infrared thermal imaging of human skin. IEEE Eng Med Biol Mag. 2002;21(6):41-8. https://doi.org/10.1109/MEMB.2002.1175137
5. Wishart GC, Campisi M, Boswell M, Chapman D, Shackleton V, Iddles S, et al. The accuracy of digital infrared imaging for

breast cancer detection in women undergoing breast biopsy. Eur J Surg Oncol. 2010;36(6):535-40. https://doi.org/10.1016/j.ejso.2010.04.003
6. Ng EY, Ung LN, Ng FC, Sim LS. Statistical analysis of healthy and malignant breast thermography. J Med Eng Technol. 2001;25(6):253-63. https://doi.org/10.1080/03091900110086642
7. Sterns EE, Zee B, SenGupta S, Saunders FW. Thermography. Its relation to pathologic characteristics, vascularity, proliferation rate, and survival of patients with invasive ductal carcinoma of the breast. Cancer. 1996;77(7):1324-8. https://doi.org/10.1002/(SICI)1097-0142(19960401)77:7<1324::AID-CNCR15>3.0.CO;2-3
8. Hovinen M, Siivonen J, Taponen S, Hanninen L, Pastell M, Aisla AM, et al. Detection of clinical mastitis with the help of a thermal camera. J Dairy Sci. 2008;91(12):4592-8. https://doi.org/10.3168/jds.2008-1218
9. Wolfrum A, Kummel S, Theuerkauf I, Pelz E, Reinisch M. Granulomatous mastitis: a therapeutic and diagnostic challenge. Breast Care (Basel). 2018;13(6):413-418. https://doi.org/10.1159/000495146
10. Aydın HO, Baykal A, Konan A, Kaynaroğlu V. Idiopathic granulomatous mastitis: factors influencing recovery and recurrence. The European Research Journal 2019;5(5):768-75. https://doi.org/10.18621/eurj.424016
11. Hasbahceci M, Kadioglu H. Use of imaging for the diagnosis of idiopathic granulomatous mastitis: a clinician's perspective. J Coll Physicians Surg Pak. 2018;28(11):862-7. https://doi.org/10.29271/jcpsp.2018.11.862
12. Fazzio RT, Shah SS, Sandhu NP, Glazebrook KN. Idiopathic granulomatous mastitis: imaging update and review. Insights Imaging. 2016;7(4):531-9. https://doi.org/10.1007/s13244-016-0499-0
13. Kocaoglu M, Somuncu I, Ors F, Bulakbasi N, Tayfun C, Ilkbahar S. Imaging findings in idiopathic granulomatous mastitis. A review with emphasis on magnetic resonance imaging. J Comput Assist Tomogr. 2004;28(5):635-41. https://doi.org/10.1097/01.rct.0000131927.82761.40
14. Yildiz S, Aralasmak A, Kadioglu H, Toprak H, Yetis H, Gucin Z, et al. Radiologic findings of idiopathic granulomatous mastitis. Med Ultrason. 2015;17(1):39-44. https://doi.org/10.11152/mu.2013.2066.171.rfm
15. Ayadi M, Aljumaah RS, Samara EM, Alshaikh MA, Caja G. Use of infrared thermography for mastitis detection in lactating dairy camels (Camelus dromedarius). In: Proceedings of the International Camel Conference. Camel Publishing House; 2015. p. 55-60.
16. Colak A, Polat B, Okumus Z, Kaya M, Yanmaz LE, Hayirli A. Short communication: early detection of mastitis using infrared thermography in dairy cows. J Dairy Sci. 2008;91(11):4244-8. https://doi.org/10.3168/jds.2008-1258
17. Wollowski L, Bertulat S, Kossatz A, Heuwieser W. Short communication: Diagnosis and classification of clinical and subclinical mastitis utilizing a dynamometer and a handheld infrared thermometer. J Dairy Sci. 2019;102(7):6532-9. https://doi.org/10.3168/jds.2018-15509
18. Kawali AA. Thermography in ocular inflammation. Indian J Radiol Imaging 2013;23(3):281-3. PMID: 24347863.
19. Denoble AE, Hall N, Pieper CF, Kraus VB. Patellar skin surface temperature by thermography reflects knee osteoarthritis severity. Clin Med Insights Arthritis Musculoskelet Disord. 2010;3:69-75. https://doi.org/10.4137/CMAMD.S5916
20. Saxena AK, Willital GH. Infrared thermography: experience from a decade of pediatric imaging. Eur J Pediatr. 2008;167(7):757-64. https://doi.org/10.1007/s00431-007-0583-z
21. Chanmugam A, Langemo D, Thomason K, Haan J, Altenburger EA, Tippett A, et al. Relative temperature maximum in wound infection and inflammation as compared with a control subject using long-wave infrared thermography. Adv Skin Wound Care. 2017;30(9):406-14. https://doi.org/10.1097/01.ASW.0000522161.13573.62
22. Toutouzas K, Benetos G, Drakopoulou M, Bounas P, Tsekoura D, Stathogiannis K, et al. Insights from a thermography-based method suggesting higher carotid inflammation in patients with diabetes mellitus and coronary artery disease. Diabetes Metab. 2014;40(6):431-8. https://doi.org/10.1016/j.diabet.2014.05.005
23. Castillo-Martinez C, Valdes-Rodriguez R, Kolosovas-Machuca ES, Moncada B, Gonzalez FJ. Use of digital infrared imaging in the assessment of childhood psoriasis. Skin Res Technol. 2013;19(1):e549-51. https://doi.org/10.1111/j.1600-0846.2011.00611.x
24. Shahari S, Wakankar A. Color analysis of thermograms for breast cancer detection. 2015 International Conference on Industrial Instrumentation and Control. 2015. p. 1577-81. https://doi.org/10.1109/IIC.2015.7151001
25. Prasad S, Jaiprakash P, Dave A, Pai D. Idiopathic granulomatous mastitis: An institutional experience. Turk J Surg. 2017;33(2):100-3. https://doi.org/10.5152/turkjsurg.2017.3439
26. Han B, Choe Y, Park J, Moon W, Ko YH, Yang J-H, et al. Granulomatous mastitis: Mammographic and sonographic appearances. AJR Am J Roentgenol. 1999;173(2):317-20. https://doi.org/10.2214/ajr.173.2.10430126
27. Sarigoz T, Ertan T, Topuz O, Sevim Y, Cihan Y. Role of digital infrared thermal imaging in the diagnosis of breast mass: A pilot study: Diagnosis of breast mass by thermography. Infrared Physics & Technology. 2018;91:214-9. https://doi.org/10.1016/j.infrared.2018.04.019
28. Zeng J, Lin L, Deng F. Infrared thermal imaging as a nonradiation method for detecting thermal expression characteristics in normal female breasts in China. Infrared Physics & Technology. 2020;104:103125. https://doi.org/10.1016/j.infrared.2019.103125
29. Habel LA, Capra AM, Oestreicher N, Greendale GA, Cauley JA, Bromberger J, et al. Mammographic density in a multiethnic cohort. Menopause. 2007;14(5):891-9. https://doi.org/10.1097/gme.0b013e318032569c

Original Article

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Penetrating Injuries of Peripheral Vascular Structures: Short -Term Follow-up Study

Periferik Vasküler Yapıların Delici Kesici Alet Yaralanmalarının Kısa Dönem Takibi

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ABSTRACT

Objective: *Penetrating vascular injuries are medical conditions that we often come across and require urgent treatment. Early diagnosis and treatment play a big role reducing the mortality and morbidity in patients suffering from penetrating vascular injuries.*
Method: *We retrospectively observed 168 patients who were operated by us between January 2016 and September 2019 because of peripheral vascular injuries. Demographic features, clinical findings at diagnosis and follow-up and 3rd month arterial duplex ultrasound (DUS) findings were evaluated.*
Results: *In our study, 244 vascular structures were repaired in 168 patients. The most commonly injured vascular structure was femoral artery in 54 (22%) cases. Other injured vascular structures were radial artery in 47 (19%), superficial femoral vein in 33 (14%), deep femoral vein in 28 (11%), ulnar artery in 23 (9%), brachial artery in 21 (9%), popliteal artery in 12 (5%), posterior tibial artery in 9 (4%) and the anterior tibial artery in 3 (1%) cases When it comes to surgical techniques, while primary repair was performed in 57 (23%), and end-to-end anastomosis in 92 patients (38%). As a graft material saphenous vein was used in 60 (25%) and PTFE (polytetraflorethylene) in 35 patients (14%). Two patients (1.19%) with femoral artery repair had suffered from compartment syndrome and fasciotomy had to be done. In a patient with bone fracture accompanied to vascular injury, amputation was performed by the orthopedic clinic due to severe osteomyelitis and necrosis after discharge. At follow-up control after 3 months, stenosis above 70% was not detected with arterial duplex ultrasound (DUS) in any patient and no intervention was required.*
Conclusion: *Immediate arrival of patients with penetrating injuries to the hospitals and approach to the patient in consideration of vascular injury in the emergency rooms are significantly important in reducing limb loss and mortality.*

Keywords: *peripheral vessel, vascular injury, penetrating injury*

ÖZ

Amaç: *Delici kesici alet yaralanmaları (DKAY) sık gördüğümüz ve acil tedavi gerektiren bir durumdur. Erken tanı ve tedavi bu hasta grubunda mortalite ve morbiditeyi azaltır.*
Yöntem: *Çalışmamızda 2016 Ocak ile 2019 Eylül arasında DKAY nedeniyle ameliyat ettiğimiz 168 hastayı retrospektif olarak inceledik. Hastaların demografik özellikleri, tanı ve izlem sırasındaki klinik bulguları ile 3. ay doppler ultrason bulguları değerlendirildi.*
Bulgular: *Çalışmamızda 168 hastada, 244 vasküler yapı onarımı yapıldı. Elli dört olgu (%22) ile en sık yaralanan vasküler yapı femoral arter olarak saptandı. Yaralanan diğer vasküler yapılar sırasıyla 47 olguda (%19) radial arter, 33 olguda (%14) yüzeyel femoral ven, 28 olguda (%11) derin femoral ven, 23 olguda (%9) ulnar arter, 21 olguda (%9) brakial arter, 12 olguda (%5) popliteal arter, 9 olguda (%4) posterior tibial arter ve 3 olguda (%1) anterior tibial arterdi. Cerrahi teknik olarak 57 hastada (%23) primer onarım, 92 hastada (%38) uç-uca anastomoz tercih edildi, 60 hastada (%25) safen ven greft ve 35 hastada (%14) PTFE (polytetrafloretilen) greft interpozisyonu uygulandı. Femoral arter tamiri yapılan 2 hastada (%1.19) kompartman sendromu gelişti ve fasyotomi ihtiyacı oldu. Kemik fraktürü ve vasküler yaralanması olan bir hastaya ise taburculuk sonrası osteomyelit ve nekroz nedeniyle ortopedi kliniği tarafından amputasyon uygulandı. Üçüncü ay doppler kontrollerinde hiçbir hastada %70 üzeri darlık saptanmadı ve herhangi bir girişim gereksinimi olmadı.*
Sonuç: *Delici kesici alet yaralanması olan hastaların hastaneye vakit kaybetmeden ulaşması ve acil serviste vasküler yaralanma şüphesi ile yaklaşım, uzuv kaybını ve mortaliteyi azaltmak için önemlidir.*

Anahtar kelimeler: *periferik damar, damar yaralanması, kesici alet yaralanması*

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INTRODUCTION

Penetrating injuries happen to be more common in patients with low socioeconomic status. Penetrating injuries are more common than firearm-related injuries. The features of the sharp objects (length, thickness, sharpness) play a key role in the degree of damage in the injuries. Course of treatment may vary depending on the body area damaged with the penetrating injury. When the penetrating injuries happen to be in thorax and abdomen, they are considered as multiple traumas and require a multidisciplinary approach as well. Penetrating injuries in isolated limbs are more common and nerve, muscle and bones might be damaged along with the vascular injuries. In such cases, since the operation and follow-up period can become more complex than usual, after evaluating the patient with the relevant clinics, multidisciplinary approach should be considered. Quickly determining whether or not there is a vascular pathology and consulting the patient to vascular surgery for its treatment is really important for decreasing the mortality and morbidity of the patient with penetrating injuries.

MATERIAL and METHOD

This study is a descriptive retrospective study in which we examined 250 patients who applied to the emergency service of Bakirkoy Dr. Sadi Konuk Research and Training Hospita Training and Research Hospital between January 2016 and September 2019 and operated due to peripheral vascular injuries. We only included patients with peripheral vascular injuries in this study. Patients with penetrating injuries to abdominal and thorax, patients with gunshot wounds and missing 3rd month follow-up results were excluded from the study. All these mentioned information of 168 patients were accessed and these patients were included in the study. Cases with multiple vessel injuries were also included in the study and counted as one patient, but when it comes to total number of vessels, we counted each vascular structure individually. A total of 168 patients and 244 vascular repair structures were included in the study.

All of the patients were operated in consideration of BT angiography findings. The repair methods were chosen according to mechanism of vascular injures and severity of vascular damage. While the primary repair was the first choice in simple injuries where vascular integrity was not impaired, end-to-end anastomosis was preferred with the fragmented injuries. Also, if end-to-end anastomosis cannot be performed; saphenous vein or PTFE graft interposition methods became our first choice. When a graft was required for repair, the diameter of the native vessel has been the primary guide for our graft choices. Saphenous vein graft was preferred firstly and if the diameter of the saphenous vein graft was not suitable, then the PTFE graft which is suitable for the native vessel diameter was chosen for interposition, and 6/0 or 7/0 prolene sutures were used for the anastomosis and repair.

Patients who had venous intervention with saphenous vein or PTFE graft interposition, were treated both with warfarin and low- molecular weight heparin (LMWH). We aimed to keep the INR (international normalized ratio) around 2-2.5. When the INR value reached to the therapeutic dose, LMWH treatment was stopped. In patients who had arterial intervention, dual anti-platelet therapy (aspirin and clopidogrel) was prescribed.

In these patients, we had checked and observed the affected vascular structures and the procedures applied to these structures (repair methods), postoperative pulsations of the affected extremities, the amount of erythrocyte suspension used during all process, the follow-up during hospitalization time, whether there was a pathology in the vascular structures in imaging procedure performed after 3 months, whether there was a patient applied to the emergency service during these 3 months and whether there was any pathology during the 3 month follow-up period. During the follow-up, it was checked whether there were any emergency applications and whether any pathology developed during the follow-up period.

The study protocol was approved by the local ethics committee. The data were analyzed with SPSS v21. Descriptive statistical categorical variables were expressed in frequency (n) and percentage (%), while numerical variables were expressed in mean standart deviation.

RESULTS

A total of 168 patients and 244 vascular repair structures were included in the study. Most of (n=129 :77%) of the patients included in the study were male, and 39 (23%) of them were female. Mean age of the patients was 28.34±8.42 years. While 91 (37%) of the injured vascular structures were on the upper limbs and 153 (63%) on the lower limbs. The injured vascular structures in the upper limbs were radial artery in 47 (19%), ulnar artery in 23 (9%), and brachial artery in 21 (9%) patients. The injured vascular structures in the lower limbs were femoral artery in 54 (22%), superficial femoral vein in 33 (14%), deep femoral vein in 28 (11%), popliteal vein in 14 (6%), popliteal artery in 12 (5%), posterior tibial artery in 9 (4%) and anterior tibial artery in 3 patients (1%).

As a treatment, vascular structures were not sutuıred, however, the primary repair or end-to-end anastomosis were first choices. Primary repair was performed in 57 of the injured vascular structures , and end- to -end anastomosis was carried on in 92 vessels. Sixty cases were repaired with saphenous vein graft and 35 of them with PTFE graft interposition.

Graft thrombosis developed in the upper extremities of 2 patients (3.33%) who had saphenous vein graft interposition procedure in the brachial artery. While embolectomy was performed in one of them and in the other patient revision of the surgery was made by removing the saphenous graft and re-interposing with saphenous vein graft.

PTFE graft thrombosis developed in 3 patients (8.57%). In one patient thrombosis developed in the popliteal vein, and in the other two patients in the femoral artery. Graft thrombosis in the popliteal vein was treated by interposing the saphenous vein graft instead of the prosthetic graft. One of the patients with graft thrombosis in the femoral artery was treated by embolectomy and the other by replacing PTFE graft. with saphenous graft. Complication of thrombosis occurred in all patients before discharge and no patient needed amputation.

Two cases with femoral artery injury who applied relatively late to our clinic had a fasciotomy because the patients suffered from compartment syndrome. These patients were transferred to the orthopedic clinic for follow-up and treatment. Bone fracture was accompanied by only one of the all cases, and this patient was amputated by the orthopaedic clinic due to osteomyelitis developed 2 months after discharge. The mean amount of erythrocyte suspension

Table 1. Demographic, operative and postoperative data.

Gender	Male Female	129 (77%) 39 (23%)
Age	28.34±8.42	
Injured vascular structure	Femoral artery Radial artery Superficial femoral vein Deep femoral vein Ulnar artery Brachial artery Popliteal vein Popliteal artery Posterior tibial artery Anterior tibial artery	54 (22%) 47 (19%) 33 (14%) 28 (11%) 23 (9%) 21 (9%) 14 (6%) 12 (5%) 9 (4%) 3 (1%)
Vascular repair technique	Primary repair End to end anastomosis Saphen vein interposition PTFE graft interposition	57 (23%) 92 (38%) 60 (25%) 35 (14%)
Erythrocytes suspension Transfusion (unit)	1.4±0.8	
Complication	PTFE graft thrombosis Saphen vein graft thrombosis Compartment syndrome Fasciotomy Amputation	3 (8.57%) 2 (3.33%) 2 (1.19%) 2 (1.19%) 1 (0.59%)
Hospital stay (day)	3.88±2.14	

Table 2. Third month duplex ultrasound (DUS) results.

Repaired Vascular Structure	No Stenosis	0-50% Stenosis	50-70% Stenosis	>70% Stenosis
Femoral artery	49 (91%)	4 (7%)	1 (2%)	-
Radial artery	45 (96%)	1 (2%)	1 (2%)	-
Ulnar artery	22 (96%)	1 (4%)	-	-
Brachial artery	17 (81%)	3 (14%)	1 (5%)	-
Popliteal artery	11 (92%)	1 (8%)	-	-
Posterior tibial artery	8 (89%)	1 (11%)	-	-
Anterior tibial artery	3 (100%)	-	-	-
Superficial femoral vein	27 (82%)	5 (15%)	1 (3%)	-
Deep femoral vein	21 (75%)	5 (18%)	2 (7%)	-
Popliteal vein	11 (79%)	2 (14%)	1 (7%)	-

used was 1.4±0.8 units (Table 1).
Patients with nerve injuries had sensory and/or motor defects. These patients were consulted to the neurosurgery and neurology clinics and treated in compliance with their recommendations.

Patients were checked up at postoperative 3rd month. A DUS was performed to detect restenosis. The postoperative 3rd month total patency rate was 87%. Postoperative 3rd month patency rates for repaired radial artery (n=45: 96%) ulnar artery (n=22:96%), brachial artery (n=17: 81%), femoral artery (n=49:91%) superficial femoral vein (n=27: 82%), deep femoral vein (n=21:75%), popliteal vein (n=11: 79%), popliteal artery (n=11: 92%), posterior tibial artery (n=8: 89%) and anterior tibial artery (n=3:100%) were as indicated. None of the patients had stenosis above 70% and no intervention was required (Table 2).

DISCUSSION

Vascular injuries are medical problems that require immediate treatment. When not treated early, they can be fatal secondary to bleeding. The damage in the vascular structure and the severity of the injury should be diagnosed and treated as quickly and appropriately as possible. Regardless of the course of treatment, the follow-up period of the patient is also essential. Vascular injuries can occur in five different patterns; intimal injuries (subintimal hematoma, flap), total wall defects (bleeding, hematoma, pseudoaneurysm), loss of vascular integrity (bleeding or total occlusion), arteriovenous fistula and spasm [(1)]. In addition, arterial examination and imaging can be misleading due to hematoma. Firstly, we must determine the entry and exit areas of the injury. After that, we must perform a physical examination in accordance with the anatomy of the injured area. In this physical examination, while we may find serious problems such as active bleeding, severe hematoma, thrill, loss of pulsation, paleness, coldness, paraesthesia and paralysis, it is also possible to encounter mild hematoma, unilateral loss of pulsation or no symptoms at all. The patients' condition will determine the process until the operation [(2)].

Contrast-enhanced computed tomography (CECT) is the imaging method when evaluating the patient with penetrating injuries. Arterial duplex ultrasound (DUS) does not play a role in the evaluation of patients suffering from such injuries (1,2). CECT is the gold standard [(3)]. In CT (computed tomography), the presence of arterial extravasation of the contrast agent, narrowed image of arterial lumen or its complete disappearance, pseudoaneurysm and arteriovenous fistula should be carefully evaluated [(4)] (Figure 1). Venous and late phase images should also be taken after arterial imaging due to the possibility of the damage in the venous structures, in all patients

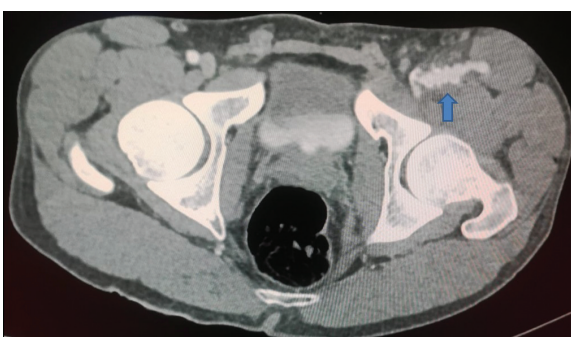

Figure 1(→). Arterial phase axial CT angiography image. Contrast extravasation (arrow) in the left common femoral artery due to penetrating injury.

if possible. After the clinical evaluation and CT imaging, we should decide whether the patient needs an intervention or not. If intervention is required, it is necessary to decide whether endovascular or surgical intervention will be performed. To prevent blood lose in patients with especially severe bleeding, tourniquet should be applied to the proximal part of the injury. On the other hand, tourniquet should be carefully loosened to allow the flow while the CT imaging was performed, otherwise the images may be misleading as there will be no contrast transition to the distal tourniquet. If the vascular structures are visible, applying direct pressure to these structures will reduce blood loss, mortality and morbidity. Penetrating injuries mostly happen to be in the upper extremities (73%) and usually together with tissue and nerve injuries (78%) [5].

Leyland et al. reported that, in their study conducted between 1981 and 2003, 66.6% of patients with penetrating injuries were at the ages of 15-34 and 53.7% were male [6]. In the study of Karger et al., it is seen that male to female ratio was 3.64 and 48% of the patients were at 21-40 years old [7]. In the study of Köksal et al., 60.5% of the cases were under the age of 30 and 94.4% were male [8]. In our study, 77% of the cases were male, 23% female, and the average age was 28.34±8.42 years, similar to other studies.

In the study of Wong et al., the average time of hospitalization was 10.4 days, while it was 4.64 days in Köksal's study, and 3.88 days in our study [8,9]. Since Wong et al. included in their study not only patients with penetrating injuries but also patients with multiple traumas, the hospitalization time of their patients was longer.

In the studies of Boström et al. with more than 1000 patients, the mortality rate was 3.4% [10]. Jacob AO et al. found mortality rate as 2.26% in their study, which included 1500 penetrating injury cases [11]. While the mortality rate was 5.6% in the study of Köksal et al., there was no mortality in our study. The most important reason for this is that, while only vascular injuries in the extremities were included in our study, the other studies had included cases with abdominal and/or thoracal penetrating injury. Patients deceased independent of penetrating injury were also included in Boström's study. Since our study had a retrospective design, 250 patients were examined, but only 168 patients were included in the study because they attended follow up visits after discharge . The mortality rate of 82 uncooperative patients without follow-up data was therefore unknown.

Edema and compartment syndrome are the most common complications after delayed vascular repair, due to longer ischemic period of the tissue. Raised pressure within the compartments of an injured extremity following reperfusion can cause mechanical injury to muscle and nerve, exacerbating the initial ischemic insult. This can be avoided by prompt application of prophylactic fasciotomy in high-risk limbs [12].

Flint et al. reported that 27.2% of patients undergoing vascular repair developed compartment syndrome and had fasciotomy [13]. In the study of Tunenir et al., 5.6% of the patients needed fasciotomy [14]. Perkins's metanalysis with 971 patients presented that performing prophylactic fasciotomy did not make a significant difference compared to those who did not, in terms of complications [15]. In 2 patients (1.19%) in our study, compartment syndrome and the need for fasciotomy occurred. In the metanalysis published by Perkins et al., 1384 of 2416 limbs affected were accompanied by nerve injury [15]. In our study, 41 of 168 patients had nerve injuries. Although fasciotomy does have risks, it is an important surgical adjunct to improve neuromuscular recovery following vascular injury and reperfusion, supported by research and clinical observation [16]. Ligation of the vessels in the treatment of venous injuries especially in the lower extremity increases the risk of secondary amputation 6 times [15]. Therefore, in our clinic, all venous injuries in deep venous system were approached and treated either by primary repair or interposition with saphenous vein or prosthetic grafts. To ensure the patency of these vascular structures, appropriate anticoagulant agents were prescribed for the patients, and therapeutic levels INR (2-2.5) were targeted.

Twenty –six studies and 1184 injuries were included in the metanalysis of Perkins et al. and it was found that the risk of amputation was lower in patients with the saphenous vein grafts compared to the

prosthetic grafts [15]. In our study, PTFE grafts were used in 35 (14%) patients, other repairs were performed with primary repair or saphenous vein grafts. The most important reason for not using saphenous vein was that the diameter of the native vein to be repaired was clearly incompatible. The other factor we believe is that the preparing the saphenous vein graft prolongs the process even for a small period of time for the limb that needs urgent perfusion.

In the study of Shackford et al., it was observed that the amputation rate was high especially in the patients older than 55 years and the secondary amputation was needed mostly in women [17]. In our study, the male patients were more numerous and patients happened to be in a younger age. In our study, one patient who had a bone injury along with the vascular injury, suffered from soft tissue infection and osteomyelitis, which resulted in below- the-knee amputation. The risk of an amputation caused by secondary reasons may last years, therefore, the secondary amputation rates may seem relatively lower. However, patients were followed-up for only 3 months in our study.

Although, penetrating injuries mostly involved upper limbs in 73% of the cases [5], in our study lower limb injuries 63% were more common.

Study limitations

The shortcomings of our study are its retrospective design, lack of comparison between vascular repair methods, and relatively shorter follow-up period of 3 months.

CONCLUSION

Immediate arrival of patients with penetrating injuries to the hospitals and consideration of vascular injury in the emergency rooms are significantly important in reducing limb loss and mortality rates. The multidisciplinary management of these patients is as important as the correct diagnosis.

Compliance with ethical standards conflict of interest: The authors have nothing to disclose and declare no conflicts of interest. This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Ethics Committee Approval: Bakirkoy Dr. Sadi Konuk Training and Research Hospital has received approval from the Clinical Research Ethics Committee (2020/167).

Conflict of interests: The authors declare no conflict of interest.

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REFERENCES

1. Feliciano DV. Evaluation and treatment of vascular injuries. In: Browner BD, Jupiter JB, Levine AM, Trafton PG, Krettek C, editors. Skeletal trauma: Basic science, management and reconstruction. Philadelphia, PA: Saunders Elsevier; 2009. p. 323-40.
2. Dennis JW, Frykberg ER, Veldenz HC, Huffman S, Menawat S. Validation of non-operative management of occult vascular injuries and accuracy of physical examination alone in penetrating extremity trauma; 5 to 10 year follow-up. J Trauma. 1998;44(2):243-52. https://doi.org/10.1097/00005373-199802000-00001
3. Inaba K, Potzman J, Munera F, McKenney M, Munoz R, Rivas L, et al. Multi-slice CT angiography for arterial evaluation in the injured lower extremity. J Trauma. 2006;60(3):502-6. https://doi.org/10.1097/01.ta.0000204150.78156.a9
4. Miller-Thomas M, West C, Cohen A. Diagnosing traumatic arterial injury in the extremities with CT Angiography: pearls and pitfalls. Radiographics. 2005;25 Suppl 1:S133-42. https://doi.org/10.1148/rg.25si055511
5. Franz RW, Skytta CK, Shah KJ, Hartman JF, Wright ML. A five-year review of management of upper extremity arterial injuries at an urban level trauma center. Ann Vasc Surg. 2012;26(5):655-64. https://doi.org/10.1016/j.avsg.2011.11.010
6. Leyland AH. Homicides involving knives and other sharp objects in Scotland, 1981-2003. J Public Health (Oxf). 2006;28(2):145-7. https://doi.org/10.1093/pubmed/fdl004.
7. Karger B, Niemeyer J, Brinkmann B. Suicides by sharp force: typical and atypical features. Int J Legal Med. 2000;113(5):259-62. https://doi.org/10.1007/s004149900093
8. Köksal O, Ozdemir F, Bulut M. Analysis of patients with stabbing injuries who applied to emergency Department of Uludag University Hospital. Uludag University Faculty of Medicine Science. 2009;35(2):63-7. Available from: https://dergipark.org.tr/tr/download/article-file/420831
9. Wong K, Petchell J. Severe trauma caused by stabbing and firearms in metropolitan Sydney, New South Wales, Australia. ANZ. ANZ J Surg. 2005;75(4):225-30. https://doi.org/10.1111/j.1445-2197.2005.03333.x
10. Boström L, Heinius G, Nilsson B. Trends in the incidence and severity of stabwounds in Sweden 1987-1994.

Eur J Surg. 2000;166(10):765-70. https://doi.org/10.1080/110241500447380
11. Jacob AO, Boseto F and Ollapallil J. Epidemic of Stab Injuries: An Alice Springs Dilemma. ANZ J Surg. 2007;77(8):621-5. https://doi.org/10.1111/j.1445-2197.2007.04174.x
12. Percival TJ, White JM, Ricci MA. Compartment syndrome in the setting of vascular injury. Perspect Vasc Surg Endovasc Ther. 2011;23(2):119-24. https://doi.org/10.1177/1531003511401422
13. Flint LM, Richardson JD. Arterial injuries with lower extremity fracture. Surgery. 1983;93(1 Pt 1):5-8. PMID: 6849188.
14. Tünerir B, Beşoğlu Y, Yavuz T. Peripheral artery injuires and results of treatment. Turkish Journal of Thoracic and Cardiovascular Surgery. 1998;6(2):151-4. Available from: http://tgkdc.dergisi.org/uploads/pdf/pdf_TGKDC_257.pdf
15. Perkins ZB, Yet B, Glasgow S. Meta-analysis of prognostic factors for amputation following surgical repair of lower extremity vascular trauma. Br J Surg. 2015;102(5):436-50. https://doi.org/10.1002/bjs.9689
16. Percival TJ, Rasmussen TE. Reperfusion strategies in the management of extremity vascular injury with ischaemia. Br J Surg. 2012;99 Suppl 1:66-74. https://doi.org/10.1002/bjs.7790
17. Shackford SR, Kahl JE, Calvo RY, Shackford MC, Danos LA, Davis JW, et al. Limb salvage after complex repairs of extremity arterial injuries is independent of surgical specialty training. J Trauma Acute Care Surg. 2013;74(3):716-23. https://doi.org/10.1097/TA.0b013e3182827035

Original Article

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Evaluation of Metabolic and Nutritional Status of Children with Autism Spectrum Disorders: Results of a Single Center in Turkey

Otizm Spektrum Bozukluğu Olan Çocukların Metabolik ve Beslenme Durumlarının Değerlendirilmesi: Türkiye'de Tek Bir Merkezin Sonuçları

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ABSTRACT

Objective: *The aim of this study is to evaluate the presence of inherited metabolic disordes and metabolic dysfunction and also biomarkers of nutritional status in pediatric patients with autism spectrum disorders.*
Method: *Biochemical and metabolic screening analyzes of 239ASD diagnosed children who were admitted to the Pediatric Metabolic Diseases Outpatient Clinic in Bakırköy Dr. Sadi Konuk Training and Research Hospital between October 2014 and November 2017, were evaluated rectospectively. ASD diagnosis were done according to DSM-5 (APA, 2013) criteria.*
Results: *The mean age of the participants was 56±34 months (19 -168 months), and 77.4% of them were male. IMD was present in 3,4% (n: 8) which those were two mitochondrial diseases and the one each hyperprolinemia type 1(HPI-1), guanidinoacetatemethyltransferase (GAMT) deficiency, mild phenylketonuria, 3-methyl crotonyl CoA carboxylase deficiency (3-MCC), middle-chain fatty acid oxidation defect (MCAD), classic type homocystinuria (cystathionine ϐ-synthase deficiency). Mitochondrial dysfunction sign was present in % 5,4 (n: 13) of ASD cases. VitB12 level was high in 38% (n: 79) and low in 1% (n: 2) of all patients. The 25-OH vitamin D level was in normal range in 28.5% (n: 55) and high in 71.5% (138) of cases. Folate level was high in 8.2% (n: 17) of cases and no participant had low levels of folate.*
Conclusion: *Evaluation of patients' metabolic profiles and nutrient levels with a standard panel in autism spectrum disorders will provide recognition of treatable metabolic diseases and acquired metabolic disorders.*

Keywords: *Autism spectrum disorders, inherited metabolic disorders, nutrition*

ÖZ

Amaç: *Bu çalışmanın amacı çocuk otizm spectrum bozukluğu hastalarında kalıtsal metabolik hastalık ve metabolik disfonksiyonun varlığı ile beslenme durumunun biyobelirteçlerini değerlendirmektir.*
Yöntem: *Ekim 2014-Kasım 2017 tarihleri arasında Bakırköy Dr. Sadi Konuk Eğitim ve Araştırma Hastanesi Çocuk Pediatrik Metabolik Hastalıklar Polikliniğine başvuran 239 ASD tanısı almış çocuğun biyokimyasal ve metabolik tarama analizleri rektospektif olarak incelendi.*
Bulgular: *Katılımcıların ortalama yaşı 56 ay±34 ay (19-168 ay) olup, bunların % 77,4'ü erkekti. iki vaka mitokondriyal hastalık ve her birinden birer Hiperprolinemi tip 1(HPI-1), guanidinoasetatemetiltransferaz (GAMT) eksikliği, hafif fenilketonüri, 3-metil krotonil CoA karboksilaz eksikliği (3-MCC), orta zincirli yağ asidi oksidasyon defekti (MCAD), klasik tip homosistinüri (sistatiyonin ϐ-sentaz eksikliği) olacak şekilde vakaların %3,4'ine (n: 8) kalıtsal metabolik hastalık tanısı konuldu. ASD olgularının % 5,4'ünde (n: 13) mitokondriyal disfonksiyon bulgusu mevcuttu. VitB12 düzeyi tüm hastaların% 38'inde (n: 79) yüksek ve % 1'inde (n: 2) düşüktü. 25-OH D vitamini düzeyi olguların% 28.5'inde (n: 55) normal,% 71.5'inde (138) yüksekti. Olguların % 8.2'sinde (n: 17) folat düzeyi yüksekti ve hiçbir katılımcının folat düzeyi düşük bulunmadı.*
Sonuç: *Otizm spectrum bozukluklarında hastaların metabolik profilleri ve nutrient düzeylerinin standart bir panel ile değerlendirilmesi, tedavi edilebilir metabolik hastalıklar ve edinsel metabolik bozuklarının tanınmasını sağlayacaktır.*

Anahtar kelimeler: *Otizm spektrum bozuklukları, kalıtsal metabolik bozukluklar, beslenme*

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INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by impairments in reciprocal social communication and a tendency to engage in repetitive stereotyped patterns of behaviors, interests, and activities and restricted interests [1]. Until the 1980's it was considered as a rare disorder seen approximately 4 per 10,000 children [2]. Recent estimates provided by the US Center for Disease Control and Prevention show that ASD affects 168 per 1000 children aged 8 years [3]. Increased public awareness of the disorder and access to the specialists, increased diagnostic ability of the psychiatrists for mild forms of ASD and changes in the diagnostic criteria are among the reported reasons of this increasing frequency. On the other hand a real increase possibly inherent to environmental factors cannot be excluded [4].

Today the exact etiology of ASD remains poorly understood. A wide range of genetic variations and interactions of gene-gene and gene-environment are suggested to be responsible in ASD pathophysiology [5]. Patient's individual features such as genetic background, sex, the intrauterine environment, and early life experience including neonatal infections are thought to influence the phenotypic expression of the genetic factors [6]. Moreover it has been suggested that possible interactions among pleiotropic genetic factors increase individual vulnerability to environmental inputs, and finally lead to development of multiple systemic comorbidities including metabolic derangement [7]. Several inherited metabolic diseases (IMD), such as phenylketonuria, homocysistinuria, mitochondrial diseases and cerebral creatinine deficiency, have been found to be associated with ASD (Table 1). IMD has been reported in 2-5 % of patients with ASD [8,9]. In last decades researches have focused on metabolic problems including abnormal levels of some vitamines and biomarkers of mitochondrial dysfunction (MD) and oxidative stress, besides the presence of an exact or overt metabolic disease [10-13]. Mitochonrial dysfunction may be primary or secondary. Primary mitochondrial dysfunction refers to mitochondrial dysfunction caused by a defect in a gene directly involved in the function of mitochondrial systems which impair ATP production and antioxidant pathways. Secondary mitochondrial dysfunction refers to some genetic, metabolic diseases other than mitochondrial disease and environmental toxicants which affect the mitochondrial systems indirectly. Rossignol & Frye have shown that the presence of abnormal biomarker values, such as higher pyruvate and/or lactate levels, which indicate mitochondrial dysfunction are more frequently seen in ASD than in normal population, and this value is even much higher than the prevalence of metabolic disease [14]. Moreover Shoffner et al. investigated 28 ASD patients with a comorbid mitochondrial disease and showed that in this particular group of ASD patients regression history which commonly occurs after fever, is much more common than others without a mitochondrial disease [15]. The autistic regression may be a result of underlying but not overt MD which renders children vulnerable to external factors causing regression into ASD clinic [14]. Because today no definitive treatment for core symptoms of ASD is present and the only treatment method is educational approaches, finding out a treatable etiologic cause of metabolic disease or dysfunction especially in younger ASD patients, will significantly improve the prognosis of ASD.

Most of previous studies on ASD and metabolic dysfunction comorbidity have small sample sizes and they often examine a limited number of metabolic biomarkers. Moreover not all studies have demonstrated abnormal levels of biomarkers. Kosinovsky et al. have shown that none of the ASD cases has incre-

Table 1. Inherited metabolic disease presented with autism spectrum disorder.

Phenylketonuria	Cerebral Folate deficiency
Homocysistinuria	Pridoxine deficiency
Mitochondrial diseases	Propionic acidemia
Cerebral creatinine deficiency	Urea cycle defects
Mucopolysaccharidosis Type III	Adenylsuccinate lysase deficiencies
L-2-OH glutaric aciduria	Succinate semialdehyde dehydrogenase deficiency
Lesch Nyhan Syndrome	Dihydropyrimidine dehydrogenase deficiency
Smith-Lemli Opitz Syndrome	Cerebrotendinous xanthomatosis
Congenital glycosylation defect	Neuronal ceroid lipofuscinoses

ased levels of plasma ammonia, plasma amino acids, plasma lactic acid and pyruvic acid and urinary organic acid [16]. As far as we know, incidence of IMD in Turkish ASD patients has been examined in only one study which detected 9 cases with IMD among 300 ASD patients [17]. However this study has not examined the metabolic dysfunction other than a spesific disease state.

In this current study we aimed to evaluate the presence of IMD and metabolic dysfunction and also biomarkers of nutritional status in pediatric ASD patients. For this purpose, we planned to investigate biochemical (urea, ALT, AST, ammonia, lactate, uric acid, creatinine, vitamin B_{12}, ferritin, total homocysteine (tHcy), vitamin D and folic acid) and metabolic screening (amino acids, carnitine / acylcarnitine profile and urine organic acids) analysis in a large sample of children with ASD diagnosis. We hypothesized that both the diagnosis of a specific IMD and abnormal levels in biomarkers indicating metabolic dysfunction were more frequent in patients diagnosed as ASD.

MATERIAL and METHODS

Participants

The study included children with diagnosis of ASD who were admitted to our Pediatric Metabolic Diseases Outpatient Clinic between October 2014 and November 2017. A total of 314 ASD patients were admitted to the clinic within this time period, and the study was completed with 239 cases after exclusion of cases with incomplete data. All patients had been referred from the child and adolescent psychiatry outpatient clinic of the same hospital, where they received the diagnosis of ASD according to DSM-5 (APA, 2013) criteria. The study was approved by the local Ethics Committiee with protocol number: 2018-06-13.

Procedures

Data related to sociodemographic, clinical and laboratory information including biochemical and metabolic screening analysis of participants were obtained from their files.

Biochemical analysis

Urea, ALT, AST, ammonia, lactate levels were analyzed by enzymatic method; uric acid by colorimetric method; and creatinine by modified Jaffe method. Vitamin B12, ferritin, total homocysteine (tHcy), 25-OH vitamin D was analyzed by immunenzymatic method; and folic acid by competitive binding principle method by Roche.

Metabolic screening analysis

Quantitative analysis of amino acids was performed by liquid chromotography - mass spectrometry (LC-MS-MS), and carnitine / acylcarnitine profile was analyzed by Tandem mass spectrometry (Tandem MS) method, and urine organic acids by gas chromatography-mass spectrometry (GC-MS) method by redox.

Statistical analysis

SPSS for Windows 23.0 version was used for the statistical analyses. Mann-Whitney U test was used for continuous variables in paired groups. The difference analysis was evaluated by chi-square test in categorical variables with two or more groups. Statistical significance was accepted as $p < 0.05$.

RESULTS

A total of 239 pediatric ASD patients were included in the study., and 77.4% of them were male. The mean age of the participants was 56±34 months (age range was 19 -168 months).

Biochemical analysis

Blood urea level was low in 11.3% (n:24) of patients. These patients with low blood urea had normal levels of ammonia and glutamine. Blood uric acid level was low in 1.4% (n:3) and high in 7.2% (n:15) of all cases, however these abnormal levels were not associated with an IMD.

Blood creatinine level was low in 4.7% (n:10) and high in 4.7% (n:10) of patients. One ASD patient was diagnosed as guanidinoacetate methyltransferase (GAMT) deficiency. Based on low creatinine levels, the diagnosis was made by molecular analysis.

Vitamin B12 level was high in 38% (n:79) and low in 1% (n:2) of all patients. The 25-OH vitamin D level was within normal range in 28.5% (n:55) and high in 71.5% (138) of cases. Folate level was high in 8.2% (n:17) of cases and no participant had low levels of folate.

Homocysteine level was higher than normal limits in

Table 2. Biochemical results of the patients.

	medium±ss n (%)	Median (min-max)
Urea (mg/dl)	23,59±6,67 ↓ 24 (%11,3) N 185 (%86,9) ↑ 4 (%1,9)	23 (6-46)
Uric acid (mg/dL)	3,47±0,93 ↓ 3 (%1,4) N 190 (%91,3) ↑ 15 (%7,2)	3,4 (1,2-8,1)
	0,35±0,09 ↓ 10 (%4,7) N 192 (%90,6) ↑ 10 (%4,7)	0,35 (0,12-0,69)
ALT (IU/L)	16,44±7,93 N 205 (%97,6) ↑ 5 (%2,4)	15 (6-58)
AST (IU/L)	32,98±11,01 N 189 (%90,9) ↑ 19 (%9,1)	30,5 (14-72)
TSH (µIU/L)	2,64±1,34 N 201 (%96,2) ↑ 8 (%3,8)	2,35 (0,64-9,5)
FT4 (ng/dL)	1,31±0,18 N 35 (%16,7) ↑ 174 (%83,3)	1,31 (0,63-1,7)
B12 (pg/mL)	507,76±320,96 ↓ 2 (%1) N 127 (%61,1) ↑ 79 (%38)	451 (117-1725)
Ferritin (µ/L)	34,39±32,66 ↓ 66 (%32,5) N 136 (%67) ↑ 1 (%0,5)	28 (4,6-359)
Folat (ng/mL)	11,81±8,45 N 190 (%91,8) ↑ 17 (%8,2)	11 (3,5-112)
25 OH vitamin D (ng/mL)	28,65±13,54 N 55 (%28,5) ↑ 138 (%71,5)	26 (3,7-70)
Homocysteine (µmol/L)	6,67±2,47 ↓ 42 (%20,6) N 161 (%78,9) ↑ 1 (%0,5)	6 (0-15)
Laktate (µmg/dL)	18,69±10,87 N 132 (%67,7) ↑ 62 (%31,8)	15,8 (0,01-68)
Ammonia (µgr/dL)	66,44±27,63 N 166 (%84,3) ↑ 26 (%13,2)	61 (12-209)

ALT: alanine transaminase, AST: aspartate transaminase, TSH: Thyroid stimulating hormone FT4: FreeT4

one (0.5%) patient. This patient had high methionine level but normal vitamin B12 and folic acid levels, and was diagnosed as classic type homocystinuria (cystathionine β-synthase deficiency).

Blood lactate value was high in 31.8% (n:62) and urinary lactate level was high in 7.8% (n:4) of cases. Two of these cases were diagnosed as mitochondrial disease. High blood ammonia level was present in 13.2% (n:26) of cases. However, these elevations were not associated with an IMD (Table 2).

Metabolic screening analysis

Urine organic acid evaluation showed that increased pyruvic acid level was present in 9,8% (n:5) and high levels of 5-oxoproline in 15.7% (n:8) of cases. Methylglutaric acid was elevated in 3 (5%) patients including 2 patients diagnosed as mitochondrial disease and the third patient had mitochondrial dysfunction. Ethylmalonic acid level was (indicating mitochondrial dysfunction) high in 6.7 % (n:4) of cases. The 3-hydroxy-propionic acid level was elevated in 18.3% (n: 11) of cases. Mitochondrial dysfunction was detected in 3 cases (Table 3).

Table 3. Urine organic acid results of the patients.

(µmol/L)	medium±ss n (%)	Median (min-max)
2-Hydroxy-isovaleric acid	0,23±0,14 N 60 (%100)	0,2 (0,2-0,88)
3-Hydroxy-propionic acid	25,04±29,59 N 49 (%81,7) ↑ 11 (%18,3)	12 (4-142)
3-Hydroxy-isovaleric acid	9,49±7,22 ↓ 58 (%96,7) N 2 (%3,3)	5,8 (2,96-44,2)
3-Methyl glutaconic acid	5,49±8,18 N 57 (%95) ↑ 3 (%5)	6,65 (3,5-38)
Ethylmalaonic acid	6,49±7,22 N 56 (%93,4) ↑ 4 (%6,6)	6,65 (3,5-38)
Piruvic acid	14,31±16,5 N 44 (%86,3) ↑ 5 (%9,8)	11 (4,32-117)
Laktic acid (mmol/mol)	56,47±96,69 N 14 (%27,5) ↑ 4 (%7,8)	24 (4,5-429)
5-Oxoproline	124,33±407,93 N 43 (%84,3) ↑ 8 (%15,7)	53 (26-2960)

Table 2. Blood aminoacid and acylcarnitine profiles of the cases.

(µmol/L)	medium±ss n (%)	Median (min-max)
Phenylalanine	78,62±20,41 N 96 (%74,4) ↑ 33 (%25,6)	75 (35-149)
Proline	220,74±106,45 N 122 (%94,6) ↑7 (%5,4)	193 (95-833)
Glutamin	526,95±172,3 ↓ 6 (%4,7) N 117 (%90,7) ↑ 6 (%4,7)	531 (169-1052)
Alanine	410,5±172,72 ↓ 5 (%3,9) N 94 (%72,9) ↑ 30 (%23,3)	395 (27-942)
Glycine	298,63±146,42 N 77 (%62,1) ↑ 39 (%31,5)	272,5 (29,6-969)
C0	31,68±10,66 N 158 (%100)	29 (12-65)
C3 (propionyl)	1,52±0,81 N 158 (%100)	1,39 (0,34-5,45)
C4 (butyryl)	0,24±0,2 N 157 (%99,4) ↑ 1 (%0,6)	0,2 (0,01-1,49)
C4-OH (3-OH butyryl)	0,13±0,11 N 158 (%100)	0,1 (0-0,66)
C5 (isovaleryl)	0,14±0,09 N 158 (%100)	0,12 (0,03-0,7)
C5-OH (3-OH isovaleryl)	0,21±0,14 N 157 (%99,4) ↑ 1 (%0,6)	0,18 (0,01-1,18)
C10:1 (decanoyl)	0,11±0,1 N 157 (%99,4) ↑ 1 (%0,6)	0,09 (0-0,99)
C14:1 (tetradecanoyl)	0,07±0,08 N 158 (%100)	0,04 (0,01-0,45)
C14-OH (3-OH tetradecanoyl)	0,02±0,07 N 157 (%99,4) ↑ 1 (%0,6)	0,01 (0,01-0,91)

C: carnitine

Quantitative evaluation of serum amino acid profiles showed that; blood phenylalanine level was above normal limits in 25.6 % (n:33) of cases. Among them one patient was diagnosed as mild type phenylketonuria and two were diagnosed as hyperphenylalaninemia. High blood proline levels were present in 5.4 % (n:7) of cases; one of them was diagnosed as hyperprolinemia type1 (HPI). Mitochondrial dysfunction was detected in 3 cases. High blood alanine level was present in 23.3 % (n:30) of cases, and among them 4 cases had mitochondrial dysfunction. Increased blood glycine level was present in 39 (31.5%) cases but these increased levels were not associated with an IMD (Table 4).

Evaluations of carnitine/acylcarnitine profile demonstrated abnormal biomarker levels associated with a specific disease in two cases. One case (0.6%) had high levels of C5-OH (3-OH isovaleryl) carnitine and was diagnosed as 3-MCC (3-methyl crotonyl CoA carboxylase deficiency). The other case (0.6%) had high levels of C8, C10: 1 (decanoil) C10: 2 carnitine and was diagnosed as middle-chain fatty acid oxidation defect. One patient (0.6%) had high levels of C14: 2 (tetradecadienoyl) and 3 cases (1.9%) had high levels of C14-OH (3-OH tetradecadienoyl) carnitine but these elevated levels were not associated with a metabolic disease (Table 4).

Biochemical and metabolic screening analyzes of participants showed that IMD was present in 3.4 % (n:8) and mitochondrial dysfunction in % 5,4 (n:13) of ASD cases. Overall metabolic disarrangement was present in 8,8 % of all participants.

DISCUSSION

Autism spectrum disorder is an important neurodevelopmental disorder with increasing frequency impairing the quality of life of the patients and their families. Many studies have demonstrated that IMD may present with autism symptoms. The diagnosis of underlying IMD may be delayed in patients without clinical findings other than autism findings [9]. In these ASD patients who have the chance of medical treatment, the detection of the underlying medical disease early in life significantly affects the outcome of ASD.

In this current study we found that IMD was present in 3.4% and abnormal biomarker levels indicating mitochondrial dysfunction not related to an overt disease in 5.4 % of cases. Our findings related to IMD frequency are compatible with previous reports [8,9,17]. It has been reported that in ASD patients pre-

sence of abnormal levels of mitochondrial biomarkers is much more common than presence of MD [14]. Our findings support that mitochondrial dysfunction, even it is not an overt disease state, plays an important role in the etiopathogenesis of ASD.

Biochemical analysis revealed abnormal findings related to levels of blood urea, uric acid and creatinine in some patients. Twenty-four (11.3%) patients had decreased blood urea levels but their blood ammonia and / or glutamine levels were within normal range. Blood uric acid levels were abnormal in 8,6% of cases; three (1.4%) patients had low levels and fifteen (7.2%) patients had high levels of blood uric acid, but these abnormal levels were not associated with OCD. Further analysis was not performed in these patients with abnormal blood uric acid levels because none of them had clinical findings and persistent abnormal levels which are the criteria for recommendation for further analysis for uric acid metabolism disorders. Decreased urea levels are not only seen in urea cycle disorders but it is also observed also secondary to malnutrition [18]. Decreased urea levels and abnormal blood uric acid levels might be related to abnormal nutritional patterns related to restrictive and selective type food intake seen in ASD patients. For example insufficient protein intake due to abnormal feeding might be the cause of low urea levels in our ASD patients. Blood creatinine level was low in 4.7% (n:10) and high in 4.7% (n:10) of the cases. One patient was diagnosed with GAMT deficiency which is the most common creatinine metabolism defect. In approximately 80% of cases with GAMT deficiency any additional systemic finding other than behavior disorders such as autistic behavior and self-harm may not be present [19]. Since the treatment response and prognosis of these diseases depend on the age at which treatment is initiated, early diagnosis is an important issue.

Assessment of levels of vitamins among ASD patients showed that; in terms of vitamin B12 levels 38 % of the patients had increased and 1% had low levels of vitamin B12. Among the participants 8.2% had high folate level. None of them had low folate nor low vitamin D levels. One patient had increased homocysteine levels with normal levels of vitamin B12 and folic acid, and was diagnosed as classic homocystinuria (cystathionine β-synthase deficiency) because of high methionine levels. Contrary to expectations, B12, folic acid and vitamin D deficiency were not observed in our patients. Researchers suggest that deficiencies of these vitamins are important factors in the pathogenesis of ASD [20-22]. However in recent decade researches have demonstrated that in ASD patients increased oxidative stress due to metabolic or environmental effects may cause elevation of B12, which can explain our finding of elevated vitamin B12 levels [23,24]. Moreover our study was done in a clinical sample rather than in a population-based sample. The reason why we could not find any deficiency in vitamin B12, folic acid and vitamin D levels may be due to the fact that these deficiencies have been treated in previous visits to physicians.

In last decades many researches have focused on the mitochondrial function in the pathophysiology of ASD. It is known that mitochondrial dysfunction may lead to autism [25]. Several studies have demonstrated that mitochondrial dysfunctions reduce intracellular energy production, increase oxidative stress, and lead to a decrease in neurogenesis and postsynaptic neurotransmitter release [11-14,25,26]. In a recent epidemiological study, the probability of development of autism in the presence of mitochondrial disease has been found to be 550-770 times higher than in the normal population [15]. In another study conducted with 25 ASD patients with coexisting mitochondrial dysfunction, authors found that these specific groups of ASD patients had at least some physical examination findings suggesting mitochondriopathy in contrast to patients with idiopathic autism. Clinical aspects of mitochondrial dysfunction in autism spectrum disorder (ASD) include unusual neurodevelopmental regression in clinic presentation, especially if triggered by an inflammatory event, gastrointestinal symptoms, seizures, motor delays, fatigue and lethargy [25,27]. Therefore, it is appropriate to examine the clues of metabolic disease more carefully in the clinical evaluations of children with ASD diagnosis, especially in patients with regression history. However in our study in 13 ASD patients (5.4 %) with abnormal biochemical or metabolic screening results any of these defined clinical/physical signs were not present. Some ASD diagnosed children with comorbid mitochondrial dysfunction, may have no clinical/physical finding and no abnormality in the levels of biomarkers such as lactate.

Mitochondrial dysfunction does not always occur as a result of genetic defect, rather it can be acquired secondary to adverse environmental effects which induce oxidative stress [28]. Moreover other genetic-metabolic diseases and environmental factors (food, radio, etc.) as well as primary mitochondrial diseases, can cause mitochondrial dysfunction. According to different hypothesis, oxidative stress due to mitochondrial dysfunction and poor ATP production may contribute to Rett Syndrome which is associated with autism clinic [29]. Moreover stressors, such as dehydration, fever and infection can lead to a functional decline and neurodegenerative regression in individuals with underlying but not overt MD [25]. Some patients with ASD phenotypes clearly show some clinical features of the genetic-based primary MD [30]. These include an elevated plasma L-lactate, urine metabolites (such as ethylmalonic acid, 3-methylglutaric acid) biochemical evidence of mitochondrial dysfunction, elevated pyruvate, and alanine levels. Besides, a history of regression in ASD manifestations and presence of multiorgan disorders should alert clinicians to the possibility of MD in autism [27]. Thus, the identification of children with ASD/MD is important since each type of IMD or MD associated with different metabolites has its own specific treatment [11].

Evaluations of blood aminoasit and carnitine / acylcarnitine profile revealed that increased blood phenylalanine levels were present in 25.6% (n:33) cases. Among these cases one patient was diagnosed as mild type phenylketonuria and two cases as hyperphenylalaninemia. C5-OH (3-OH isovaleryl) carnitine was detected in 0.6% (n:1) of the cases who were diagnosed as 3-MMC. Higher levels of C8 (octanoyl) and C10: 1 (decanoyl) C10: 2 (dekadodienoyil) carnitine were found in 0.6% (n:1) of cases who was diagnosed as mid-chain fatty acid oxidation defect (MCAD).

The association of ASD with late diagnosed PKU is well known. It has been reported that in patients with phenylketonuria diet therapy has a corrective effect on the autism symptoms [31,32]. The 3-MCC is generally diagnosed in older ages and manifests signs of psychosocial regression and fatigue (Cho et al 2016). MCAD is the most common fatty acid oxidation disorder. Although patients present with hypoketotic hypoglycemia, a significant proportion of patients with genetic diagnosis remain lifelong asymptomatic [33,34]. MCAD is a common disease in the population and can be observed without any symptoms. In order to determine the relationship between MCAD and ASD, studies should be done in larger groups.

Study limitations

Our study was not a prospective study, and had a retrospective design without any control group. Because information related to autism severity and its clinic (whether the patient had autism regression) were not included in the files of all patients the relationship between metabolic dysfunction and these two conditions could not be examined in this study.

CONCLUSION

Recently the researches on the etiopathogenesis of ASD have focused on the metabolic disarrengements. The results of our autism panel examination in pediatric ASD patients showed that IMD was present in 3,4% and mitochondrial dysfunction in 5,4% of participants. Overall 8,8% of all ASD patients had abnormal metabolic findings. Today ASD has no medical treatment for its core symptoms. Finding out an underlying curable metabolic condition will allow an important chance for medical treatment of ASD, at least for reducing its clinical severity. A standard metabolic screening program for ASD has not been established yet. We think by the recent progresses in diagnostic methods we will see better the actual higher frequency of metabolic problems associated with ASD. In order to understand whether these metabolic problems are whether cause or result of ASD, studies conducted with elaborate and large samples are needed.

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REFERENCES

1. Maenner MJ, Rice CE, Arneson CL, Cunniff C, Schieve LA, Carpenter LA, et al. Potential impact of DSM-5 criteria on autism spectrum disorder prevalence estimates. JAMA Psychiatry. 2014;71(3):292-300. https://doi.org/10.1001/jamapsychiatry.2013.3893
2. Fombonne E. Epidemiology of autistic disorder and other pervasive developmental disorders. J Clin Psychiatry. 2005;66 Suppl 10:3-8. PMID: 16401144.
3. Baio J, Wiggins L, Christensen DL, Maenner MJ, Daniels J, Warren Z, et al. Prevalence of Autism Spectrum Disorder Among Children Aged 8 Years - Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2014. MMWR Surveill Summ. 2018;67(6):1-23. https://doi.org/10.15585/mmwr.ss6706a1
4. Palmieri L, Persico AM. Mitochondrial dysfunction in autism spectrum disorders: cause or effect? Biochim Biophys Acta. 2010;1797(6-7):1130-7. https://doi.org/10.1016/j.bbabio.2010.04.018
5. Yoo H. Genetics of autism spectrum disorder: current status and possible clinical applications. Exp Neurobiol. 2015;24(4):257-72. https://doi.org/10.5607/en.2015.24.4.257
6. Constantino JN, Charman T. Diagnosis of autism spectrum disorder: reconciling the syndrome, its diverse origins, and variation in expression. Lancet Neurol. 2016;15(3):279-91. https://doi.org/10.1016/S1474-4422(15)00151-9
7. Barone R, Alaimo S, Messina M, et al. A Subset of patients with autism spectrum disorders show a distinctive metabolic profile by dried blood spot analyses. Front Psychiatry. 2018;9:636. https://doi.org/10.3389/fpsyt.2018.00636
8. Ververi A, Vargiami E, Papadopoulou V, et al. Clinical and laboratory data in a sample of greek children with autism spectrum disorders. J Autism Dev Disord. 2012;42(7):1470-6. https://doi.org/10.1007/s10803-011-1414-7
9. Schiff M, Benoist JF, Aissaoui S, et al. Should metabolic diseases be systematically screened in nonsyndromic autism spectrum disorders? PLoS One. 2011;6(7): e21932. https://doi.org/10.1371/journal.pone.0021932
10. Esparham AE, Smith T, Belmont JM, Haden M, Wagner LE, Evans RG, Drisko JA. Nutritional and metabolic biomarkers in autism spectrum disorders: an exploratory study. Integr Med (Encinitas). 2015;14(2):40-53. PMID: 26770138.
11. Frye RE, Delatorre R, Taylor H, Slattery J, Melnyk S, Chowdhury N, James SJ. Redox metabolism abnormalities in autistic children associated with mitochondrial disease. Transl Psychiatry. 2013;3(6):e273. https://doi.org/10.1038/tp.2013.51
12. Frustaci A, Neri M, Cesario A, Adams JB, Domenici E, Dalla Bernardina B, Bonassi S. Oxidative stress-related biomarkers in autism: systematic review and meta-analyses. Free Radic Biol Med. 2012;52(10):2128-41. https://doi.org/10.1016/j.freeradbiomed.2012.03.011
13. Dhillon S, Hellings JA, Butler MG. Genetics and mitochondrial abnormalities in autism spectrum disorders: a review. Curr Genomics. 2011;12(5):322-32. https://doi.org/10.2174/138920211796429745
14. Rossignol DA, Frye RE. A review of research trends in physiological abnormalities in autism spectrum disorders: immune dysregulation, inflammation, oxidative stress, mitochondrial dysfunction and environmental toxicant exposures. Mol Psychiatry. 2012;17(4):389-401. https://doi.org/10.1038/mp.2011.165
15. Shoffner J, Hyams L, Langley GN, et al. Fever plus mitochondrial disease could be risk factors for autistic regression. J Child Neurol. 2010;25(4):429-34. https://doi.org/10.1177/0883073809342128
16. Kosinovsky B, Hermon S, Yoran-Hegesh R, Golomb A, Senecky Y, Goez H, Kramer U. The yield of laboratory investigations in children with infantile autism. J Neural Transm (Vienna). 2005;112(4):587-96. https://doi.org/10.1007/s00702-004-0198-8
17. Kiykim E, Zeybek CA, Zubarioglu T, et al. Inherited metabolic disorders in Turkish patients with autism spectrum disorders. Autism Res. 2016;9(2):217-23. https://doi.org/10.1002/aur.1507
18. Wijburg FA, Nassogne MC. Disorders of the urea cycle and related enzymes. In: Saudubray JM, van den Berghe G, Walter JH, editors. Inborn Metabolic Diseases Diagnosis and Treatment. 5th ed. 2012. p. 298-324. https://doi.org/10.1007/978-3-642-15720-2_20
19. Braissant O, Henry H. AGAT, GAMT and SLC6A8 distribution in the central nervous system, in relation to creatine deficiency syndromes: a review. J Inherit Metab Dis. 2008;31(2):230-9. https://doi.org/10.1007/s10545-008-0826-9
20. Schmidt RJ, Hansen RL, Hartiala J, Allayee H, Schmidt LC, Tancredi DJ, et al. Prenatal vitamins, one-carbon metabolism gene variants, and risk for autism. Epidemiology. 2011;22(4):476-85. https://doi.org/10.1097/EDE.0b013e31821d0e30
21. Grant WB, Soles CM. Epidemiologic evidence supporting the role of maternal vitamin D deficiency as a risk factor for the development of infantile autism. Dermatoendocrinol. 2009;1(4):223-8. https://doi.org/10.4161/derm.1.4.9500
22. Meguid NA, Hashish AF, Amwar M, Sidhom G. Reduced serum levels of 25-hydroxy and 1,25-dihydroxy vitamin D in Egyptian children with autism. J Altern Complement Med. 2010;16(6):641-5. https://doi.org/10.1089/acm.2009.0349
23. McCaddon A, Hudson P, Ellis D, Hill D, Lloyd A. Effect of supplementation with folic-acid on relation between plasma homocysteine, folate, and vitamin B12. Lancet. 2002;360(9327):173. https://doi.org/10.1016/S0140-6736(02)09395-9
24. Hooshmand B, Solomon A, Kåreholt I, et al. Associations between serum homocysteine, holotranscobalamin, folate and cognition in the elderly: a longitudinal study. Intern Med. 2012;271(2):204-12. https://doi.org/10.1111/j.1365-2796.2011.02484.x
25. Rossignol DA, Frye RE. Melatonin in autism spectrum disorders: a systematic review and meta-analysis. Dev Med Child Neurol. 2011;53(9):783-92. https://doi.org/10.1111/j.1469-8749.2011.03980.x
26. Ghanizadeh A. Increased glutamate and homocysteine and decreased glutamine levels in autism: a review and strategies for future studies of amino acids in autism. Dis Markers. 2013;35(5):281-6.

https://doi.org/10.1155/2013/536521
27. Weissman JR, Kelley RI, Bauman ML, et al. Mitochondrial disease in autism spectrum disorder patients: a cohort analysis. PLoS One. 2008;3(11):e3815. https://doi.org/10.1371/journal.pone.0003815
28. Niyazov DM, Kahler SG, Frye RE. Primary mitochondrial disease and secondary mitochondrial dysfunction: ımportance of distinction for diagnosis and treatment. Mol Syndromol. 2016;7(3):122-37. https://doi.org/10.1159/000446586
29. Heilstedt HA, Shahbazian MD, Lee B. Infantile hypotonia as a presentation of Rett syndrome. Am J Med Genet. 2002;111(3):238-42. https://doi.org/10.1002/ajmg.10633
30. Haas RH. Autism and mitochondrial disease. Dev Disabil Res Rev. 2010;16(2):144-53. https://doi.org/10.1002/ddrr.112
31. Herrero E, Aragon MC, Gimenez C, et al. Inhibition by phenylalanine of tryptophan transport by synaptosomal plasma membrane vesicles: implications in the pathogenesis of phenylketonuria. J Inherit Metab Dis. 1983;6(1):32-5. https://doi.org/10.1007/BF02391190
32. Mazlum B, Anlar B, Kalkanoğlu-Sivri S, Karlı-Oğuz K, Özusta Ş, Ünal F. Late-diagnosed phenylketonuria case presenting with autism spectrum disorder in early childhood. Turk J Pediatr. 2016;58(3):318-22. https://doi.org/10.24953/turkjped.2016.03.016
33. Iafolla AK, Thomson Jr. RJ, Roe CR, et al. Medium-chain acilCoA dehidrogenase deficiency: clinical course in 120 affected children. J Pediatr. 1994;124(3):409-15. https://doi.org/10.1016/S0022-3476(94)70363-9
34. Andresen BS, Bross P, Udvari S, et al. Tr molecular basis of MCAD deficiency in compound heterozygous patients- is there a correlation between genotype and phenotype? Hum Mol Genet. 1997;6(5):695-707. https://doi.org/10.1093/hmg/6.5.695

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Our Surgical Experience in Anomalous Opening of the Common Bile Duct §

Koledok Açılım Anomalilerindeki Cerrahi Deneyimlerimiz

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ABSTRACT

Objective: *The treatment of anomalous opening of the common bile duct opening is performed by endoscopic or surgical methods. In addition to endoscopic procedures, indications of surgical treatment, surgical procedures and complications related to these methods are aimed to be presented in the light of the literature.*
Method: *Patients who underwent surgical treatment for anomalous opening of the common bile duct between November 2014 and August 2018 were analyzed retrospectively. Demographic characteristics, presenting symptoms, diagnostic methods, treatment procedures, complications and postoperative follow-up information were analyzed.*
Results: *A total of 8 patients were included in the study. The mean age was 64.3 years. Male, and female patients constituted 75% (n=6) and 25% (n=2) of the study population. The most common presenting symptoms were abdominal pain (50%) and biliary colic (25%). Cholestatic enzymes were found to be high in 75% of the patients. Fifty-one patients had hyperbilirubinemia. Ectopic biliary drainage was most commonly (75%) located at the bulbus and the second most common (25%) localization was the post-bulbar region. The most common surgical procedure was hepaticojejunostomy (62.5%) and choledochoduodenostomy was performed in 25% of the cases.*
Conclusion: *Surgical treatment methods can be used in addition to endoscopic methods in the treatment of anomalous common bile duct opening. Caution should be exercised in patients undergoing surgical treatment for anastomotic leakage, duodenal ulcer bleeding and bleeding from the area of sphincterotomy.*

Keywords: *choledochus, ectopic opening, surgical treatmant*

ÖZ

Amaç: *Koledok açılım anomalisinin tedavisi endoskopik ya da cerrahi yöntemler ile yapılmaktadır. Endoskopik girişimlerin yanısıra cerrahi tedavi endikasyonları, uygulanan cerrahi yöntemler ve bu yöntemlere ait komplikasyonların literatür eşliğinde sunulması amaçlanmıştır.*
Yöntem: *Kliniğimizde Kasım 2014 ile Ağustos 2018 tarihleri arasında koledok açılım anomalisi nedeni ile cerrahi tedavi uygulanan olgular retrospektif olarak analiz edilmiştir. Hastaların demografik özellikleri, başvuru semptomları, tanı yöntemleri, tedavi prosedürleri, komplikasyonlar ve postoperatif dönemdeki takip bilgileri analiz edildi.*
Bulgular: *Çalışmamıza toplam 8 hasta dahil edildi. Ortalama yaş 64,3 idi. Erkek cinsiyet %75 (n=6), kadın cinsiyet %25 (n=2) idi. En sık başvuru semptomu karın ağrısı (%50) ve bilyer kolik (%25) idi. Kolestaz enzimleri hastaların %75'inde yüksek bulundu. Yüzde elli olguda ise hiperbilluribinemi mevcuttu. Ektopik bilyer drenaj en sık (%75) bulbus, ikinci sıklıkta ise (%25) postbulber bölgede idi. En sık uygulanan cerrahi işlem (%62,5) hepatikojejunostomi, %25 olguda ise koledokoduodenostomi idi.*
Sonuç: *Koledok açılım anomalisi tedavisinde endoskopik yöntemlerin yanısıra cerrahi tedavi yöntemleri de uygulanabilmektedir. Cerrahi tedavi uygulanan hastalarda anastomoz kaçağı, duodenal ülser kanaması ve sfinkterotomi alanından kanama açısından dikkatli olunmalıdır.*

Anahtar kelimeler: *koledok, ektopik açılım, cerrahi tedavi*

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INTRODUCTION

The common bile duct enters the second part of the duodenum from the side of the medial wall and opens into the ampulla of Vater. It may open to the third or fourth part of the duodenum and, less frequently, to the bulbus, stomach or pylorus [1-4]. Anatomic anomalies related to the opening of the common bile duct into the gastrointestinal tract have been further diagnosed through the widespread use of Endoscopic Retrograde Cholangiopancreatography (ERCP). The etiology of anomalous opening of the common bile duct is unknown and considered to be the result of an error during embryonic development of the biliary system [5].

The ectopic opening of the common bile duct to the duodenum was first described by Vesalius in 1543. In this case, one of the double bile ducts opened to the gastric antrum and the other to the duodenum [6]. In the largest case series in the literature, ectopic biliary drainage anomaly was reported in 53 of 12,158 cases who underwent ERCP (0.43%) [1].

Although congenital anomalies of the common bile duct are more common in childhood, they can also be diagnosed in adulthood. While anomalous opening of the common bile duct in adulthood can be identified incidentally, they can also be diagnosed based on symptoms such as biliary stone, recurrent attacks of cholangitis, duodenal ulcer and/or duodenal obstruction [7].

It was thought that the risk of bleeding and perforation was high in endoscopic procedures due to the defective sphincter structure and lack of intramural part of CBD leaning to the duodenum. Therefore, surgical treatment was adopted as the primary treatment modality. Nowadays, with the development of endoscopic intervention technologies and increasing experience on the part of endoscopists, endoscopic treatment methods have gained more importance [1,8,9].

In this article, we aimed to present our experience with patients who underwent surgical treatment due to anomalous opening of the common bile duct in the light of the literature.

MATERIAL and METHOD

Eight patients who underwent surgical treatment at the General Surgery Clinic of Çukurova University Faculty of Medicine due to anomalous opening of the common bile duct between November 2014 and August 2018 were included in the study. Ethical approval numbered 88/16 and dated 05.03.2019 was received from the Çukurova University Faculty of Medicine Ethics Committee. Patient files and hospital information system records were examined and a database was created. The cases were analyzed retrospectively. Follow-up data was supported with telephone interviews with the patients. Patients under 18 years of age and patients whose records could not be reached were excluded from the study.

Patients' demographic characteristics, Body Mass Indexes (BMIs), American Society of Anesthesiologists (ASA) scores, presenting symptoms, preoperative amylase and bilirubin levels, previous abdominal surgery, and diagnostic methods, treatment procedures, intraoperative, and postoperative complications, postoperative hospital stay, re-operations, postoperative 90-day mortality, 90-day unplanned re-admission and mean follow-up times were analyzed.

Endoscopic Retrograde Cholangiopancreatography (ERCP) was performed using the standard technique with the aid of therapeutic duodenoscope (Olympus, TJF 240, Japan) with the patient in the prone position. Anomalous opening of the common bile duct was defined as the absence of a papilla or papilla-like structure in normal anatomical localization in the second part of the duodenum and inability to obtain cholangiogram when the contrast agent was given from the orifice in the ampulla [6]. Sphincterotomy was not performed in cases with choledocholithiasis accompanied by ectopic biliary drainage due to a high risk of perforation.

Endoscopic examination revealed cases with gastric and/or duodenal ulcer, duodenal deformity or stenosis, and these cases were recorded.

Anastomotic leakage was defined as the deterioration of anastomotic integrity detected by clinical and radiological imaging methods.

Wound infection was defined as superficial or deep surgical site infection on the incision site according to the definition of Centers for Disease Control and Prevention (CDC) [10].

Unscheduled re-operation was accepted as a surgical intervention under general, spinal or epidural anesthesia within 30 days after primary surgery, according to the definition of the National College of Surgeons National Surgical Quality Improvement Program (ACS-NSQIP) [11].

Hydration and IV antibiotherapy were applied to patients with cholangitis at the first admission. After determining the etiology of cholangitis, the treatment strategy was redefined.

All patients were treated with low- molecular weight heparin, prophylactic antibiotherapy and compression stockings during induction of anesthesia as antithrombotic therapy.

Follow-up

In the early follow-up after the patients were discharged, they were followed up at in the outpatient clinics for certain periods in terms of wound location and other postoperative complications. In addition to laboratory tests, imaging methods, if available, were preferred according to the symptoms. Subsequent follow-ups were carried out at 6-month intervals in the first year and at yearly intervals after the 2nd year.

Surgical Technique

The patients were operated under endotracheal general anesthesia, with the patient in a slightly inverse Trendelenburg position. After insertion of a central catheter, nasogastric tube, urinary catheter, and skin preparation, right subcostal incision was made. Hepatoduodenal ligament was dissected in the Roux-N-Y hepaticojejunostomy technique. After visualization of the common bile duct from the hilar region to the periampullary region, the right and left hepatic ducts were cut from their bifurcation and distal parts. The distal common bile duct stump was sutured and closed. The jejunum was cut with the help of a stapler, 40 cm distally from the Treitz ligament. Retrocolic anastomosis of the distal jejunum at the distal 40th cm was made with the bifurcation of the posterior wall with continuous 5-0 prolene sutures, while the anastomosis of the anterior wall was made with 5-0 prolene sutures with individual hepaticojejunostomies. Jejenojejunal anastomosis was performed manually, double-layered side by side with 3/0 vicryl and 3/0 silk sutures at the distal 60th cm l. In the portoenterostomy technique, common bile duct was excised above the bifurcation and small bowel anastomosis was performed similarly. Anastomosis of the liver capsule was performed with 6-0 prolene sutures to form a Roux-N-Y portoenterostomy. In the technique of choledochoduodenostomy, the duodenum was mobilized by the Kocher maneuver and the common bile duct was released. Common bile duct was opened with vertical incision then duodenum was opened transverse incision. The lumen of the common bile duct was irrigated. Stone was extracted in patients with choledocholithiasis. Then, anastomosis of choledochoduodenostomy was completed with 4-0 prolene sutures. Gastroenterostomy was performed by the Bilroth II procedure with 3-0 vicryl and 3-0 silk sutures placed retrocolically on the posterior aspect of the stomach. Silicone drains were placed in the Winslow and Douglas cavities in all patients.

Statistical Evaluation

Data were analyzed using IBM SPSS Statistics for Windows, version 24 (IBM Corp., Armonk, N.Y., USA). Categorical variables were summarized as numbers and continuous variables as mean and standard deviation (median and minimum-maximum, where necessary).

RESULTS

A total of 8 patients were included in the study. The mean age was 64.3 years. Male , and female patients constituted 75% (n=6) and 25% (n=2) of the study population. The mean body mass index was 27.6 and the mean ASA score was 2. The most common comorbid diseases were diabetes mellitus (75%) and hypertension (75%).

The most common symptoms were abdominal pain (50%) and biliary colic (25%), but there were also patients presenting with jaundice and fever. Cholestatic liver enzymes were found to be high in 75% of the patients. Leukocytosis was detected in

62.5% of the patients. Fifty percent had hyperbilirubinemia. Four patients had a history of cholecystectomy and one patient had a history of liver resection due to a benign disease.

As concomitant diseases at diagnosis, CBD stone was detected in 5, GB stone in 2, duodenal ulcer in 5 and gastric ulcer in 1 patient. Ectopic biliary drainage was most commonly located at the bulbus (75%), and the second most common localization (25%) was the post-bulbar region (Table 1).

Table 1. Demographics and clinics of patients.

	Mean	Median	Range	Minumum-maximum
Age (years)	64.3	64,5	44	43-87
BMI	27.6	27.65	17,93	22.67-40.6
Sex				**n**
Male				6
Female				2
ASA				
1				0
2				6
3				2
4				0
Comorbidities				
Diabetes Mellitus				3
Hypertension				3
Asthma				1
Coronary artery disease				1
Clinicalcharacteristics Symptoms				
Abdominal pain				4
Biliary pain				2
Icterus				1
Fever/chills				1
Laboratory findings				
Leukocytosis				5
Jaundice				4
Increased serum ALP-GGT				6
Previousabdominalsurgery				
Cholecystectomy				4
Liver resection				1
Concomitantdiseases at diagnosis				
CBD stone				5
GB stone				2
Duodenal ulcer				5
Gastric ulcer				1
Ectopic biliary drainage to				
Bulbus				6
Post bulbar				2

ALP: Alkaline phosphatase, GGT: Gamma-glutamyltransferase
CBD: Common bile duct, GB: gallbladder

Cholangiographic examination revealed common bile duct dilation in all patients. Half of the patients had choledocholithiasis. Intrahepatic biliary dilation was present in 37.5% of the cases (Table 2).

Hepaticojejunostomy was the most common surgical procedure (62.5%). Two patients who underwent

Table 2. Cholangiographic findings of patients.

Variable	n
Common bile ductus dilation (>10 mm)	8
Common bile duct stone	4
Intrahepatic Bile Duct Dilation	3
Pneumobilia	2
Hook shaped CBD	2
Pancreatic duct visualization	2

CBD: Common bile duct

hepaticojejunostomy had not cholecystectomy before. Another common operation (25%) was choledochoduodenostomy. One of the patients underwent choledochoduodenostomy and gastroenterostomy in the same session due to the presence of a stenotic ulcer. Additionally, one patient underwent hepaticojejunostomy and gastroenterostomy in the same session. Case-based causes such as patient's age, comorbid factors, and less invasive techniques compared to hepaticojejunostomy were effective in the success of choledochodoudenostomy. With the increased experience of our clinic on choledochal anomalies, hepaticojejunostomy is applied more frequently only one of the cases in our study underwent portoenterostomy. In this case, dilation compatible with the choledochal cyst was detected during the operation which necessitated choledochal excision and portoenterostomy (Table 3).

The mean operative time was 165 min. in one patient, intraoperative iatrogenic small bowel injury developed and primary repair was performed and no complications developed. Postoperative anastomotic leakage developed in two patients and biliary leakage was resolved with medical treatment. Postoperative upper gastrointestinal bleeding developed in one patient which was controlled by endoscopic intervention. In one case, anastomotic leak was followed up medically, upper GIS bleeding occurred and surgical treatment was applied. During follow-up, the patient died of myocardial ınfarction (Table 3).

The mean postoperative hospital stay was 37 days. The causes of re-admission to the hospital within 90 days were wound infection (37.5%) and jaundice (12.5%). Any etiologic factor could not be detected by imaging and other methods in the patient who applied with jaundice. The mean follow-up period was 28 months (Table 3).

Table 3. Operative procedures, surgical morbidity, and clinical outcome.

	n
Surgicalapproach	
Hepaticojejunostomy	2
Hepaticojejunostomy with cholecystectomy	2
Hepaticojejunostomy with gastroenterostomy	1
Choledochoduodenostomy with cholecystectomy	1
Choledochoduodenostomy with gastroenterostomy	1
Portoenterostomy	1
Intraoperative Complication	
Iatrogenic small bowel injury	1
Postoperative complication	
Anastomosis leak	1
GIS bleeding	1
Anastomosis leak + GIS bleeding	1
Evisceration	1
Clavien-Dindo	
1	1
2	3
3	2
4	1
5	1
Reoperation	
GIS bleeding with anastomosis leakage	1
Evisceration	1
Postoperative 90-day mortality	1
90-day readmission	
Wound infection	2
Icterus	1

	Mean	Median	Range	Minumum-maximum
Mean operative time (min)	165	145	255	105-360
Postoperative Length of hospital stay (day)	37	28	82	8-90
Mean follow up (month)	28	26	47	6-53

GIS: Gastrointestinalsystem

DISCUSSION

The most important feature of the extrahepatic biliary system is the frequent occurrence of anatomic variations. The CBD typically enters the posteromedial aspect of the second portion of the duodenum. Sometimes the papilla of Vater may end in atypical anatomic localization, including the stomach, pyloric duct, duodenal bulb, and the third or fourth part of the duodenum. This very rarely seen condition is called ectopic biliary drainage [3]. Abnormal opening of the common bile duct to the duodenum has been reported in a limited number of cases. The incidence of common bile duct anomaly was found to be 0.43-1.05% in large series in the literature [1,2].

The etiology of ectopic biliary drainage is unknown and it is thought to be caused by abnormalities seen during embryological development.

In the literature, it is reported that 50-95% of cases with ectopic biliary drainage are seen in the male sex. This might bring to mind an etiologic relation with Y chromosome-induced embryonic abnormality [1,3,7,8]. In our series, male gender dominance was present in support of the findings in the literature.

Patients with anomalous choledochal openings become symptomatic in the 5th or 6th decades of life with symptoms such as cholangitis and upper gastrointestinal bleeding [1,2,8]. Although the age range of the patients was between 36-78 years in our series, the median age at diagnosis was 55 years which was consistent with the literature.

Biliary colic pain is the most common presenting symptom in ectopic common bile duct openings and this symptom is present in 95-100% of the patients during presentation [1,8]. In a study by A. Taş et al., presenting symptoms were determined to be biliary colic (100%), cholangitis (60%), acute renal failure (10%), previous bleeding (20%), and liver abscess (20%) [5]. The presenting symptoms in our series were abdominal pain (50%) and biliary colic (25%).

Ectopic opening of the CBD is commonly associated with biliary tract diseases. Gallstone, choledocholithiasis, obstructive jaundice, cholangitis, pancreatitis and peptic ulcer are complications of ectopic CBD openings [1,2,7,8]. Malfunction of the valve mechanism is blamed for the occurrence of these complications. The dysfunction of this mechanism may allow intestinal bacteria and gastric contents to enter the biliary system, causing recurrent attacks of cholangitis, transient obstruction and liver abscess [2,12]. In our series, one patient presented with cholangitis. Elevation of cholestatic enzymes was detected in 75% of the patients. Leukocytosis was present in 50% of the cases. Half of the patients in the study had a history of cholecystectomy due to cholelithiasis. Cholelithiasis was detected in 2 of 4 cases without cholecystectomy and choledocholithiasis was found in 62.5% of all cases.

In many series in the literature, it has been stated that the ectopic opening was localized mostly in the bulbus part of the duodenum [2,3,5,13]. In 75% of the cases in our series common biler duct opened in bulbus and in 25% of the case post-bulbar opening was detected .

Biliary system pathologies are frequently reported in patients with ectopic choledochal opening [1,2,5,8]. In their series, Taş et al. found common bile duct dilation in all of their patients. Deformed CBD, such as hook-shaped CBD, has been described as a predominant feature of ectopic orifice of papilla in the literature. The hook-shaped configuration may result from acute angulation of the CBD caused by premature drainage of bile into the bulb [8]. In the series of Lee et al, cholangiography showed this hook-shaped configuration of the distal CBD in all patients. They therefore argued that the hook-shaped configuration of the distal CBD should be considered as a characteristic finding for ectopic choledochal opening in patients without any history of abdominal surgery other than simple cholecystectomy [8]. In our series, all patients had common bile duct dilation and 37.5% of the patients had choledochal dilation accompanied by intrahepatic dilation. In 25% of our patients with ectopic common bile duct opening, hook shaped appearance was detected in the common bile duct. Although ERCP is widely used in the diagnosis of ectopic common bile duct opening, there are publications in the literature stating that endoscopic ultrasonography (EUS) will also be useful [14].

Coexistence of common bile duct anomaly and duodenal ulcer is a common condition. Easy passage of duodenal content into the biliary tract (duodenobiliary reflux) and reflux of the bile to the duodenum without sphincteric control can cause biliary tract diseases, duodenal ulcer and ulcer complications [1,2,5]. Diseases of duodenal mucosa (ulcers and deformation/stenosis) and biliary system diseases are closely related to the location and morphology of the ectopic opening [1,2,5,8]. In our series, 62.5% of the patients had duodenal ulcer and 2 patients had a history of duodenal ulcer surgery.

Treatment of ectopic biliary drainage is associated with biliary pathology and complications of peptic ulcer [3]. Most symptomatic patients are treated using surgical or endoscopic methods. If choledocholithiasis is accompanied by abnormal opening of the common bile duct into the stomach or duodenum, endoscopic treatment may fail and surgical intervention may be necessary. The intramural portion of the duct is not fully developed in these patients [8]. Therefore, there is a high risk of perforation and/or bleeding during endoscopic sphincterotomy, so endoscopic sphincterotomy is not recommended in these patients. There is no consensus regarding the treatment of asymptomatic ectopic choledochal openings [1,3,8].

In the study performed by Disibeyaz et al, endoscopic approach was the primary treatment method. Fifty-two percent of the patients with dilated CBD (with or without stone) were treated with endoscopic methods and 16% of them with surgical methods [1]. In the same study, surgical treatment was performed in patients with stenosis in the distal common bile duct or stones larger than 2 cm that could not be extracted by endoscopic intervention. Thirty-two percent of the patients with dilated CBD who were symptomatic but had not choledocholithiasis became asymptomatic with supportive medical treatment [1]. However, recurrent cholangitis episodes developed in 13-20% of patients that underwent endoscopic treatment.

Surgery is the main treatment modality in most of the case reports in the literature. Choledochoduodenostomy, choledochotomy with stone extraction and hepaticojejunostomy are frequently reported surgical methods. In the series of Lee et al., 72% of the patients were treated surgically and the most common surgical method was choledochoenterostomy [8]. In our series, patients underwent hepaticojejunostomy (n=5), portoenterostomy (n=1), and choledochoduodenostomy (n=2). In one patient, choledochoduodenostomy and gastroenterostomy procedure was performed due to stenosis resulting from duodenal ulcer. We performed gastroenterostomy on one of the patients who underwent hepaticojejunostomy due to ulcer-related stenosis. The main factors that determine the operation technique are anatomical location of the common bile duct opening, associated biliary pathologies, previous ulcer surgery and ulcer complications.

In one case, iatrogenic small bowel injury developed during bridectomy and primary repair was performed, and any postoperative complications were not observed. Postoperative anastomotic leakage developed in two, postoperative GIS bleeding in one and evisceration in one patient. The patient who had GIS bleeding was treated with the endoscopic method. Primary repair was performed on the patient who developed evisceration. One patient who developed anastomotic leakage was followed up medically and the fistula regressed in the follow-up. One patient who was followed up for anastomotic leakage developed massive ulcer bleeding. Since the bleeding could not be stopped by endoscopic procedures, the patient was operated on. In the operation, the bleeding ulcer was repaired with primary sutures, and gastroduodenal artery ligation and gastroenterostomy were performed. In the postoperative period, open abdomen was followed up for a long time. Although bile leakage and GIS hemorrhage were controlled during the follow-up, exitus developed due to myocardial infarction. In this case, we think that hemostatic therapies administered due to GIS bleeding had an effect on the development of myocardial ınfarction in the background of coronary heart disease. We think that recurrent inflammation of the common bile duct and consequent deterioration of wound healing are effective in postoperative anastomotic leakage. We estimate that bleeding secondary to duodenal ulcer developing in the postoperative period is due to the change in duodenal pH level resulting from applied bilioenteric diversion.

According to Clavien 2-3 classification used in the scoring of postoperative complications, our postoperative complication rate was 62.5%. The mean postoperative hospital stay was 37 days, and the main reason for the long hospital stay was anastomotic leakage, ulcer bleeding and wound infection. Wound infection and jaundice were the most common causes of unplanned re-admissions to the hospital within 90 days. The patient with jaundice was followed up medically and bilirubin levels returned to normal levels. Patients with wound infection were controlled with daily dressings. There were no deaths due to biliary system disease at a mean follow-up of 27.8 months. Myocardial infarction was the cause of mortality in one patient.

In conclusion, the ectopic opening of CBD into the duodenum is a rare anomaly that can cause incidental findings as well as pathologies in the biliary system and duodenal region. This anomaly should be kept in mind especially in patients with recurrent cholangitis, biliary colic, choledocholithiasis and / or recurrent duodenal ulcer. In addition to endoscopic methods, surgical treatment methods can be applied. Patients undergoing surgical treatment should be closely monitored for postoperative anastomotic leakage, bleeding arising from duodenal ulcer and sphincterotomy site. There are limited number of studies in the literature regarding surgical treatment of common bile duct anomalies. Prospective studies are needed to compare the long-term results of surgical and endoscopic treatment methods.

Ethics Committee Approval: Approval was received from the local Ethics Committee (date: 03.05.2019 decision no. 88/16).
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REFERENCES

1. Disibeyaz S, Parlak E, Cicek B, et al. Anomalous opening of the common bile duct into the duodenal bulb: endoscopic treatment. BMC Gastroenterol. 2007;7(1):26. https://doi.org/10.1186/1471-230X-7-26
2. Sezgin O, Altintas E, Ucbilek E. Ectopic opening of the common bile duct into various sites of the upper digestive tract: a case series. Gastrointest Endosc. 2010;72(1):198-203. https://doi.org/10.1016/j.gie.2010.02.012
3. Saritas U, Senol A, Ustundag Y. The clinical presentations of ectopic biliary drainage into duodenal bulbus and stomach with a thorough review of the current literature. BMC Gastroenterol. 2010;10:2. https://doi.org/10.1186/1471-230X-10-2
4. Dacha S, Wang XJ, Qayed E. A case of an ectopic ampulla of vater in the pyloric channel. ACG Case Rep J. 2014;1(3): 161-3. https://doi.org/10.14309/crj.2014.37
5. Taş A, Kara B, Ölmez S, Yalçın MS, Öztürk NA, Saritas, B. Retrospective analysis of cases with an ectopic opening of the common bile duct into duodenal bulb. Adv Clin Exp Med. 2018;27(10):1361-4. https://doi.org/10.17219/acem/69691
6. Quintana EV, Labat R. Ectopic drainage of the common bile duct. Ann Surg. 1974;180(1):119-23. https://doi.org/10.1097/00000658-197407000-00018
7. Parlak E, Dişibeyaz S, Cengiz C, et al. Ectopic opening of the common bile duct and duodenal stenosis: an overlooked association. BMC Gastroenterol. 2010;10:142. https://doi.org/10.1186/1471-230X-10-142
8. Lee SS, Kim MH, Lee SK, et al. Ectopic opening of the common bile duct in the duodenal bulb: clinical implications. Gastrointest Endosc. 2003;57(6):679-82.

https://doi.org/10.1067/mge.2003.210
9. Lee W, Park JH, Kim JY, et al. A case of gallbladder cancer combined with ectopic individual opening of pancreatic and bile ducts to the duodenal bulb. Korean J Hepatobiliary Pancreat Surg. 2015;19(3):121-4. https://doi.org/10.14701/kjhbps.2015.19.3.121
10. Horan TC, Gaynes RP, Martone WJ, Jarvis WR, Emori TG CDC definitions of nosocomial surgical site infections, 1992: a modification of CDC definitions of surgical wound infections. Infect Control Hosp Epidemiol. 1992;13(10):606-8. https://doi.org/10.1017/S0195941700015241
11. American College of Surgeons. User Guide for the 2012 ACS NSQIP Participant Use Data File; 2013. Available from: https://www.facs.org/-/media/files/quality-programs/nsqip/ug12.ashx
12. Lee HJ, Ha HK, Kim MH, et al. ERCP and CT findings of ectopic drainage of the common bile duct into the duodenal bulb. AJR Am J Roentgenol. 1997;169(2):517-20. https://doi.org/10.2214/ajr.169.2.9242767
13. Sezgin O, Altıntaş E, Üçbilek E. Ectopic opening of the common bile duct into the tomach. Turk J Gastroenterol. 2010;21(2):163-7. https://doi.org/10.4318/tjg.2010.0076
14. Krstic M, Stimec B, Krstic R, Ugljesic M, Knezevic S, Jovanovic I. EUS diagnosis of ectopic opening of the common bile duct in the duodenal bulb: a case report. World J Gastroenterol. 2005;11(32):5068-71. https://doi.org/10.3748/wjg.v11.i32.5068

Original Article

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The Clinicopathologic Features and the Factors Associated with the Survival in Light -Chain Amyloidosis Patients: A Single Center Descriptive Study

Hafif Zincir Amiloidozlu Hastalarda Klinikopatolojik Özellikler ve Sağkalım ile İlişkili Faktörler: Tek Merkezden Tanımlayıcı Çalışma

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ABSTRACT

Objective: *To present the clinicopathologic features and assess the factors related to the survival in light- chain amyloidosis (AL) patients.*
Method: *All the patients with AL diagnosis being followed-up in the hematology department were recruited in the study. Clinicopathologic data were obtained. Factors related with overall survival (OS) including systemic inflammatory response markers were analyzed.*
Results: *In 16 AL patients, the estimated OS was 58.6±10.8 months, with a-5-year- survival rate of 52.1%. While, 43.8% of the patients died during the study period. Gastrointestinal and respiratory complaints were the most frequent symptoms. Myocardial and renal biopsies were amyloid positive in 31.3% and 25% of the patients respectively. Myeloma was diagnosed in 18.8% and amyloid was positive in 31.3% of the bone marrow biopsies. There was no difference between surviving and deceased patients with respect to laboratory findings including systemic inflammatory markers. Only immunoglobulin M was significantly lower in the deceased patients and IgM was found to be the only factor independently associated with OS. Lower IgM levels were associated with decreased OS. An IgM value of 75.4 mg/dL was found as a cut-off value with a sensitivity and specificity of 71.4% and 66.7% respectively for the prediction of survival status.*
Conclusion: *AL is a rare, progressive, systemic disease with a wide spectrum of clinical presentations. The disease most commonly presents with gastrointestinal and respiratory complaints. IgM level seems to be an independent predictor of survival and may be used as a prognostic marker.*

Keywords: *light chain amyloidosis, AL, Immunoglobulin M, neutrophil- to- lymphocyte ratio, platelet -to- lymphocyte ratio, systemic inflammatory response markers*

ÖZ

Amaç: *Hafif zincir amiloidozlu (AL) hastalarda klinikopatolojik özelliklerin ortaya konması ve sağ kalım ile ilişkili faktörlerin değerlendirilmesi.*
Yöntem: *Hematoloji kliniğinde takip edilen tüm AL tanısı almış hastalar çalışmaya dahil edildi. Klinikopatolojik veriler toplandı. Hayatta olan hastalar ile ölmüş olan hastalar karşılaştırıldı. Toplam sağ kalım ile ilişkili faktörler, sistemik inflamatuvar belirteçler de dahil olmak üzere analiz edildi.*
Bulgular: *Çalışmaya dahil edilen 16 hastada tahmin edilen toplam sağ kalım 58,6±10,8 ay ve 5 yıllık sağ kalım %52,1 olarak bulundu. Çalışma süresinde hastaların %43,8'i öldü. En sık görülen şikayetler gastrointestinal ve respiratuvar semptomlardı. Miyokard ve renal biyopsilerde amiloid hastaların sırasıyla %31,3 ve %25'inde pozitif olarak bulundu. Kemik iliği değerlendirmesinde hastaların %18,8'inde miyelom tespit edildi ve amiloid kemik iliği biyopsilerinin %31,3'ünde pozitif idi. Hayatta kalan ve ölen hastalar arasında sistemik inflammatuvar belirteçler açısından fark yoktu. Sadece immunoglobulin M'nin ölen hastalarda daha anlamlı olarak düşük olarak bulundu ve IgM toplam sağ kalım ile bağımsız olarak ilişkili tek faktör olarak bulundu. Daha düşük IgM seviyeleri azalmış toplam sağ kalım ile ilişkili idi. IgM için 75,4 mg/dL eşik değerinin hayatta kalma ön görüsü için %71,4 sensitivite ve %66,7 spesifisiteye sahip olduğu bulundu.*
Sonuç: *AL geniş bir klinik yelpazeye sahip, nadir görülen, ilerleyici, sistemik bir hastalıktır. Hastalık en sık gastrointestinal ve respiratuvar şikayetlerle kendini gösterir. IgM sağ kalım için bağımsız bir ön gördürücü olarak görülmektedir ve prognostik bir belirteç olarak kullanılabilir.*

Anahtar kelimeler: *hafif zincir amiloidoz, AL, immunoglobulin M, nötrofil lenfosit oranı, platelet lenfosit oranı, sistemik inflamatuar yanıt belirteçleri*

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INTRODUCTION

Light-chain amyloidosis (AL) is defined as "primary amyloidosis" which results from extra-cellular deposition of fibril-forming monoclonal immunoglobulin light chains or a fragment of the light chain usually produced by a clonal population of plasma cells in the bone marrow. It is the most common form amyloidosis with an incidence estimated to be around 1 to 4.5 cases per 100,000 [1]; however, the studies from autopsies have suggested a higher incidence [2]. In USA, about 2500 patients are diagnosed as having AL every year [3]. It has been reported to be seen 5-10 times less frequently than multiple myeloma with an incidence rate similar to that of Hodgkin's lymphoma or chronic myelogenous leukemia [4,5].

AL amyloidosis is usually a systemic disease characterized by multiple organ and tissue involvement as the pathologic, insoluble fibrils may deposit extracellularly in various tissues and organs. Deposition may impair the function of the affected organ or tissue. Any organ may be effected, heart, kidney, liver and nervous system being the most commonly involved ones. In United Kingdom AL constitutes 68% of all the amyloid cases and heart and kidney are the most frequently affected organs, followed by liver and nervous system [6-9]. Therefore, the disease spectrum is broad, ranging from mild symptoms to life threatening disorders including restrictive cardiopathy which is considered to be responsible from the mortality in most of the cases [8].

There are relatively few studies on this rare systemic disease. Because symptoms are nonspecific and early diagnosis and treatment prolongs survival, studies that present clinical features are important. In this study we aimed to present the clinicopathologic features and assess the factors related to the survival in AL patients.

MATERIAL and METHOD

A single-center retrospective, non-randomized study was conducted in Başkent University Hospital Department of hematology. All the patients with the diagnosis of amyloidosis who were followed-up in the hematology department between January 2014 and July 2019 were recruited. Patients younger than 18 years and whose diagnosis was not light-chain amyloidosis were excluded. A total of 16 patients were identified. Data were obtained from the hospital's electronic database and patients' files. Institution's ethical approval was obtained for the study (KA19/284).

The diagnosis of amyloidosis was suspected when there were clinical signs and symptoms and when immunoglobulin free light- chain abnormalities were detected in immunofixation blood tests. Noninvasive diagnostic criteria of the consensus for amyloid-related organ involvement were used for evaluation [10,11]. However the definitive diagnosis was based on the examination of the histological samples. Tissue biopsies were obtained from the involved organs whenever possible. The evaluation processes included upper gastrointestinal endoscopy, colonoscopy, cardiac biopsy, renal biopsy, bone and bone marrow biopsies. Light microscopic examination finding of amorphous extracellular Congo- red positive deposits, which display characteristic dichroism and apple green birefringence under polarized light confirmed the definitive diagnosis. In order to exclude multiple myeloma, bone marrow assessment was performed.

Chemotherapy consisted of vincristine, doxorubicin, dexamethasone (VAD), bortezomib, cyclophosphamide, dexamethasone (VCD), rituximab, bortezomib, lenalidomide, dexamethasone (R-BORD) and colchium.

The main outcome to be assessed was overall survival which was calculated from the time of diagnosis to death from any cause or the last follow-up. The laboratory and pathologic results of the deceased and surviving patients were compared.

Statistical analysis was performed with SPSS version 22 (demo version, IBM). Nominal data were expressed as percentages. Normality of data were tested with one sample Kolmogorov-Smirnov and or Shapiro-Wilk tests. Normally distributed continuous data were expressed as means±standard deviation, and non-normally distributed continuous data as median (interquartile range [IQR]). Mean survival time was shown as mean±standard error (95% confidence interval [95% CI]). Chi-square, t test, and Mann-Whitney U tests were used where appropriate. Kaplan-Meier analysis was performed for survival

analysis. Comparisons between groups with respect to survival were performed using the log-rank test. Cox regression analysis was used to determine the independent predictors of OS. A receiver operating characteristic (ROC) curve was constructed to find a cut-off value whenever appropriate. A p value of ≤ .05 was considered statistically significant.

RESULTS

The clinicopathologic characteristics of all the patients were depicted in Table 1. The mean age was 63.4±6.8 years with a male to female ratio of 1:1.29 (62.4±5.8 years in deceased patients vs 64.1±5.8 years in survivors, p=0.638). Seven patients (43.75%) died during the study period. The most common reason for death was restrictive cardiomyopathy associated with amyloidosis (n:3/7) followed by amyloidosis associated chronic renal failure (n:2/7). One patient died due to congestive heart failure which was not associated with amyloidosis and one patient died due to gastrointestinal bleeding. Gastrointestinal complaints were

Table 1. Clinicopathological features of light chain amyloidosis patients.

Age (D)	Sex	Syptoms	Biopsy	Bone Marrow	Serum IF	Chemotherapy	Ex (R)	OS
67	F	Backache, dyspepsia, generalized bone pain	Stomach	IgG lambda myeloma	IgG lambda MG	VCD	+(CRF)	1
73	F	Leg swelling, numbness weight loss, hoarseness bruising on body	Myocard Lympho node	Amyloid (-) <10% atypical plasma cells	IgG lambda MG	-	-	28
59	M	Weakness, lymphadenopathy on neck	Myocard	Amyloid (+)	IgM kappa MG	RBORD	-	96
63	F	Imbalance	Kidney	-	Suspected staining	Colchium on ilbda column	-	67
67	F	Edema and echymosis on ligs, papillary atrophy on tongue, hepatomegaly,	Liver, stomach	Kappa light chain myeloma, 15% atypical plasma cells, Amploid (+)	No gammopathy	-	-	28
56	F	Nausea, dyspepsia, Weight loss, effusion in knees Abdominal swelling	Kidney	80% plasma cell infiltration, myeloma	Lambda light chain	VAD	+(CRF)	1
59	M	Shortness of breath, numbness of feet, polyneuropathy, pleuresia, hypertrophic cardiomyopathy	Kidney	5-10% plasma cells	IgG lambda MG	VCD	+(CHF)	60
52	F	Gastrointestinal bleeding	-	Amploid (+) few plasma cells	No gammopathy	-	+(GIB)	2
63	M	Diarrhea, weight loss, Shortness of breath	Myocard	Amyloid (+), 0.4% plasma cells	No gammopathy	-	-	27
65	F	Shortness of breath, restrictive cardiomyopathy	Duodenum	15% Atypical plasma cells	Lambda light chain	-	+(CHF)	8
61	F	Anorexia, nausea, vomitting	Iliopsoas muscle	Kappa light chain, plasma cell infiltration	Kappa light chain	VAD, VCD, Revlimid	+(CHF)	24
54	M	Shortness of breath, pretibial edema	Myocard, fat tissue	10% plasma cells	IgG lambda MG	VCD, autotransplant	-	28
61	M	Weight loss, constipation, neck pain	Lymph node, Tonsil	Normal	IgG lambda MG	VCD	-	93
70	M	Shortness of breath	-	Amyloid (+)	No gammopathy	-	-	66
67	F	Dyspepsia, abdominal pain, leg swelling, muscle weakness	Kidney	8-9% plasma cell	Lambda light chain	Colchium	-	64
77	M	Shortness of breath, abdominal pain, hepatosplenomegaly	Myocard, colon	Normal	No gammopathy	VD	+(CHF)	1

M: male, F: Female, Age(R): Age at diagnosis, SerumIF: serum immunofixation, Ex (R): Exitus (Reason), Ig: immunoglobulin, MG: monoclonal gammopathy, VAD: Vincristine, doxorubicin, dexamethasone, VCD: bortezomib, cyclophosphamide, dexamethasone, R-BORD: Rituximab, bortezomib, lenalidomide, dexamethasone, CRF: Chronic renal failure, CHF: Congestive heart failure, GIB: Gastrointestinal bleeding.

the most common symptoms (50%) followed by shortness of breath (37.5%), swelling/edema of the lower extremities (31.3%) and weight loss (25%). Myocardial and renal biopsies were amyloid- positive in 31.3% and 25% of the patients respectively. Bone marrow analyses resulted in detection of myeloma in 18.8% and amyloid-positivity in 31.3% of the bone marrow biopsy specimens. Immunoglobulin (IgG) lambda monoclonal gammopathy was found to be the most common form (50%) in immunofixation blood tests. Various treatment regimens were given, VCD being the most common treatment regimen (Table 1). Autologous stem cell transplantation was performed in one patient.

The laboratory findings of the patients are depicted in Table 2. Except IgM, no significant difference was found in the laboratory results between the surviving and deceased patients (median [IQR]: 83 [1016.1] vs 65 [54], p=0.042). Although not significant neutrophil-to- lymphocyte ratio, LDH, creatinine and C-Reactive protein were found to be higher and sedimentation rate was lower in the deceased patients (Table 2). Proteinuria was present in 8 patients with a median value of 350 mg/L/day (IQR: 1797.5 mg/dL). The most common monoclonal gammopathy in the urine immune electrophoresis was found to be kappa light chain (n:4). Correlation analysis showed a significant correlation between OS and LDH and IgM (Spearman's rho correlation coefficient: -0.673, p=0.006 for LDH, Spearman's rho correlation coefficient: 0.586, p=0.0017 for IgM). When the systemic involvement were grouped as gastrointestinal, cardiac, renal and lymph node involvements, and mortality rates were compared with respect to system involved, it was found that death occurred in cases with gastrointestinal (67%), cardiac (42.7%), lymph node (50%) and renal (33.3%) involvement (χ^2 test, p=0.860).

The estimated cumulative OS was 58.6±10.8 months in the studied population (Figure 1). One-, 2- and 5-year OS rates were 68.8%, 62.5% and 52.1% respectively. To determine the factors associated with the OS a Cox regression analysis was carried out. LDH, IgM, NLO, creatinine, C-reactive protein and sedimentation were included in the analysis (Table 3). IgM was found

Table 2. Comparison of the laboratory findings of deceased and surviving patients (Data expressed as mean±standard deviation or median (interquartile range)).

	Surviving (n:9)	Deceased (n:7)	p
Hemoglobin (g/dL)[a]	11.8±2.8	11.3±1.8	0.638
Sedimentation (mm/hr)[b]	54 (61.5)	27 (28)	0.315
Lactic Dehyrogenase (U/L)[b]	197 (121.5)	266 (192)	0.054
Creatinine (mg/dL)[b]	1 (1.23)	3.6 (2.93)	0.174
Calcium (mg/dL)[a]	8.9±0.9	9.4±0.9	0.349
β2microglobulin (mg/dL)[b]	3.2 (13.4)	1.8 (17.78)	1
Platelet Lymphocyte Ratio[b]	121.1 (73.6)	100.7 (113.7)	0.536
Neutrophil Lymphocye Ratio[b]	1.95 (1.39)	3.21 82.26)	0.42
C-Reactive Protein (mg/L)[b]	11.2 (15.9)	17.7 (29.1)	0.232
Mean Platelet Volume (fl)[a]	7.9±1.9	8.2 ±1.2	0.810
Red Cell Distribution Width (%)[a]	15.6±2.9	15.5±1.7	0.955
Immunoglobulin G (mg/dL)[b]	879 (1172.5)	703 (721)	0.606
Immunoglobulin A (mg/dL)[b]	139 (297)	90 (214)	0.351
Immunoglobulin M (mg/dL)[b]	83 (1016.1)	65 (54)	0.042*

*[a]: Student t test; [b]: Mann - Whitney U test, *: statistically significant*

Table 3. Cox regression model for identification of factors related to overall survival: Analysis of maximum likelihood estimates.

	β	p	Hazard Ratio 95 % CI	
Immunoglobulin M	-0.231	0.048*	0.794	0.632 - 0.998
LDH at treatment	-0.048	0.054	0.953	0.908 - 1.001
Sedimentation rate	-0.287	0.056	0.750	0.559 - 1.008
Creatinine	3.382	0.055	29.42	0.924 - 936.85
C reactive protein	0.043	0.105	1.044	0.991 - 1.100
NLR	-1.689	0.075	0.185	0.029 - 1.189

*NLR: Neutrophil to lymphocyte ratio, LDH: Lactic dehydrogenase, *: statistically significant*

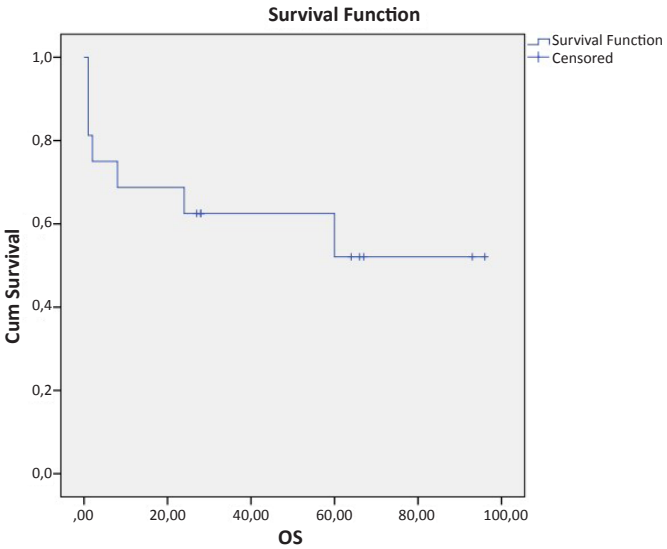

Figure 1. The overall survival (OS) in patients with AL amyloidosis.

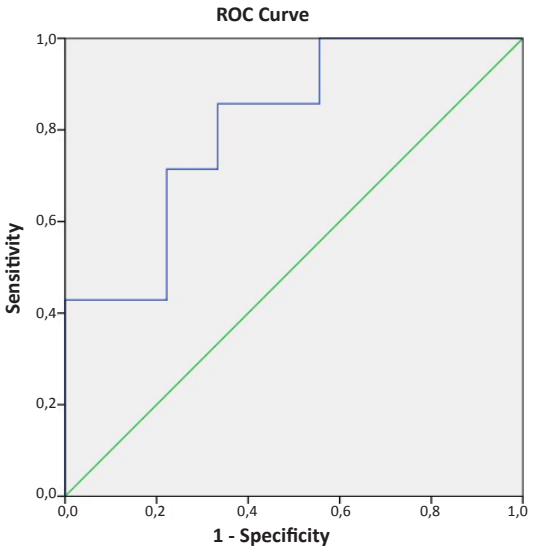

Figure 2. ROC curve for Immunoglobulin M with respect to survival.

to be an independent predictor of OS with a hazard ratio of 0.794 (p=0.048). A ROC analysis was carried out to find a cut- off value for IgM to predict survival (Figure 2). Area under the curve was found to be 0.810 with a standard error of 0.110 (p=0.039, 95% CI: 0.595-1.00). An IgM value of 75.4 mg/dL was found as a cut-off value with a sensitivity and specificity of 71.4% and 66.7% respectively for prediction of survival status.

DISCUSSION

Amyloidosis is a relatively rare disorder and currently there are very few epidemiologic data from our country. This study was conducted in order to present the relevant data on AL amyloidosis and to draw attention to this subtle, progressive disease which can be controlled and treated with early diagnosis.

The main challenge is the diagnosis of this systemic disease because it may affect almost every organ and therefore there are no specific symptoms and signs which in turn causes delay in the diagnosis. The most common symptom was found to be gastrointestinal symptoms including dyspepsia, nausea, diarrhea which was seen in 50% of the patients. Respiratory symptoms, swelling/edema of the lower extremities and weight loss are the other most frequently observed symptoms. Heart and the kidneys are reported to be the most frequently affected organs [12,13]. Cardiac symptoms develop in 50% of the AL amyloidosis patients due to deposition of amyloid in heart resulting in progressive restrictive cardiomyopathy and cardiac involvement is considered to be the major cause of mortality with a 5-year survival of <10% [13,14]. In the present study 3 of the 7 deceased patients had cardiac involvement and the 5-year survival among patients with cardiac involvement was 35.7%. The main cause of death in these patients was restrictive cardiomyopathy associated with amyloidosis. Peripheral neuropathy is also a common presenting symptom, seen in about 50% of the patients [7]. Autonomic nervous system involvement may result in delayed gastric emptying and intestinal motility disorders and may be another cause of the gastrointestinal symptoms which were most frequently detected in the present study. Because there are no specific diagnostic imaging, blood or urine tests [15], it is recommended that AL amyloidosis should be suspected in patients with non-diabetic nephrotic syndrome, non-ischemic cardiyomyopathy, polyneuropathy with monoclonal protein, hepatomegaly, increased ALP with normal liver imaging or when a monoclonal gammopathy is present in a patient with unexplained edema, weight loss, fatigue or paresthesia [16-18].

The most common type of gammopathy in AL amyloidosis is reported to be the lambda isotype which has a 3:1 ratio to kappa [19]. In the present study

there were 9 lambda and 2 kappa gammopathies at a 4.5/1 ratio which is not different from that reported in the literature. AL amyloidosis and lambda light -chain gammopathy have been reported to have a tendency for renal involvement due to the interaction of mesangial cells derived from the 6a germ-line gene [20]. In the present study in 66.7% (n:2/3) of the only lambda light- chain cases renal involvement was detected.

In AL amyloidosis bone marrow biopsy is performed to exclude multiple myeloma or other disorders such as Waldenström's macroglobulinemia [21]. In bone marrow examination, amyloid deposits were detected in 31.3% of the patients and the plasma cell burden was less than 15% in most of the cases. In three cases (18.8%) multiple myeloma was diagnosed and this was also similar to the literature reporting presence of multiple myeloma in 10-15% of AL amyloidosis patients [12].

Systemic inflammatory response markers including NLR, PLR, CRP, RDW and MPV were also analyzed and the levels of these markers were similar between the surviving and deceased patients. RDW and MPV were assessed previously in AA amyloidosis; however, their levels have not been studied in AL amyloidosis up to now [22]. Erdem et al. showed that MPV was decreased and RDW was increased in AA amyloidosis due to chronic inflammation [22]. The MPV and RDW values found in AA patients were similar to those of our AL patients. NLR and PLR values have not been reported for AL amyloidosis before. Elevated levels of these markers have been reported to be associated with many clinical conditions including cardiovascular diseases, malignancies, cirrhosis, ulcerative colitis and adverse events in pregnancy [23-26]. In their study Uslu et al showed that a NLR value >2.21 was associated with development of amyloidosis in Familial Mediterranean Fever patients [23]. The normal values of NLR in 60-69 year-old Turkish men and women were reported to be 2.41±1.54 and 2.09±1.4 respectively [27], which is lower than the mean NLR (2.8±1.8) in this study. NLR was even higher in the deceased patients. The PLR values in 60-69 year- old Turkish healthy patients have been reported to be 101±82 and this value was again lower than 118.8±49.3 which was found in AL amyloidosis patients in this study [28]. As is indicated, NLR and PLR levels increase in AL amyloidosis patients, however they are not independent predictors of survival.

The only significant factor related to the overall survival was found to be Ig M in this studied population. All IgG, A and M levels were lower in deceased patients; only IgM was a significant marker for survival. There is evidence that levels of immunoglobulins are correlated with prognosis and spread of disease in some cancer types [29]. Ig plays an important role in humoral immunity and Tsavardis et al showed that gastric cancer patients with increased levels of IgM have a longer survival [29]. Low gammaglobulins have been purposed as risk factors for development of lymphoma in Sjögren's syndrome [30,31]. In other studies low IgM but not IgG was associated with progression to lymphoma in Sjögren's syndrome [32,33]. These results show that low IgM levels are associated with poor prognosis in various clinical situations. In the present study we have showed for the first time that AL amyloidosis may be one of these disorders. Lower IgM levels may be associated with lower OS. It is true that the pathophysiology of these diseases is different from that of AL. However, in the literature it has been shown that IgM fraction in intravenous IgGAM can play a distinct role in controlling inflammatory and autoimmune diseases and it can reduce oxidative stress which is associated with heart failure, cardiomyopathy and myocarditis [34-40]. From this point of view, it would be logical to assume a protective role of IgM especially in cardiac diseases including heart failure. In the present study the most common cause of death was restrictive cardiomyopathy and low IgM levels were associated with OS. This finding needs to be assessed in additional studies performed with greater number of AL amyloidosis cases in order to establish the prognostic value of IgM in such patients.

In addition to the drawbacks related its retrospective design, the main limitation of this study was the small sample size due to the relative rarity of the investigated disorder. The main strength of the study was that it was conducted in a single tertiary center and all the diagnostic and treatment procedures were uniform.

In conclusion AL amyloidosis is a rare, progressive, systemic disease with a wide spectrum of clinical

presentations. The most important challenge is the diagnosis. The disease most commonly presents with gastrointestinal and respiratory complaints. Except MPV, systemic inflammatory response markers are slightly elevated. Ig M level seems to be an independent predictor of survival and may be used as a prognostic marker.

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REFERENCES

1. Gertz MA, Lacy MQ, Dispenzieri A. Amyloidosis: recognition, confirmation, prognosis, and therapy. Mayo Clin Proc. 1999;74(5):490-4. https://doi.org/10.4065/74.5.490
2. Skinner M, Sanchorawala V, Seldin DC, Dember LM, Falk RH, Berk JL, et al. High-dose melphalan and autologous stem-cell transplantation in patients with AL amyloidosis: An 8-year study. Ann Intern Med. 2004;140(2):85-93. https://doi.org/10.7326/0003-4819-140-2-200401200-00008
3. Dubrey SW, Cha K, Skinner M, LaValley M, Falk RH. Familial and primary (AL) cardiac amyloidosis: echocardiographically similar diseases with distinctly different clinical outcomes. Heart. 1997;78(1):74-82. https://doi.org/10.1136/hrt.78.1.74
4. Desport E, Bridoux F, Sirac C, Delbes S, Bender S, Fernandez B, et al. Al amyloidosis. Orphanet J Rare Dis. 2012;7:54. https://doi.org/10.1186/1750-1172-7-54
5. Gertz MA, Lacy MQ, Dispenzieri A. Amyloidosis. Hematol Oncol Clin North Am. 1999;13(6):1211-33. https://doi.org/10.1016/S0889-8588(05)70122-
6. D'Aguanno V, Ralli M, Artico M, Russo FY, Scarpa A, Fiore M, et al. Systemic amyloidosis: a contemporary overview. Clin Rev Allergy Immunol. 2019 Aug 2. https://doi.org/10.1007/s12016-019-08759-4
7. Wechalekar AD, Gillmore JD, Hawkins PN. Systemic amyloidosis. Lancet. 2016;387(10038):2641-54. https://doi.org/10.1016/S0140-6736(15)01274-X
8. Merlini G, Bellotti V. Molecular mechanisms of amyloidosis. N Engl J Med. 2003;349(6):583-96. https://doi.org/10.1056/NEJMra023144
9. Sipe JD, Benson MD, Buxbaum JN, Ikeda S, Merlini G, Saraiva MJ, et al. Amyloid fibril protein nomenclature: 2012 recommendations from the Nomenclature Committee of the International Society of Amyloidosis. Amyloid. 2012;19(4):167-70. https://doi.org/10.3109/13506129.2012.734345
10. Gertz MA, Comenzo R, Falk RH, Fermand JP, Hazenberg BP, Hawkins PN, et al. Definition of organ involvement and treatment response in immunoglobulin light chain amyloidosis (AL): a consensus opinion from the 10th International Symposium on Amyloid and Amyloidosis, Tours, France, 18-22 April 2004. Am J Hematol. 2005;79(4):319-28. https://doi.org/10.1002/ajh.20381
11. Gertz MA, Merlini G. Definition of organ involvement and response to treatment in AL amyloidosis: an updated consensus opinion. Amyloid 2010;17(Suppl 1):48-9. Available from: https://www.researchgate.net/publication/285021111
12. Kyle RA, Gertz MA. Primary systemic amyloidosis: Clinical and laboratory features in 474 cases. Semin Hematol 1995;32:45-9. PMID: 7878478.
13. Falk RH. Diagnosis and management of the cardiac amyloidoses. Circulation. 2005;112(13):2047-60. https://doi.org/10.1161/CIRCULATIONAHA.104.489187
14. Shi J, Guan J, Jiang B, Brenner DA, Del Monte F, Ward JE, et al. Amyloidogenic light chains induce cardiomyocyte contractile dysfunction and apoptosis via a non-canonical p38alpha MAPK pathway. Proc Natl Acad Sci USA. 2010;107(9):4188-93. https://doi.org/10.1073/pnas.0912263107
15. Chee CE, Lacy MQ, Dogan A, Zeldenrust SR, Gertz MA. Pitfalls in the diagnosis of primary amyloidosis. Clin Lymphoma Myeloma Leuk. 2010;10(3):177-80. https://doi.org/10.3816/CLML.2010.n.027
16. Mabru M, Dacher JN, Bauer F. Left ventricular hypertrophy: Cardiac magnetic resonance may help differentiate amyloidosis from hypertrophic cardiomyopathy. Arch Cardiovasc Dis. 2010;103(1):55-6. https://doi.org/10.1016/j.acvd.2008.11.006
17. Perfetto F, Moggi-Pignone A, Livi R, Tempestini A, Bergesio F, Matucci-Cerinic M. Systemic amyloidosis: A challenge for the rheumatologist. Nat Rev Rheumatol. 2010;6(7):417-29. https://doi.org/10.1038/nrrheum.2010.84
18. Gertz MA. Immunoglobulin light chain amyloidosis diagnosis and treatment algorithm 2018. Blood Cancer J. 2018;8(5):44. https://doi.org/10.1038/s41408-018-0080-9
19. Sanchorawala V. Light-chain (AL) amyloidosis: diagnosis and treatment. Clin J Am Soc Nephrol. 2006;1(6):1331-41. https://doi.org/10.2215/CJN.02740806
20. Comenzo RL, Zhang Y, Martinez C, Osman K, Herrera GA. The tropism of organ involvement in primary systemic amyloidosis: contributions of Ig V(L) germ line gene use and clonal plasma cell burden. Blood. 2001;98(3):714-20. https://doi.org/10.1182/blood.V98.3.714
21. Sanchorawala V, Blanchard E, Seldin DC, O'Hara C, Skinner M, Wright DG. AL amyloidosis associated with B-cell lymphoproliferative disorders: Frequency and treatment outcomes. Am J Hematol. 2006;81(9):692-5. https://doi.org/10.1002/ajh.20635
22. Erdem E, Erdem D, Dilek M, Kaya C, Karataş A, Kut E, et al. Red cell distribution width and mean platelet volume in amyloidosis. Clin Appl Thromb Hemost. 2014;20(3):334-7. https://doi.org/10.1177/1076029612462761
23. Uslu AU, Deveci K, Korkmaz S, Aydin B, Senel S, Sancakdar E, et al. Is neutrophil/lymphocyte ratio associated with subclinical inflammation and

amyloidosis in patients with familial Mediterranean fever? Biomed Res Int. 2013;2013:185317. https://doi.org/10.1155/2013/185317
24. Balta S, Ozturk C. The platelet-lymphocyte ratio: A simple, inexpensive and rapid prognostic marker for cardiovascular events. Platelets. 2015;26(7):680-1. https://doi.org/10.3109/09537104.2014.979340
25. Celikbilek M, Dogan S, Ozbakır O, Zararsız G, Kücük H, Gürsoy S, et al. Neutrophil-lymphocyte ratio as a predictor of disease severity in ulcerative colitis. J Clin Lab Anal. 2013;27(1):72-6. https://doi.org/10.1002/jcla.21564
26. Toprak E, Bozkurt M, Çakmak BD, Özçimen EE, Silahlı M, Yumru AE, et al. Platelet-to-lymphocyte ratio: A new inflammatory marker for the diagnosis of preterm premature rupture of membranes J Turk Ger Gynecol Assoc. 2017;18(3):122-6. https://doi.org/10.4274/jtgga.2017.0028
27. Aydın İ, Ağıllı M, Aydın FN, Kurt YG, Çaycı T, Taş A, et al. Farklı yaş gruplarında nötrofil/lenfosit oranı referans aralıkları. Gülhane Tıp Derg. 2015;57:414-8. https://doi.org/10.5455/gulhane.166398
28. Yurtdaş M, Özdemir M, Aladağ N. Investigation of neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio and mean platelet volume in patients with compensated heart failure. JAREM. 2018;8:67-71. https://doi.org/10.5152/jarem.2018.1632
29. Tsavaris N, Tsigalacis D, Kosmas C, Koufos C, Vaiopoulos G, Tzivras M, et al. Preliminary evaluation of the potential prognostic value of serum levels of immunoglobulins (IgA, IgM, IgG, IgE) in patients with gastric cancer. Int J Biol Markers. 1998;13(2):87-91. PMID: 9803356. https://doi.org/10.1177/172460089801300204
30. Talal N, Bunim JJ. The development of malignant lymphoma in the course of Sjögren's syndrome. Am J Med. 1964;36:529-40. https://doi.org/10.1016/0002-9343(64)90101-9
31. Bijlsma JW, Burmester G, da Silva JA, et al, editors. EULAR compendium on rheumatic diseases. London: BMJ Publishing Group; 2009. p. 322.
32. Kimman J, Bossuyt X, Blockmans D. Prognostic value of cryoglobulins, protein electrophoresis, and serum immunoglobulins for lymphoma development in patients with Sjögren's syndrome. A retrospective cohort study. Acta Clin Belg. 2018;73(3):169-181. https://doi.org/10.1080/17843286.2017.1373966
33. Voulgarelis M, Skopouli FN. Clinical, immunologic, and molecular factors predicting lymphoma development in Sjögren's syndrome patients. Clin Rev Allergy Immunol. 2007;32(3):265-74. https://doi.org/10.1007/s12016-007-8001-x
34. Maisch B, Alter P. Treatment options in myocarditis and inflammatory cardiomyopathy: Focus on i. v. immunoglobulins. Herz. 2018;43(5):423-430. https://doi.org/10.1007/s00059-018-4719-x
35. Grönwall C, Silverman GJ. Natural IgM: Beneficial autoantibodies for the control of inflammatory and autoimmune disease? J Clin Immunol. 2014;34 Suppl 1(01):S12-21. https://doi.org/10.1007/s10875-014-0025-4
36. Kishimoto C, Shioji K, Kinoshita M, Iwase T, Tamaki S, Fujii M, et al. Treatment of acute inflammatory cardiomyopathy with intravenous immunoglobulin ameliorates left ventricular function associated with suppression of inflammatory cytokines and decreased oxidative stress. Int J Cardiol. 2003;91(2-3):173-8. https://doi.org/10.1016/S0167-5273(03)00002-0
37. Gullestad L, Aass H, Fjeld JG, et al. Immunomodulating therapy with intravenous immunoglobulin in patients with chronic heart failure. Circulation. 2001;103(2): 220-5. https://doi.org/10.1161/01.CIR.103.2.220
38. Aukrust P, Yndestad A, Ueland T, et al. The role of intravenous immunoglobulin in the treatment of chronic heart failure. Int J Cardiol. 2006;112(1):40-5. https://doi.org/10.1016/j.ijcard.2006.05.015
39. Nussinovitch U, Shoenfeld Y. Intravenous immunoglobulin-indications and mechanisms in cardiovascular diseases. Autoimmun Rev. 2008;7(6):445-52. https://doi.org/10.1016/j.autrev.2008.04.001
40. Walpen AJ, Laumonier T, Aebi C, Mohacsi PJ, Rieben R. IgM enriched intravenous immunoglobulin inhibits classical pathway complement activation, but not bacterial killing by human serum. Xenotransplantation. 2004;11(2):141-8. https://doi.org/10.1046/j.1399-3089.2003.00098.x

Original Article

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Is the Use of a Stapler for Ileocolic Anastomosis Linked to Anastomotic Leakage?

İleokolik Anastomozda Stapler Kullanımı Anastomoz Kaçağı ile İlişkili midir?

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ABSTRACT

Objective: *The effect of stapler use on anastomotic leakage (AL) is uncertain in right hemicolectomy and ileocolic anastomosis. In this study, the effect of hand -sewn or assisted anastomosis performed in our center on anastomotic leakage will be investigated and short-term mortality results will be presented.*
Method: *The present study was planned as a retrospective review of the medical charts of patients who underwent right hemicolectomy and an ileocolic anastomosis for the treatment of right colon cancer. Patients 18 years and older, elective surgeries were included in the study. Emergency surgeries were excluded. Ileocolic anastomosis performed with staples or using hand-sewn technique was recorded. Anastomotic leakage and mortality rates at 1., and 6. postoperative months were compared.*
Results: *Of the 101 patients who underwent right hemicolectomy for a right colon adenocarcinoma, 66 (65.3%) underwent hand-sewn anastomosis and 35 (34.7%) ileocolic anastomosis using staplers. Anastomotic leakage developed in one patient (1.5%) in the hand-sewn group and in two patients (5.7%) in the stapled anastomosis group, although the difference was not statistically significant (p=0.23). Mortality rates at 1 month and 6 months did not differ significantly between the groups.*
Conclusion: *The present study shows that a stapled ileocolic anastomosis after a right hemicolectomy doesn't increase the risk of anastomotic leakage, and also has not any unfavorable effect on mortality in the short term.*

Keywords: *right hemicolectomy, ileocolic anastomosis, anastomotic leakage*

ÖZ

Amaç: *Sağ hemikolektomi ve ileokolik anastomozda stapler kullanımının anastomoz kaçağı (AK) üzerine etkisi belirsizdir. Bu çalışmada, merkezimizde, anastomozunstapler yardımlı veya elle yapılmış olmasının anastomoz kaçağı üzerine etkisi araştırılacak ve kısa dönem mortalite sonuçları sunulacaktır.*
Yöntem: *Bu çalışma, retrospekif bir çalışma olarak, sağ kolon veya hepatic fleksura kanseri nedeniyle sağ hemikolektomi ve ileokolik anastomoz yapılan hastaların dosyalarının taranması şeklinde planlandı. On sekiz yaş ve üzeri hastalar, elektif ameliyatlar çalışmaya dâhil edildi. Acil ameliyatlar çalışma dışı bırakıldı. İleokolik anastomoz tekniği stapler ile veya elle dikiş olarak kayıt edildi. AK, 1. ve 6. aydaki mortalite oranları bu iki grupta karşılaştırıldı.*
Bulgular: *Ocak 2013 ile Aralık 2018 arasında sağ kolon adenokarsinoması nedeniyle sağ hemikolektomi yapılan toplam 101 hastadan 66 (65,3%) hastaya elle dikiş ile anastomoz yapılırken 35 (34,7%) hastanın ileokolik anastomozu stapler ile yapıldı. Postoperative komplikasyon gelişimi ve patolojik sonuçlar bakımından gruplar arası istatiksel olarak fark yoktu. Elle dikiş anastomozda 1 (1,5%), stapler anastomozda ise 2 (5,7%) hastada AK gelişti, ancak bu fark istatiksel olarak anlamlı değildi (p=0,23). Sadece intraoperative kan transfüzyonu anastomoz kaçağı için risk faktörü olarak saptandı (p=0,002). Bir aylık ve 6 aylık mortalite oranlarında da gruplar arasında istatiksel olarak anlamlı fark yoktu.*
Sonuç: *Bu çalışma bize gösterdiki, sağ hemikolektomi sonrası ileokolik anastomozdastapler kullanımı AK riskini arttırmamaktadır. Ayrıca kısa dönem mortalite üzerine de olumsuz etkisi yoktur. Son yıllarda yapılmış ve AK için stapleri suçlayıcı çalışmaların aksine biz ileokolik anastomozda staplerin güvenle kullanılabileceğini düşünüyoruz.*

Anahtar kelimeler: *sağ hemikolektomi, ileokolik anastomoz, anastomoz kaçağı*

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INTRODUCTION

Anastomotic leakage continues to be an important cause of morbidity and mortality following colonic resection. The rates of anastomotic leakage remain stable, despite advances in surgical techniques for the treatment of colon cancer. Previous studies report anastomotic leakage rates of as high as 7.5% following colon cancer surgery [1-3].

Various risk factors have been described for anastomotic leakage, and studies have endeavored to improve techniques of anastomosis [4-8]. The risk factors for anastomotic leakage may be related to the patient or the surgical technique. Previous studies have identified the male gender, obesity, smoking, poor nutritional status and diabetes mellitus as risk factors for anastomotic leakage [6,9,10].

A large number of studies have been conducted on techniques of anastomosis. Studies on the development of anastomotic leakage comparing stapled and hand-sewn ileocolic anastomoses have yielded controversial results. In a Cochrane review of 825 patients with colon cancer conducted in 2011, the rate of anastomotic leakage was found to be lower in those undergoing stapled anastomosis than in those undergoing hand-sewn anastomosis [11]. In a Danish study involving 1,414 patients, the rate of anastomotic leakage was found to be higher in patients undergoing stapled anastomosis [12].

According to current data, the effect of stapled anastomosis in a right hemicolectomy on anastomotic leakage remains uncertain. The present study evaluates the effect of stapled versus hand-sewn anastomosis on anastomotic leakage in patients undergoing right hemicolectomy and an ileocolic anastomosis in our center, and presents mortality outcomes in the short term.

MATERIALS and METHOD

The present retrospective cohort study is a review of patients who underwent right hemicolectomy and ileocolic anastomosis due to cancer of the right colon and hepatic flexure in a gastroenterological surgery clinic. The study was approved by the local ethics committee of the hospital (number 2019.2/10-161). Patients aged 18 years and older who underwent right hemicolectomy and ileocolic anastomosis due to cancer of the right colon and hepatic flexure (with or without hepatic metastasectomy), patients with an Eastern Cooperative Oncology Group (ECOG) performance status of ≤3 and those undergoing elective surgery were included in the study. Patients with a pathological diagnosis other than adenocarcinoma, patients undergoing R1/R2 resection or palliative and emergency surgery were excluded.

The data of the patients who underwent surgery between January 2013 and December 2018 were retrieved from the hospital automated system and the archived records. All operations were performed by or under the supervision of gastroenterological surgeons. Patients with anastomotic leakage, those with a confirmed diagnosis of anastomotic leakage who required radiological imaging or surgery after developing symptoms of peritonitis within 30 days of surgery were not included in the study.

The patients were first divided into two groups according to the anastomotic technique employed (stapled or hand-sewn). Then, the demographic data and mortality rates of the patients at postoperative 1 month and 6 months in both groups were compared. The body mass index (BMI), hematocrit (Hct), albumin, creatinine and C-reactive protein (CRP) levels of patients within one week prior to surgery were recorded. Patients who had smoked in the last one month were classified as active smokers. End-to-side or side-to-side anastomoses were performed using both stapled and hand-sewn techniques [13,14]. In the hand-sewn technique, anastomosis was performed using a double-layer suture in a standard manner. Extracorporeal anastomosis was performed in all laparoscopic interventions.

Patients who had previously undergone abdominal surgery for any reason, and those who had received neoadjuvant chemotherapy were recorded. Charlson's comorbidity index (CCI) was used to define comorbidity [15]. In addition, length of hospital stay, preoperative FEV1, Nutritional Risk Screening (NRS 2002) and American Society of Anesthesiologists (ASA) scores were recorded. Data on open versus laparoscopic approach, operation time and perioperative blood transfusion were recorded. Standard

right hemicolectomy involved the ligation of the right branches of the middle colic vessels, while extended right hemicolectomy involved the ligation of the middle colic vessels at their origins. The pathological stage of the patients was determined according to the guidelines of the 8th edition of the American Joint Committee on Cancer (AJCC) Colon and Rectum Staging Manual.

Continuous variables with normal distribution were presented as mean±standard deviation (SD). Categorical variables were expressed as numbers and percentages. The clinical variables were compared using a Student's t-test and a Pearson's Chi-square test. A Mann-Whitney U test was used to compare continuous variables. A p value less than 0.05 was considered statistically significant in all tests. The statistical analysis was performed using the SPSS version 25.0 (IBM, Armonk, NY) software package.

RESULTS

A total of 101 patients underwent right hemicolectomy due to adenocarcinoma of the right colon between January 2013 and December 2018, while 66 (65.3%) patients underwent hand-sewn, and 35 (34.7%) stapled ileocolic anastomosis. Of the study patients, 52.5% were male, the mean BMI was 27.7 kg/m^2, 30.6% were smokers and 20.7% had a past history of abdominal surgery.

The CCI was ≥1 in 45.6% of the patients, and this rate was higher in the stapled than in the hand-sewn anastomosis group (p=0.011). Preoperative FEV1 was lower in the stapled anastomosis group (p=0.003), while other parameters that could be related to complications did not differ significantly between the groups. A comparison of the demographic data and clinical characteristics of patients of the stapled and hand-sewn anastomosis groups is presented in Table 1.

Table 1. Baseline characteristics of patients and method of anastomosis.

	Number of patients (n=101)	Hand-sewn anastomosis (n=66)	Stapled anastomosis (n=35)	p value
Age (years); mean±SD	61.1±13.5	60.7±13.0	61.9±14.5	0.44
Gender; n (%)				
Male	53 (52.5)	34 (51.5)	19 (54.2)	
Female	48 (47.5)	32 (48.5)	16 (45.8)	
BMI (kg/m^2); mean±SD	27.7±4.5	28.0±4.5	27.2±4.3	0.65
Smoker; n (%)	31 (30.6)	22 (33.3)	9 (25.7)	0.43
CCI; n (%)				0.011
0	55 (54.4)	42 (63.6)	13 (37.2)	
≥1	46 (45.6)	24 (36.4)	22 (62.8)	
NRS-2002 score; n(%)				0.16
0	51 (50.4)	30 (45.5)	21 (60)	
≥1	50 (49.6)	36 (54.5)	14 (40)	
Abdominal operation history; n (%)	21 (20.7)	14 (21.2)	7 (20)	0.88
FEV1 (%); mean±SD	95.3±19.6	97.5±15.7	91.0±25.1	0.003
ASA grade; n (%)				0.28
1-2	39	28	11	
3-4	62	38	24	
Hematocrit (%); mean±SD	32.15±5.40	33.12±5.51	30.36±4.76	0.33
Albumin; mean±SD	3.97±0.46	3.99±0.48	3.92±0.41	0.53
C-reactive protein >0,35 mg/dl (yes); n (%)	24 (23.7)	17 (25.7)	7 (20)	0.51
Creatinin >1,0 mg/dl (yes;) n (%)	16 (15.8)	12 (18.1)	4 (11.4)	0.37

SD, standard deviation; BMI, body mass index; CCI, charlson comorbidity index; NRS-2002, nutrition risk screening-2002; ASA, American society anesthesiologists

Table 2. Surgical procedure and postoperative course.

	Number of patients (n=101)	Hand-sewn anastomosis (n=66)	Stapled anastomosis (n=35)	p value
Surgical approach; n (%)				
Open	81 (80.1)	57 (86.3)	24 (68.5)	0.033
Laparoscopic	20 (19.9)	9 (13.7)	11 (31.5)	
Procedure; n (%)				0.71
Standard right hemicolectomy	80 (79.2)	53 (80.3)	27 (77.1)	
Extended right hemicolectomy	21 (21.8)	13 (19.7)	8 (22.9)	
Type of anastomoses; n (%)				
End-to-side	42 (41.5)	40 (60.6)	2 (5.7)	<0.001
Side-to-side	59 (58.5)	26 (39.4)	33 (94.3)	
Intraoperative transfusion (yes); n (%)	24 (23.7)	13 (19.6)	11 (31.4)	0.18
Surgical duration (min); mean±SD	189±57	180.6±46.1	204.8±71.3	<0.001
Complication (yes); n (%)	54 (53.4)	34 (51.5)	20 (57.1)	0.58
Anastomotic leak (yes); n (%)	3 (2.97)	1 (1.5)	2 (5.7)	0.23
Lenght of hospital stay (day); mean±SD	9.9±5.9	9.7±5.8	10.3±6.1	0.55
pT-stage; n(%)				0.08
T1-T2	8 (7.9)	3 (4.6)	5 (14.3)	
T3-T4	93 (92.3)	63 (95.4)	30 (85.7)	
pN-stage; n (%)				0.22
N0	58 (57.4)	35 (53)	23 (65.7)	
N1-2	43 (42.6)	31 (47)	12 (34.3)	
Pathologic stage; n (%)				0.54
1-2	56 (55.6)	34 (51.5)	22 (62.8)	
3	41 (40.5)	29 (43.9)	12 (34.2)	
4	4 (3.9)	3 (4.6)	1 (3)	

A laparoscopic approach was used in 19.9% of the patients. In total, 21.8% of the patients underwent an extended right hemicolectomy. Hand-sewn anastomosis most commonly performed for end-to-side anastomosis (60.6%), and stapled anastomosis for side-to-side anastomosis (94.3%) (p<0.001). There was no statistically significant difference between the groups in terms of intraoperative blood transfusions, postoperative complications and the results of pathological examinations. The operating time was longer in the stapled anastomosis group (p<0.001). Anastomotic leakage developed in one patient in the hand-sewn (1.5%) and in two patients in the stapled anastomosis group (5.7%), however the difference between the groups was not statistically significant (p=0.23). Details regarding surgery and patient outcomes are presented in Table 2.

Anastomotic leakage was detected in a total of three patients, without any relationship with advanced age, gender, operation time, laparoscopic approach, smoking status or anastomosis technique. Only intraoperative blood transfusion was found to be a risk factor for anastomotic leakage (p=0.002). The data on anastomotic leakage is presented in Table 3.

Furthermore, two patients developed anastomotic leakage and underwent repeat surgery and an end ileostomy. Of these, one patient was followed up with a conservative approach, and the fistula in this patient closed spontaneously within 30 days of surgery.

One patient in the hand-sewn anastomosis group died within 30 days of surgery, however when compared to the stapled anastomosis group (p=0.46), the difference between groups was not statistically significant. Furthermore, two patients in the hand-

Table 3. Incidence and univariate analysis of factors associated with anastomotic leak.

	n	(%)	p value
Age; years			
<65	2/56	(3,5)	0.691
≥65	1/45	(2,2)	
Gender			
Male	2/53	(3,7)	0.617
Female	1/48	(2)	
Intraoperative blood transfusion			
Yes	3/24	(12,5)	0.002
No	0/77	(0)	
Operation time, min			
≥192	2/34	(5,8)	0.219
<192	1/67	(1,5)	
Surgical approach			
Open	2/81	(2,4)	0.550
Laparoscopic	1/20	(5)	
Abdominal operation history			
No	3/80	(3,7)	0.368
Yes	0/21	(0)	
CCI≥1			
No	1/55	(1,8)	0.456
Yes	2/46	(4,3)	
Smoker			
No	3/70	(4,2)	0.242
Yes	0/31	(0)	
Anastomotic technique			
Hand-sewn	1/66	(1,5)	0.237
Stapled	2/35	(5,7)	
Type of anastomoses			
End-to-side	1/42	(2,3)	0.768
Side-to-side	2/59	(3,3)	

CCI: charlson comorbidity index

sewn anastomosis group died within 6 months of surgery, while no mortality was recorded in the stapled anastomosis group, and again the difference between the two groups was not statistically significant (p=0.29).

DISCUSSION

The present study shows that neither the stapled nor hand-sewn ileocolic anastomosis approach to the treatment of right colon cancer poses a risk for the development of anastomotic leakage. The overall rate of anastomotic leakage was 2.9% in the study, and the use of stapler did not increase the rate of anastomotic leakage. A statistical analysis identified only intraoperative blood transfusion as being related to the development of anastomotic leakage. A homogeneous group was created in our study to include only patients with right colon cancer. All operations were performed with the purpose of achieving a cure, and in line with oncological principles.

There is a lack of consensus in the literature regarding the stapled ileocolic anastomosis. In a Cochrane review of 955 patients undergoing ileocolic anastomosis in 2007, stapled end-to-end anastomosis was reported to reduce the risk of anastomotic leakage, and the use of a stapler, as a popular approach at the time in surgical interventions, prevails today [16]. The same study did not identify any difference between the groups in terms of other anastomotic complications. Today, the situation is reversed for ileocolic anastomosis, which has been associated with low rates of anastomotic leakage, while the use of stapler is regularly implicated in anastomotic leakage. Some recent studies have suggested that the use of stapler in ileocolic anastomosis increases the risk of anastomotic leakage [6,10,17,18].

Aside from the anastomosis technique, male gender, smoking status and intraoperative blood transfusion were also identified as risk factors for anastomotic leakage following right hemicolectomy [12,17]. In a Swedish study on a large series of patients, Gustafsson et al. reported male gender and emergency surgery, in addition to stapled anastomosis to be the risk factors for anastomotic leakage after ileocolic anastomosis. Similarly, a Danish study involving 1,414 patients reported anastomotic leakage in 3.2% of patients, while smoking and the stapled anastomosis approach were identified as risk factors for anastomotic leakage [12]. The present study evaluated smoking status, operation time and Charlson's comorbidity index and their relationship with healing following anastomosis, and found that while these parameters may be risky for wound healing, they did not pose a risk for anastomotic leakage.

Different risk factors have been identified for anastomotic leakage in ileocolic anastomosis in various studies, and the stapled anastomosis technique has been most commonly implicated. Stapled anastomosis is an accepted technique worldwide due to its ease of use, while the use of the stapled approach in

ileocolic anastomosis has been linked to the development of anastomotic leakage. However any explanation has not yet been provided as to how it increases the rate of anastomotic leakage [12]. Although these studies include large patient groups, they cannot be regarded as a substitute for randomized and controlled trials. The same study also found that long-term survival is independent of the anastomosis technique, which was corroborated by a further study [19]. A few studies, however, have reported the benefits of stapled anastomosis regarding overall survival [20]. The present study, therefore, has evaluated survival in the short term rather than in the long term. It was noted that the use of a stapler had no effect either on the development of anastomotic leakage or mortality in the short term (postoperative 1 and 6 months).

The present study has some limitations, the first of which is its retrospective design, because the anastomosis technique is selected by the surgeon based on the condition of the patient. Dividing the patients into stapled and hand-sewn anastomosis groups can be seen as a simple grouping technique, however other data related to the anastomosis technique is reported in the present study. The number of patients was relatively low when compared to the large series assessed in previous studies, and a logistic regression analysis was not possible due to the low rates of anastomotic leakage.

The present study shows that the use of stapler in ileocolic anastomosis procedures following a right hemicolectomy does not increase the risk of anastomotic leakage, and has not any unfavorable effect on mortality in the short term. In contrast to the recent studies implicating the stapled approach in anastomotic leakage, the present study suggests that a stapler may be used in ileocolic anastomosis. That said, the authors believe that randomized and controlled studies are required to obtain more reliable data.

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REFERENCES

1. Bakker IS, Grossmann I, Henneman D, Havenga K, Wiggers T. Risk factors for anastomotic leakage and leak-related mortality after colonic cancer surgery in a nationwide audit. Br J Surg. 2014;101(4):424-32. https://doi.org/10.1002/bjs.9395
2. Krarup PM, Nordholm-Carstensen A, Jorgensen LN, Harling H. Anastomotic leak increases distant recurrence and long-term mortality after curative resection for colonic cancer: a nationwide cohort study. Ann Surg. 2014;259(5):930-8. https://doi.org/10.1097/SLA.0b013e3182a6f2fc
3. Takahashi H, Haraguchi N, Nishimura J, Hata T, Yamamoto H, Matsuda C, et al. The Severity of anastomotic leakage may negatively impact the long-term prognosis of colorectal cancer. Anticancer Res. 2018;38(1):533-9. https://doi.org/10.21873/anticanres.12255
4. Jestin P, Pahlman L, Gunnarsson U. Risk factors for anastomotic leakage after rectal cancer surgery: a case-control study. Colorectal Dis. 2008;10(7):715-21. https://doi.org/10.1111/j.1463-1318.2007.01466.x
5. Matthiessen P, Hallbook O, Andersson M, Rutegård J, Sjödahl R. Risk factors for an astomotic leakage after anterior resection of the rectum. Colorectal Dis. 2004;6(6):462-9. https://doi.org/10.1111/j.1463-1318.2004.00657.x
6. Jessen M, Nerstrøm M, Wilbek TE, Roepstorff S, Rasmussen MS, Krarup PM. Risk factors for clinical anastomotic leakage after right hemicolectomy. Int J Colorectal Dis. 2016;31(9): 1619-24. https://doi.org/10.1007/s00384-016-2623-5
7. Aday U, Gundes E, Ciyiltepe H, Cetin DA, Deger KC, Gulmez S, et al. Does antiaggregant administration lead to early diagnosis in proximal colon cancer? North Clin Istanb. 2017;4(2):173-9. https://doi.org/10.14744/nci.2017.80148
8. Pramateftakis MG, Vrakas G, Hatzigianni P, Tsachalis T, Matzoros I, Christoforidis E, et al. The hand sewn anastomosis after colon resection due to colonic cancer. Tech Coloproctol. 2010;14 Suppl 1:S57-9. https://doi.org/10.1007/s10151-010-0612-1
9. García-Granero E, Navarro F, CerdánSantacruz C, Frasson M, García-Granero A, Marinello F, et al. Individual surgeon is an independent risk factor for leak after double-stapled colorectal anastomosis: An institutional analysis of 800 patients. Surgery. 2017;162(5):1006-16. https://doi.org/10.1016/j.surg.2017.05.023
10. Frasson M, Granero-Castro P, RamosRodríguez JL, Flor-Lorente B, Braithwaite M, MartíMartínez E, et al. Risk factors for anastomotic leak and postoperative morbidity and mortality after elective right colectomy for cancer: results from a prospective, multicentric study of 1102 patients. Int J Colorectal Dis. 2016;31(1):105-14. https://doi.org/10.1007/s00384-015-2376-6
11. Choy PY, Bissett IP, Docherty JG, Parry BR, Merrie A, Fitzgerald A. Stapled versus hand sewn methods for ileocolic anastomoses. Cochrane Database Syst Rev. 2011;(9):CD004320. https://doi.org/10.1002/14651858.CD004320.pub3
12. Nordholm-Carstensen A, SchnackRasmussen M, Krarup PM. Increased leak rates following stapled versus hand sewn ileocolic anastomosis in patients with right-sided colon cancer: a nationwide cohort study. Dis Colon Rectum. 2019;62(5): 542-8. https://doi.org/10.1097/DCR.0000000000001289
13. Steichen FM. The use of staplers in anatomical side-to-side and functional end-to-end enteroanastomoses. Surgery. 1968;64(5):948-53. PMID: 5687844.
14. Meagher AP, Wolff BG. Right hemicolectomy with a linear cutting stapler. Dis Colon Rectum. 1994;37(10):1043-5. https://doi.org/10.1007/BF02049322
15. Charlson M, Wells MT, Ullman R, King F, Shmukler C. The

Charlson comorbidity index can be used prospectively to identify patients who will incur high future costs. PLoS One. 2014;9(12):e112479. https://doi.org/10.1371/journal.pone.0112479
16. Choy PY, Bissett IP, Docherty JG, Parry BR, Merrie AE. Stapled versus hand sewn methods for ileocolic anastomoses. Cochrane Database Syst Rev. 2007;(3):CD004320. https://doi.org/10.1002/14651858.CD004320.pub2
17. Gustafsson P, Jestin P, Gunnarsson U, Lindforss U. Higher frequency of anastomotic leakage with stapled compared to hand sewn ileocolic anastomosis in a large population-based study. World J Surg. 2015;39(7):1834-9. https://doi.org/10.1007/s00268-015-2996-6
18. 2015 European Society of Coloproctology collaborating group. The relationship between method of anastomosis and anastomotic failure after right hemicolectomy and ileo-caecal resection: an international snapshot audit. Colorectal Dis. 2017;19(8):e296-311. https://doi.org/10.1111/codi.13646
19. Wolmark N, Gordon PH, Fisher B, et al. A comparison of stapled and hand sewn anastomoses in patients undergoing resection for Dukes' B and C colorectal cancer. An analysis of disease-free survival and survival from the NSABP prospective clinical trials. Dis Colon Rectum. 1986;29(5):344-50. https://doi.org/10.1007/BF02554128
20. Akyol AM, McGregor JR, Galloway DJ. Recurrence of colorectal cancer after sutured and stapled large bowel anastomoses. Br J Surg. 1991;78(11):1297-300. https://doi.org/10.1002/bjs.1800781107

Original Article

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Dosimetric Comparison of Volumetric Modulated Arc Therapy (VMAT) and Helical Tomotherapy for Hippocampal-Avoidance Prophylactic Whole Brain Radiotherapy (HA-PWBRT) in Small Cell Lung Cancer; Treatment Plans Based Study

Küçük Hücreli Akciğer Kanserinde Hipokampüs-Korumalı Profilaktik Tüm Beyin Radyoterapisinin (HA-PWBRT) Volumetrik Ark Tedavisi ve Helikal Tomoterapinin Dozimetrik Karşılaştırması; Tedavi Planlarına Dayalı Çalışma

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ABSTRACT

Objective: *To carry out a dosimetric evaluation of two different advanced radiotherapy modalities prophylactic-whole brain radiotherapy with hippocampal avoidance in the treatment of patients with small cell lung cancer (SCLC).*
Method: *Computed tomography with fused magnetic resonance images of patients who were diagnosed with SCLC without brain metastasis were combined and re-planned with volumetric-modulated arc (VMAT) and also helical tomotherapy (n=10). Doses of 25 Gy were prescribed in 10 fractions for the whole brain volume. Planning was performed according to RTOG 0933 criteria for hippocampal avoidance (Dmax ≤16Gy, D100% ≤9Gy). Planning target volume (PTV), homogeneity index (HI), conformity index (CI), minimum dose (Dmin) maximum dose (Dmax) and dose for organs at risk (OARs) were compared.*
Results: *In the analysis of PTV data, no significant difference was found between the helical tomotherapy and VMAT for D95 values, the minimum, maximum and mean values of PTV brain doses were higher in the VMAT arm (p=0,000). When comparing PTV HI and CI values, VMAT was significantly superior in CI value (p=0.033), but there was no significant superiority in HI values (p=0.499). When compared statistically, and radiation doses delivered to hippocampus were evaluated it was seen that helical tomotherapy provided significantly better hippocampal protection than VMAT (right: 6.43Gy vs 8.94Gy; p=0.000; left: 6.10Gy vs 8.96Gy; p=0.000), mean (right 7.60Gy vs 11.42Gy; p=0.000; left: 7.56Gy vs 11.70Gy; p =0.000) and maximum (right: 15.83Gy vs 20.8Gy; p=0.003; left: 15.99Gy vs 20.13Gy; p=0.009). When analyzing lens doses, it was observed that the mean and maximum dose values of both right and left lenses were much lower in helical tomotherapy (mean right: 4.52Gy vs 12,34Gy; p=0,000 and left: 4.66Gy vs 11.69Gy; p=.,000; maximum right: 3.41Gy vs 10.54Gy; p=0.00 and left: 3.53Gy vs 10.10Gy; p=0,000).*
Conclusion: *Acceptable treatment plans have been developed in both radiotherapy methods. Both hippocampus avoidance region and lens doses were found to be significantly superior in helical tomotherapy when compared with VMAT.*

Keywords: *dosimetry, hippocampal avoidance, VMAT, helical tomotherapy*

ÖZ

Amaç: *Küçük hücreli akciğer kanseri hastalarının tedavisinde hipokampüs koruması ile profilaktik-tüm beyin radyoterapisinin iki farklı ileri radyoterapi tekniği ile dozimetrik olarak değerlendirmesi amaçlanmaktadır.*
Yöntem: *Beyin metastazı olmayan küçük hücreli akciğer kanseri teşhisi konulan, 10 hastanın bilgisayarlı tomografi ile manyetik rezonans görüntüleri ile birleştirilerek hacimsel ark tedavisi (VMAT) ve helikal tomoterapi ile yeniden planlandı. Tüm beyin hacmine 10 fraksiyonda 25 Gy dozu reçete edildi. Hipokampüs koruması için RTOG 0933 kriterlerine göre planlama yapıldı (Dmax ≤16Gy, D100% ≤9Gy). Planlanan hedef hacmi (PTV), homojenite indeksi (HI), uygunluk indeksi (CI), maksimum doz (Dmax), minimum doz (Dmin) ve risk altındaki organların (OAR) aldıkları dozlar karşılaştırıldı.*
Bulgular: *PTV verilerinin analizinde, helikal tomoterapi ve VMAT arasında D95 değerleri için anlamlı bir fark bulunmadı (p=0,141), VMAT kolunda PTV beyin dozlarının minimum, maksimum ve ortalama değerleri daha yüksekti (p=0,000). PTV; HI ve CI değerleri karşılaştırıldığında, VMAT CI değerinde önemli ölçüde üstündü (p=0.033), ancak HI değerlerinde önemli bir üstünlük yoktu (p=0.499). İstatistiksel olarak karşılaştırıldığında, hipokampus minimum (Sağ: 6,43Gy&8,94Gy; p=0,000; Sol: 6,10Gy&8,96Gy; p=0,000), ortalama (Sağ:7,60Gy&11,40Gy; p=0,000; Sol: 7,56Gy&11,70Gy; p=0,000) ve maksimum (Sağ: 15,83Gy&20,8Gy; p=0,003; Sol: 15,99Gy&20,13Gy; p=0,009) değerlerine bakıldığında helikal tomoterapi, VMAT'den önemli ölçüde daha iyi hipokampüs koruması sağladığı görüldü. Lens dozları analiz edildiğinde, sağ ve sol lenslerin ortalama ve maksimum doz değerlerinin helikal tomoterapide çok daha düşük olduğu gözlendi (Ortalama Sağ: 4,52Gy&12,34Gy;p=0,000 ve Sol :4,66Gy &11,69Gy; p=0,000; Maksimum Sağ:3,41Gy&10,54Gy; p=0,000 ve Sol: 3,53Gy&10,10Gy; p=0,000).*
Sonuç: *Her iki radyoterapi yönteminde de kabul edilebilir tedavi planları geliştirilmiştir. Hem hipokampus korunması hem de lens dozları helikal tomoterapide VMAT'den anlamlı olarak daha üstün olduğu görüldü.*

Anahtar kelimeler: *dozimetri, hipokampus koruması, VMAT, helikal tomoterapi*

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INTRODUCTION

PProphylactic whole brain irradiation (PWBRT) that decreases brain metastasis is administered as a treatment for patients with limited stage small cell lung cancer (SCLC) [1] and also patients with extensive-stage SCLC who respond to systemic therapy [2]. PWBRT is widely used in patients with SCLC. The findings in a study showed that PWBRT could prolong survival of the patients and only a mild reaction to chemotherapy could be observed [3]. Using PWBRT for patients with non-small-cell lung cancer (NSCLC) has recently taken considerable attention because PWBRT is likely to lessen the incidence of brain metastasis, but PWBRT does not prolong survival of the patients [4,5].

Whole brain radiotherapy (WBRT) is mainly administered for patients who have many brain metastases that arose from various malignancies [6]. The development of WBRT cerebellar dysfunction, short-term memory and reduced ability to learn may lead to various side effects, such as disorders in neurocognitive functions [7,8]. The hippocampus has a vital effect on memory consolidation and emotional learning [9,10]. In the subgranular zone, the interruption of neurogenesis may bring on damaged memory [11-14]. The harm that arises from radiation to the hippocampus unfavourably affects cognitive function [15-17].

With the novel radiation technologies like dynamic intensity-modulated radiation therapy (dIMRT) and step-and-shoot intensity-modulated radiotherapy (sIMRT) volumetric-modulated arc therapy (VMAT), and helical tomotherapy that were used to prevent the loss of neuronal stem cells arising from radiation exposure. Hippocampal- avoidance (HA) radiation techniques are developing day by day. We should note that the first data in the literature that was obtained from helical tomotherapy or LINAC-based intensity-modulated radiotherapy (IMRT) have already verified practicality of the method used [18,19]. Sood et al.'s findings showed the practicality of WBRT using VMAT both to spare the hippocampus and also decrease dose to OARs significantly (e.g., parotid glands, cochleae, scalp and ear canal) [20]. Tomotherapy brings a homogeneous dose delivery to the whole brain and evades the hippocampus in a conformal manner [21]. However, surprisingly, in the literature, it is still not conclusive which approach is the most proper HA-PWBRT approach for VMAT and helical tomotherapy for patients with SCLC, which should be further investigated. To shed light into the literature, in this study, a dosimetric comparison has been made between VMAT and helical tomotherapy to investigate the technical advantages regarding the two treatment modalities in HA-PWBRT.

MATERIAL and METHODS

Study design

This study was approved by Ethics committee of SBU Kartal Dr Lütfi Kırdar Training and Research Hospital (2020/514/174/3). This study was a radiotherapy planning study aimed at testing the HA-WBRT planning technique with VMAT and helical tomotherapy.

Population:

Ten patients who were previously treated with HA-WBRT, according to the RTOG 0933 protocol, using helical tomotherapy whose treatment was re-planned with VMAT technique and compared to helical tomotherapy treatment plans.These ten patients who were treated between September 2018 and February 2019 and who did not have brain metastasis by magnetic resonance imaging (MRI) were included in the study. All patients had been histologically diagnosed with small cell lung cancer (SCLC), seven of them were in limited stage and 3 were in extensive stage

Simulation and contouring

In this study, computed tomography (CT) data were obtained following RTOG 0933 criteria. All of the patients in this study had MRI with axial T2-weighted and gadolinium contrast-enhanced T1-weighted sequences for hippocampus contouring with slice thickness not extending 1.5 mm. To obtain a CT simulation with a slice thickness of 1.25 mm, the patients were immobilized in the supine position. The MRI images were semi-automatically fused to the simulation CT by the radiation oncologist in the Eclipse planning system (Varian Medical Systems, Palo Alto, CA, USA). Contouring was performed with 2D and 3D brushes on axial images. Contours for targets and other normal structures which encompassed the brainstem, eyes, cord, chiasm, brain, optic nerves lenses, and external patient contour w/

immobilization devices, were contoured in treatment contouring system SomoVision (Varian Medical Systems, Palo Alto, CA,USA)

Hippocampus was contoured taking into account of RTOG shaping guides. The hippocampus head and amygdala are located in the gray matter on the other side of the temporal horn. At the level of the pons and pituitary gland, lower part of the hippocampus was located. Contours were ended at the splenium of the corpus callosum (Clinical target volume-hippocampal avoidance: CTV-HA). Planning target volume-hippocampal avoidance (PTV-HA) zone was produced with 5 mm contour widening from CTV-HA. The whole brain PTV with the hippocampal avoidance region was created by subtracting this PTV-HA region from the brain parenchyma to C1 and C2. The same radiation oncologist defined the contours and the contours were also peer reviewed. Afterwards, the contours in sagittal, coronal and axial projections were rechecked by a neuroradiologist. Before starting treatment, according to the protocol, the dose up to 100% of the hippocampal dose should not be more than 9 Gy, the maximal hippocampal dose should not be more than 16 Gy. It was stated that 100% of the hippocampus that exceeded 10 Gy and the maximum hippocampal dose that exceeded17 Gy were unacceptable and re-planning was required.

HA-PWBRT Planning technique

Volumetric ARC (VMAT) technique:

The VMAT plans were created with the Trilogy (Varian Medical Systems Inc, Palo Alto, USA) linac with a maximum rate of 600 MU / min with 120 high resolution multi-leaf collimator (MLC) with a central width of 2.5 mm in 10x10 areas and 5 mm leaf width in others. The VMAT technique offers single or multiple volumetric modulation arcs with varying portal speeds, dose rates and MLC leaf movement speeds to achieve optimum target coverage. Treatment plans were optimized and calculated using Eclipse (Varian Medical Systems, Inc) TPS version 13.7.0 and an Anisotropic Analytical Algorithm (AAA). Each plan for this study consisted of six non-planar arcs with collimator angle rotations of 30 and 330 degrees, gantry angles of 181 to 179 and 181 to 340 degrees, and couch rotations of 10, 270 and 350 degrees. We used the Arc Geometry Tool to create arcs. The Progressive Resolution Optimizer (version 13.7.10) used for VMAT optimization [22].

Helical Tomotherapy Technique:

The helical Tomotherapy plans were created for the treatment planning workstation (Precision 1.1.0.0; Accuray,, Sunnyvale, CA, USA) with the Collapsed Cone Convolution / Superposition algorithm using 6MV unflattened photon beam by 64 dual multi-leaf collimators). All helical tomotherapy plans are designed for each patient: 2.5 cm-dynamic jaw and plans have a step value in the range of 0.172 to 0.225 and a modulation factor in the range of 1.6 to 3 [23].

Statistical analysis:

Statistical analysis and comparisons between the treatment plans of two different irradiation techniques were performed using a one-way Analysis of Variance (ANOVA) and SPSS Version 21 statistical software (IBM, USA) In order to be statistically significant, p values must be below $p < .05$ [24].

RESULTS

In the study, HA-PWBRT radiotherapy plans of 10 male patients were compared. The mean age of patients receiving radiotherapy treatment was 56.3 (range; 47-68) years. Seven patients had limited stage SCLC and 3 patients extensive-stage SCLC.The mean total brain volume was 1396 cc, volumes of the right, and left hippocampi were 1.55 cc, and 1.59 cc, respectively. For the treatment of HA- PWBRT irradiation, the total treatment dose for each patient was planned to be 25 Gy/ 10 fractions (Table 1).

Table 1. Patient characteristics of HA-PWBRT.

Gender of Total Patients	**Male 10 /10**
Pathology of the Total Patients	Limited- Stage SCLC 7/10 Extensive-Stage SCLC 3/10
Average Age of Total Patients	56.3 (47-68)
Total Treatment Dose	25Gy/10 fractions
Total Brain Volume Mean of Total Patients	1396 cc
Hippocampus Right Volume Mean of Total Patients	1.55 cc
Hippocampus Left Volume Mean of Total Patients	1,59 cc

Table 2. Treatment characteristics of the HA-PWBRT with Helical Tomotherapy &VMAT.

Structure	Dosimetric Parameter	Helical Tomotherapy	VMAT	p value
PTV	D95	23,01	23,77	*p=0.141*
	minimum	5,99	8,99	***p=0,000***
	maximum	28,34	33,75	***p=0,000***
	mean	26,52	28,75	***p=0,000***
	HI	0,39	0,37	*p=0.499*
	CI	1,17	1,09	***p=0.033***
Right Hippocampus	minimum	6,13	8,94	***p=0,000***
	maximum	15,83	20,80	***p=0,003***
	mean	7,60	11,42	***p=0,000***
Left Hippocampus	minimum	6,10	8,96	***p=0,000***
	maximum	15,99	20,13	***p=0,009***
	mean	7,56	11,70	***p=0,000***
Right Lens	maximum	3,41	10,54	***p=0,000***
	mean	4,52	12,34	***p=0,000***
Left Lens	maximum	3,53	10,19	***p=0,000***
	mean	4,66	11,69	***p=0,000***

Dose-volume values obtained from patient plans were compared using two different radiotherapy treatment methods.In this study, the hippocampus (left-right); lens (left-right); Planning target volume (PTV); maximum, minimum and mean dose, the dose of 95% of the volume (D95) PTV, homogeneity index (HI) and conformity index (CI) were analyzed and two radiotherapy techniques were compared in terms of these parameters.

As a result of the analyzes, in the comparison of two modalities it is seen that the average of minimum (tomotherapy TPS; Right: 6,13 Gy; Left: 6,10 Gy & VMAT TPS; Right: 8,94Gy; Left: 9,96 Gy p right=0,000& p left: 0,000), mean (Tomotherapy TPS; Right: 7,6 Gy; Left:6,10 Gy & VMAT TPS; Right: 11,4Gy; Left: 11,7 Gy; p right=0,000& p left: 0,000) and maximum (Tomotherapy TPS; Right: 15.8 Gy; Left: 15,8 Gy & VMAT TPS; Right: 20,8Gy; Left: 20,13Gy; p right=0,003& p left: 0,009) dose values of both right and left hippocampus regions were lower than the values obtained from tomotherapy treatment planning system (TPS) (Table 2). When compared statistically, according to the p values of hippocampus minimum (Right p=0,000; Left p=0,000), mean (Right p=0,000; Left p=0,000) and maximum (Right p=0.003; Left p=0.009) shows that tomotherapy TPS provides significantly better hippocampal protection than VMAT TPS (Table 2, Figure 1, 2).

For the analysis of the lens doses it was observed that mean and maximum doses of both right and left lenses were lower than the values obtained from helical tomotherapy TPS. The maximal and mean doses were as follows: right lens; 3,41Gy,4,52Gy, and 10,54 Gy,12,34Gy and left lens; 3,53Gy,4,66Gy and 10,19Gy,11,69Gy for helical tomotherapy and VMAT, respectively. Statistically, both p values of the right lens (mean p=0,000, maximum p=0,000) and the left lens (mean p=0,000, maximum p=0,000) reveal that helical tomotherapy TPS lens protection is significantly superior than the VMAT TPS (Figure 1, 2).

In the analyzes of the PTV data, there is no significant difference (p=0,141) for the D95 values between tomotherapy and VMAT TPS. On the other hand for the PTV minimum (p=0,000), maximum (p=0,000) and mean (p=0,000) values were significantly higher in the VMAT TPS arm. When PTV HI and CI values were compared, VMAT TPS was significantly superior (p=0.033) in the CI value, but no significant superiority was detected in HI values (p=0.499) (Table 2).

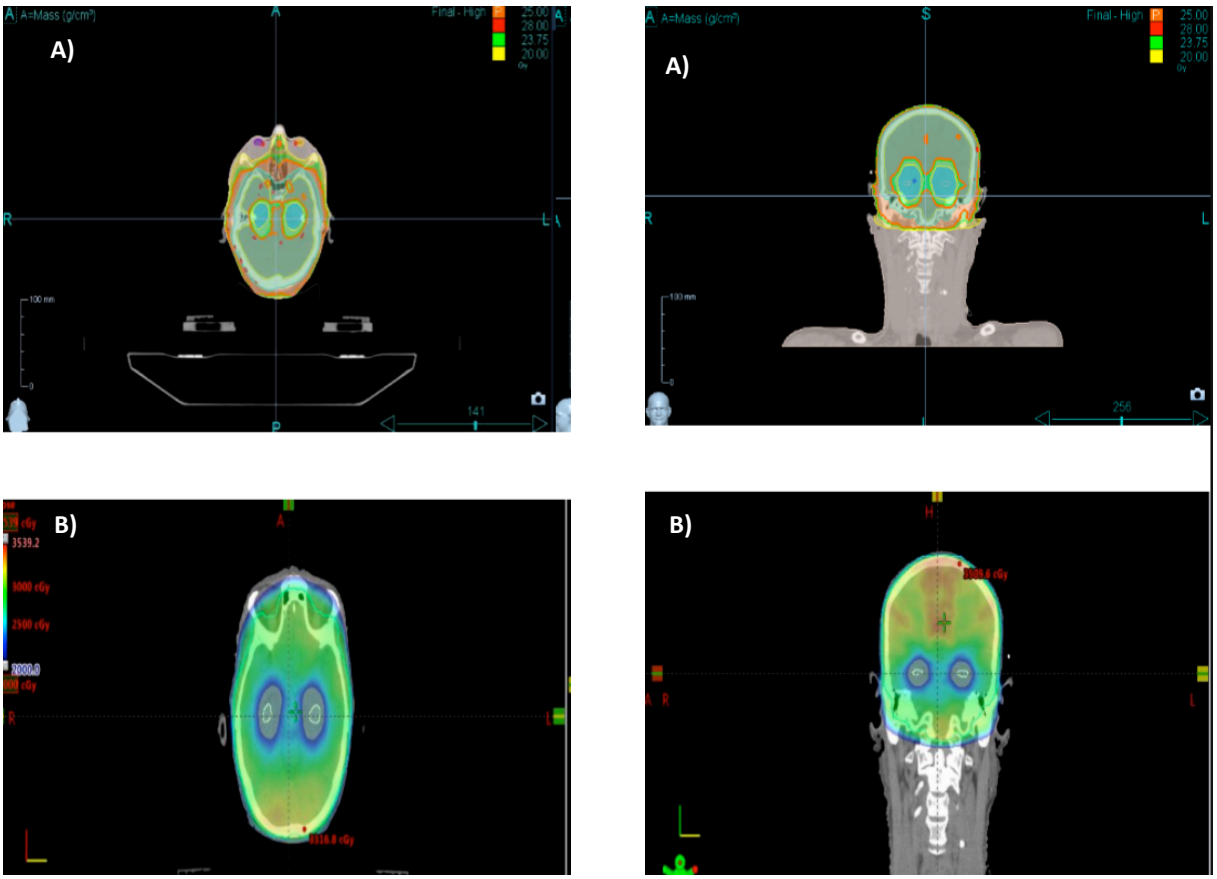

Figure 1. Color wash comparison of dose distributions for two modalities in a representative patient.
A; Helical Tomotherapy,
B; VMAT: Volumetric-Modulated Arc Therapy

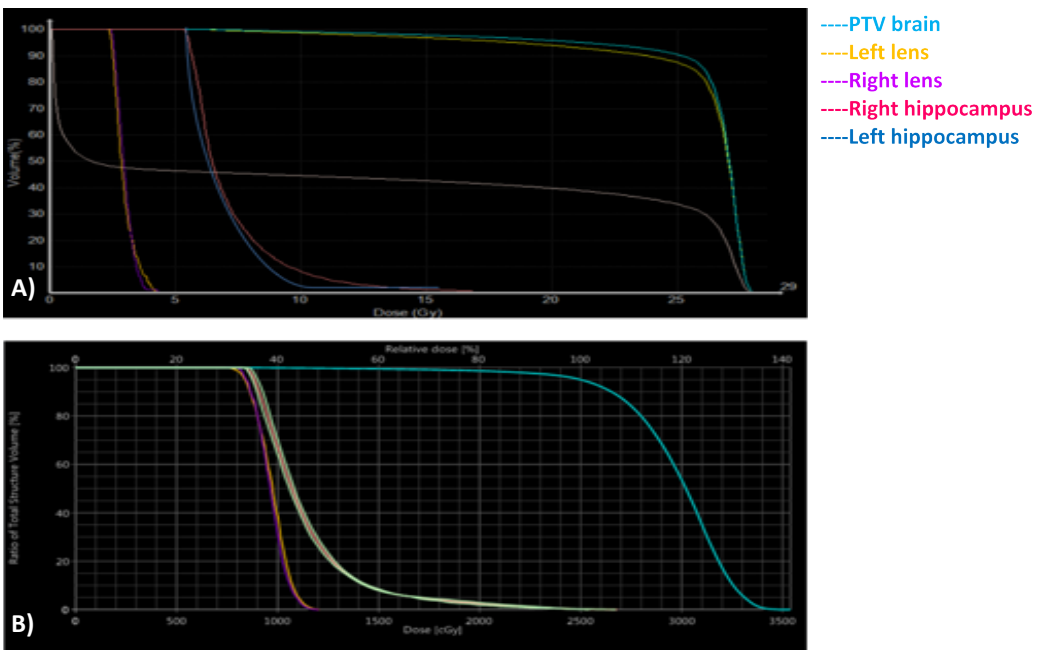

Figure 1. Color wash comparison of dose distributions for two modalities in a representative patient.
A; Helical Tomotherapy,
B; VMAT: Volumetric-Modulated Arc Therapy

DISCUSSION

The degree of the harm that arises from radiation doses delivered to the organs at risk (OARs) has been overlooked in the literature. Thanks to the improvement in the sIMRT, dIMRT, VMAT and helical tomotherapy, recently, we can reduce neurocognitive toxicity and deficits of the OARs Studies have reported that harm to the hippocampus that arises from radiation exposure may have a vital effect on the cognitive decline of the patients. Deficits in learning and memory were seen in the patients that were administered whole brain radiotherapy which were discussed, and suggested to be associated with the hippocampal injury [25]. We highlighted the dosimetric benefits of two advanced radiotherapy modalities (VMAT and helical tomotherapy TPS) with the hippocampus and OARs so as to use this treatment of PWBRT in the present study.

In our study, the PTV minimum (p=0,000), maximum (p=0,000) and mean (p=0,000) values were significantly higher in the VMAT TPS arm. When the PTV HI and CI values were compared , VMAT TPS was found to be statistically significantly superior (p=0.033) in the CI value, but without any significant superiority in HI values (p=0.499). Helical Tomotherapy TPS can provide a better dose distribution in the PTV brain compared to the VMAT TPS, as Cozzi et al. reported [26].

The helical tomotherapy and IMRT in hippocampal sparing whole brain radiotherapy were investigated by Gondi et al. to compare their effectiveness. Their findings showed that the target volume coverage and OAR-sparing were comparable in the two techniques. However, the median dose of 5.5 Gy and the maximum dose of 12.8 Gy were received by the hippocampus. For helical Tomotherapy, it was 7.8 and for IMRT, it was 15.3 Gy [19]. In the present study, the same results were found. Considering the radiation doses received by hippocampus, minimum (tomotherapy TPS; Right: 6.13 Gy; Left: 6.10 Gy & VMAT TPS; Right: 8.4Gy ; Left:9.96 Gy p right=0.000& p left: 0.000), mean (Tomotherapy TPS; Right:7.6 Gy; Left: 6.10 Gy, and VMAT TPS; Right: 11.4Gy; Left: 11.7 Gy; p right=0.000& p left:0.000) and maximum (Tomotherapy TPS; Right: 15.8 Gy ; Left:15.8 Gy , and VMAT TPS; Right: 20,8Gy; Left:20,13Gy; p right= 0,003& p left: 0,009) doses show that helical tomotherapy TPS provides significantly better hippocampal protection than VMAT TPS.

Rong et al. [24] assessed the potential dosimetric differences among three modalities, namely IMRT, helical tomotherapy and VMAT, in providing HA-WBRT. When we evaluated hot spots to plan target volume (PTV), and compared with helical tomotherapy, the average dose administered to 2% of PTV (D2%) for IMRT was not superior to VMAT given that both IMRT and VMAT indicated a significantly higher D2%. In the present study, PTV minimum and maximal doses were 8.99Gy, 5,99 Gy and 33.75 Gy, 28,34 Gy for VMAT and helical tomotherapy TPS, respectively. With the same results, Rong et al. showed that PTV provided better dose distribution in helical tomotherapy TPS than VWAT TPS.

Rong et al. showed that helical tomotherapy had a considerably better homogeneity index (HI) of 0.15, and 0.03 when they compared with IMRT and VMAT (24). However, in the present study, we could not find any difference in HI (0.39&0,37 p=0,5), but in the conformity index (CI), VMAT TPS has better coverage than helical tomotherapy TPS. In terms of hippocampal avoidance, in their study, Rong et al. calculated that IMRT (8.7 Gy) and VMAT (8.6 Gy) had a higher mean dose when compared to helical tomotherapy (8.0 Gy) [24]. In the present study, helical tomotherapy and VMAT TPS right (7,6Gy and 11.4Gy) and left (7.56Gy and 11.7Gy) hippocampal doses are better dose calculation than VMAT TPS (p=0,000). In this study, helical tomotherapy and VMAT TPS were used for the calculation of the right (7.6Gy and 11.4Gy) and left (7.56Gy and 11.7Gy) hippocampal doses, respectively. As a result, helical tomotherapy has better dose distribution than VMAT TPS (p=0,000).

When the OAR-sparing impacts were evaluated, VMAT delivered higher doses on lenses than helical tomotherapy. The maximal doses delivered were as follows: right lens 3.41Gy vs 10.54 Gy and left lens 3.53Gy vs 10.19Gy for helical tomotherapy and VMAT, respectively. In our study for both lenses, the maximum and mean VMAT TPS doses were significantly higher than helical tomotherapy (p=0,000), as shown by Rong et al. [24]. Besides sparing the hippocampus on helical tomotherapy HA-PWBRT plans, for helical tomotherapy plan optimization, to decre-

ase dose to OARs without making a compromise with the HA-PWBRT-PTV coverage, the doses to OARs were administered. When compared with VMAT TPS, statistically significant dose reductions ($p < 0.001$) to OARs were detected. Sood et al. calculated mean dose to the hippocampus as 8.4Gy, and the maximum dose as 15.6Gy without making a compromise with the WB-PTV coverage in line with the RTOG guidelines [18,20]. Our findings showed considerable dose reductions on other OARs, including both right and left lenses compared with VMAT TPS. However, in this study, VMAT TPS provided conformity and dose homogeneity as those found in helical tomotherapy, but higher lens and hippocampal doses were calculated compared to helical tomotherapy TPS.

Gondi et al. conducted research on the effectiveness regarding only helical tomotherapy and the IMRT methods. Their findings showed that treatment modalities were effective for WBRT and there were not any differences about the effectiveness level in two methods [19]. However, in this study, our findings showed that helical tomotherapy TPS was significantly more efficacious in decreasing the minimum hippocampal dose than VMAT TPS. The findings obtained in this study are compatible with published research that indicated that helical tomotherapy indicated an enhanced potential for the selective sparing of tissue in comparison with VMAT TPS.

In their study, Jiang et al. showed that 4 planning modalities (sIMRT, dIMRT, VMAT and Tomo) satisfied the RTOG 0933 protocol dose compatibility criteria for hippocampal sparing. Using the planning modalities reported above with an acceptable mean PTV brain coverage (88.2%-92.6), delivery of similar mean doses to the hippocampi were accomplished [27]. In our study, in the comparison of two modalities (VMAT and helical tomotherapy), it is seen that the average of minimum, mean and maximum doses received by both right and left hippocampal regions are lower in helical tomotherapy treatment planning system (TPS). Also, they concluded that the inner ear dose was reduced to Dmean ≤15Gy in all four planning modalities [27].

The dose of the lens during cranial radiotherapy depends on many factors. Patient-dependent factors are the distance between age and lens and the lateral bone quantus, and treatment-related factors as radiation quality, total dose, patient set-up errors, dose rate, and radiotherapy technique. The dose limit is considered to be about 4-8 Gy in adults and is especially important in patients undergoing prophylactic cranial irradiation [28,29]. Adult lens may tolerate a total of 5 Gy in radiotherapy applied in fractions; 50% probability is about 15 Gy, which will cause clinical problems in vision. In our study, both the lens doses were observed , and mean and maximum doses lower in helical tomotherapy TPS. Statistically, both doses of the right (maximum dose 3,41Gy) and the left lenses (maximum dose 3,53Gy) reveal that, lens protection in helical tomotherapy TPS is significantly superior to the VMAT TPS.

Ongoing randomized multicenter phase III trial (NCT02397733) performed with patients with SCLC to be received PCI treatment or PCI with hippocampus prevention with intensity-modulated radiation therapy or volumetric modulated arc therapy so as to to assess the potential effects of PCI with hippocampal avoidance for the neurocognitive function and life quality of the patients with SCLC [30]. The doses that were administered in their study were the same doses we have administered in the present study as follows: maximum dose was 16 Gy and the hippocampus optimum D100% dose was 9 Gy.

Conclusions

Our data showed that all two HA-PWBRT modalities (VMAT and helical tomotherapy) are likely to produce acceptable treatment plans with satisfactory PTV brain coverage. In this retrospective study, it has been found that helical tomotherapy not only protects the hippocampus better than VMAT TPS in HA-PWBRT but also significantly reduces lens doses.
Abbreviations

CI: conformity index; CT: computed tomography; CTV: clinical target volume; Dmax: maximum dose; Dmin: minimum dose; DVH: dose–volume histogram; HI: homogeneity index; MRI: magnetic resonance imaging; NSCLC: non-small cell lung cancer; OARs: dose to organs at risk; PTVbrain: planning clinical target volume; VMAT: volumetric-modulated arc therapy; HA-PWBRT: Hippocampal Avoidance

Prophylactic Whole brain radiotherapy, TPS: Treatment Planning System

Ethics Committee Approval: This study approved in SBU Kartal Dr Lütfi Kırdar Training and Research Hospital 2020/514/174/3).

Conflict of Interest: None declared.

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Informed Consent: Informed consent was not obtained since the study is radiotherapy planned based.

REFERENCES

1. Auperin A, Arriagada R, Pignon JP, et al. Prophylactic cranial irradiation for patients with small-cell lung cancer in complete remission. Prophylactic cranial irradiation overview collaborative group. N Engl J Med. 1999;341(7):476-84. https://doi.org/10.1056/NEJM199908123410703
2. Slotman BJ, Faivre-Finn C, Kramer GW, et al. Prophylactic cranial irradiation in extensive small cell lung cancer. N Engl J Med. 2007;357(7):664-72. https://doi.org/10.1056/NEJMoa071780
3. Arruda Viani G, Campiolo Boin A, Yuri Ikeda V, et al. Thirty years of prophylactic cranial irradiation in patients withsmall cell lung cancer: a meta-analysis of randomized clinical trials. J Bras Pneumol. 2012;38(3):372-81. https://doi.org/10.1590/S1806-37132012000300013
4. Pöttgen C, Eberhardt W, Grannass A, et al. Prophylactic cranial irradiation inoperable stage IIIA non-small-cell lung cancer treated with neoadjuvant chemoradiotherapy: results from a German multicenter randomized trial. J Clin Oncol. 2007;25(31):4987-92. https://doi.org/10.1200/JCO.2007.12.5468
5. Gore EM, Bae K, Wong SJ, et al. Phase III comparison of prophylactic cranial irradiation versus observation in patients with locally advanced non-small-cell lung cancer: primary analysis of radiation therapy oncology group study RTOG 0214. J Clin Oncol. 2011;29(3):272-8. https://doi.org/10.1200/JCO.2010.29.1609
6. Nussbaum ES, Djalilian HR, Cho KH, Hall WA. Brain metastases. Histology, multiplicity, surgery, and survival. Cancer. 1996;78(8):1781-8. PMID: 8859192. https://doi.org/10.1002/(SICI)1097-0142(19961015)78:8<1781::AID-CNCR19>3.0.CO;2-U
7. De Angelis LM, Delattre JY, Posner JB. Radiation-induced dementia in patients cured of brain metastases. Neurology. 1989;39(6):789-96. https://doi.org/10.1212/WNL.39.6.789
8. Roman DD, Sperduto PW. Neuropsychological effects of cranial radiation: current knowledge and future directions. Int J Radiat Oncol Biol Phys. 1995;31(4):983-98. https://doi.org/10.1016/0360-3016(94)00550-8
9. Marsh J, Gielda B, Herskovic A, et al. Sparing of the hippocampus and limbic circuit during whole brain radiation therapy: a dosimetric study using helical tomotherapy. J Med Imaging Radiat Oncol. 2010;54(4):375-82. https://doi.org/10.1111/j.1754-9485.2010.02184.x
10. Raineki C, Holman PJ, Debiec J, et al. Functional emergence of the hippocampus in context fear learning in infant rats. Hippocampus. 2010;20(9):1037-46. https://doi.org/10.1002/hipo.20702
11. Jeneson A, Mauldin KN, Hopkins RO, et al. The role of the hippocampus in retaining relational information across short delays: the importance of memory load. Learn Mem. 2011;18(5):301-5. https://doi.org/10.1101/lm.2010711
12. Hannula DE, Tranel D, Cohen NJ. The long and the short of it: relational memory impairments in amnesia, even at short lags. J Neurosci. 2006;26(32):8352-9. https://doi.org/10.1523/JNEUROSCI.5222-05.2006
13. Deng W, Saxe MD, Gallina IS, et al. Adult-born hippocampal dentate granule cells undergoing maturation modulate learning and memory in the brain. J Neurosci. 2009;29(43):13532-42. https://doi.org/10.1523/JNEUROSCI.3362-09.2009
14. Hamilton GF, Murawski NJ, St. Cyr SA, et al. Neonatal alcohol exposure disrupts hippocampal neurogenesis and contextual fear conditioning in adult rats. Brain Res. 2011;1412:88-101. https://doi.org/10.1016/j.brainres.2011.07.027
15. Chang EL, Wefel JS, Hess KR, et al. Neurocognition in patients with brain metastases treated with radiosurgery or radiosurgery plus whole-brain irradiation: a randomised controlled trial. Lancet Oncol. 2009;10(11):1037-44. https://doi.org/10.1016/S1470-2045(09)70263-3
16. Gondi V, Pugh SL, Tome WA, et al. Preservation of memory with conformal avoidance of the hippocampal neural stem-cell compartment during whole-brain radiotherapy for brain metastases (RTOG 0933): a phase II multi-institutional trial. J Clin Oncol. 2014;32(34):3810-6. https://doi.org/10.1200/JCO.2014.57.2909
17. Tang FR, Loke WK, Khoo BC. Post natal irradiation-induced hippocampal neuropathology, cognitive impairment and aging. Brain Dev. 2017;39(4):277-293. https://doi.org/10.1016/j.braindev.2016.11.001
18. Ghia A, Tomé W, Thomas S, et al. Distribution of brain-metastases in relationtothehippocampus: implications for neurocognitive functional preservation. Int J Radiat Oncol Biol Phys. 2007;68(4):971-7. https://doi.org/10.1016/j.ijrobp.2007.02.016
19. Gondi V, Tolakanahalli R, Mehta MP, et al. Hippocampal-sparing whole brain radiotherapy: a ''How-To'' technique, utilizing helical tomotherapy and LINAC based intensity modulated radiotherapy. Int J Radiat Oncol Biol Phys. 2010;78(4):1244-52. https://doi.org/10.1016/j.ijrobp.2010.01.039
20. Sood S, Pokhrel D, McClinton C, et al. Volumetric-modulated arc therapy (VMAT) for whole brain radiotherapy: not only for hippocampal sparing, but also for reduction of dose to organs at risk. Med Dosim. 2017;42(4):375-83. https://doi.org/10.1016/j.meddos.2017.07.005
21. Gutiérrez AN, Westerly DC, Tomé WA, et al. Whole brain radiotherapy with hippocampal avoidance and

simultaneously integrated brain metastases boost: a planning study. Int J Radiat Oncol Biol Phys. 2007;69(2):589-97. https://doi.org/10.1016/j.ijrobp.2007.05.038
22. Bijina T K, Ganesh KM, Pichandi A, et al. Cyberknife, helical tomotherapy and rapid arc sib-sbrt treatment plan comparison for carcinoma prostate. Asian Pac J Cancer Prev. 2020;21(4):1149-1154. https://doi.org/10.31557/APJCP.2020.21.4.1149
23. Zhang J, Peng Y, Ding S, et al. Comparison of different combinations of irradiation mode and jaw width in helical tomotherapy for nasopharyngeal carcinoma. Front Oncol. 2020;10:598. https://doi.org/10.3389/fonc.2020.00598
24. Rong Y, Evans J, Xu-Welliver M, et al. Dosimetric evaluation of intensity-modulated radiotherapy, volumetric modulated arc therapy, and helical tomotherapy for hippocampal-avoidance whole brain radiotherapy. PLoS One. 2015;10(4):e0126222. https://doi.org/10.1371/journal.pone.0126222
25. Monje ML, Mizumatsu S, Fike JR, et al. Irradiation induces neural precursor-cell dysfunction. Nat Med. 2002;8(9):955-62. https://doi.org/10.1038/nm749
26. Cozzi L, Clivio A, Bauman G, et al. Comparison of advanced irradiation techniques with photons for benign intracranial tumours. Radiother Oncol. 2006;80(2):268-73. https://doi.org/10.1016/j.radonc.2006.07.012
27. Jiang A, Sun W, Zhao F, et al. Dosimetric evaluation of four whole brain radiation therapy approaches with hippocampus and inner ear avoidance and simultaneous integrated boost for limited brain metastases. Radiat Oncol. 2019;14(1):46. https://doi.org/10.1186/s13014-019-1255-7
28. Henk JM, Whitelocke RA, Warrington AP, Bessell EM. Radiation dose to the lens and cataractformation. Int J Radiat Oncol Biol Phys. 1993;25(5):815-20. https://doi.org/10.1016/0360-3016(93)90310-R
29. Hempel M, Hinkelbein W. Eye sequelae following external irradiation. Recent Results Cancer Res. 1993;130: 231-6. https://doi.org/10.1007/978-3-642-84892-6_20
30. de Dios NR, Couñago F, López JL, et al. Treatment Design and Rationale for a Randomized Trial of Prophylactic Cranial Irradiation With or Without Hippocampal Avoidance for SCLC: PREMER Trial on Behalf of the Oncologic Group for the Study of Lung Cancer/Spanish Radiation Oncology Group-Radiation Oncology Clinical Research Group. Clin Lung Cancer. 2018;19(5):e693-7. https://doi.org/10.1016/j.cllc.2018.05.003

Original Article

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Risk Factors Affecting Postoperative Morbidity in Laparoscopic Treatment of Perforated Appendicitis, a Single- Center Experience

Perfore Apandisitlerin Laparoskopik Tedavisinde Morbidite Gelişimi Üzerine Etkili Risk Faktörleri, Tek Merkez Deneyimi

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ABSTRACT

Objective: *Postoperative morbidity may occur more in laparoscopic treatment of perforated appendicitis than simple appendicitis. In this study, we aimed to investigate the risk factors affecting the development of morbidity in laparoscopic treatment of perforated appendicitis.*
Method: *The files of patients who underwent laparoscopic appendectomy due to perforated appendicitis were analysed retrospectively. Finding of perforation has been documented by surgeons who performed surgery. Information on the patients such as age, gender, Charlson Comorbidity Index (CCI), body mass index (BMI), ASA scores, symptom onset time, time between hospital admission and surgery, surgical findings, perforation sites, type of surgery, stump closure materials, white blood cell counts, pathology results and postoperative morbidities were recorded. Data were compared between patients with and without morbidity, and multivariate regression analysis of variables with significant p value was performed.*
Results: *The rate of morbidity development in laparoscopic treatment of perforated appendicitis was 22.14% (66/298). In multivariate regression analysis, the onset of symptoms longer than 72 hours, proximal perforation, grade 5 diffuse peritonitis in surgical finding according to Disease Severity Score (DSS), conversion from laparoscopic to open surgery and gangrene or necrosis in histopathological finding were found to be effective risk factors in the development of morbidity. (p=0.013, odds ratio=1,455, p=0.010, odds ratio=2.009, p=0.002, odds ratio=2.648, p=0.014, odds ratio=6.537, p=0.003, odds ratio=1.843; respectively).*
Conclusion: *The development of postoperative morbidity in laparoscopic treatment of perforated appendicitis is associated with late admission development of diffuse peritonitis, conversion to open surgery, proximal perforation and presence of necrosis. According to odds ratio, the risk factor with the highest probability of developing morbidity was found to be conversion to open surgery. We think that patients diagnosed with perforated appendicitis should be operated on as early as possible, routinely placing a drain should be avoided, and laparoscopic approach should be preferred as much as possible to reduce the morbidity rates.*

Keywords: *perforated appendicitis, laparoscopy, morbidity*

ÖZ

Amaç: *Perfore apandisitlerin laparoskopik tedavisinde postoperatif morbidite basit apandisitlere göre daha fazla gelişmektedir. Biz bu çalışmada, perfore apandisitlerin laparoskopik tedavisinde morbidite gelişimine etki eden risk faktörlerini araştırmayı amaçladık.*
Yöntem: *Perfore apandisit nedeniyle laparoskopik apendektomi yapılan hastaların dosyaları retrospektif olarak incelendi. Perforasyon bulgusu ameliyatı yapan cerrahlar tarafından belgelenmiştir. Çalışmaya dahil edilen hastaların yaşı, cinsiyeti, Charlson Komorbidite Indeksi (CCI), vücut kitle indeksi (VKİ) ASA skorları, semptom başlangıç ve hastane başvuru ile ameliyat arasında geçen süreleri, ameliyat bulguları, perforasyon yerleri, ameliyat şekilleri, güdük kapatma materyalleri, lökosit değerleri, patoloji sonuçları ve postoperatif morbiditeleri kayıt altına alındı. Veriler morbidite gelişen ve gelişmeyen hastalarda karşılaştırıldı ve p değeri anlamlı çıkan değişkenlerin multivariate regresyon analizi yapıldı.*
Bulgular: *Perfore apandisitlerin laparoskopik tedavisinde mobidite gelişme oranı %22.14 (66/298) olarak izlenmiştir. Multivariate regresyon analizinde semptom başlangıcının üzerinden 72 saat geçmesi, ameliyat bulgularına göre perforasyon yerinin radiks olması, DSS'ye göre ameliyat bulgusunda grade 5 diffuz peritonit tablosu olması, laparoskopiden açığa dönülmesi ve post operatif histopatolojik bulguda gangren veya nekroz olması post operatif morbidite gelişimi üzerine etkili risk faktörleri olarak bulunmuştur (p=0.013, olasılık oranı=1,455; p=0.010, olasılık oranı=2.009; p=0.002, olasılık oranı=2.648; p=0.014, olasılık oranı=6.537; p=0.003, olasılık oranı=1.843; sırasıyla).*
Sonuç: *Perfore apandisitlerin laparoskopik tedavisinde post operatif morbidite gelişimi geç başvuru, diffüz peritonit gelişimi, açığa dönüş, radiks perforasyonu ve nekroz varlığı ile ilişkilidir. Olasılık oranlarına göre morbidite gelişme ihtimali en yüksek olan risk faktörü açığa dönüş olarak bulunmuştur. Apandisit perforasyonu tanısı alan hastaların olabildiğince erken ameliyat edilmesi, rutin dren yerleştirilmemesi ve morbidite gelişimini önlemek için mümkün olduğunca laparoskopik yaklaşım uygulanması gerektiğini düşünüyoruz.*

Anahtar kelimeler: *perfore apandisit, laparoskopi, morbidite*

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INTRODUCTION

Acute appendicitis is the most common cause of sudden abdominal pain and emergency operations that require surgery, and is one of the most common abdominal surgical procedure performed by general surgeons [1]. Appendectomy is the gold standard in treatment [2]. Although acute appendicitis is usually treated successfully with early diagnosis and appropriate approach, perforation may occur in 16-39% of the cases, and may lead to life-threatening complications [3,4]. Perforated appendicitis have more morbidities and complications such as 3 times more frequent hospital stays, higher costs and 2.3 times greater number of fatalities than simple appendicitis [5,6].

While open appendectomy was preferred for treatment in the 1990s, laparoscopic appendectomy became the gold standard in the 2000s [7,8]. Laparoscopic appendectomy (LA) is an effective treatment method that can be applied safely in simple appendicitis treatment. The laparoscopic approach is superior to open appendectomy (OA) in terms of postoperative surgical site infections, analgesia requirement, average length of hospital stay, return to work, and overall recovery [9,10]. However, its role in complicated appendicitis is controversial due to more frequent intra-abdominal abscess development and longer operative times [11,12]. Despite the technical developments in LA, postoperative intra-abdominal abscesses are bothersome for both surgeons and patients. In the laparoscopic approach, perforated appendicitis, obesity and young age are possible risk factors for the development of intra-abdominal abscess after appendectomy [13,14].

In this study, we aimed to determine the factors that may affect the development of postoperative morbidity in the laparoscopic treatment of perforated appendicitis.

MATERIAL and METHODS

The files of patients who underwent laparoscopic appendectomy between January 2017 and January 2020 were retrospectively reviewed. Patients older than 18 years of age and with the surgical finding of perforated appendicitis were included in the study. Patients younger than 18 years of age and who had no evidence of perforated appendicitis were excluded from the study. Information on the patients such as age, gender, Charlson Comorbidity Index (CCI), body mass index (BMI), American Society of Anesthesiologists (ASA) scores, onset of symptoms and time between hospital admission and surgery, surgical findings, perforation sites, surgery types, stump closure materials, leukocytes counts, pathology results and morbidities were recorded. Disease Severity Score (DSS) was used for classification according to the surgical findings [15]. According to the surgical findings, the patients were divided into 3 groups as perforated appendicitis with localized fluid (Grade 3), with regional abscess (Grade 4), and with diffuse peritonitis (Grade 5). Surgical site infection, prolonged ileus, and cardiopulmonary complications occurred within the first 30 days postoperatively were considered surgical morbidity. The parameters recorded in patients with and without morbidity were compared and the risk factors affecting the development of morbidity were determined by performing a multivariate risk analysis of the parameters that showed a significant difference in the p value.

This study was carried out in accordance with the 1964 Helsinki Declaration and its recent amendments. Written consent was obtained from all participants. Permission was obtained from the local ethics committee (Ref. Nr:2020/274)

Statistical Analysis

SPSS (Statistical Package for the Social Sciences) 24. program (IBM, Armonk, NY) was used for statistical analysis. While evaluating the study data, descriptive statistical methods (average, standard deviation, median, frequency, ratio, minimum, maximum) as well as the Independent sample t test for the comparisons of normally distributed parameters in two groups, and the Mann-Whitney U test for the comparisons of two groups that did not show normal distribution were used The Pearson Chi-Square test was used for the analysis of qualitative data. Multivariate regression analysis of factors affecting the development of morbidity was performed. Significance was evaluated at $p<0.01$ and $p<0.05$ levels.

RESULTS

It was determined that a total of 1302 patients

underwent laparoscopic appendectomy between January 2017 and January 2020. Of these patients, 298 (22.88%) had signs of perforation. 197 (66.1%) of the patients were male and 101 (33.9%) of them were female. The mean age was 40.7. BMI was 27.3 kg / m^2. The mean Charlson comorbidity index score was 0.93. The ASA score of 48 (16.10%) patients was 3-4. Mean symptom onset time was 2.53±1.14 days. The mean time from hospital admission to surgery was determined as 10.16±4.16 hours. Mean WBC countswere 16.5 103/µL.

When we examined the surgical findings, perforation of appendicitis was found in the distal appendix in 139 (46.64%) and the proximal appendix in 159 (53.36%) cases While 190 (53.69%) patients had DSS grade 3 localized fluid, 38 (12.75%) DSS grade 4 localized abscess and 70 (23.48%) DSS grade 5 diffuse peritonitis. Conversion to open surgery required in 18 (6.04%) patients. The reasons for conversion were determined as exploration difficulty due to adhesions and the revealing of the appendix radix as a result of appendix lysis. Partial cecum resection was performed in 21 (7.04%) of the patients because the perforation was quite proximal and there was no distance to close the appendix stump. When we examined the stump closure materials, it was found that hemo-o-lok clips were used in 244 (81.87%), endostapler (Ethicon flex 60 mm) in 23 (7.71%) , and sutures in 31 (10.40%) patients. It was determined that an abdominal drain was placed in only 245 (82.21%) of 298 patients, (Table 1).

According to final histopathological evaluation, the patients had acute inflamed appendicitis (n=67), 93 phlegmonous appendicitis (n=93), gangrenous or necrotic appendicitis (n=130), grade 1 neuroendocrine tumour (n=3), mucinous neoplasia (n=3), and mucocele (n=2). In the lumen of the appendix of 67 patients, fecaloid was detected (Table 1).

Morbidity was observed in 66 (22.14%) patients. Surgical site infection developed in 44 (14.76%) of these patients. Of the patients who developed surgical site infection, superficial wound infection was observed in 8, deep wound infection in 4, and organ / space surgical site infection in 32 patients. Prolonged ileus developed in 16 patients and atelectasis in 6 patients. Diagnoses of patients with organ / space

Table 1. General and perioperative characteristics of patients.

Mean age±SD (Min/Max)	40.72± 16.87 (18-85)
Gender, n (%)	
Female	101 (33.9%)
Male	197 (66.1%)
Mean BMI±SD	27.3±3.81 kg/m^2
Mean Charlson comorbidity index±SD (Min/Max)	0.93± 1.72 (0-8)
ASA scores, n (%)	
1-2	250 (83.90%)
3-4	48 (16.10%)
Mean WBC	16.5 10^3/uL
Mean time interval between symptoms onset and surgery (day)±SD	2.53±1.14
Mean time interval hospital application and surgery (hours) ±SD (Min/Max)	10.16±4.16 (3-22)
Intraoperative finding according to Diasease Severity Score (DSS), n (%)	
Grade 3 perforated with localized free fluid	190 (53.69%)
Grade 4 perforated with a regional abscess	38 (12.75%)
Grade 5 perforated with diffuse peritonit	70 (23.43%)
Site of perforation, n (%)	
Proksimal(radix)	159 (53.35%)
Distal (apex ve corpus)	139 (46.65%)
Stump closure material, n (%)	
Endoclip	244 (81.88%)
Suture	31 (10.40%)
Endostapler	23 (7.72%)
Abdominal drain, n (%)	
Yes	245 (82.21%)
No	53 (17.79%)
Conversion to open surgery	18 (6.04%)
Partial cecal resection	21 (7.04%)
Mean operating time (min) ±SD (Min/Max)	82.25± 28.08(24-190)
Histopathological findings, n (%)	
Acute inflamated	67 (22.48%)
Phlegmonous	93 (31.20%)
Gangrenous and necrosis	130 (43.62%)
Mucosel	2 (0.67%)
NET (Grade 1)	3 (1.00%)
Mucinous neoplasm	3 (1.00%)
Complications, n (%)	
Surgical site infection	
superficial	8 (2.68%)
deep	4 (1.34%)
organ/space	32 (10.73%)
Prolonged ileus	16 (5.36%)
Atelectasis	6 (1.67%)
Total morbidity	66 (22.14%)
Clavien-Dindo classification, n (%)	
Grade 1	30 (10.06%)
Grade 2	18 (6.04%)
Grade 3	18 (6.04%)
3a	7 (2.34%)
3b	11 (3.69%)
Re-operation	11 (3.69%)

Table 2. Comparison of patients characteristics and perioperative datas according to development of morbidity

Variables	Morbidity (-) (n=232)	Morbidity (+) n=66)	P value
Mean age	40,42±15,68	41,82±20,63	[b]0,553
Gender			
Female	82 (35.3%)	19 (28.8%)	[a]0,321
Male	150 (64.7%)	47 (71.2%)	
Mean BMI	27,01±3,74	28,33±3,95	[b]0,013*
Mean CCI	0,8±1,51	1,39±2,27	[c]0,124
ASA score			
1-2	202 (80.8%)	48 (19.2%)	[a]0,002**
3-4	30 (62.5%)	18 (37.5%)	
Mean WBC	16013,96±10565,12	18420,61±25778,21	[c]0,907
Mean time interval between symptoms onset and surgery(day)	2,39±0,93	3,05±1,46	[c]0,001**
Time interval between symptoms onset and surgery			
<72h	135 (58.18%)	25 (37.88%)	[c]0,001**
>72h	97 (41.8%)	41 (62.12%)	
Mean time interval hospital application and surgery(hour)	10,06±4,25	10,53±3,84	[b]0,419
Intraoperative finding according to Diasease Severity Score (DSS)			
Grade 3	168 (72.4%)	22 (33.3%)	[a]0,001**
Grade 4	25 (10.8%)	13 (19.7%)	
Grade 5	39 (16.8%)	31 (47%)	
Site of perforation			
Proximal	122 (52.6%)	17 (25.75%)	[a]0,001**
Distal	110 (47.4%)	49 (74.25%)	
Stump closure material			
Endoclip	201(86.6%)	43 (65.2%)	[a]0,001**
Suture	16 (6.9%)	15 (22.72%)	
Endostapler	15 (6.5%)	8 (12.12%)	
Abdominal drain			
Yes	184 (79.3%)	61 (92.4%)	[a]0,014*
No	48 (20.7%)	5 (7.6%)	
Laparoscopically completed	227 (81.1%)	53 (18.9%)	[a]0,001**
Conversion to open surgery	5 (27.8%)	13 (72.8%)	
Partial caecum resection			
Yes	217 (93.5%)	61 (90.9%)	[a]0,462
No	15 (6.5%)	5 (9.1%)	
Mean operating time	78,37±25,81	95,92±31,53	[b]0,001**
Histopathological findings			
Acute inflamated	51 (21.98%)	16 (24.24%)	[a]0,003**
Phlegmanous	83 (35.77%)	10 (15.15%)	
Gangrenoz and necrosis	91 (39.22%)	39 (59.09%)	
Fecaloid			
Yes	185 (79.74%)	46 (69.69%)	[a]0,085
No	47 (20.76%)	20 (30.30%)	

*[a]Pearson Chi-Square, [b]Independent Sample t testi, [c]Mann Whitney U Testi *p<0,05,**p<0,001*

Tablo 3. Multivariate regression analysis of variables that are significant in the morbidity.

	Multivariate p value	Odd's Ratio	95% C.I	
			Lower	Upper
ASA score 3-4	0.953	0.986	0.616	1.578
Conversion to open surgery	0.014	6.537	1.470	29.065
Proximal perforation of appendicitis (radix)	0.010	2.009	1.179	3.424
Stump closure material (suture)	0.829	0.939	0.527	1.671
Abdominal drain	0.183	2.006	0.720	5.593
DSS Grade 5 diffuse peritonitis	0.002	2.648	1.216	3.986
Gangrenous or necrotic appendicitis	0.003	1.843	1.223	2.777
Mean BMI	0.135	1.070	0.979	1.169
Mean operating time	0.060	1.012	1.000	1.024
Symptom onset (>72h)	0.013	1.455	1.082	1.958

surgical site infection were confirmed by computed tomography (CT) of the abdomen and intra-abdominal abscess was observed in all 32 patients. The mean abscess size of these patients was 5.43±2.32 cm. While 16 patients were treated with antibiotics only, drains were placed in 7 patients by interventional radiology, and 9 patients were re-operated. Antibiotic treatment was performed to patients with abscess of <5 cm. Interventional drainage was planned first for patients with> 5 cm abscess, but drainage could not be performed in 9 patients because the access was not suitable for placing the drain, and these patients were re-operated. Two patients with deep wound infection were operated due to evisceration, and a total of 11 patients underwent reoperation. According to Clavien Dindo classification, there were 30 patients who received only fluid therapy, electrolyte replacement and wound care in Grade 1, 18 patients received antibiotic treatment in Grade 2, and 18 patients underwent invasive drainage and reoperation in Grade 3. Grade 4 and 5 complications were not observed (Table 1). Mortality did not occur in any of our patients.

High BMI, higher ASA score, symptom onset time longer than 72 hours, conversion to open surgery, proximal perforation in appendicitis, use of sutures as stump closure material, DSS grade 5 diffuse peritonitis as surgical finding, insertion of a drain, presence of gangrenous or necrotic appendicitis as pathological findings and longer operative time were found to be significant as factors affecting the morbidity (Table 2).

When the multivariate regression analysis was perrformed, the symptom onset time longer than 72 hours, conversion to open surgery, proximal perforation of appendicitis, the DSS grade 5 diffuse peritonitis, gangrenous or necrotic appendicitis were found to be statistically significant factors effecting postoperative morbidity (Table 3).

DISCUSSION

Mortality and morbidity are higher in perforated appendicitis than in non-perforated appendicitis [16]. Studies have shown that perforated appendicitis is one of the most effective risk factors for the development of morbidity after appendectomy [17,18]. In a study, Frazee et al. reported total morbidity was 20% after surgery for perforated appendicitis and intraabdominal abscess was the most common cause of morbidity with an incidence of 11 percent [19]. Intra-abdominal abscess is the most common complication after perforated appendicitis and occurs in 14-18% of postoperative patients [20]. In the study by Guy et al., it was observed that intraabdominal abscess developed in 9% of the cases with perforated appendicitis after laparoscopic appendectomy [21]. In this study, postoperative morbidity developed in 22.14% (66/298), and intraabdominal abscess in 10.93% (32/298) of the cases with similar rates reported in the studies in the literature. However, in the literature, it is unclear what factors caused such higher rates of morbidity development in perforated appendicitis after laparoscopy.

In a study , Asarias et al., reported that older age has an impact on the development of postoperative morbidity in perforated appendicitis and that the probability of intraabdominal abscess formation increased by 30% with each decade of life [22]. In the study of Ming

et al., it was observed that infections developed more frequently on the postoperative wound site in males with complicated appendicitis [23].

In a study, Guy et al. reported that gender and age are not effective in the development of morbidity [21]. In this study, we determined that gender and age have no effect on the development of morbidity. In a retrospective study of 2076 patients, increased morbidity development rates were observed after laparoscopic appendectomy in patients with an ASA score of 2 and above [18]. In this study, the morbidity rate was found to be higher in patients with ASA 3-4, but ASA 3-4 had not any significant effect on morbidity in the multivariate regression analysis.

In a cohort study of 4618 patients, diabetes mellitus was found to be effective in the development of intraabdominal abscess after laparoscopic appendectomy [17]. In the study of Cho et al., the comorbidity of the patients was found to be ineffective in the development of morbidity after laparoscopic appendectomy [24]. In this study, mean values of Charlson Comorbidity Indexes were found to be similar between both groups.

It has been found that BMI has no effect on postoperative morbidity in previous studies [17,18,24]. In our study, it was found that postoperative morbidity was significantly more frequently detected in overweight patients while it was found to be insignificant in multivariate analysis. The reason for the higher incidence of morbidity in patients with high BMI can be explained by the higher occurrence of wound site infections due to fat necrosis and thicker subcutaneous adipose tissue.

The time from the onset of symptoms to surgery is one of the important variables for the development of morbidity after laparoscopic appendectomies. In the study performed by Lasek et al., in comparisons made between 48 hours before and after surgery, intraabdominal abscess developed more frequently in patients who were operated after 48 hours [17]. Similarly, in a study, Fair et al. found a higher rate of postoperative complications in appendectomies performed after 48 hours [25]. In this study, the duration of symptom onset of patients with morbidity was significantly longer than those without morbidity. In the multivariate analysis, interventions made 72 hours after the onset of symptoms were found to be an effective risk factor in the development of morbidity.

In the study of Dijk et al., it was concluded that operating patients up to 24 hours after hospital admission had no effect on postoperative morbidity [26]. In our study, all patients were operated within 24 hours after admission, and the time from hospital admission to surgery was similar in those with and without morbidity. It has been stated that preoperative CRP and WBC values have no effect on the development of morbidity in the laparoscopic treatment of perforated appendicitis [21]. In our study, preoperative WBC values were similar between those who did, and didi not develop morbidity.

Although perforated appendicitis was determined as a risk factor for the development of morbidity after appendectomy, morbidity development rates were not compared according to the perforation sites. In this study, it was observed that postoperative complications developed more frequently in appendicitis perforated from the radix area compared to perforations from the distal appendix area such as the corpus and apex. In multivariate regression analysis, radix perforations were identified as an effective risk factor for morbidity after laparoscopic treatment in perforated appendicitis. In the study of Garst et al., it was stated that as the DSS scores increased, postoperative morbidity increased significantly after appendectomy [15]. In the study of Guy et al., It was observed that after laparoscopic treatment of perforated appendicitis, surgical findings according to DSS did not affect the development of morbidity [21]. In this study, the morbidity rates of patients who were grade 5 according to DSS, (cases with diffuse peritonitis), were found to be significantly higher than other grades, and in the multivariate regression analysis, it was found to be a risk factor affecting postoperative morbidity.

In the study of Lasek et al., it was stated that conversion to open surgery had no effect on postoperative morbidity [17]. In the study of Andert et al., postoperative morbidity was observed more frequently in conversion from laparoscopy and it was found to be a risk factor for the development of morbidity in multivariate analysis [27]. In this study, conversion was

found to be a effective factor for morbidity, and in the multivariate analysis, and an effective risk factor in the development of postoperative morbidity. The reason for this can be explained by the fact that the majority of the cases of conversion from laparoscopy was DSS grade 5, the appendix was perforated from the radix, and superficial and deep facial wound infections due to the subumbilical midline incision were observed more frequently.

In the studies where stump closure materials were compared, no difference was found between use of endoloop, suture, endostapler, and endoclip in terms of postoperative morbidity and intra-abdominal abscess development [28-31]. In this study, although the postoperative morbidity rate was higher when the closure of the stump was performed with sutures, it was found to be insignificant as a risk factor on morbidity in multivariate analysis.

In a study, Castro et al. found no difference between patients with and without drains in terms of postoperative morbidity development in patients who had undergone laparoscopic treatment for perforated appendicitis [32]. A recent Cochrane analysis found that placing drains in perforated appendicitis did not reduce the risk of morbidity, even increased 30-day morbidity with very little evidence [33]. In this study, postoperative morbidity was observed more frequently in patients with a drain, but placement of a drain was not found as a risk factor affecting morbidity in multivariate regression analysis.

In the study of Lasek et al. it was determined that the operation times were longer in patients who developed intra-abdominal abscess after laparoscopic appendectomy. [17]. In this study, operation time was significantly longer in patients who developed postoperative morbidity. In the multivariate regression analysis, it was found to be an insignificant factor.

In a study by Guy et al. it was bserved that histopathological findings after laparoscopic treatment of perforated appendicitis were not risk factors for the development of morbidity [21]. In this study, it was observed that morbidity rate was higher in patients with histopathological findings of gangrene or necrosis findings, and it was determined as a significant risk factor for the development of morbidity in multivariate analysis. It was determined that the presence of fecaloid in the lumen did not increase morbidity.

This study has some limitations. Retrospective design of this study is the major limitation. Furthermore, this case series represented a complex, heterogeneous patient population dispersed over a significant period of time. However, as the study was conducted in a tertiary referral center, the high volume of patients underwent laparoscopy for perforated appendicitis so the results and the statistical analysis might be considered as reliable and valuable.

CONCLUSION

In this study, the onset of symptoms longer than 72 hours, proximal perforation, surgical finding of grade 5 diffuse peritonitis according to Disease Severity Score (DSS), conversion to open surgery, gangrene or necrosis in histopathological finding were found to be effective risk factors in the development of morbidity in multivariate regression analysis. Conversion to open surgery was found to be the variable with the highest risk factor for the development of morbidity according to odds ratio.. We think that patients diagnosed with perforated appendicitis should be operated on as early as possible, routine placiement of a drain should be avoided, and laparoscopic approach should be preferred as much as possible to reduce the morbidity rates.

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REFERENCES

1. Sauerland S, Lefering R, Neugebauer EA. Laparoscopic versus open surgery for suspected appendicitis. Cochrane Database Syst Rev. 2002;(1):CD001546. https://doi.org/10.1002/14651858.CD001546
2. Cao J, Tao F, Xing H, et al. Laparoscopic Procedure is Not Independently Associated With the Development of Intra-

Abdominal Abscess After Appendectomy. Surg Laparosc Endosc Percutan Tech. 2017;27(5):409-14. https://doi.org/10.1097/SLE.0000000000000460
3. Al-Omran M, Mamdani M, McLeod RS. Epidemiologic features of acute appendicitis in Ontario, Canada. Can J Surg. 2003;46(4):263-8. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3211626/
4. Fike FB, Mortellaro VE, Juang D, et al. The impact of postoperative abscess formation in perforated appendicitis. J Surg Res. 2011;170(1):24-6. https://doi.org/10.1016/j.jss.2011.03.038
5. Barrett ML, Hines AL, Andrews RM. Trends in Rates of Perforated Appendix, 2001-2010. Available from: https://www.hcup-us.ahrq.gov/reports/statbriefs/sb159.pdf (cited 2018 April 1).
6. Andersson RE. Short and long-term mortality after appendectomy in Sweden 1987 to 2006. Influence of appendectomy diagnosis, sex, age, co-morbidity, surgical method, hospital volume, and time period. A national population-based cohort study. World J Surg. 2013;37(5):974-81. https://doi.org/10.1007/s00268-012-1856-x
7. Golub R, Siddiqui F, Pohl D. Laparoscopic versus open appendectomy: a meta-analysis. J Am Coll Surg. 1998;186(5):545-53. https://doi.org/10.1016/S1072-7515(98)00080-5
8. Andersson RE. Short-term complications and longterm morbidity of laparoscopic and open appendicectomy in a national cohort. Br J Surg. 2014;101(9):1135-42. https://doi.org/10.1002/bjs.9552
9. Frazee RC, Roberts JW, Symmonds RE, et al. A prospective randomized trial comparing open versus laparoscopic appendectomy. Ann Surg. 1994;219(6):725-8; discussion 728-31. https://doi.org/10.1097/00000658-199406000-00017
10. Guller U, Hervey S, Purves H, et al. Laparoscopic versus open appendectomy: outcomes comparison based on a large administrative database. Ann Surg. 2004;239(1):43-52. https://doi.org/10.1097/01.sla.0000103071.35986.c1
11. Sauerland S, Jaschinski T, Neugebauer EA. Laparoscopic versus open surgery for suspected appendicitis. Cochrane Database Syst Rev. 2010;(10):CD001546. https://doi.org/10.1002/14651858.CD001546.pub3
12. Gorter RR, Eker HH, Gorter-Stam MA, et al. Diagnosis and management of acute appendicitis. EAES consensus development conference 2015. Surg Endosc. 2016;30(11):4668-90. https://doi.org/10.1007/s00464-016-5245-7
13. Ming PC, Yan TY, Tat LH. Risk factors of postoperative infections in adults with complicated appendicitis. Surg Laparosc Endosc Percutan Tech. 2009;19(3):244-8. https://doi.org/10.1097/SLE.0b013e3181a4cda2
14. Luo CC, Chien WK, Huang CS, et al. National trends in therapeutic approaches and outcomes for pediatric appendicitis: aTaiwanese nationwide cohort study. Pediatr Surg Int. 2015;31(7):647-51. https://doi.org/10.1007/s00383-015-3718-8
15. Garst GC, Moore EE, Banerjee MN, et al. Acute appendicitis: a disease severity score for the acute care surgeon. J Trauma Acute Care Surg. 2013;74(1):32-6. https://doi.org/10.1097/TA.0b013e318278934a
16. Humes DJ, Simpson J. Acute appendicitis. BMJ. 2006; 333(7567):530-4. https://doi.org/10.1136/bmj.38940.664363.AE
17. Lasek A, Pędziwiatr M, Wysocki M, et al. Risk factors for intra-abdominal abscess formation after laparoscopic appendectomy-results from the Pol-LA (Polish Laparoscopic Appendectomy) multicenter large cohort study. Wideochir Inne Tech Maloinwazyjne. 2019;14(1):70-8. https://doi.org/10.5114/wiitm.2018.77272
18. Tartaglia D, Fatucchi LM, Mazzoni A, et al. Risk factors for intra-abdominal abscess following laparoscopic appendectomy for acute appendicitis: a retrospective cohort study on 2076 patients. Updates Surg. 2020 Apr 27. https://doi.org/10.1007/s13304-020-00749-y
19. Frazee R, Abernathy S, Davis M, et al. Fast track pathway for perforated appendicitis. Am J Surg. 2017;213(4):739-41. https://doi.org/10.1016/j.amjsurg.2016.08.006
20. Beek MA, Jansen TS, Raats JW, et al. The utility of peritoneal drains in patients with perforated appendicitis. Springerplus. 2015;4:371. https://doi.org/10.1186/s40064-015-1154-9
21. Guy S, Wysocki P. Risk factors for intra-abdominal abscess post laparoscopic appendicectomy for gangrenous or perforated appendicitis: A retrospective cohort study. International Journal of Surgery Open. 2018;10:47-54. https://doi.org/10.1016/j.ijso.2017.12.003
22. Asarias JR, Schlussel AT, Cafasso DE, et al. Incidence of postoperative intraabdominal abscesses in open versus laparoscopic appendectomies. Surg Endosc. 2011;25(8):2678-83. https://doi.org/10.1007/s00464-011-1628-y
23. Ming PC, Yee Yan TY, Tat LH. Risk factors of postoperative infections in adults with complicated appendicitis. Surg Laparosc Endosc Percutan Tech. 2009;19(3):244-8. https://doi.org/10.1097/SLE.0b013e3181a4cda2
24. Cho J, Park I, Lee D, Sung K, Baek J, Lee J. Risk factors for postoperative intra-abdominal abscess after laparoscopic appendectomy: analysis for consecutive 1,817 experiences. Dig Surg. 2015;32(5):375-81. https://doi.org/10.1159/000438707
25. Fair BA, Kubasiak JC, Janssen I, et al. The impact of operative timing on outcomes of appendicitis: a National Surgical Quality Improvement Project analysis. Am J Surg. 2015;209(3):498-502. https://doi.org/10.1016/j.amjsurg.2014.10.013
26. van Dijk ST, van Dijk AH, Dijkgraaf MG, Boermeester MA. Meta-analysis of in-hospital delay before surgery as a risk factor for complications in patients with acute appendicitis. Br J Surg. 2018;105(8):933-45. https://doi.org/10.1002/bjs.10873
27. Andert A, Alizai HP, Klink CD, et al. Risk factors for morbidity after appendectomy. Langenbecks Arch Surg. 2017;402(6):987-93. https://doi.org/10.1007/s00423-017-1608-3
28. Swank HA, van Rossem CC, van Geloven AAW, et al. Endostapler or endoloops for securing the appendiceal stump in laparoscopic appendectomy: a retrospective cohort study. Surg Endosc. 2014;28(2):576-83. https://doi.org/10.1007/s00464-013-3207-x
29. Al-Temimi MH, Berglin MA, Kim EG, et al. Endostapler versus Hem-O-Lok clip to secure the appendiceal stump and mesoappendix during laparoscopic appendectomy. Am J Surg. 2017;214(6):1143-8. https://doi.org/10.1016/j.amjsurg.2017.08.031
30. Mannu GS, Sudul MK, Bettencourt-Silva JH, et al. Closure methods of the appendix stump for complications during laparoscopic appendectomy. Cochrane Database Syst Rev. 2017;11(11):CD006437. https://doi.org/10.1002/14651858.CD006437.pub3
31. Ceresoli M, Tamini N, Gianotti L, et al. Are endoscopic loop ties safe even in complicated acute appendicitis? A systematic review and meta-analysis. Int J Surg. 2019;68:40-7. https://doi.org/10.1016/j.ijsu.2019.06.011
32. Aneiros Castro B, Cano I, García A, et al. Abdominal drainage after laparoscopic appendectomy in children: an endless controversy? Scand J Surg. 2018;107(3):197-200. https://doi.org/10.1177/1457496918766696
33. Li Z, Zhao L, Cheng Y, et al. Abdominal drainage to prevent intra-peritoneal abscess after open appendectomy for complicated appendicitis. Cochrane Database Syst Rev. 2018;5(5):CD010168. https://doi.org/10.1002/14651858.CD010168.pub3

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Hydroxychloroquine Use on Healthcare Workers Exposed to COVID-19 -A Pandemic Hospital Experience

COVİD -19 Temas Öyküsü Olan Sağlık Çalışanlarında Hidroksiklorokin Kullanımı; Bir Pandemi Hastanesi Deneyimi

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ABSTRACT

Objective: *As healthcare professionals play a role in combating the COVID-19 outbreak, the risk of disease exposure and illness increases. In our study, we aimed to measure the effectiveness of post-contact use in order to protect the healthcare professionals who work very intensively during the outbreak.*
Method: *A total of 208 healthcare workers who applied to Employee Health Unit section between the dates 25 March-25 April 2020 with a history of contact with COVID-19 patients were included in the study. Employees were evaluated in low, moderate and high risk groups according to the Contact Risk Algorithm included in the Evaluation of Healthcare Workers Guidelines with COVID-19 theme. Three-day hydroxychloroquine treatment was initiated to 138 healthcare professionals who were considered as high risk. The treatment regimen was arranged as 2x400 mg on the first day and 2x200 mg on the 2nd and 3rd days. The COVID-19 positivity rates were analyzed according to the contact risk groups*
Results: *There was a statistically significant difference between occupational groups according to contact risk groups (p<0.01); the rate of contact risk of nurses in the middle, and the doctors in the high risk groups was found to be significantly higher. When the COVID-19 positivity rates were analyzed according to the contact risk groups, the COVID-19 positivity rates were 9.4% in the high-, 16.3% in the moderate and 14.3% in the low-risk groups. The contact was found to be related to the COVID-19 test, and the positivity rate from contact with the patient was found to be significantly high (p<0.01).*
Conclusion: *Recommendations about hydroxychloroquine for postexposure prophylaxis vary. Hydroxychloroquine can be a possible effective agent in postexposure prophylaxis. We think that conducting similar studies on larger samples can provide significant benefits to individuals and public health.*

Keywords: *COVID-19, contact, healthcare workers, hydroxychloroquine*

ÖZ

Amaç: *Sağlık çalışanları COVID-19 salgını ile mücadelede rol aldıkça hastalığa maruz kalma ve hastalanma riskleri artmakta. Çalışmamızda salgın surecinde cok yoğun şekilde çalışan sağlık çalışanlarını korumak adına temas sonrası proflaksinin etkinliğini ölçmeyi amaçladık.*
Yöntem: *Çalışan sağlığı birimine 25 Mart-25 Nisan 2020 tarihleri arasında COVID-19 hasta ile temas öyküsü ile başvuran 208 sağlık çalışanı çalışmaya dahil edildi. COVID-19 temasi olan Sağlık Çalışanlarının Değerlendirilmesi Rehberinde yer alan temas riski algoritmasına göre düşük, orta ve yüksek risklerde değerlendirildi. Yüksek riskli olarak değerlendirilen 138 sağlık çalışanına ise 3 günlük hidroksiklorokin tedavisi başlandı. Tedavi rejimi ilk gün 2x400 mg 2. ve 3. günlerde 2x200 mg olacak şekilde düzenlendi. Temas riski gruplarına göre COVID-19 pozitiflik oranları analiz edildi.*
Bulgular: *Temas risk gruplarına göre meslek grupları incelendiğinde; orta risk grubunun hemşirelerin, yüksek risk grubunda ise doktorların oranı anlamlı düzeyde yüksek olarak saptanmıştır. Temas risk gruplarına göre COVID-19 pozitiflik oranları incelendiğinde yüksek risk grubunda COVID-19 pozitiflik oranı %9,4 iken, orta risk grubunda bu oran %16,3 ve düşük risk grubunda %14,3 olarak saptanmıştır. Temas ise COVID-19 test ile ilişkili saptanmış olup hasta ile temastan gelen pozitiflik oranı anlamlı düzeyde yüksek bulunmuştur (p<0,01).*
Sonuç: *Maruziyet sonrası profilaksi için Hidroksiklorokin hakkındaki öneriler değişiklik göstermektedir. Hidroksiklorokinin, temas sonrası profilakside olası etkili bir ajan olabileceğini düşünmekteyiz. Daha büyük örneklemler üzerinden benzer çalışmaların yapılmasının bireylere ve halk sağlığına önemli faydalar sağlayacağını düşünüyoruz.*

Anahtar kelimeler: *COVID-19, temas, sağlık çalışanları, hidroksiklorokin*

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INTRODUCTION

Initially a new respiratory virus infection was identified in Wuhan city, Hubei, China, after the number of patients with acute severe respiratory infections increased on December 31, 2019. Since then the outbreak has spread rapidly to other states in China and other countries around the world [1]. In January 2020, the causative agent was identified as a novel coronavirus (2019-nCoV) that had not been previously detected in humans by the World Health Organization (WHO). Thereafter, the name of the 2019-nCoV disease was accepted as COVID-19 and the virus was named as SARS-CoV-2 due to its similarity to the SARS CoV [2]. Findings related to fever, cough, dyspnea and bilateral pneumonic infiltration were detected in cases. Since the COVID-19 epidemic began in the world, as of May 19, 2020, 316,289 deaths were reported. Currently, there are 4,735,622 confirmed cases in 212 countries and regions [3]. As of the same date, total number of 151,615 cases were reported by the Turkish Ministry of Health [4].

Coronaviruses are enveloped and single-stranded RNA viruses of the Coronaviridae family and are known as "crown viruses" due to their crown-like protrusions on their outer surfaces [5]. Similar to SARS-CoV [26], 2019-nCoV uses angiotensin converting enzyme 2 (ACE2) receptor binding motif (RBM) as receptor [6]. The entry of the virus into the host cells occurs after contact with these specific ACE2 receptors either by endocytosis or membrane fusion. The viral genome must be released for the replication of viruses entering the host cell [1,7]. The main routes of transmission for the 2019-nCoV infection are by airborne droplets and direct contact [8]. The incubation period is usually 3-7 days and can extend up to 14 days [9]. Personal protective measures, proper isolation and early diagnosis are essential to prevent the spread of infection. Hospital-acquired infection poses a risk to both inpatients and hospital staff.

In a clinical series presenting 138 adult patients with 2019-nCoV pneumonia, it was emphasized that 41% of patients were suspected to be infected by nosocomial transmission in hospital [10]. As healthcare professionals play a role in the treatment of this disease, their risks of exposure to disease increase. At the same time this situation carries the risk of decreasing health manpower in the fight against the COVID-19 outbreak. China had reported 3387 infected healthcare workers in Hubei Province until February 25, 2020, and at least 18 of these healthcare workers died [11]. As of 04.30. 2020, according to the statement of the Turkish Ministry of Health, 7428 employees were infected among 1 million 100 thousand health workers. The rate of this number in average cases is reported to be close to 6.5 percent [12].

Up to now no specific effective antiviral therapy for COVID-19 has been found. Although most COVID-19 patients have mild or moderate prognosis, up to 5-10% of these can contract a disease with a severe, potentially life-threatening course. Yet, there is an urgent need for effective drugs and vaccination [13]. Chloroquine and hydroxychloroquine are aminoquinolines, which have been used to treat malaria and autoimmune diseases. Besides their antimalarial effects, these drugs possess immunomodulatory effects allowing their use for the treatment of autoimmune conditions [14]. It has been suggested that HCQ treatment for SARS-CoV-2 infection is more effective in the treatment with its higher lung, blood and plasma concentrations, which reach therapeutic levels within a short time. It is recommended to use 400 mg of HCQ sulfate twice a day for a day and then 200 mg twice a day for a further 4 days [1,15].

Schwartz J. et al. suggested strategies such as pre-exposure or post-exposure prophylaxis to prevent viral transmission, especially for healthcare workers exposed to SARS-CoV-2 [11]. Paglitano et al. also indicated in their studies that hydroxychloroquine can be effective in preventing respiratory invasion in healthcare workers exposed to SARS-CoV-2 and also reported that prophylactic administration of hydroxychloroquine may be beneficial in healthcare professionals participating in high-risk procedures such as intubation, endotracheal aspiration, and bronchoscopy in patients with COVID-19 [16]. Indian Ministry of Health also recommended hydroxychloroquine at a dose of 2x400 mg on the first day, and then once a week prophylaxis for suspected or verified asymptomatic healthcare workers who treated patients with COVID-19 [17]. The Health Care Assessment Guideline, whose theme is COVID-19, organized by the Science Board of the Turkish Ministry of Health, also proposed prophylaxis to

healthcare workers who came into contact with the COVID-19 patient. In this guideline, healthcare professionals who come into contact with COVID-19 patients are categorized according to the actions they take during contact [18].

In our study, we evaluated the early results of the 3-day prophylactic treatment with hydroxychloroquine which was used for those who were considered high-risk contacts by evaluating the healthcare workers who applied to the Occupational Health and Safety unit of our hospital with a history of COVID-19 contact.

MATERIAL and METHOD

A total of 208 health workers (doctors, nurses, technicians, pharmacists, clinical secretaries and cleaning staff) who applied to Occupational Health Unit between 25 March - 25 April 2020, were included in the study after being classified according to their ages, genders, professions, work units, additional diseases and smoking statuses.

Healthcare workers in contact with a patient diagnosed with Covid-19, aged 18 and older, with negative serology and PCR (-) at day 0, females with negative pregnancy test who signed the informed consent forms were included in the study. Employees were evaluated in low, moderate and high risk groups according to the contact risk algorithm included in the Health Care Assessment Guideline with COVID-19 contact [18]. According to guide, the working group is risk-free if the healthcare personnel use the medical mask all their personal protective equipment appropriately; low risk if not using gloves, gown and glasses; moderate risk if the personnel wears a medical mask with goggles and N95 indication; the risk was considered to be high if they did not use any masks. The risk was considered to be high if they did not use any masks. Healthcare workers in contact with a patient diagnosed with Covid-19 without a medical mask were considered to be at high risk if they did not use any medical masks or N95. Employees classified as risk-free were not included in the study by explaining the precautions they should take. According to the Ministry of Health COVID-19 theme Health Assessment Guideline [19] the laboratory algorithm was applied depending on the contact health worker risk categories was followed. Symptoms were monitored for 14 days for healthcare workers included in the low risk class, and active symptom monitoring was performed for those with medium risk; If symptom developed, he was directed to PCR test on the day of symptom, if not on the 7th day. In addition to the follow-up of symptoms, 3-day hydroxychloroquine treatment was initiated for 138 healthcare workers who were considered to be at high-risk. The treatment was arranged as 2x400 mg on the first day and 2x200 mg on the 2nd and 3rd days. PCR results of the healthcare workers evaluated according to the contact risk were followed on the 7th day; the efficacy of prophylaxis was observed in people who received hydroxychloroquine treatment by classifying the information obtained from PCR (+), and PCR (-) cases.

Statistical Analysis

NCSS (Number Cruncher Statistical System) 2007 Statistical Software (Utah, USA) program was used for statistical analysis. While evaluating the study data, Shapiro- Wilk test and box plot charts were used for descriptive statistical methods (mean, standard deviation, median, frequency, ratio) as well as for the normal distribution of variables. One-way Anova test was used in comparing the groups with normally distributed variables, Bonferroni test was employed in determining the group that caused the difference; and Student t test was used in the evaluation of the two groups. In comparison of qualitative data, Pearson Chi-Square test, Fisher's Exact test and Fisher-Freeman Halton test were used. Significance was evaluated at the level of $p<0.05$.

RESULTS

The study was conducted on a total of 208 healthcare workers at Bakirkoy Sadi Konuk Research and Training Hospital between March, 25 2020 and April, 25 2020. The ages of the employees varied between 20 and 64, and the average age was 35.18±9.49 years. While,28.4% of the employees were men and 71.6% were women.

Examination of the employees distribution based on contact status revealed that 10.1% (n=21) were in the low- risk; 23.6% (n=49) in the moderate and 66.3% (n=138) in the high -risk groups.

Table 1. Clinicopathological features of light chain amyloidosis patients.

			Middle			
		Total	Low risk	Middle Risk	High risk	p
Age	**Min-Ort±SD**	35,94±9,81	38,57±11,04	31,54±6,53	35,94±9,80	[a]0,005**
Gender	**Men**	59 (28,4)	4 (19)	14 (28,6)	41 (29,7)	[b]0,600
	Women	149 (71,6)	17 (81)	35 (71,4)	97 (70,3)	
Occupation	**Doctor**	53 (25,5)	0 (0)	5 (10,2)	48 (34,8)	[c]0,001**
	Nurse/Midwife	84 (40,4)	6 (28,6)	28 (57,1)	50 (36,2)	
	Asst. Health Employee	13 (6,3)	1 (4,8)	3 (6,1)	9 (6,5)	
	Personel	27 (13,0)	7 (33,3)	5 (10,2)	15 (10,9)	
	Other Employees	31 (14,9)	7 (33,3)	8 (16,3)	16 (11,6)	
Covid 19 Results	**Covid 19 (-)**	182 (87,5)	18 (85,7)	38 (77,6)	126 (91,3)	[b]0,042*
	Covid-19 (+)	26 (12,5)	3 (14,3)	11 (22,4)	12 (8,7)	
Contact	**Patient**	43 (20,7)	2 (9,5)	6 (12,2)	35 (25,4)	[b]0,062
	Health Employee	165(79,3)	19 (90,5)	43 (87,8)	103 (74,6)	
Smoker (Cigarette)		37 (17,8)	5 (23,8)	2 (4,1)	30 (21,7)	[c]0,007**
Additional Illnesses		75 (36,1)	7 (33,3)	8 (16,3)	60 (43,5)	[b]0,003**

** [a]Oneway ANOVA test, [b]Pearson ki kare test, [c]Fisher Freeman Halton test*
***p<0,01*

Table 2. Evaluations of descriptive properties according to Covid-19 positivity.

		Middle		
		Covid 19 (+) (n=26)	Covid 19 (-) (n=182)	p
Age	**Min-Ort±SD**	34,48±9,62	35,29±9,50	[d]0,691
Gender	**Men**	7 (11,9)	52 (88,1)	[b]0,862
	Women	19 (12,8)	130 (87,2)	
Occupation	**Doctor**	3 (5,7)	50 (94,3)	[c]0,075
	Nurse/Midwife	9 (10,7)	75 (89,3)	
	Asst. Health Employee	1 (7,7)	12 (92,3)	
	Personel	6 (22,2)	25 (80,6)	
	Other Employees	7 (25,9)	20 (74,1)	
Contact	**Patient**	11 (25,6)	32 (74,4)	[b]0,008**
	Health Employee	15 (9,1)	150 (90,9)	
Cigarette		3 (11,5)	34 (18,7)	[b]0,583
Additional Illnesses		13 (50,0)	62 (34,1)	[b]0,113

[b] Pearson chi square test, [c]Fisher Freeman Halton test, [d]Student t test
***p<0,01*

There was a statistically significant difference between the ages by contact risk groups (p<0.01). The average age of the moderate risk group was significantly lower than the low, and high risk groups, respectively p=0.013; p=0.015; p<0.05). There was no significant difference between the average age of low and high risk groups.

There was a statistically significant difference between occupational groups according to contact risk groups (p<0.01). Significantly higher number of nurses were found in the moderate risk group, while doctors were significantly more numerous in the high risk group. Greater number of staff and other employees were also found in the low risk group.

There was a significant difference between COVID-19 positivity rates according to contact risk groups (p<0.05). While the COVID-19 positivity rate was 8.7% in the high risk group, this rate was 22.4% in the moderate risk group and 14.3% in the low risk group (Table 1).

According to COVID-19 test results, no statistically significant difference was found between age and gender distributions (p>0.05) No statistically significant difference was found between COVID-19 test results among the vocational groups of health personnel (p>0.05) (Table 2).

Contact risk was found to be related to the COVID-19 test –positivity rates , and the positivity rate from contact with the patient was found to be significantly high (p <0.01) (Figure 1).

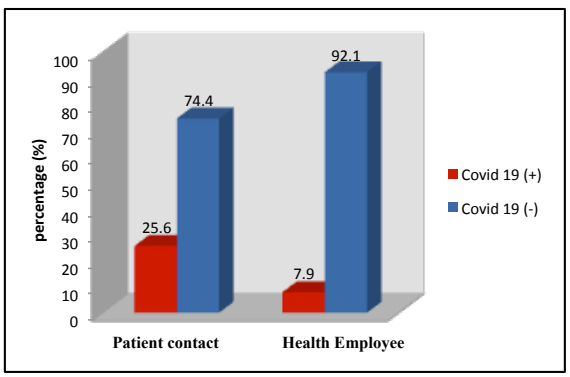

Figure 1. Covid-10 test results distribution by contact status.

DISCUSSION

During the epidemic, healthcare workers are concerned about the health of both themselves and their families. Healthcare workers who are accidentally infected are an important source of infection because they can transmit the virus to their families and patients receiving medical care for different reasons, and to other healthcare professionals they work with.

As one of the first pandemic hospitals with high patient admission in Turkey, supply of personal protective equipment and necessary training for this equipment were provided from the first day. According to the Guidelines for Assessment of Healthcare Professionals with the COVID-19 theme organized by the Ministry of Health Science Board of our country, when the hydroxychloroquine prophylaxis was applied to the group with high risk after contact with COVID-19 patients, the COVID-19 test positivity rate was lower in the group which received prophylaxis than the group that did not. COVID-19 test positivity rate was statistically and significantly lower than those in the low and moderate risk groups (p<0.05). However, we could not say that this completely depends on the application of prophylaxis, since we did not have a control group.

Twenty-six cases of our healthcare professionals were COVID-19 test positive and their ages varied between 20 and 64, and their mean age was 34.48±9.62 years. Seven of healthcare workers with COVID-19 test positivity were men and 19 of them were women. Similarly, the risk factors of 72 healthcare workers who had COVID-19 disease in the tertiary hospital in Wuhan, where the COVID-19 epidemic started, were analyzed and 39 health workers were reported to be working in general departments, and their ages ranged between 21-66 years [20].

Contact is one of the main transmission ways of SARS-CoV-2. Contagion from patients to healthcare workers is generally the result of contamination of the hands of healthcare workers after touching the patients, and hand hygiene is considered as the most important prevention measure that healthcare workers can take. When the contact history was examined, the positivity rates from the contact with COVID-19 patients were found to be significantly higher (p<0.01). The results emphasize the importance of hand hygiene after contact with COVID-19 patients, similar to other studies [20,21].

According to the contact stories, no significant difference was found between the average ages of the low and high risk groups. It was observed that the highest age average was in the low-risk group and the lowest age average was in the moderate -risk group.

The reason for this may be that the senior healthcare professionals might have used the prevention methods more properly as a consequence of their experience.

When the occupational groups were examined

according to the contact risk groups; the number of nurses in the moderate and of the doctors in the high risk group was found to be significantly higher. Depending on the intensive working conditions of the healthcare workers during the epidemic,these higher rates may be associated with greater contact load. Similar to our study in a prospective, observational cohort study in the UK and the USA frontline healthcare workers were found to be at higher risk of reporting COVID-19 test positivity [19].

There are different opinions about the application of hydroxychloroquine prophylaxis in healthcare professionals and its protocols. In a post-exposure prophylaxis and preoperative treatment study for COVID -19 conducted by Lother et al. hydroxychloroquine prophylaxis was planned to be applied to asymptomatic healthcare workers or household contacts exposed to COVID -19 cases within four days so as to prevent serious complications of the disease. Hydroxychloroquine was administered in 200 mg tablets for a total dose of 3.800 mg for 5 consecutive days. Results of the study are expected [22].

Some evidence is known about the effectiveness of hydroxychloroquine against this virus and some clinical data to support the effectiveness of this drug in the prophylaxis of infection. However, controlled clinical studies on this subject are ongoing.

In our study the rates of nurses and doctors in risk groups were found to be significantly higher. The contact was found to be related to the COVID -19 test, and the positivity rate from contact with the patient was found to be significantly high. The COVID-19 pandemic, which affects the whole world, continues, health professionals are also working intensively in this process. As contact with patients increases, healthcare professionals' COVID-19 test positivity rates will also increase. We think that conducting similar studies on larger samples to protect healthcare workers can provide significant benefits to individuals and public health.

Limitations of our study can be stated as follows: 1) long-term results of our study are not yet available 2) follow-up of the participants still continues, 3) lack of a control group in the high-risk group that did not receive prophylaxis. In the guideline published by the Turkish Ministry of Health, prophylaxis is recommended for health workers with high-risk contact history. Therefore, there is no control group in the high-risk group who did not receive prophylaxis. The low number of samples can also be considered as a limitation.

Ethics Committee Approval: For our study, the approval number 2020/144 was obtained from the ethics committee of University of Health Sciences, Bakırköy Dr. Sadi Konuk Training and Research Hospital.
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Conflict of interest: The authors declare that they have no conflict of interest.
Informed Constent: All subjects provided informed consent to participate in the study.

REFERENCES

1. Yao X, Ye F, Zhang M, Cui C, Huang B, Niu P, et al. In Vitro Antiviral Activity and Projection of Optimized Dosing Design of Hydroxychloroquine for the Treatment of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Clin Infect Dis. 2020;71(15):732-9. https://doi.org/10.1093/cid/ciaa237
2. World Health Organization. Novel Coronavirus (2019-nCov) Situation Report-1 21 January 2020. Available from: https://apps.who.int/iris/bitstream/handle/10665/330760/nCoVsitrep21Jan2020-eng.pdf?sequence=3&isAllowed=y
3. World Health Organization. Coronavirus (COVID-19) disease dashboard. Available from: https://covid19.who.int/ (cited 2020 May 20).
4. Turkish Ministry of Health. Coronavirus dashboard. Available from: https://covid19.saglik.gov.tr/ (cited 2020 May 20).
5. Virology: Coronaviruses. Nature. 1968;220:650. https://doi.org/10.1038/220650b0
6. Wan Y, Shang J, Graham R, Baric RS, Li F. Receptor recognition by novelcoronavirus from Wuhan: An analysis based on decade-long structural studies of SARS. J Virol. 2020;94(7):e00127-20. https://doi.org/10.1128/JVI.00127-20
7. Tang T, Bidon M, Jaimes JA, et al. Coronavirus membrane fusion mechanism offers a potential target for antiviral development. Antiviral Res. 2020;178:104792. https://doi.org/10.1016/j.antiviral.2020.104792
8. Han Q, Lin Q, Jin S, You L. Coronavirus 2019-nCoV: A brief perspective from the front line. J Infect. 2020;80(4):373-7. https://doi.org/10.1016/j.jinf.2020.02.010
9. Backer JA, Klinkenberg D, Wallinga J. Incubation period of 2019 novel coronavirus (2019-nCoV) infections among travellers from Wuhan, China, 20-28 January 2020. Euro Surveill. 2020;25(5):2000062. https://doi.org/10.2807/1560-7917.ES.2020.25.5.2000062
10. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020;323(11):1061-9. https://doi.org/10.1001/jama.2020.1585
11. Schwartz J, King C-C, Yen M-Y. Protecting Healthcare Workers During the Coronavirus Disease 2019 (COVID-19) Outbreak: Lessons From Taiwan's Severe Acute Respiratory Syndrome

Response. Clin Infect Dis. 2020;71(15):858-60. https://doi.org/10.1093/cid/ciaa255
12. Turkish Ministry of Health information meeting with WHO. Available from: https://www.saglik.gov.tr/TR,65370/bakan-koca-dsoye-turkiyenin-covid-19-mucadelesini-anlatti.html (cited 2020 April 30).
13. Hoffmann C. Treatment. In: Kamps BS, Hoffmann C, editors. Covid Reference, Edition 2020-2. SteinHauser Verlag; Amedeo, Germany; 2020. Available from: https://www.covidreference.com (cited 2020 April 12).
14. Schrezenmeie E, Dörner T. Mechanisms of action of hydroxychloroquine and chloroquine: implications for rheumatology. Nat Rev Rheumatol. 2020;16(3):155-66. https://doi.org/10.1038/s41584-020-0372-x
15. Lu CC, Chen MY, Lee WS, Chang YL. Potential therapeutic agents against COVID-19: What we know so far. J Chin Med Assoc. 2020;83(6):534-6. https://doi.org/10.1097/JCMA.0000000000000318
16. Pagliano P, Piazza O, De Caro F, Ascione T, Filipelli A. Is Hydroxychloroquine a Possible Postexposure Prophylaxis Drug to Limit the Transmission to Healthcare Workers Exposed to Coronavirus Disease 2019? Clin Infect Dis. 2020;71(15):887-8. https://doi.org/10.1093/cid/ciaa320
17. Rathi S, Ish P, Kalantri A, Kalantri S Hydroxychloroquine prophylaxis for Covid-19 contacts in India. Lancet Infect Dis. 2020;S1473-3099(20)30313-3. https://doi.org/10.1016/S1473-3099(20)30313-3
18. Turkish Ministry of Health Guidance to COVID-19. Available from: https://covid19.saglik.gov.tr/Eklenti/37692/0/covid19-temasiolansaglikcalisanlarinindegerlendirilmesipdf.pdf (cited 2020 March 25).
19. Nguyen LH, Drew DA, Joshi AD, et al. Risk of COVID-19 among frontline healthcare workers and the general community: a prospective cohort study [Preprint]. medRxiv. 2020 May 25;2020.04.29.20084111. https://doi.org/10.1101/2020.04.29.20084111
20. Ran L, Chen X, Wang Y, et al. Risk factors of healthcare workers with corona virus disease 2019: a retrospective cohort study in a designated hospital of Wuhan in China. Clin Infect Dis. 2020;ciaa287. https://doi.org/10.1093/cid/ciaa287
21. Lu W, Danni Y, Xinlan W, Yujuan C, You L, Huai Y. Correlation between hand hygiene compliance and nosocomial infection in medical staff. Chinese Journal of Disinfection 2014;31(11):1237-8.
22. Lother SA, Abassi M, Agostinis A, Bangdiwala AS, Cheng MP, Drobot G, et al. Post-exposure prophylaxis or pre-emptive therapy for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2): study protocol for a pragmatic randomized-controlled trial. Can J Anaesth. 2020;67(9):1201-11. https://doi.org/10.1007/s12630-020-01684-7

Original Article

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Assessment of Knowledge Levels of Nurses Working in Surgical Clinics About ERAS Protocol

Cerrahi Kliniklerde Çalışan Hemşirelerin ERAS Protokolüne Yönelik Bilgi Düzeylerinin İncelenmesi

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ABSTRACT

Objective: *The aim of the study was to assess the knowledge levels of nurses working in surgical clinics about ERAS protocol.*
Method: *The study was carried out as a descriptive study. The sample of the study consisted of 127 surgical unit nurses who were not on leave or sick leave during the study period and who were willing to participate. A data collection form consisting of two sections and 47 questions developed by the researchers was used to collect the data. The necessary ethical and institutional approvals were obtained before the study.*
Results: *In the study 84.25% of the nurses stated that they did not know about the ERAS protocol, 88.97% indicated that the institution where they were working did not implement ERAS practices, 99.21% said that they did not follow any publication on the ERAS protocol,and 99.21% expressed that they did not receive any training on the ERAS protocol.*
Conclusion: *It was determined that most of the surgical nurses in the study did not know about the ERAS protocol and that ERAS protocol were not implemented in the clinic where they were working. In line with these results, we can recommend that surgical nurses follow the current developments and evidence-based guidelines on the ERAS protocol. Organization of trainings for the implementation of ERAS practices and ensuring the participation of nurses can help increase their knowledge levels in this regard.*

Keywords: *ERAS, nursing, information, enhanced recovery after surgery protocol*

ÖZ

Amaç: *Çalışmanın amacı cerrahi kliniklerde çalışan hemşirelerin ERAS protokolüne ilişkin bilgi düzeylerinin incelemektir.*
Yöntem: *Çalışma tanımlayıcı olarak gerçekleştirildi. Araştırmanın örneklemini ise çalışmanın yapıldığı dönemde izinli/ raporlu olmayan ve araştırmaya katılma konusunda istekli olan 127 cerrahi birim hemşiresi oluşturdu. Verilerin toplanmasında araştırmacılar tarafından geliştirilen iki bölüm ve toplam 47 sorudan oluşan veri toplama formu kullanıldı. Çalışmaya başlamadan önce gerekli etik ve kurum izni alındı.*
Bulgular: *Çalışmada hemşirelerin %84.25'i "ERAS protokolünü bilmediklerini, %88,97'si çalıştıkları klinikte ERAS protokolü uygulamalarına yer verilmediğini, %99,21'i ise "ERAS protokolüne yönelik herhangi bir yayını takip etmediğini, %99,21'i ERAS protokolünü içeren herhangi bir eğitim almadığını belirtti.*
Sonuç: *Çalışmadaki cerrahi hemşirelerin çoğunluğunun ERAS protokolünü bilmedikleri ve çalıştıkları klinikte ERAS protokolü uygulamalarına yer verilmediği belirlendi Bu sonuçlar doğrultusunda cerrahi hemşirelerinin ERAS protokolüne yönelik güncel gelişmeleri ve kanıta dayalı rehberleri takip etmeleri, eras protokolü uygulamalarına yönelik eğitimlerin düzenlenmesi ve hemşirelerin katılımlarının sağlanması ile hemşirelerin bilgi düzeylerinin arttırılması önerilebilir.*

Anahtar kelimeler: *ERAS, hemşirelik, bilgi, cerrahi sonrası hızlandırılmış iyileşme protokolü*

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INTRODUCTION

With the recent developments in surgical practices and anesthesia methods, in particular, there has been a significant increase in the number of patients who are undergoing surgery [1]. Due to this serious increase in patient population, the treatment and care protocols of hospitals are inadequate, so progress should be made with evidence-based practices instead of traditional approaches [1,2]. It is known that recovery after surgery can be accelerated and mortality due to surgery can be reduced with an up-to-date approach on treatment and care in surgery and evidence-based practices. One of such evidence-based practices is the Enhanced Recovery After Surgery (ERAS) protocol, also known as the Fast Track Surgery (FTS) protocol, developed by the ERAS Society [3,4]. The ERAS protocol can be used for colorectal, gynecological and thoracic operations and other complex procedures [5]. The ERAS protocol requires a patient-centered, evidence-based and interdisciplinary approach to reduce patients' response to surgical stress, optimize their physiological functions and facilitate surgical recovery [6-11]. It is reported in the literature that ERAS has contributed to patient outcomes, reduced postoperative complications, accelerated recovery and supported early discharge [12-15]. Today, many evidence shows that the ERAS protocol reduces hospital stay by 2-3 days and morbidity and complication rates by 30-50%. Correspondingly, it leads to a decrease in the cost of health services for both the institution and the patient [16-20].

The components of the ERAS protocol include the preoperative, perioperative and postoperative periods. The preoperative period includes practices such as consultancy prior to admission, loading of liquid and carbohydrates, not prolonging fasting periods, not performing or selectively performing intestinal preparation, using antibiotic prophylaxis, thromboprophylaxis without premedication. The perioperative period includes practices such as using short-acting anesthetic agents, applying mid-thoracic epidural anesthesia/analgesia, avoiding drainage, salt and water loading and ensuring normothermia (heating the body, using heated intravenous fluids). The postoperative period includes practices such as applying mid-thoracic epidural anesthesia/analgesia, not using nasogastric tubes, preventing nausea and vomiting, avoiding salt and water loading, early removal of catheters, early initiation of oral feeding, using non-opioid oral analgesia, early mobilization, stimulating bowel movements and inspection of results and compliance with the protocol [5,10,21].

However, the literature shows low rates of postoperative care in accordance with the ERAS protocol. McLeod et al. [22] determined the obstacles for the implementation of ERAS to be the lack of workforce, hospital resources and participation and poor communication between team members. Successful implementation of ERAS depends on nurses to accept the use of this protocol and anesthesiologists and physicians to be in collaboration [6]. Considering the research on the implementation of ERAS, it is seen that most institutions focus on the perspective and effect of physicians' roles. However, taking into account that patient care is interdisciplinary in ERAS, nurses have a key role in overcoming ERAS implementation barriers and ensuring compliance with the protocol [11]. Understanding the role of nurses in the implementation of the ERAS protocol is important for future research. Although the global literature includes many studies on the ERAS protocol, there are only a limited number of studies on implementation involving the roles of nurses or its relationship with nursing [23]. There are also a limited number of reviews and researches by nurses on the ERAS protocol in Turkey [4,10,24-28]. In line with the information in the literature, the purpose of this study was to assess the knowledge levels of nurses working in surgical clinics about ERAS protocol and to contribute to the literature.

MATERIAL and METHOD

This study was carried out as a descriptive study between October 2018-March 2019 in the surgical units (orthopedics, plastic surgery, eye surgery, otolaryngology, urology, general surgery, neurosurgery and cardiovascular surgery) of one state, one city and one university hospital.

The target population of the study consisted of 360 nurses working in the surgical units (orthopedics, plastic surgery, eye surgery, otolaryngology, urology, general surgery, neurosurgery and cardiovascular

surgery) of a State Hospital, a City Hospital and a University Hospital in the same city in Turkey. The sample of the study consisted of 127 surgical unit nurses working in the surgical units of a State Hospital, a City Hospital and a University Hospital in the same city hospital between October 2018-March 2019, who were not on leave and were willing to participate. The data were collected by the researchers in 10-15 minutes by face-to-face interviews with the nurses on their working days and at certain hours not interfering with their work after obtaining written and verbal permission from the participants.

A data collection form developed by researchers and consisting of two sections was used to collect data. After the form was created, it was revised in line with the expert opinions of 5 people on the ERAS protocol. The first section included 13 questions for demographic characteristics (age, marital status, gender, educational status, etc.), occupational data (total work years, work hours) and individual questions regarding ERAS. The second section consisted of 34 questions regarding the information on ERAS protocol [29-31]. The Statistical Package for the Social Sciences 25 (IBM SPSS) software was used for the statistical analyses. In data evaluation, descriptive statistical methods (mean, standard deviation, frequency, etc.) were used.

Before the study, ethical approval was received from the Ethics Committee of Balıkesir University (No: 20188/188), and institutional approvals were obtained from the state hospital and city hospital where the study was conducted. The nurses were informed that all information written on the forms would be kept by the researchers, that their answers would remain confidential and would only be used for scientific purposes. The nurses gave verbal and written permission regarding their willingness to participate in the study.

RESULTS

It was determined that 26.7% of the nurses participating in the study were aged between 37-42 years, 59.05% had a bachelor's degree, 45.94% were in the profession for ≥16 years, and 72.44% worked for 40-49 hours weekly (Table 1).

The statistical evaluation of the knowledge levels of the nurses regarding ERAS is given in Table 2. In the study 84.25% of the nurses stated that they did not

Table 1. Demographic characteristics of nurses working in surgical clinics (n=127).

		n	%
Age	19-24 age	24	18.9
	25-30 age	25	19.7
	31-36 age	17	13.4
	37-42 age	34	26.7
	42 age and above	27	21.3
Marital status	Married	89	70.07
	Single	38	29.93
Graduate program	Vocational School of Health	25	19.68
	Associate Degree	20	15.74
	Bachelor's Degree	75	59.05
	Postgraduate	7	5.51
Total years in the profession	Less than 1 year	5	3.94
	1-5 years	27	21.26
	6-10 years	26	20.47
	11-15 years	14	11.02
	16 years and above	55	43.31
Weekly work hours	40-49	93	73.23
	50-59	23	18.11
	60 hours and above	11	8.66
Hospital where they worked	State hospital	28	22.05
	City hospital	60	47.24
	University hospital	39	30.1

Table 2. Knowledge on ERAS protocol (n=127).

		n	%
Could you write down what you know about the ERAS protocol?	I do not know	107	84.25
	Other	20	15.75
Are there any sources where you follow the latest developments in the field of surgery?	Yes	7	5.51
	No	120	94.49
Are ERAS protocol practices implemented in your clinic?	Yes	14	11.02
	No	113	88.98
Are there any publications you follow for the ERAS protocol?	Yes	126	99.21
	No	1	0.79
Have you attended any training that includes the ERAS protocol?	Yes	126	99.21
	No	1	0.79
Do you think ERAS protocol practices are useful?	Yes	27	21.26
	No	2	1.58
	I do not know	98	77.16

know about the ERAS protocol, 88.97% stated that the institution where they were working did not implement ERAS practices, 99.21% indicated that they did not follow any publication on the ERAS protocol, 99.21% reported that they did not receive any training on the ERAS protocol, and %77.16 expressed that they did not know whether ERAS practices were useful.

The numbers and percentages of the responses to the questions for the preoperative, perioperative and postoperative parts of the ERAS protocol are given in Table 3. For the preoperative part, 95.27% of the surgical nurses stated that the item "Patient counseling and education should begin at the first visit and should continue throughout the surgical procedure" was "correct", and 74.80% stated that the item "clear fluids can be taken up to 2 hours before surgery" was "incorrect".

For the perioperative part, the item "Risk factors should be evaluated for nausea and vomiting after surgery" was found to be the most correct answer by 90.55%, and the item "Short-acting anesthetics should be used" was the most incorrect answer by 19.68% of the participants. For the postoperative part, the item "Catheters should be removed as soon as possible" was found to be the most correct answer by 81.88%, and "Oral feeding should be provided in the early postoperative period" was the most incorrect answer by 27.55% of the participants.

DISCUSSION

For enhanced recovery after surgery and the successful implementation of the ERAS protocol, it is important for nurses to have high awareness and knowledge about ERAS, in addition to all other healthcare team members. It is reported in the literature that there are gaps in the training of healthcare professionals in terms of ERAS protocols and the implementation of these protocols [32]. Conn et al. [33] examined the experience of practitioners in successfully implementing postoperative recovery for elective colorectal surgery. In their qualitative study on 26 healthcare workers, they showed that most surgeons and anesthesiologists knew about the principles of ERAS, but most nurses did not know about the ERAS protocol. Similarly, Ince and Celebi [27] and Guzel and Yava [28], Kirik [34], Gustafsson et al. [35] found that most nurses did not have sufficient knowledge about ERAS in perioperative care. We also determined that most of the nurses did not have information about the ERAS protocol in this study. This finding is similar to the literature, and we think that it may be due to the continuation of traditional practices rather than evidence-based practices such as ERAS in Turkey and in some other countries, and because nurses do not receive any training on ERAS and follow publications in this regard.

Successful implementation of the ERAS protocol is possible only through the collaboration of a team of surgeons, anesthesiologists and nurses [36]. Herbert

Table 3. Knowledge levels regarding ERAS protocol.

Preoperative Period	Correct (%)	Incorrect (%)	Undecided (%)
1. Patient counseling and education should begin at the patient's first visit and should continue throughout the surgical procedure.	95.27	0	4.73
2. Patients should receive detailed education about the ERAS protocol with all team members.	71.65	3.15	25.20
3. Smoking, alcohol use and presence of anemia should be routinely investigated in the preoperative period	92.91	0	6.29
4. The patient should stop smoking at least 4 weeks before the intervention.	62.99	7.09	29.92
5. The patient should stop alcohol use at least 4 weeks before the intervention.	69.29	7.09	23.62
6. Blood glucose level should be kept at an optimum level.	87.40	2.36	10.24
7. Intestinal cleaning performed before surgery is effective in reducing infection rates.	73.23	8.66	18.11
8. Solid foods can be taken up to 6 hours before surgery.	24.41	65.35	10.24
9. Heterogeneous liquids (juice) can be taken up to 4 hours before surgery.	20.47	67.72	11.81
10. Clear fluids can be taken up to 2 hours before surgery.	12.60	74.80	12.60
11. Administration of carbohydrate fluids until the midnight before surgery accelerates recovery in the postoperative period.	11.81	34.65	53.54
12. The use of routinely applied long-acting sedatives should be avoided 12 hours before surgery.	48.04	7.87	44.09
13. Short-acting anxiolytics should be used before surgery.	30.71	18.11	51.18
14. Thromboembolism prophylaxis should be started the day before surgery.	39.37	23.62	37.01
15. Nutritional status should be evaluated, and nutritional support should be provided if NRS-2002/SGD-C score is above 3.	72.44	0	27.56
16. Short-acting anesthetics should be used.	41.73	19.69	38.58
17. Risk factors for nausea and vomiting after surgery should be evaluated.	90.55	1.58	7.87
18. Drainages, tubes and catheters should be used limitedly and only if necessary and should be removed as soon as possible.	81.89	2.36	15.75
19. Patients should be heated 10-20 minutes before surgery to ensure normothermia.	51.18	16.54	32.28
20. Antimicrobial prophylaxis should be done intravenously an hour before incision.	69.29	5.51	25.20
21. Patients should be given fluids (colloids and crystalloids) so that their cardiac functions remain optimal.	77.17	3.93	18.90
22. Advanced hemodynamic monitoring should be used for easy monitoring of fluid therapy and effective oxygen transport in the perioperative period.	62.21	8.66	29.13
23. Mid-thoracic epidural anesthesia/analgesia should be used.	35.43	14.96	49.61
24. Low-molecular-weight heparin should be used for postoperative thromboembolism prophylaxis.	56.69	7.09	36.22
25. Antiemetic prophylaxis should be performed to reduce nausea and vomiting after surgery.	74.80	3.94	21.26
26. Catheters should be removed as soon as possible.	81.89	0.79	17.32
27. High energy fluids after surgery should contain protein/carbohydrate.	59.06	10.24	30.71
28. Balanced crystalloid solutions should be used instead of 0.9% sodium chloride to prevent hyperchloremic acidosis.	38.58	8.66	52.76
29. Patients should be ensured to chew gums to prevent distension and constipation after surgery.	30.71	26.77	42.52
30. Controlled insulin therapy and regular blood glucose monitoring should be performed to prevent the development of hypoglycemia in patients with severe hyperglycemia.	74.02	4.72	21.26
31. Opioid use should be reduced after surgery.	57.48	4.72	37.80
32. A multimodal pain relief method should be used to control pain.	57.48	1.58	40.94
33. Oral feeding should be provided in the early postoperative period.	53.54	27.56	18.90
34. Patients should be kept out of bed for 2 hours on the day of surgery and 6 hours a day until discharge.	62.22	13.37	24.41

et al. [37] stated that ERAS is a strong evidence-based practice, but it has a slow transition to practice in clinics. Ament et al. [38] stated that the communication, institutional culture and structural features of clinics (circulation of employees) are common issues related to the applicability and sustainability of the ERAS protocol. Most of the nurses participating in this study stated that ERAS practices were not included in the clinic where they worked. This finding shows that the institutional culture and structural features of the clinics may have been effective in the lack of implementation of ERAS in the clinics where the nurses worked.

The ERAS protocol covers both preoperative, perioperative and postoperative periods. This protocol includes practices such as preoperative patient education and counseling, prevention of prolonged hunger due to surgery through nutrition, standardized analgesic and anesthetic regimens and early mobilization [23,39-41].

Educating patients before the surgery about issues such as the surgical team, possible complications and their management, pain management, etc. is the most important component of the ERAS protocol [23,42]. Inci and Celebi [27] found that the knowledge of nurses on preoperative training and counseling was compatible with ERAS. Similarly, in this study, the fact that the nurses knew that patients should be

educated in the preoperative period is compatible with the ERAS protocol.

The 2011-2017 guidelines of the American Society of Anesthesiologists (ASA) states that it is sufficient to stop the consumption of solid foods six hours before the operation and that of clear liquids two hours before the operation [43]. Patients undergoing surgery should be given 800 ml of liquid food rich in carbohydrates until the midnight before surgery and 400 ml 2–3 hours before surgery. This practice has been shown to improve postoperative well-being, reduce insulin resistance and significantly shorten hospital stay [44]. In the study of Inci and Celebi [27], the knowledge levels of nurses regarding fasting times were not found to be compatible with the ERAS protocol. In the study of Kankilic and Tuna [45], it was found that only 4.2% of healthcare professionals performed practices in line with the fasting recommendations of the ERAS protocol. Similarly, in this study, the knowledge levels of nurses regarding fasting times before surgery were not found to be compatible with the ERAS protocol. This result may be related to the continuation of the traditional attitudes of nurses working in surgical clinics regarding fasting times and their lack of sufficient knowledge in this regard.

Postoperative nausea-vomiting should be prevented, because it can restrict the oral feeding of patients in the early period. For this purpose, the use of agents that can induce vomiting should be avoided during the surgery, and combined antiemetic agents should be used (highly evidenced, strongly recommended) [35,44]. In this study, the knowledge levels and practices of nurses regarding nausea and vomiting were compatible with the ERAS protocols. This may suggest that nurses are conscious about risk factors in preventing nausea and vomiting after surgery and that antiemetic drugs are routinely applied during operations. A wide variety of agents are used to reduce preoperative anxiety. In practices similar with the ERAS protocol, long-acting premedication agents should be avoided [46]. In this study, it was seen that nurses had limited knowledge on the use of short-acting anesthetics.

It is recommended that urinary catheters should be removed in the early period due to their disadvantages such as urinary infection and restriction of mobilization [1,35]. In the current study, we observed that the practices of nurses regarding the removal of catheters were in accordance with the ERAS protocol. This may be due to the fact that the early removal of catheters is a routine practice performed in the clinics. The transition to oral feeding in the early postoperative period reduces both hospital stay and infection risk. However, early oral feeding may increase the risk of vomiting and may lead to problems such as delay in mobilization, pulmonary problems and bloating when a multimodal treatment is not applied [47]. In the study of Inci and Celebi [27], it was observed that nurses had low levels of knowledge regarding transition to oral feeding in the postoperative period. Similarly, in this study, we observed that the practices of nurses regarding transition to oral feeding in the postoperative period were in line with the ERAS protocols.

Limitations: The study was limited to State Hospital, City Hospital and University Hospital in the same city.

CONCLUSIONS

In conclusion, we determined that most of the surgical nurses in the study did not know about the ERAS protocol, that ERAS practices were not included in the clinics where they were working, that they did not follow any publications regarding the ERAS protocol, that they received no training including the ERAS protocol and that they did not know whether ERAS practices were useful. It was seen that the nurses had limited knowledge levels regarding the intake of clear fluids up to 2 hours before surgery, the preference of using short-acting anesthetics and the transition to oral feeding in the early postoperative period. In line with these results, we can recommend that surgical nurses follow the current developments and evidence-based guidelines on the ERAS protocol. Organization of trainings for the implementation of ERAS practices and ensuring the participation of nurses can help increase their knowledge levels in this regard. Considering the literature, it is seen that there are limited international and national publications on the topic. Thus, further studies with larger sample groups and different study types can be planned.

Ethics Committee Approval: Ethical approval was received from the Ethics Committee of Balıkesir

University (No: 20188/188), and institutional approvals were obtained from the state hospital and city hospital where the study was conducted.

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REFERENCES

1. Demirhan I, Pınar G. Enhanced recovery after surgery and nursing practices enhanced recovery and nursing. Yıldırım Beyazıt Üniversitesi Sağlık Bilimleri Fakültesi Hemşirelik E-Dergisi. 2014;(2)1:43-53. Available from: http://hdergi.ybu.edu.tr/index.php/e-dergi/article/view/62
2. Batdorf NJ, Lemaine V, Lovely JK, Ballman KV, Goede WJ., Martinez-Jorge J, et al. Enhanced recovery after surgery in microvascular breast reconstruction. J Plast Reconstr Aesthet Surg. 2015;68(3):395-402. https://doi.org/10.1016/j.bjps.2014.11.014
3. Abdikarim I, Cao XY, Li SZ, Zhao YQ, Taupyk, Y, Wang Q. Enhanced recovery after surgery with laparoscopic radical gastrectomy for stomach carcinomas. World J Gastroenterol. 2015;21(47):13339-44. https://doi.org/10.3748/wjg.v21.i47.13339
4. Cilingir D, Candas B. Enhanced recovery after surgery protocol and nurse's role. Journal of Anatolia Nursing and Health Sciences. 2017;20(2):137-43. Available from: https://dergipark.org.tr/tr/download/article-file/348666
5. Persico M, Miller D, Way C, Williamson M, O'Keefe K, Strnatko D, et al. Implementation of enhanced recovery after surgery in a community hospital: an evidence-based approach. J Perianesth Nurs. 2019;34(1):188-197. https://doi.org/10.1016/j.jopan.2018.02.005
6. Hübner M, Addor V, Slieker J, Griesser AC, Lécureux E, Blanc C, et al. The impact of an enhanced recovery pathway on nursing workload: a retrospective cohort study. Int J Surg. 2015;24(Pt A):45-50. https://doi.org/10.1016/j.ijsu.2015.10.025
7. Feldheiser A, Aziz O, Baldini G, Cox BPBW, Fearon K CH, Feldman LS, et al. Enhanced recovery after surgery (ERAS) for gastrointestinal surgery, part 2: consensus statement for anaesthesia practice. Acta Anaesthesiol Scand. 2016;60(3):289-334. https://doi.org/10.1111/aas.12651
8. Bray MS, Appel AL, Kallies KJ, Borgert AJ, Zinnel BA, Shapiro SB. Implementation of an enhanced recovery after surgery program for colorectal surgery at a community teaching hospital. WMJ. 2017;116(1):22-6. Available from: https://wmjonline.org/wp-content/uploads/2017/116/1/22.pdf
9. Watson DJ. The role of the nurse coordinator in the enhanced recovery after surgery program. Nursing. 2017;47(9):13-7. https://doi.org/10.1097/01.NURSE.0000522018.00182.c7
10. Aksoy A, Vefikulucay Yılmaz D. A new approach to evidence based practices in gynecological surgery: ERAS protocol and nursing. Turkiye Klinikleri J Nurs Sci. 2018;10(1):49-58. https://doi.org/10.5336/nurses.2017-56268
11. Brown D, Xhaja A. (2018). Nursing perspectives on enhanced recovery after surgery. Surg Clin North Am. 2018;98(6):1211-21. https://doi.org/10.1016/j.suc.2018.07.008
12. Adamina M, Kehlet H, Tomlinson GA, Senagore AJ, Delaney CP. Enhanced recovery pathways optimize health outcomes and resource utilization: a meta-analysis of randomized controlled trials in colorectal surgery. Surgery. 2011;149(6):830-40. https://doi.org/10.1016/j.surg.2010.11.003
13. Hughes MJ, McNally S, Wigmore SJ. Enhanced recovery following liver surgery: a systematic review and meta-analysis. HPB (Oxford). 2014;16(8):699-706. https://doi.org/10.1111/hpb.12245
14. Miller TE, Thacker JK, White WD, Mantyh C, Migaly J, Jin J, et al. Reduced length of hospital stay in colorectal surgery after implementation of an enhanced recovery protocol. Anesth Analg. 2014;118(5):1052-61. https://doi.org/10.1213/ANE.0000000000000206
15. Scott MJ, Miller TE. Pathophysiology of major surgery and the role of enhanced recovery pathways and the anesthesiologist to improve outcomes. Anesthesiol Clin. 2015;33(1):79-91. https://doi.org/10.1016/j.anclin.2014.11.006
16. Archibald LH, Ott MJ, Gale CM, Zhang J, Peters MS, Stroud GK. Enhanced recovery after colon surgery in a community hospital system. Dis Colon Rectum. 2011;54(7):840-5. https://doi.org/10.1007/DCR.0b013e31821645bd
17. Melnyk M, Casey RG, Black P, Koupparis AJ. Enhanced recovery after surgery (ERAS) protocols: time to change practice?. Can Urol Assoc J. 2011;5(5):342-8. https://doi.org/10.5489/cuaj.11002
18. Chandrakantan A, Gan TJ. Demonstrating value: a case study of enhanced recovery. Anesthesiol Clin. 2015;33(4):629-50. https://doi.org/10.1016/j.anclin.2015.07.004
19. Stowers MD, Lemanu DP, Hill AG. Health economics in enhanced recovery after surgery programs. Can J Anaesth. 2015;62(2):219-30. https://doi.org/10.1007/s12630-014-0272-0
20. Thiele RH, Rea KM, Turrentine FE, Friel CM, Hassinger TE, Goudreau BJ, et al. Standardization of care: impact of an enhanced recovery protocol on length of stay, complications, and direct costs after colorectal surgery. J Am Coll Surg. 2015;220(4):430-43. https://doi.org/10.1016/j.jamcollsurg.2014.12.042
21. Bernard H, Foss, M. Patient experiences of enhanced recovery after surgery (ERAS). Br J Nurs. 2014;23(2):100-6. https://doi.org/10.12968/bjon.2014.23.2.100
22. McLeod RS, Aarts MA, Chung F, Eskicioglu C, Forbes SS, Conn LG, et al. Development of an enhanced recovery after surgery guideline and implementation strategy based on the knowledge-to-action cycle. Ann Surg. 2015;262(6):1016-25. https://doi.org/10.1097/SLA.0000000000001067
23. Mendes DIA, Ferrito CRDAC, Gonçalves MIR. Nursing ınterventions in the enhanced recovery after surgery®: scoping review. Rev Bras Enferm. 2018;71(suppl 6):2824-32. https://doi.org/10.1590/0034-7167-2018-0436
24. Kabatas MS, Ozbayır T. Enhanced recovery after surgery (ERAS) protocols after colorectal surgery: a systematic review. Gümüşhane University Journal of Health Sciences. 2016;5(3):120-32. Available from: https://dergipark.org.tr/tr/download/article-file/220051
25. Unlü H. Nursing care in elderly patients who were total hip or knee arthroplasty with rapid recovery protocol. Turkiye Klinikleri J Surg Nurs-Special Topics. 2017;3(2):143-50. Available from: https://www.turkiyeklinikleri.com/article/en-hizli-iyilesme-protokolu-ile-total-kalca-veya-diz-artroplastisi-yapilan-yasli-hastalarda-hemsirelik-bakimi-78855.html
26. Tuna PT, Kursun S. ERAS and nursing care for colon surgery. Dokuz Eylül Üniversitesi Hemşirelik Fakültesi Elektronik Dergisi. 2018;11(2):180-88. Available from: https://dergipark.org.tr/tr/download/article-file/752812
27. Celebi E, Ilce A. Determination of knowledge levels of nurses working ın surgical clinics on ERAS protocols. In: 3. International, 11. National Turkish Surgical and Operating Room Nursing Congress Book. 2019. p. 392-400.
28. Guzel N, Yava A. The determination of knowledge and attitudes on enhanced recovery after surgery protocol of the nurses who working on surgical units. Journal of Zeugma Health

Sciences. 2019;1(1):15-23. Available from: https://sbf.hku.edu.tr/wp-content/uploads/2020/01/Cerrahi-kliniklerinde-%C3%A7al%C4%B1%C5%9Fan-hem%C5%9Firelerin-ERAS-enhanced-recovery-after-surgery-protokol%C3%BCneili%C5%9Fkin-bilgi-ve-tutumlar%C4%B1n%C4%B1nbelirlenmesi.pdf
29. Lassen K, Coolsen MM, Slim K, Carli F, de Aguilar-Nascimento JE, Schäfer M, et al. Guidelines for perioperative care for pancreaticoduodenectomy: enhanced recovery after surgery (ERAS®) Society recommendations. World J Surg. 2013; 37(2):240-58. https://doi.org/10.1007/s00268-012-1771-1
30. Elias KM. Understanding enhanced recovery after surgery guidelines: an introductory approach. J Laparoendosc Adv Surg Tech A. 2017;27(9):871-5. https://doi.org/10.1089/lap.2017.0342
31. American Association of Nurse Anesthetists. Enhanced recovery after surgery. Available from: https://www.aana.com/practice/clinical-practice-resources/enhanced-recovery-after-surgery (cited 2019 April 22).
32. Austin J. The effect of an education plan on nursing ıntervention compliance with inpatient post-operative colorectal surgical patients using enhanced recovery after surgery (ERAS) protocols [doctoral dissertation]. USA: University of Kentucky, College of Nursing; 2019.
33. Conn LG, McKenzie M, Pearsall EA, McLeod RS. Successful implementation of an enhanced recovery after surgery programme for elective colorectal surgery: a process evaluation of champions' experiences. Implement Sci. 2015;10:99. https://doi.org/10.1186/s13012-015-0289-y
34. Kırık MS. Kolorektal ameliyatlarda klinik alanda ameliyat öncesi sırası ve sonrası uygulamaların ERAS protokolüne uygunluğunun karşılaştırılması [master's thesis]. Gaziantep: Sanko Üniversitesi, Sağlık Bilimleri Enstitüsü; 2018.
35. Gustafsson UO, Scott MJ, Hubner M, Nygren J, Demartines N, Francis N, et al. Guidelines for perioperative care in elective colorectal surgery: enhanced recovery after surgery (ERAS) society recommendations. World J Surg. 2019;43(3):659-95. https://doi.org/10.1007/s00268-018-4844-y
36. Bozkırlı BO, Gündogdu RH, Ersoy PE, Akbaba S, Temel H, Sayın T. ERAS protokolü kolorektal cerrahi sonuçlarımızı etkiledi mi?. Turkish Journal of Surgery. 2012;28(3):149-52. https://doi.org/10.5152/UCD.2012.05
37. Herbert G, Sutton E, Burden S, Lewis S, Thomas S, Ness A, et al. Healthcare professionals' views of the enhanced recovery after surgery programme: a qualitative investigation. BMC Health Serv Res. 2017;17(1):617. https://doi.org/10.1186/s12913-017-2547-y
38. Ament SM, Gilliseen F, Moser A, Maessen JM, Dirksen CD, von Meyenfeldt MF, et al. Factors associated with sustainability of 2 quality improvement programs after achieving early implementation success. A qualitative case study. J Eval Clin Pract. 2017;23(6):1135-43. https://doi.org/10.1111/jep.12735
39. Nelson G, Altman A, Nick A, Meyer L, Ramirez PT, Achtari C, et al. Guidelines for preand intraoperative care in gynecologic/oncology surgery: enhanced recovery after surgery (ERAS®) society recommendations. Gynecol Oncol. 2016;140(2):313-22. https://doi.org/10.1016/j.ygyno.2015.11.015
40. Gan TJ, Scott M, Thacker, J, Hedrick T, Thiele RH, Miller TE. American society for enhanced recovery: Advancing enhanced recovery and perioperative medicine. Anesth Analg. 2018;126(6):1870-3. https://doi.org/10.1213/ANE.0000000000002925
41. Dort JC, Farwell DG, Findlay M, Huber GF, Kerr, P, Shea-Budgell MA, et al. Optimal perioperative care in major head and neck cancer surgery with free flap reconstruction: a consensus review and recommendations from the enhanced recovery after surgery society. JAMA Otolaryngol Head Neck Surg. 2017;143(3):292-303. https://doi.org/10.1001/jamaoto.2016.2981
42. Tezber K, Aviles C, Eller M, Cochran A, Iannitti D, Vrochides D, et al. Implementing enhanced recovery after surgery (ERAS) program on a specialty nursing unit. J Nurs Adm. 2018;48(6):303-9. https://doi.org/10.1097/NNA.0000000000000619
43. Gok F, Yavuz Van Giersbergen, M. Preoperative fasting: a systematic review. Pamukkale Medical Journal. 2018;11(2): 183-94. https://doi.org/10.5505/ptd.2017.50490
44. ERAS Türkiye Derneği. Available at: http://eras.org.tr/page. (cited 2020 April 5).
45. Kankılıc R, Tuna A. An investigation of preoperative and postoperative nutrition, pain and early mobilisation practices in TUR-P surgery in relation to the ERAS protocol. KSU Medical Journal. 2019;14(2):69-74. https://doi.org/10.17517/ksutfd.484635
46. Umari M, Falini S, Segat M, Zuliani M, Crisman M, Comuzzi L, et al. Anesthesia and fast-track in videoassisted thoracic surgery (VATS): from evidence to practice. J Thorac Dis. 2018;10(Suppl 4):S542-54. https://doi.org/10.21037/jtd.2017.12.83
47. Gundogdu RH. Cerrahi iyileşmenin hızlandırılması için modern teknikler. In: Eti Aslan F, editor. Cerrahi bakım vaka analizleri ile birlikte. Ankara: Akademisyen Tıp Kitabevi; 2016. p. 455-70.

Original Article

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Comparison of Effectiveness of Jet Nebulizer and Mesh Nebulizer in Epinephrine Inhalation Therapy of Children with Acute Bronchiolitis*

Akut Bronşiolitli Çocuklarda Epinefrin İnhalasyon Tedavisinde Jet Nebülizatör ile Mesh Nebülizatörün Etkinliğinin Karşılaştırılması

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ABSTRACT

Objective: *Inhaled bronchodilators are commonly used in the treatment of patients hospitalized with the diagnosis of acute bronchiolitis. The mesh nebulizer, developed in recent years, allows to deliver the smaller particles of drugs to the distal airways. The aim of this retrospective study is to compare the effectiveness of mesh nebulizers with jet nebulizers in terms of clinical improvement, length of hospital stay and intensive care requirement.*
Method: *The study was conducted in Haseki Research and Training Hospital. Seventy-five pediatric patients between 1-24 months of age who were hospitalized with the diagnosis of acute bronchiolitis were included in the study. Forty patients were treated with jet nebulizer and 35 patients were treated with mesh nebulizer. The responses to the treatment were evaluated with duration of hospitalization, changes in heart rates, respiratory rates and Wang respiratory scores at 24th and 48th hours of therapy and requirement of intensive care.*
Results: *Respiratory syncytial virus was the most commonly isolated viral pathogen (37.3%), followed by rhinovirus. Wang respiratory scores were significantly decreased in patients using mesh nebulizers at the 24th (p<0.001) and 48th hours (p<0.001) of treatment. Respiratory and heart rates were significantly improved at the 48th hours of therapy (p=0.026, p=0.023, respectively). The patients who were treated with jet nebulizer had longer hospital stay than those treated with mesh nebulizer (p=0.006).*
Conclusion: *It was concluded that mesh nebulizer contribute to rapid improvement in acute respiratory failure, shortened the duration of hospitalization and may decrease the requirement of intensive care in patients with acute bronchiolitis.*

Keywords: *bronchiolitis, inhalation therapy, epinephrine, nebulized bronchodilators*

ÖZ

Amaç: *Akut bronşiyolit tanısıyla hastaneye yatan hastalarda, tedavi olarak inhale bronkodilatörler sıklıkla kullanılmaktadır. Son yıllarda geliştirilen mesh nebülizatör, ilaçların daha küçük partiküller halinde distal hava yollarına iletilmesini sağlamaktadır. Bu geriye dönük çalışmanın amacı, mesh nebülizörler ile jet nebülizörlerin etkinliğini klinik düzelme, hastanede kalış süresi ve yoğun bakım ihtiyacı açısından karşılaştırmaktır.*
Yöntem: *Bu çalışma Haseki Eğitim ve Araştırma Hastanesi'nde yapıldı. Akut bronşiolit tanısı ile hastaneye yatırılan 1-24 ay arası 75 çocuk çalışmaya alındı. Kırk hasta jet nebülizatör ile 35 hasta mesh nebülizatörle tedavi edildi. Tedaviye yanıt hastanede kalış süresi, tedavinin 24 ve 48. saatlerindeki kalp, solunum sayıları ve Wang solunum skorundaki değişiklik ile yoğun bakım gereksinimi açısından değerlendirildi.*
Bulgular: *En sık izole edilen viral etken respiratuar sinsisyal virüs (%37,3) idi onu rhinovirüs takip ediyordu. Mesh nebülizatör kullanan hastalarda tedavinin 24. (p<0.001) ve 48. (p<0.001) saatlerinde Wang solunum skoru anlamlı olarak düşüktü. Tedavinin 48. saatinde solunum ve kalp hızları belirgin olarak düzeldi (p=0,026, p=0,023, sırasıyla). Jet nebülizatörle tedavi olan hastaların hastanede kalış süreleri mesh nebülizatör ile tedavi edilenlere göre daha uzundu (p=0,006).*
Sonuç: *Akut bronşiolitli hastalarda, mesh nebülizörünün akut solunum yetmezliğinde hızlı iyileşmeye katkıda bulunduğu, hastanede yatış süresini kısalttığı ve yoğun bakım gereksinimini azaltabileceği sonucuna varılmıştır.*

Anahtar kelimeler: *bronşiolit, inhalasyon tedavisi, epinefrin, nebülize bronkodilatörler*

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INTRODUCTION

Acute bronchiolitis, commonly seen in children under two years, causes obstruction of distal airways. It is the most common lower respiratory tract disease in childhood [1-3]. Viral infections mostly cause this disease and the most common pathogen is respiratory syncytial virus (RSV) [1-3]. The disease is classified as mild, moderate, and severe due to clinical signs [3]. While mild cases can be followed up on an outpatient clinics, babies with bronchiolitis lasting less than 3 months and have a moderate to severe bronchiolitis should be hospitalized [1,3]. In some periods these patients accumulate in emergency departments, lock the pediatric intensive care units and bring an economic burden to the health system [1,2].

Treatment of acute bronchiolitis consists of supportive treatments based on oxygenation and hydration in children [1-3]. Some patients need bronchodilators to reduce edema of the small airways [3]. Jet nebulizer (JN) is one of the easiest, almost effective, inexpensive device in inhalation therapy and is widely used [4]. However, it has been shown that the concentration of the nebulized drug reaching into the small airways decreases due to the increased respiratory rate [5]. A new technologic device, named mesh nebulizer (MN), has been developed to overcome this obstacle [6-9]. It is made of palladium element, and contains one thousand holes in a diameter of 5 mm that vibrate 128,000 times per second [6]. It enables to deliver drug particles into the distal airways of the lung [8]. It has been claimed that it accelerates recovery and shortens hospital stay [8,9].

The aim of this study is to compare the effectiveness of JN and MN in clinical recovery, hospital stay and intensive care requirement in patients who were diagnosed with bronchiolitis.

MATERIAL and METHOD

This retrospective study was conducted in S.B.Ü. Haseki Training and Research Hospital and was approved by the local ethical board (No:2019-18). The children under two years of age who diagnosed with acute bronchiolitis and hospitalized were included in the study. Data of patients were collected from the hospital's registration system. The severity of the disease was determined using Wang respiratory score [10]. In this scoring system there are four parameters; respiratory rate, wheezing, retraction and general condition. The total score ranges from 0 to 12. In order to design equal groups, in terms of the severity of the disease, the patients who had Wang respiratory score of 7 at admission and received only epinephrine inhalation were enrolled. All patients were scored according to the clinical records at admission, at 24th hours and 48th hours of treatment. The dose of epinephrine was determined as 0.1 ml/kg/ dose (1 mg/1 ml) (1: 1000 Adrenaline) due to the routine protocol of our department. Every child received six doses of epinephrine in a day, extra doses were not needed. The hypoxic patients were excluded from the study , only the ones who had oxygen saturations above 92% were selected. Additional oxygen therapy was not given during the treatment except for the patients who were transferred to intensive care unit. We formed the groups according to the type of nebulizer. Group 1 used JN (CGR-1002®, CGR Medical Ltd, Istanbul, Turkey) and Group 2 used MN (Aerogen Solo®, Aerogen Ltd, Galway, Ireland). Sterile masks were used for inhalation therapy in every child in the MN group. The infected materials of MN was removed with surface disinfectant containing didecyldimethylammonium chloride and left in disinfectant which contains non-corosive quarterner amonium carbonate, non-ionic surface active material and enzymatic complex.

The demographic features such as age, sex, breastfeeding in the first 6 month of life, consanguinity, history of atopy and exposure of smoke were noted. Length of hospitalization, steroid or magnesium use, fever (if body temparature above 38.5°C), presence of acute phase reactants, and results of nasal swabs were noted. Respiratory and heart rates, Wang respiratory scores at admission and after 24th-48th hours of therapy were compared according to nebulizer's type. Also nasal swabs and acute phase reactants were evaluated in groups. Since routine use of pulse oximeter was not available in all patients in pediatric wards, we could not obtained oxygen saturation values of the patients.

Exclusion criteria

Patients who had history of prematurity, and recurrent wheezing, pneumonia, chronic lung diseases

such as asthma, congenital heart disease and who received inhalation therapy other than epinephrine were excluded.

Statistical analysis: To analyze the data SPSS 15.0 for Windows was used. The categorical variables were given as frequencies and percentages for continuous variables as mean and standard deviations (SD). Comparison of the data which did not show normal distribution, were analyzed with Mann-Whitney U test. Chi- square test was used for comparing categorical variables. Statistically significant p value was accepted at <0.05.

Based on previous studies with 95% confidence intervals, the sample size was determined to be n1=n2=35, N=70. The level of statistical significance was established as 0.05 with 95% statistical power.

RESULTS

The study was performed on 75 patients diagnosed with acute bronchiolitis including 42 (56%) girls and 33 (44%) boys, whose ages ranged from 1 to 23 months. The inhalation therapy with epinephrine was given to 40 patients with a JN, and 35 patients with a MN. The characteristic features of the groups were detailed in Table 1. There was no statistically significant difference between the age and sex of the children in groups (p>0.05). The history of breastfeeding, consanguinity, exposure to smoking and the presence of atopic individuals in the family showed no statistically significant difference between groups (p>0.05). There was no statistically significant difference in the number of febrile episodes and the use of steroid and magnesium therapy during hospitalization between groups (Table 2). RSV was observed in nasal swabs of 37.3% of the patients who participated in the study. Rhinovirus, one of the most common viruses after RSV, was detected in 25.3% of patients. There was no statistically significant difference in the comparative respiratory panel results examined for the control of the homogeneity of the groups (p>0.05) (Table 3). There were no significant differences between the groups in terms of the presence of viral agents, acute phase reactants and blood counts (p>0.05).

Table 2. Viral etiologies of the study groups.

	Nebulizer type	
Virus n (%)	**Jet nebulizer**	**Mesh nebulizer**
Rhinovirus	9 (22.5)	10 (28.6)
Parainfluenza	3 (7.5)	4 (11,4)
Coronavirus	0 (0.0)	1 (2.9)
Respiratory syncytial virus	15 (37.5)	13 (37.1)
Human metapneumovirus	1 (2.5)	3 (8.6)
Human bocavirus	2 (5.0)	0 (0.0)
Adenovirus	2 (5.0)	2 (5.7)
Influenza A	1 (2.5)	1 (2.9)
Influenza B	1 (2.5)	0 (0.0)
Negative	6 (15.0)	1 (2.9)

Table 3. Comparison of receiving magnesium and steroid in study groups.

	Nebulizer type		
	Jet nebulizer	**Mesh nebulizer**	**p value**
Magnesium (i.v) n (%)	9 (22.5)	13 (37.1)	0.785
Systemic steroid n (%)	12 (30.0)	10 (28.6)	0.892

p<0.05 accepted statistically significant

We did not find any statistically significant difference in the mean respiratory rates at admission and 24th hour of the treatment (p>0.05). The respiratory rate was significantly lower at 48th hour after treatment in MN group than JN group (p=0.026). The children's

Table 1. Characteristic features of the groups.

	Nebulizer type		
	Jet nebulizer	**Mesh nebulizer**	**p value**
Age (month) Median (IQR)	6.0 (7.0)	5.0 (8.0)	0.970
Sex (male) n (%)	19 (47.5)	14 (40.0)	0.514
Breastfeeding first 6 months n (%)	25 (62.5)	24 (68.6)	0.582
Consanguinity n (%)	13 (32.5)	5 (14.3)	0.065
Exposure to smoking n (%)	16 (40.0)	17 (48.6)	0.456
History of familial atopy n (%)	10 (25.0)	7 (20.0)	0.606

Standard deviation: SD, IQR: Interquartile range, p<0.05 accepted statistically significant

Table 4. Comparison of clinical variables of the groups.

	Nebulizer type		
	Jet nebulizer	**Mesh nebulizer**	**p value**
Fever n (%)	5 (12.5)	8 (22.9)	0.237
Respiratory rate (Mean±SD)			
Admission	49.9±6.5	48.1±6.3	0.271
24th hour	41.6±5.9	41.4±6.9	0.926
48th hour	38.4±5.0	35.4±5.6	0.026
Heart rate (Mean±SD)			
Admission	133.6±11.5	131.6±10.9	0.492
24th hour	125.9±12.0	123.9±10.6	0.398
48th hour	121.9±11.4	116.3±10.6	0.023
Wang scores (Mean±SD)			
Admission	7.00±0.00	7.0±0.00	1,000
24th hour	6.00±0.00	5.7±0.5	<0.001
48th hour	4.80±0.41	4.23±0.43	<0.001
Duration of hospitalization Median (IQR)	7.0 (3.0)	5.0 (4.0)	0.006

Standard deviation: SD, IQR: Interquartile range, p<0.05 accepted statistically significant

heart rates were not significantly different in two groups at admission and at 24th hour of therapy (p>0.05), otherwise heart rates decreased in MN group at the 48th hours of treatment (p=0,023). The mean Wang respiratory scores of patients using JN was statistically significantly higher than patients using MN at 24th and 48th hours (p<0.001). The mean hospitalization time of patients using JN was statistically significantly higher than patients using MN (p=0.006) (Table 4). We also detected that five children needed intensive care in JN group (12.5%) during the treatment course. However, in MN group no one was treated in the intensive care unit.

DISCUSSION

As far as we know, this is the first study that compares the efficacy of inhaled epinephrine using JN vs MN in children with acute bronchiolitis. In this study, we figured out that nebulized epinephrine treatment with a MN, significantly improved the disease severity scores at the 48th hour of management, increased the recovery rates and reduced the duration of hospitalization.

Acute bronchiolitis seems mostly in the winter period in our country and crowds emergency clinics. Many studies show that the most common factor in acute bronchiolitis is RSV [2,11,12]. RSV was found in the rate of 20-63% in infants under the age of two years in Turkey [11,13]. In the present study, RSV was the most frequently isolated viral pathogen (37.3%) followed by rhinovirus.

The main approach in the management of acute bronchiolitis is supportive therapy providing oxygenation and hydration [3,14,15]. However, beta-2 agonists, epinephrine, corticosteroid and antiviral treatments are also used in daily practice due to the severity of the disease [14]. In a meta-analysis conducted by Garrison et al. systemic and inhaled corticosteroids have been shown to have no favorable effect in the treatment of hospitalized infants with acute bronchiolitis [15]. The frequency of receiving systemic steroid was found to be 48% in the study by Offer et al. [16]. In our study, systemic corticosteroids were used in 29.3% of cases who did not respond to epinephrine. Although there was no statistically significant difference between the two groups, 54.5% of the patients were in the jet nebulizer group. Likewise, the effectiveness of intravenous magnesium has not been proven. It can be tried in patients who do not improve despite supportive treatment [17]. In our study, magnesium was used in 29.3% of the cases who did not respond to epinephrine and steroid. We found no statistically significant difference between the groups in terms of magnesium treatment.

Recently, the most popular therapy is inhalation of epinephrine [18-22]. It was reported that epinephrine is

more effective in achieving recovery compared to other bronchodilator drugs [20,23]. The mucosal edema has an important role in respiratory obstruction. The use of combined alpha and beta-adrenergic agonists instead of beta-2 agonist may be more beneficial in the treatment of acute bronchiolitis, and studies have been focused on this topic [20]. Hartling et al. reported a meta-analysis that regarded the use of inhaled epinephrine in the treatment of acute bronchiolitis to improve clinical signs and oxygenation of the patients in the emergency room [22]. The effectiveness of different agents in acute bronchiolitis is not clear, studies are ongoing in this regard.

The inhalation technique is also noteworthy to enhance the efficacy of the drugs. Different devices can be used in nebulization therapy. The nebulizer types are ultrasonic nebulizer, JN and MN [24,25]. MN creates vibration with the help of electrical energy. The drug passes through a mesh and becomes volatile [24]. MNs are more effective than the other two models of nebulizers, and the vast majority of drugs reach the distal airways in the form of microaerosels with a diameter of 0.4 to 4.4 µm [24,26,27]. However, droplet size is > 5 µm in JN [28]. The amount remaining in the chamber of MN is also very few compared to other nebulizers [24,25]. In inhalation treatments using mesh technology, the distribution of aerosol drug into the airways was found to be better when evaluated by performing lung SPECT-CT [26].

It has been suggested that the drug is nebulized faster with MN than with traditional JN, and the clinicians can precisely control drug delivery into the respiratory tract [25]. There are studies comparing different types of nebulizers on children in the literature [24,25]. Dunne et al. found a decrease in hospital stay and a significant reduction in the dose of drug in patients treated with MN in the emergency department [8]. In the present study we evaluated the clinical courses of the patients. We found that respiratory rates, heart rates and Wang severity scores improved faster in the MN group than JN group. Delivery of the drug to the distal airways and removing the obstruction in the airways rapidly may be effective in correcting tachycardia and ensuring rapid recovery in the follow-up period. The silent nature of the MN can also prevent agitation in children and cause rapid effects.

Limitations of the study: One of the limitations of our study is its retrospective nature which could not allow evaluation of the acute effects of the treatments. We could not able to compare the clinical signs at 30th, 60th and 120th minutes of the hospitalization. The second one is about its cost effectiveness. Since the MN can be used in more than one patient, prospective studies are needed to evaluate the number of MNs that are used in order to perform net cost analysis.

CONCLUSION

Delivery of epinephrine using MN in acute bronchiolitis positively contributes to the recovery of clinical signs and shortening of the hospitalization time. Further large, prospective, randomized controlled studies are needed to show the effectiveness of treatment and intensive care requirement using MN in pediatric patients with acute bronchiolitis.

Ethics Committee Approval: Approval was received from the S.B.Ü. Haseki Training and Research Hospital Clinical Research Ethics Committee (2019/18, 09.10.2019).
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REFERENCES

1. Ralston SL, Lieberthal AS, Meissner HC, et al. Clinical practice guideline: the diagnosis, management, and prevention of bronchiolitis. Pediatrics. 2014;134(5): e1474-e1502. https://doi.org/10.1542/peds.2014-2742
2. Florin TA, Plint AC, Zorc JJ. Viral bronchiolitis. Lancet. 2017;389(10065):211-24. https://doi.org/10.1016/S0140-6736(16)30951-5
3. Cunningham S. Bronchiolitis. Kendig's Disorders of the Respiratory Tract in Children. 2019;420-6.e3. https://doi.org/10.1016/B978-0-323-44887-1.00024-9.
4. Song X, Hu J, Zhan S, Zhang R, Tan W. Effects of temperature and humidity on laser diffraction measurements to jet nebulizer and comparison with NGI. AAPS PharmSciTech. 2016;17(2):380-8. https://doi.org/10.1208/s12249-015-0346-5
5. Sim MA, Dean P, Kinsella J, Black R, Carter R, Hughes M. Performance of oxygen delivery devices when the breathing pattern of respiratory failure is simulated. Anaesthesia. 2008;63(9):938-40. https://doi.org/10.1111/j.1365-2044.2008.05536.x
6. Dhanani J, Fraser JF, Chan H, et al. Fundamentals of aerosol therapy in critical care. Crit Care. 2016;20(1):269.

https://doi.org/10.1186/s13054-016-1448-5
7. Sims MW. Aerosol therapy for obstructive lung diseases: device selection and practice management issues. Chest. 2011;140(3):781-8. https://doi.org/10.1378/chest.10-2068
8. Dunne RB, Shortt S. Comparison of bronchodilator administration with vibrating mesh nebulizer and standard jet nebulizer in the emergency department. Am J Emerg Med. 2018;36(4):641-6. https://doi.org/10.1016/j.ajem.2017.10.067
9. Ari A. Jet, ultrasonic, and mesh nebulizers: an evaluation of nebulizers for better clinical outcomes. Eurasian J Pulmonol. 2014;16:1-7. https://doi.org/10.5152/ejp.2014.00087
10. Wang EE, Milner RA, Navas L, Maj H. Observer agreement for respiratory signs and oximetry in infants hospitalized with lower respiratory infections. Am Rev Respir Dis. 1992;145(1):106-9. https://doi.org/10.1164/ajrccm/145.1.106
11. Hatipoğlu N, Somer A, Badur S, et al. Viral etiology in hospitalized children with acute lower respiratory tract infection. Turk J Pediatr. 2011;53(5):508-16. PMID: 22272450.
12. Øymar K, Skjerven HO, Mikalsen IB. Acute bronchiolitis in infants, a review. Scand J Trauma Resusc Emerg Med. 2014;22:23. https://doi.org/10.1186/1757-7241-22-23
13. Hacımustafaoğlu M, Çelebi S, Bozdemir SE, et al. RSV frequency in children below 2 years hospitalized for lower respiratory tract infections. Turk J Pediatr. 2013;55:130-9. PMID: 24192672.
14. King VJ, Viswanathan M, Bordley WC, et al. Pharmacologic treatment of bronchiolitis in infants and children: a systematic review. Arch Pediatr Adolesc Med. 2004;158(2):127-37. https://doi.org/10.1001/archpedi.158.2.127
15. Garrison MM, Christakis DA, Harvey E, Cummings P, Davis RL. Systemic corticosteroids in infant bronchiolitis: a meta- analysis. Pediatrics. 2000;105(4):E44. https://doi.org/10.1542/peds.105.4.e44
16. Offer I, Ashkenazi S, Livni G, Shalit I. The diagnostic and therapeutic approach to acute bronchiolitis in hospitalized children in Israel: a nationwide survey. Isr Med Assoc J. 2000;2(2):108-10. PMID: 10804929.
17. Alansari K, Sayyed R, Davidson BL, Al Jawala S, Ghadier M. IV Magnesium sulfate for bronchiolitis: a randomized trial. Chest. 2017;152(1):113-9. https://doi.org/10.1016/j.chest.2017.03.002
18. Guo C, Sun X, Wang X, Guo Q, Chen D. Network meta-analysis comparing the efficacy of therapeutic treatments for bronchiolitis in children. JPEN J Parenter Enteral Nutr. 2018;42(1):186-95. https://doi.org/10.1002/jpen.1030.
19. Sakulchit T, Goldman RD. Nebulized epinephrine for young children with bronchiolitis. Can Fam Physician. 2016;62(12):991-3. PMID: 27965333.
20. Patel H, Platt RW, Pekeles GS, Ducharme FM. A randomized, controlled trial of the effectiveness of nebulized therapy with epinephrine compared with albuterol and saline in infants hospitalized for acute viral bronchiolitis. J Pediatr. 2002;141(6):818-24. https://doi.org/10.1067/mpd.2002.129844
21. Ralston SL, Lieberthal AS, Meissner HC, et al. Clinical practice guideline: the diagnosis, management, and prevention of bronchiolitis. Pediatrics. 2014;134(5): e1474-e1502. https://doi.org/10.1542/peds.2014-2742
22. Hartling L, Fernandes RM, Bialy L, et al. Steroids and bronchodilators for acute bronchiolitis in the first two years of life: systematic review and meta-analysis. BMJ. 2011;342:d1714. https://doi.org/10.1136/bmj.d1714
23. Langley JM, Smith MB, LeBlanc JC, Joudrey H, Ojah CR, Pianosi P. Racemic epinephrine compared to salbutamol in hospitalized young children with bronchiolitis; a randomized controlled clinical trial [ISRCTN46561076]. BMC Pediatr. 2005;5(1):7. https://doi.org/10.1186/1471-2431-5-7
24. Ari A, de Andrade AD, Sheard M, AlHamad B, Fink JB. Performance comparisons of jet and mesh nebulizers using different interfaces in simulated spontaneously breathing adults and children. J Aerosol Med Pulm Drug Deliv. 2015:28(4):281-9. https://doi.org/10.1089/jamp.2014.1149
25. Soyer Ö, Kahveci M, Büyüktiryaki B, et al. Mesh nebulizer is as effective as jet nebulizer in clinical practice of acute asthma in children. Turk J Med Sci. 2019;49(4):1008-13. https://doi.org/10.3906/sag-1812-133
26. Dugernier J, Hesse M, Vanbever R, et al. SPECT-CT Comparison of lung deposition using a system combining a vibrating-mesh nebulizer with a valved holding chamber and a conventional jet nebulizer: a randomized cross-over study. Pharm Res. 2017;34(2):290-300. https://doi.org/10.1007/s11095-016-2061-7
27. Réminiac F, Vecellio L, Heuzé-Vourc'h N, et al. Aerosol therapy in adults receiving high flow nasal cannula oxygen therapy. J Aerosol Med Pulm Drug Deliv. 2016;29(2):134-41. https://doi.org/10.1089/jamp.2015.1219
28. Dhanani J, Fraser JF, Chan HK, Rello J, Cohen J, Roberts JA. Fundamentals of aerosol therapy in critical care. Crit Care. 2016;20(1):269. https://doi.org/10.1186/s13054-016-1448-5

Original Article

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Review of the Clinicopathological Features and Prognosis of the Rare Histological Types of Gastric Cancer

Mide Kanserinin Nadir Görülen Histolojik Tiplerinin Klinikopatolojik Özelliklerinin ve Prognozlarının Gözden Geçirilmesi

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ABSTRACT

Objective: *Gastric cancer consists of many histological subtypes. Prognostic value of histological types in gastric cancer has not been very well defined. In this study, we aimed to investigate the relationship between different histological types and clinicopathologic features and prognosis in gastric cancer.*
Method: *Patients whose pathological diagnosis was adenosquamous carcinoma, hepatoid adenocarcinoma, lymphoepithelioma-like carcinoma, and papillary adenocarcinoma, among the 1060 patients who underwent gastrectomy for gastric cancer between 2010-2019, were included in the study. Demographic features, clinicopathological features, oncological follow-up results and survival of the patients were analyzed.*
Results: *Group 1 (adenosquamous carcinoma) consisted of 3, Group 2 (hepatoid adenocarcinoma) of 3, Group 3 (lymphoepithelioma-like carcinoma) of 3, and Group 4 (papillary adenocarcinoma) consisted of 4 patients. The mean age of the patients included in the study was 63.3+11.76 (41-81) years. Tumors were more commonly located in the corpus in hepatoid adenocarcinoma and lymphoma-like carcinoma (67%) and in the antrum (75%) in papillary adenocarcinoma. Eight patients underwent total gastrectomy, four patients subtotal gastrectomy, and a patient with a tumor located at the gastroesophageal junction underwent proximal gastrectomy. Average tumor size (cm) was 5.11+2.23 (1.2-8) cm. Local recurrence occurred in two patients with adenosquamous carcinoma, two with hepatoid adenocarcinoma, and one with papillary adenocarcinoma. Two patients with adenosquamous carcinoma developed systemic metastasis (lung, liver), two patients with hepatoid adenocarcinoma developed peritoneal carcinomatosis, and a patient with papillary adenocarcinoma developed surrenal metastasis. Average survival was the shortest in hepatoid adenocarcinoma (17.50 months), and the longest in papillary adenocarcinoma (63 months). There was no statistical difference in survival between the groups (p: 0.445).*
Conclusion: *Rare histological types of the stomach differed in terms of their locations and prognoses. Among the rare histological types, hepatoid adenocarcinoma exhibited the most aggressive biological behavior, while patients with papillary adenocarcinoma had longer survival times.*

Keywords: *gastric cancer, rare histological type, curative resection, prognosis*

ÖZ

Amaç: *Mide kanseri birçok histolojik alt tipten oluşur. Mide kanserinde histolojik tiplerin prognostik değeri iyi tanımlanmamıştır. Bu çalışmada mide kanserinde farklı histolojik tiplerin klinikopatolojik özellikler ve prognoz ile ilişkisini araştırmayı amaçladık.*
Yöntem: *2010-2019 yılları arasında mide kanseri nedeniyle gastrektomi yapılan 1060 hasta arasından patolojik tanısı adenoskuamöz karsinom, hepatoid adenokarsinom, lenfoepitelyoma like karsinom ve papiller adenokarsinom olan hastalar dahil edildi. Hastaların demografik özellikleri, klinikopatolojik özellikleri, onkolojik takip sonuçları ve sağkalımları analiz edildi.*
Bulgular: *Grup 1 (adenoskuamöz karsinom) 3, Grup 2 (hepatoid adenokarsinom) 3, Grup 3 (lenfoepitelyoma like karsinom) 3 ve Grup 4 (papiller adenokarsinom) 4 hastadan oluşuyordu. Çalışmaya dâhil edilen hastaların yaş ortalaması 63,3±11,76 (41-81) idi. Tümörler hepatoid adenokarsinom ve lenfoma like karsinomda korpusta (%67), papiller adenokarsinom da antrumda (%75) daha sık saptandı. Sekiz hastaya total gastrektomi dört hastaya subtotal gastrektomi, gastroözofageal-EJ bileşkede yerleşmiş olan tümöre de proksimal gastrektomi uygulandı. Ortalama tümör boyutu (cm) 5,11±2,23 (1,2-8). Adenoskuamöz karsinomlu iki, hepatoid adenokarsinomlu iki ve papiller adenokarsinomlu bir hastada lokal nüks gelişti. Adenoskuamöz karsinomlu iki hastatada sistemik metastaz (akciğer, karaciğer), hepatoid adenokarsinomlu iki hastada peritoneal karsinomatozis ve papiller adenokarsinom tanılı bir hastada da sürrenal metastaz gelişti. Ortalama sağ kalım hepatoid adenokarsinomada en kısa (17,50 ay) iken papiller adenokarsinomada en uzundu. (63 ay). Gruplar arasında sağ kalım açısından istatistiksel farklılık yoktu (p: 0,454).*
Sonuç: *Midenin nadir görülen histolojik tipleri yerleşim yerleri ve prognozları açısından farklılık gösteriyordu. Nadir histolojik tipler arasından hepatoid adenokarsinom en agresif biyolojik davranış sergilerken papiller adenokarsinom diğer histolojik tiplere gore daha uzun sağ kalım sergilemişti.*

Anahtar kelimeler: *mide kanseri, nadir histolojik tip, küratif rezeksiyon, prognoz*

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INTRODUCTION

Gastric cancer is the fifth most common cancer in the world and is the third most common cause of cancer-related deaths after lung and colorectal cancer [1]. Gastric cancer is considered a heterogeneous disease. Each histological subtype of stomach cancer has different characteristics as biological behavior, therefore histological type is an important place in the individual evaluation of stomach cancer patients [2,3].

There are various histopathological classification systems for the diagnosis of gastric cancer. The most detailed classification system is the histopathological classification system made by the World Health Organization (WHO) in 2010 and updated in 2019. Unlike other systems, the WHO classification includes all other types of rarely seen gastric tumors other than gastric adenocarcinoma [4,5].

In all types of gastric cancer, the most common type of pathology is adenocarcinoma (AC) [4,6]. Primary gastric adenosquamous carcinoma (ASC) is characterized by the presence of adenocarcinoma and squamous cell carcinoma (SCC) components within the same tumor. This extremely rare histological type accounts for less than 1% of gastric carcinomas and exhibits aggressive behavior [7,8].

Hepatoid adenocarcinoma (HAC) is a rare tumor with extrahepatic origin, characterized by the imitation of the morphological phenotype of hepatocellular carcinoma (HCC). Hepatoid adenocarcinoma not only shares the morphological features of HCC, but its alpha-fetoprotein (AFP) production and immunohistochemical properties resembles HCC [9,10]. While hepatoid adenocarcinoma is seen in many organs such as the lung, pancreas, ovary, uterus and gall bladder, the most frequently affected organ is the stomach. It accounts for only 0.38 to 1.00% of all gastric cancers (GC) [9,11]. HAC is an aggressive tumor with a poor prognosis that tends to metastasize to the liver and lung at an early stage [12].

Papillary adenocarcinoma of the stomach is defined as a well-differentiated exophytic gastric carcinoma with elongated finger-like processes lined by cylindrical or cuboidal cells supported by fibromuscular connective tissue cores [6]. Papillary adenocarcinomas of the stomach make up only 6-11% of all gastric carcinoma cases. Papillary adenocarcinoma type of the stomach shows increased lymphovascular invasion, liver metastasis and poor survival compared to other adenocarcinoma subtypes [13,14].

Lymphoepithelioma-like gastric carcinoma (LELGC) is a rare gastric cancer subtype characterized by lymphocytic infiltration of the tumor stroma. This histological type, which accounts for 1.4% of all gastric carcinomas, may be associated with Epstein- Barr virus (EBV) infection or microsatellite instability (MSI). The prognosis of LELGC is better than other types of gastric carcinoma [15,16].

In this study, we aimed to discuss the surgical results and prognoses of the rarer histologic types of gastric carcinoma, which are adenosquamous carcinoma, hepatoid adenocarcinoma, papillary adenocarcinoma, and lymphoepithelioma-like carcinoma, within a 10-year period in our clinic, in the light of the literature.

MATERIALS and METHODS

After the approval of the Ethics Committee of Erciyes University Faculty of Medicine dated 10.06.2020 and numbered 2020/270, 13 patients who had the pathological diagnosis of adenosquamous carcinoma, hepatoid adenocarcinoma, papillary adenocarcinoma and lymphoepithelioma-like carcinoma, out of the 1060 patients who underwent curative surgery in our clinic between 2010-2019, were included in the study. Mixed tumors accompanied by this histological type, other histological subtypes, and patients undergoing palliative surgery were excluded from the study.

Patient files, electronic records, pathology reports, surgery reports, anesthesia follow-up forms, and nurse observation forms were examined, and a common database was created prospectively. Patients were analyzed retrospectively using this database. The population registration system was used for survival analysis.

Demographic and clinical features, tumor marker levels, tumor localizations, surgical procedure, total number of lymph nodes dissected, number of metastatic lymph nodes, tumor size, local recurrence and systemic

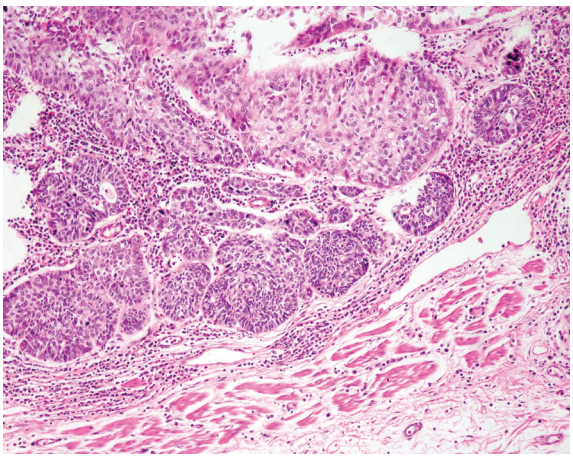


Figure 1. Adenosquamous carcinoma.

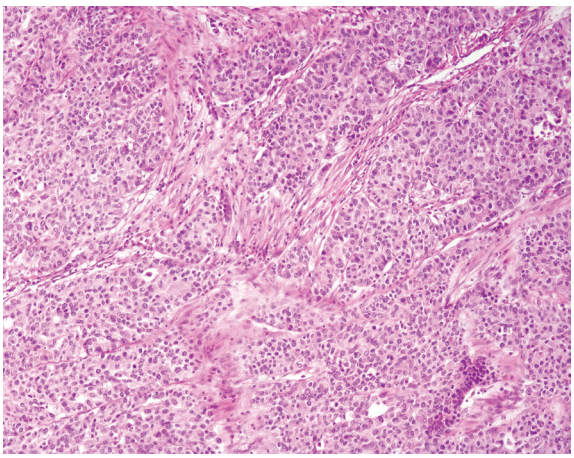


Figure 2. Hepatoid adenocarcinoma.

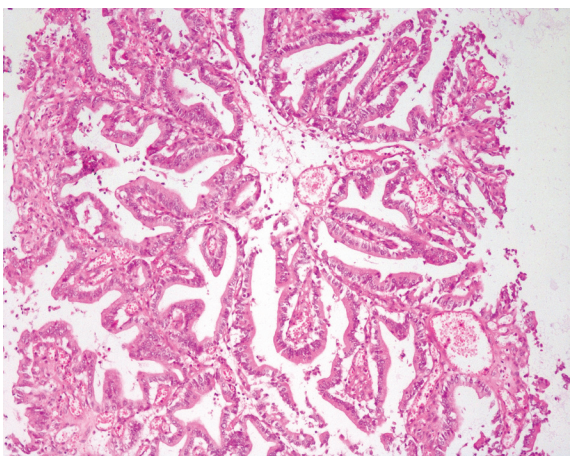


Figure 3. Papillary adenocarcinoma.

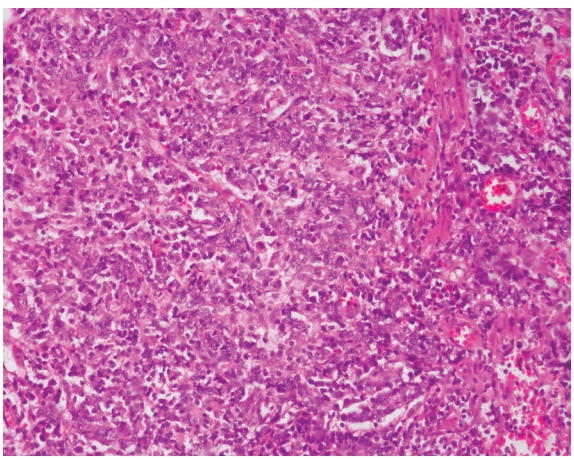


Figure 4. Lymphoepithelioma-like carcinoma.

metastasis, and mean survival times were analyzed.

Chest radiography, abdominal sonography or abdominal computed tomography scans were performed to all patients prior to the operation for tumor staging. Histological subtypes were classified using the World Health Organization (WHO) classification [4,5]. All surgical samples were reviewed by a senior pathologist from our institution (Figure 1, 2, 3, 4).

The patients were operated with conventional techniques. Total gastrectomy and subtotal gastrectomy were performed using the previously recognized and accepted Japanese Gastric Cancer Association criteria [17,18]. The location and size of the tumor were effective in choosing the type of resection.

Tumor recurrence at the hepatoduodenal ligament, celiac axis, peripancreatic region and operation site was considered as local recurrence. Peritoneal relapses and other organ metastases were considered as systemic metastases.

Overall survival time was calculated as the time from surgery to death or last follow-up. In the follow-up of the patients, evaluations were made every 3 months for the first 2 years after the operation and every 6 months afterwards.

SPSS (Statistical Package for the Social Sciences) 23.0 package program was used for statistical analysis of the data. Continuous measurements were summarized as mean and standard deviation (minimum-maximum). Kaplan-Meier analysis and Log Rank tests were used in survival analyses. Statistical significance level was taken as 0.05 in all tests.

RESULTS

Thirteen patients were included in our study. The M/F ratio of the patients was 7/6. The average age of the patients participating in the study was 63.3±11.76 (41-81) years. Tumors were most commonly located in the corpus and antrum. Eight patients underwent total gastrectomy, four patients subtotal gastrectomy, and a patient with a tumor located at the gastroesophageal junction underwent proximal gastrectomy. Average tumor size was 5.11±2.23 (1.2-8) cm. Local recurrence occurred in two patients with adenosquamous carcinoma, two with hepatoid adenocarcinoma, and one with papillary adenocarcinoma. Two patients with adenosquamous carcinoma developed systemic metastasis (lung, liver), two patients with hepatoid adenocarcinoma peritoneal carcinomatosis, and a patient with papillary adenocarcinoma surrenal metastasis. The clinical characteristics and follow-up results of the patients are shown in Table 1.

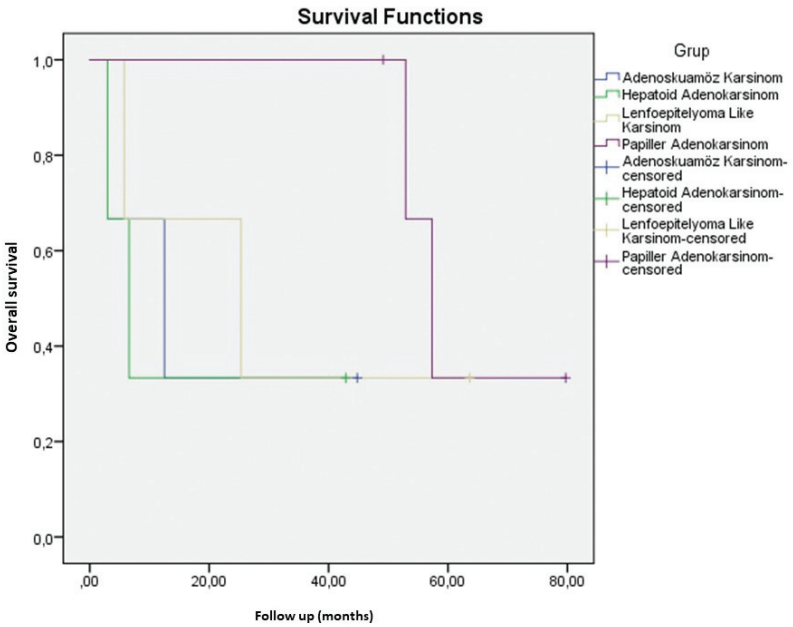
When we look at the mean survival times, hepatoid adenocarcinoma showed the worst survival with 17.50 months and papillary adenocarcinoma had the best survival with 63 months (p: 0.445). Survival analysis and curves are given in Table 2 and Graphic 1.

Table 1. Patients characteristics.

Tumor Type	Age/sex	Location	CEA/Ca19.9 (ng/ml-U/ml)	Surgical Procedure	Tumor size	Total/Metastatic lymph node	Local recurrence	Systemic metastasis	Current status
Adeno-squamous carcinoma	81/M	Small curvature	0.11/75.72	Total Gastrectomy	8	10/4	Yes	Lung	Died 3 months
Adeno-squamous carcinoma	41/F	Corpus	2/50	Subtotal Gastrectomy	6	12/0	Yes	Liver	Died 12 months
Adeno-squamous carcinoma	67/F	G-EJ	2.31/30.4	Proksimal Gastrectomy	1.2	23/0	No	No	Alive 40 months
Hepatoid adenocarcinoma	76/F	Corpus	42.16/8.47	Total Gastrectomy	8	23/8	Yes	Peritoneal Carcinomatosis	Died 7 months
Hepatoid adenocarcinoma	71/M	Corpus	314.58/19.9	Total Gastrectomy	2.5	25/0	Yes	Peritoneal Carcinomatosis	Died 3 months
Hepatoid adenocarcinoma	57/M	Small curvature	18.27/232	Total Gastrectomy	5	22/4	No	No	Alive 37 months
Lymphoepithelioma-like carcinoma	72/M	Corpus	3.74/19.54	Total Gastrectomy	5	22/8	No	No	Died 23 months
Lymphoepithelioma-like carcinoma	54/M	Corpus	2/11	Total Gastrectomy	5	33/0	No	No	Died 4 months
Lymphoepithelioma-like carcinoma	54/F	Antrum	1.24/2.64	Subtotal Gastrectomy	4.5	28/1	No	No	Alive 58 months
Papillary	69/F	Corpus	3.95/20.83	Total Gastrectomy	2	8/0	No	Surrenal	Died 57 months
Adenocarcinoma	47/M	Antrum	23.02/562.38	Subtotal Gastrectomy	6.5	15/3	Yes	No	Died 53 months
Papillary	66/F	Antrum	3.07/32.23	Subtotal Gastrectomy	4.75	18/0	No	No	Alive 76 months
Adenocarcinoma	68/M	Antrum	7/100	Total Gastrectomy	8	33/0	No	No	Alive 50 months

Table 2. Mean survival in the groups.

Groups	Mean (Mean + sd (Min Max))	Median (Mean + sd (Min Max))	p
Adeno-squamous carcinoma	20.10±10.33 (3-40.36)	12.50±7.76 (0-27.72)	0.454
Hepatoid adenocarcinoma	17.50±10.40 (3-37.90)	6.59±2.93 (0.83-12.35)	
Lymphoepithelioma-like carcinoma	31.58±13.87 (4.39-58.77)	25.34±15.97 (0-56.66)	
Papillary adenocarcinoma	63.32±6.77 (50.04-76.59)	57.32±3.59 (50.28-64.36)	



Figur 5. Overall survival in terms of the histological type.

DISCUSSION

There are many histological type classifications for gastric cancer such as Lauren classification, Ming classification and WHO classification [4,6,8,19]. Today, WHO classification is widely used worldwide.

Although the prognosis of resectable gastric cancer clearly depends on the pathological stage of the disease, controversy continues about the prognostic value of the histological type. The histological type appears to be an important clinical parameter of a tumor and is suggested as an important factor in evaluating the patient's prognosis [20]. However, controversy continues on clinicopathological features and prognostic factors.

Gastric adenosquamous carcinoma is a rare histological type of gastric cancer. Often, venous and lymphatic invasion are found in patients presenting at an advanced stage. The biological behavior of adenosquamous carcinoma has been reported to be more aggressive than adenocarcinoma. Biological behavior in gastric adenosquamous carcinoma is usually determined by its adenocarcinoma component. Hematogenous and hepatic metastases are more common in tumors which predominantly have adenocarcinoma components [21].

Because of its rarity, no standard treatment for primary gastric ASC has been established. Surgical resection remains the most commonly used treatment method. However, due to the fact that it is diagnosed at an advanced stage, surgical treatment has been shown to be applied in approximately 54% -74% of large series in the literature [7,21]. Akce et al. published a rate of 71% lymph node positivity for ASC in their study using National Cancer Database which included data of 327 patients [7]. Feng et al. found the median overall survival time to be 17 months, with 1-, 3-, and 5-year overall survival rates being 58.1%, 32.4% and 26.4%, respectively, in their study of 109 patients (21 patients from their center + 88 patients from Medline search who met inclusion criteria) with adenosquamous carcinoma undergoing R0 resection [8]. Tumor location was effective in our selection of treatment in adenosquamous tumor in our series. Lymph node involvement was detected in 33% of our patients. Compared to the literature, our lymph node involvement rate was lower. The course of these tumors was highly aggressive, and recurrent and systemic metastasis developed in 2 patients. The survival rate was 20 months on average.

Pathological diagnosis in HAC is made based on morphological features, regardless of serum AFP levels or immunohistochemical AFP staining. The primary

HAC lesion contains tubular as well as hepatoid components [12]. The scientific literature on this topic mostly includes single case reports and some small single institution patient series [12,22].

In the 328 patients series (34 patients from their center + 294 patients from Medline search) performed by Zeng et al., HAC was most commonly located in the antrum (45.6%) followed by corpus (31.3%). Distant metastasis was present at the time of diagnosis in 36.9% of the patients. Lymph node metastasis occurred in 78.4%. of the patients. The median tumor diameter was 5.5 cm [23]. Adachi et al. found a 5-year survival rate of 22% in all patients and a median survival time of 14 months, in their Japanese literature series involving 270 cases [24]. When they examined patients undergoing curative gastrectomy, they found a 5-year survival rate of 42% and a median survival time of 29 months. Survival time was affected by serum AFP level, tumor size, lymphovascular involvement, lymph node, and liver metastasis. In their series, this poor prognosis was mostly due to peritoneal spread and early recurrence in the liver [24].

Unlike the literature, HACs were located in the corpus and small curvature in our series. There was no HAC with antrum localization in our series. All patients underwent total gastrectomy. Average tumor diameter was 5 cm in our series similar to that reported in the literature reported two of our patients had lymphatic involvement. These tumors exhibited aggressive behavior and caused peritoneal carcinomatosis. Patients who developed peritoneal carcinomatosis, died early.

Lymphoepithelioma like carcinoma is defined as tumors that show histological similarity with nasopharyngeal carcinoma. The LELGC of the stomach was first described by Watanabe et al. [25] as a gastric carcinoma accompanied by a lymphoid stroma. Lymphoid stroma contains CD8- or CD4-positive T lymphocytes and CD68-positive macrophages. EBV infection is only observed in a very limited number of these infiltrating lymphocytes [26].

In the series of Tak, DH., 77% of the patients had tumors at corpus localization corpus localization [27]. Gastric LELGC is generally known to have a better prognosis than conventional gastric carcinomas. In a study comparing a LELGC series with 46 patients with adisease non-lymphoepithelioma-like carcinoma (NLELC) series with 42236, patients Park et al. found the lymphatic invasion rate to be lower (17% vs 36% p: 0.008). The frequency of lymph node involvement in LELGC was 28.3% in their series. The 5-year survival rate of patients with LELGC was 97.7% and 89.4% in the NLELC group (p:0.127) in this study, age, tumor location, depth of invasion, lymph node metastasis and venous invasion were found as prognostic indicators [28]. In their study, Tak et al. reported that postoperative recurrence or metastasis tends to occur less frequently in patients with LELGC than in patients with poorly differentiated gastric carcinoma [27]. In our series, LELGC tumors were most frequently seen in the corpus, similar to the literature. Lymph node involvement was present in two patients and the rate of involvement was higher than in the literature. Similar to the literature, there was no local recurrence or systemic metastasis in these patients. Survival rates were shorter with an average of 31 months compared to the literature.

There are several studies in the literature reporting that patients with papillary adenocarcinoma have a worse prognosis than those with other differentiated types [13]. The reliability of endoscopic submucosal dissection (ESD) has been controversial in patients with papillary adenocarcinoma because of higher rates of lymph node and liver metastasis, and a low 5-year survival rate when compared to those with non-papillary adenocarcinoma. Studies have been conducted on ESD safety in papillary carcinoma (29-30). Lee et al., in their series involving early gastric cancers-papillary (EGC-P) patients, found the most common tumor localization to be the lower third of the stomach (76.8%) and the mean tumor diameter as 3 cm in the same study [29]. Yasuda et al., in their 632 disease series comparing patients with papillary adenocarcinoma (PGC) to non-papillary gastric carcinomas (NGC), found the 5-year survival rate to be significantly lower in PGC patients compared to NGC patients (63% vs. 76%) [14]. In patients with papillary adenocarcinoma in our series, the tumor was most frequently located in the antrum, similar to the literature. Unlike the literature, average tumor diameter in our series was 5 cm, and unlike the literature, liver metastasis did not develop. Survival

rates were found to be 63 months. Only one patient had lymph node metastasis and we thought that survival was related to the tumor stage.

The most important limitation of our study was the limited number of patients, but considering the low incidence of these tumors, it was acceptable. However, we believe that the present study will contribute to the literature when we consider the limited number of studies in the literature on the combination and comparison of the results of these rare histological types.

CONCLUSION

The histological types of gastric cancer differed in terms of their location and prognosis. Among the rare histological types, hepatoid adenocarcinoma showed the most aggressive biological behavior, while, as expected, patients with papillary adenocarcinoma had a longer survival times than those with other histological types.

Ethics Committee Approval: It has been approved by the Erciyes University Clinical Research Ethics Committee (2020/270).
Conflict of Interest: There is no conflict of interest.
Funding: There are no financial supports.
Informed Consent: Because the study was retrospective, patient consent could not be obtained.

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424. https://doi.org/10.3322/caac.21492
2. Chu YX, Gong HY, Hu QY, Song QB. Adenosquamous carcinoma may have an inferior prognosis to signet ring cell carcinoma in patients with stages I and II gastric cancer. World J Gastrointest Oncol. 2020;12(1):101-12. https://doi.org/10.4251/wjgo.v12.i1.101
3. Sert OZ, Gulmez S, Uzun O, Bozkur H, Gunes OH. Gastric mixed adeno-neuroendocrine carcinoma with a large cell neuroendocrine component A case reports. Ann Ital Chir. 2019;8. PMID: 31790369.
4. Bosman FT, Carneiro F, Hruban RH, Theise ND, eds. WHO classification of tumours of the digestive system vol. 3. 4th ed. Lyon: International Agency for Research on Cancer; 2010.
5. Nagtegaal ID, Odze RD, Klimstra D, Paradis V, Rugge M, Schirmacher P, et al. The 2019 WHO classification of tumours of the digestive system. Histopathology. 2020;76(2):182-8. https://doi.org/10.1111/his.13975
6. Lauwers GY, Carneiro F, Graham DY, Curado CM. Tumours of the stomach. In: Bosman FT, Carneiro F, Hruban RH Theise ND, editors. WHO Classification of Tumours of the Digestive System. 4 th. Lyon: IARC; 2010. p. 48-58.
7. Akce M, Jiang R, Alese OB, Shaib WL, Wu C, Behera M, et al. Gastric squamous cell carcinoma and gastric adenosquamous carcinoma, clinical features and outcomes of rare clinical entities: A National Cancer Database (NCDB) analysis. J Gastrointest Oncol. 2019;10(1):85-94. https://doi.org/10.21037/jgo.2018.10.06
8. Feng F, Zheng G, Qi J, Xu G, Wang F, Wang Q, et al. Clinicopathological features and prognosis of gastric adenosquamous carcinoma. Sci Rep. 2017;7(1):4597. https://doi.org/10.1038/s41598-017-04563-2
9. Chen E-B, Wei Y-C, Liu H-N, Tang C, Liu M-L, Peng K, et al. Hepatoid adenocarcinoma of stomach: Emphasis on the clinical relationship with alpha-fetoprotein-positive gastric cancer. Biomed Res Int. 2019;2019: 6710428. https://doi.org/10.1155/2019/6710428
10. Wang Y, Sun L, Li Z, Gao J, Ge S, Zhang C, et al. Hepatoid adenocarcinoma of the stomach: a unique subgroup with distinct clinicopathological and molecular features Gastric Cancer. 2019;22(6):1183-92. https://doi.org/10.1007/s10120-019-00965-5
11. Søreide JA, Greve OJ, Gudlaugsson E, Størset S. Hepatoid adenocarcinoma of the stomach - Proper identification and treatment remain a challenge. Scand J Gastroenterol. 2016;51(6):646-53. https://doi.org/10.3109/00365521.2015.1124286
12. Søreide JA. Therapeutic approaches to gastric hepatoid adenocarcinoma: Current perspectives. Ther Clin Risk Manag. 2019;15:1469-77 https://doi.org/10.2147/TCRM.S204303
13. Yu H, Fang C, Chen L, Shi J, Fan X, Zou X, et al. Worse prognosis in papillary, compared to tubular, early gastric carcinoma. J Cancer. 2017;8(1):117-23. https://doi.org/10.7150/jca.17326
14. Yasuda K, Adachi Y, Shiraishi N, Maeo S, Kitano S. Papillary adenocarcinoma of the stomach. Gastric Cancer. 2000;3(1):33-8. https://doi.org/10.1007/PL00011687
15. Chetty R. Gastrointestinal cancers accompanied by a dense lymphoid component: an overview with special reference to gastric and colonic medullary and lymphoepithelioma-like carcinomas. J Clin Pathol. 2012;65(12):1062-5. https://doi.org/10.1136/jclinpath-2012-201067
16. Cao H, Xie J, Qian Y, Wu Y, Tang Z. Lymphoepithelioma-like gastric carcinoma treated with partial gastrectomy: Two case reports. Oncol Lett. 2019;18(1):545-52. https://doi.org/10.3892/ol.2019.10368
17. Association JGC. Japanese gastric cancer treatment guidelines 2014 (ver. 4). Gastric Cancer. 2017;20(1):1-19. https://doi.org/10.1007/s10120-016-0622-4
18. Sano T, Kodera Y. Japanese classification of gastric carcinoma: 3rd English edition. Gastric Cancer. 2011;14(2):101-12.

https://doi.org/10.1007/s10120-011-0041-5
19. Lauren P. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification. Acta Pathol Microbiol Scand. 1965;64:31-49. https://doi.org/10.1111/apm.1965.64.1.31
20. Park JM, Jang YJ, Kim JH, Park SS, Park SH, Kim SJ, et al. Gastric Cancer histology: Clinicopathologic characteristics and prognostic value. J Surg Oncol. 2008;98(7): 520-5. https://doi.org/10.1002/jso.21150
21. Chen YY, Li AFY, Huang KH, Lan YT, Chen MH, Chao Y, et al. Adenosquamous carcinoma of the stomach and review of the literature. Pathol Oncol Res. 2015;21(3):547-51. https://doi.org/10.1007/s12253-014-9890-7
22. Yang J, Wang R, Zhang W, Zhuang W, Wang M, Tang C. Clinicopathological and prognostic characteristics of hepatoid adenocarcinoma of the stomach. Gastroenterol Res Pract. 2014;2014. https://doi.org/10.1155/2014/140587
23. Zeng XY, Yin YP, Xiao H, Zhang P, He J, Liu WZ, et al. Clinicopathological characteristics and prognosis of hepatoid adenocarcinoma of the stomach: evaluation of a pooled case series. Curr Med Sci. 2018;38(6):1054-61. https://doi.org/10.1007/s11596-018-1983-1
24. Adachi Y, Tsuchihashi J, Shiraishi N, Yasuda K, Etoh T, Kitano S. AFP-producing gastric carcinoma: multivariate analysis of prognostic factors in 270 patients. Oncology. 2003;65(2):95-101. https://doi.org/10.1159/000072332
25. Watanabe H, Enjoji M, Imai T. Gastric carcinoma with lymphoid stroma. Its morphologic characteristics and prognostic correlations. Cancer. 1976;38:232-43. https://doi.org/10.1002/1097-0142(197607)38:1<232::AID-CNCR2820380135>3.0.CO;2-4
26. Fukayama M, Ushiku T. Epstein-Barr virus-associated gastric carcinoma. Pathol Res Pract. 2011;207(9):529-37. https://doi.org/10.1016/j.prp.2011.07.004
27. Tak DH, Jeong HY, Seong JK, Moon HS, Kang SH. Comparison of clinical characteristics and prognostic factors between gastric lymphoepithelioma-like carcinoma and gastric adenocarcinoma. Korean J Gastroenterol. 2013;62(5):272-7. https://doi.org/10.4166/kjg.2013.62.5.272
28. Park S, Choi M-G, Kim K-M, Kim HS, Jung S-H, Lee JH, et al. Lymphoepithelioma-like carcinoma: a distinct type of gastric cancer. J Surg Res. 2015;194(2):458-63. https://doi.org/10.1016/j.jss.2014.12.005
29. Lee HJ, Kim GH, Park DY, Kim YK, Jeon HK, Lee BE, et al. Endoscopic submucosal dissection for papillary adenocarcinoma of the stomach: is it really safe? Gastric Cancer. 2017;20(6):978-86. https://doi.org/10.1007/s10120-017-0709-6
30. Park JH, Kim JS, Kang SH, Moon HS, Sung JK, Jeong HY. Efficacy and safety of endoscopic submucosal dissection for papillary adenocarcinoma-type early gastric cancer. Medicine (Baltimore). 2019;98(25):e16134. https://doi.org/10.1097/MD.0000000000016134

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Epidemiology and the Risk Factors for Mortality in Ventilator-Associated Pneumonia

Ventilatör ile İlişkili Pnömonide Epidemiyoloji ve Mortalite ile İlişkili Risk Faktörleri

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ABSTRACT

Objective: *Ventilator-associated pneumonia (VAP) is the most common hospital-aquired infections in intensive care units (ICUs) and associated with prolonged hospital stay, increased mortality and cost. This study aims to analyse the epidemiology and the risk factors affecting 30 day-mortality in VAP.*
Method: *Adult patients with VAP were included in the study. Data were obtained from infection control commitee records. Patients were followed up for mortality until 30 days after onset of VAP or until death for the patients died within 30 days. Survivor and non-survivor groups were compared as for the predictors of mortality.*
Results: *A total of 183 VAP patients were evaluated. Early-onset VAP was observed in 16 (8.7%), and late-onset VAP in 167 (91.3%) patients. Acinetobacter baumannii was the most common cause of VAP (49.2%), followed by Pseudomonas aeruginosa (19.7%) and Klebsiella pneumoniae (13.7%). Carbapenem resistance was seen in 78 (42.6%) patients and among them, most frequently Acinetobacter baumannii (62.8%, 49/78), followed by Klebsiella pneumoniae (20.5%, 16/78), Pseudomonas aeruginosa (14.1%, 11/78) and Escherichia coli (2.6%, 2/78) were isolated. Thirty day-mortality rate was 46.4% (n=85). In univariate analysis; malignity, blood transfusion, renal replacement therapy, Higher APACHE II, SOFA and SAPS 2 scores on the day of VAP onset and Acinetobacter baumannii were found to be more common in non-survivor group. According to the Cox-regression analysis, only SOFA score on the day of VAP onset and Acinetobacter baumannii were independent predictors of mortality. Although rate of trauma patients was significantly higher in survivor group, in multivariate analysis it was not a protective factor for mortality.*
Conclusion: *The most common cause of VAP was Acinetobacter baumannii and carbapenem resistance was seen in more than half of Acinetobacter baumannii and Klebsiella pneumoniae isolates. Higher SOFA score on the day of VAP onset and Acinetobacter baumannii infections were found to be independently associated with 30-day mortality in VAP patients.*

Keywords: *ventilator-asociated pneumonia, mortality, SOFA score, Acinetobacter baumannii*

ÖZ

Amaç: *Ventilatörle ilişkili pnömoni (VİP), yoğun bakım üniteleri (YBÜ)'de en sık hastane kaynaklı infeksiyonlardan biridir ve uzun süreli hastane yatışı, artan ölüm oranı ve maliyet ile ilişkilidir. Bu çalışma, YBÜ'de VİP tanılı hastalarda epidemiyoloji ve 30 günlük mortaliteyi etkileyen risk faktörlerini irdelemeyi amaçlamaktadır.*
Yöntem: *Erişkin VİP hastaları çalışmaya dahil edildi. Hasta verileri enfeksiyon kontrol komitesi kayıtlarından elde edildi. Hastalar VİP başlangıcından 30 gün sonrasına kadar veya 30 gün içinde öldüyse ölene kadar mortalite açısından takip edildi. Yaşayan ve ölen hastalar risk faktöreri açısından karşılaştırıldı.*
Bulgular: *Çalışmaya toplam 183 VİP hastası dahil edildi. Erken başlangıçlı VİP 16 (%8,7) hastada, geç başlangıçlı VİP 167 (%91,3) hastada görüldü. En sık VİP etkeni Acinetobacter baumannii idi (%49,2), bunu Pseudomonas aeruginosa (%19,7) ve Klebsiella pneumoniae (%13,7) izledi. 78 (%42.6) hastada karbapeneme direnç görüldü. Bu hastalarda sıklık sırasına göre Acinetobacter baumannii (% 62.8, 49/78), Klebsiella pneumoniae (%20.5, 16/78), Pseudomonas aeruginosa (%14.1, 11/78) ve Escherichia coli (%2.6, 2/78) izole edildi. Çalışmada otuz günlük mortalite oranı %46,4 (n=85) olarak bulundu. Tek değişkenli analizde; ölen grupta malignite, kan transfüzyonu, renal replasman tedavisi, APACHE II, SOFA ve SAPS II skorları ve Acinetobacter baumannii sıklığının daha fazla olduğu görüldü. Cox-regresyon analizine göre, sadece VİP geliştiği andaki SOFA skoru ve Acinetobacter baumannii mortalitenin için bağımsız risk faktörü olarak bulundu. Yaşayan grupta travma hastalarının oranı anlamlı olarak daha yüksek olmasına rağmen, travma çok değişkenli analizde mortalite için koruyucu bir faktör değildi.*
Sonuç: *VİP hastalarında en sık etken Acinetobacter baumannii idi ve karbapenem direnci Acinetobacter baumannii ve Klebsiella pneumoniae izolatlarının yarısından fazlasında görüldü.VIP geliştiği andaki SOFA skoru yüksekliği ve Acinetobacter baumannii infeksiyonu VIP hastalarında 30 günlük mortalite ile bağımsız olarak ilişkili bulundu.*

Anahtar kelimeler: *ventilatörle ilişkili pnömoni, mortalite, SOFA skoru, Acinetobacter baumannii*

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INTRODUCTION

Ventilator-associated pneumonia (VAP) is defined as the pneumonia developed more than 48 hours after intubation in patients on mechanical ventilation. It is one of the most common hospital-aquired infections in intensive care units (ICUs) and associated with prolonged duration of hospital stay, increased mortality rate and cost [1-4].

VAP develops in approximately 10–40% of patients on mechanical ventilation, with large variations among ICUs. Mortality rates due to VAP has been reported varying from 20% to 50% in the literature [5-8]. Prior studies have identified the risk factors that affect the prognosis of VAP [9-11]. Multidrug-resistant (MDR) microorganisms, the severity of illness, and inadequate initial antibiotic therapy have been identified as determinants of ICU mortality in patients with VAP. Underlying diseases such as chronic obstructive pulmonary disease (COPD) are also believed to effect the mortality in VAP patients [12]. This study aims to analyse the epidemiology of VAP and identify the risk factors affecting 30 day-mortality of the patients with VAP in a tertiary care hospital ICU.

MATERIALS and METHODS

This retrospective study was conducted in a 612-bed tertiary care hospital which has a 31-bed Anesthesiology and Reanimation ICU, nine-bed neurology ICU, 16-bed coronary ICU, seven-bed cardiovascular ICU, 26-bed neonatal ICU and a 16-bed pediatric ICU. We performed the study in Anesthesiology and Reanimation ICU which accepts patients from both medical and surgical wards. The study was approved by the Ethics Committee.

Adult patients with VAP (≥18 years) who were hospitalized in ICU between January 2016 and January 2019 were included in the study. VAP is defined as a new or progressive pulmonary infiltration occurring more than 48 h after intubation in combination with at least 2 of the following criteria: temperature >38.5°C or <36.5°C; change in character of sputum (purulent or increased amount of sputum); white blood cell count >10000 cells/mm^3 or <4000 cells/mm^3 [13]. VAP is classified as early-onset VAP, occurring within 4 days of intubation, and late-onset VAP, occuring on the fifth day or later, after intubation. Respiratory samples were obtained from either endotracheal aspirate (ETA) or bronchoalveolar lavage (BAL) to determine the causative microorganism and quantitative culture cut-off points of $>10^6$ CFU/ml and $>10^4$ CFU/ml were used respectively. In cases with recurrent VAP, only the first episode was included in the study. The patients who had pneumonia at admission, multiple microorganisms in the ETA culture or the patients who did not meet the VAP criteria despite the growth of microorganisms in the ETA culture were excluded from the study. Data about demographic characteristics, underlying diseases, length of ICU stay, invasive procedures, duration of mechanical ventilation prior to VAP, and causative microorganisms were retrieved from the infection control commitee records. Acute Physiology and Chronic Health Evaluation (APACHE) II score, Sequential Organ Failure Assessment (SOFA) score and Simplified Acute Physiology Score (SAPS II) were recorded both on the admission day and the day of VAP onset. Patients were followed up for mortality until 30 days after onset of VAP or until death for the patients died within 30 days. Statistical analyses were performed by using the Statistical package for Social Sciences version 25.0 for Windows (SPSS Inc., Chicago, IL, USA). Descriptive data were presented as mean±standard deviation, frequency, median and percentage values. Categorical variables were compared using chi- square test and Fisher's Exact test. The normality of continuous variables was tested with the Kolmogorov- Smirnov test. Student's t-test was used for comparing the normally distributed continuous variables and, Mann-Whitney U test for comparing the continuous variables which were not normally distributed. Cox regression analysis was used for multivariate analysis to evaluate the independent variables associated with 30-day mortality. The "p" values less than or equal to 0.05 ($p \leq 0.05$) were considered as statistically significant.

RESULTS

A total of 183 VAP patients were enrolled in the study. Out of them, 116 (63.4%) were male and 67 (36.6%) were female with an overall mean age of 53.15±20.88 years (min: 18, max: 94). Early-onset VAP was observed in 16 (8.7%) patients and late-onset VAP in 167 (91.3%) patients. *Acinetobacter*

Table 1. Mortality associated risk factors in ventilator-associated pneumonia.

	Survivor (n=98)	Non-survivor (n=85)	p	OR	95% CI
Sex, n (%)					
Male	67 (68.4)	49 (57.6)			
Female	31 (31.6)	36 (42.4)	0.13	1.63	0.34-1.15
Age, year (mean±sd)	50.62±21.03	56.14±20.42	0.08		
Hospitalization before ICU, n (%)	40 (40.8)	40 (47.1)	0.39	1.28	0.71-2.31
Length of ICU stay, day, median (IQR)	32 (21-47)	31 (21-48)	0.85		
APACHE II on admission, median (IQR)	20 (15-25)	20 (15-26)	0.18		
SOFA on admission, median (IQR)	8 (5-10)	9 (6-11)	0.06		
SAPS II on admission, median (IQR)	45 (36-23)	48 (37-61)	0.16		
APACHE II on the day of VAP onset, median (IQR)	18 (12-23)	24 (19-28)	<0.001*		
SOFA on the day of VAP onset, median (IQR)	4 (3-7)	10 (6-3)	<0.001*		
SAPS II on the day of VAP onset, median (IQR)	39 (31-49)	48 (43-60)	<0.001*		
Duration of mechanical ventilation before VAP, day, median (IQR)	11 (7-19)	11 (7-18)	0.94		
Type of VAP, n (%)					
Early	7 (7.1)	9 (10.6)			
Late	91 (92.9)	76 (89.4)	0.41	0.65	0.23-1.82
Trauma, n (%)	34 (34.7)	15 (17.6)	0.009*	0.40	0.20-0.80
Secondary BSI, n (%)	2 (2)	5 (5.9)	0.17	3	0.56-15.88
Carbapenem resistance, n (%)	36 (36.7)	42 (49.4)	0.08	1.68	0.93-3.03
Colistin resistance, n (%)	1 (1)	3 (3.5)	0.33	3.5	0.36-34.77
Underlying diseases, n (%)					
Hypertention	24 (24.5)	25 (29.4)	0.45	1.28	0.66-2.47
Heart failure	6 (6.1)	9 (10.6)	0.27	1.81	0.61-5.33
Diabetes mellitus	13 (13.3)	14 (16.5)	0.54	1.28	0.56-2.92
Chronic renal failure	9 (9.2)	14 (16.5)	0.13	1.95	0.79-4.76
Coronary artery disease	11 (11.2)	7 (8.2)	0.49	0.71	0.26-1.92
Malignity	3 (3.1)	13 (15.3)	0.003*	5.71	1.57-20.81
COPD	8 (8.2)	5 (5.9)	0.54	0.70	0.22-2.23
Neurological diseases	7 (7.1)	4 (4.7)	0.48	0.64	0.18-2.27
Invasive procedures, n (%)					
Tracheostomy	75 (76.5)	60 (70.6)	0.36	0.73	0.38-1.42
Blood transfusion	74 (75.5)	78 (91.8)	0.003*	3.61	1.46-8.88
CVC	82 (83.7)	75 (88.2)	0.37	1.46	0.89-2.94
Nazogastric tube	97 (99)	85 (100)	1	1.87	1.63-2.15
Total parenteral nutrition	52 (53.1)	55 (64.7)	0.11	1.62	0.89-2.94
Hemodialysis/CRRT	26 (26.5)	51 (60)	<0.001*	4.1	2.22-7.75
Microorganism, n (%)					
Klebsiella pneumoniae	11 (11.2)	13 (15.3)	0.41	1.42	0.60-3.38
Acinetobacter baumannii	38 (38.8)	52 (61.2)	0.003*	2.48	1.37-4.51
Pseudomonas aeruginosa	24 (24.5)	12 (14.1)	0.07	0.5	0.23-1.08
Staphylococcus aureus	7 (7.1)	3 (3.5)	0.28	0.47	0.11-1.9

OR: Odd's ratio, sd: standard deviation, IQR: interquartile range VAP: ventilator-associated pneumonia, BSI: Bloodstream infection, CVC: Central venous catheter CRRT: continuous renal replacement therapy

baumannii was the leading cause of VAP (49.2%), followed by *Pseudomonas aeruginosa* (19.7%) and *Klebsiella pneumoniae* (13.7%). The distribution of microorganisms that caused VAP was shown in Figure 1. Carbapenem resistance was seen in 78 (42.6%) patients and among them, *Acinetobacter baumannii* (62.8%, 49/78), *Klebsiella pneumoniae* (20.5%, 16/78), *Pseudomonas aeruginosa* (14.1%, 11/78) and *Escherichia coli* (2.6%, 2/78) were isolated. The carbapenem resistance rates for each microorganizm was shown in Figure 2. Colistin resistance was seen only in *Klebsiella pneumoniae* species in 4 (2.2%) patients. Secondary bloodstream infections were seen in seven patients, four of them were infected with *Acinetobacter baumannii*, two of them with *Klebsiella pneumoniae* and one of them with *S.aureus.*

Thirty day-mortality rate was found to be 46.4% (n=85) in the study. Survivor and non-survivor groups

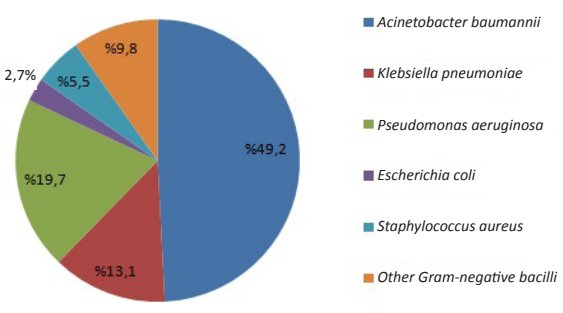

Figure 1. Distribution of bacteria that caused ventilator-associated VAP

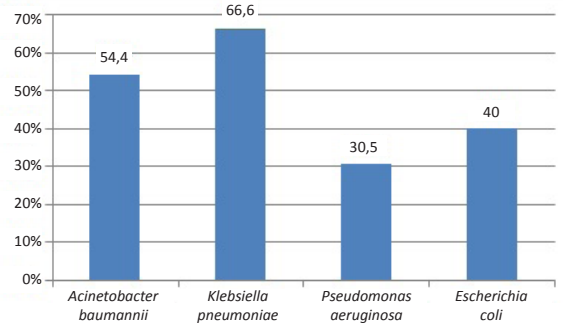

Figure 2. Carbapenem resistance rates for each bacteria.

Table 2. Cox regression analysis of risk factors for mortality in ventilator-associated pneumonia.

	Survivor (n=98)	Non-survivor (n=85)	p	OR	%95 CI
APACHE II on the day of VAP onset, median (IQR)	18 (12-23)	24 (19-28)	0.11	1.03	0.99-1.07
SOFA on the day of VAP onset, median (IQR)	4 (3-7)	10 (6-3)	0.03*	1.07	1-1.14
SAPS II on the day of VAP onset, median (IQR)	39 (31-49)	48 (43-60)	0.80	0.99	0.97-1.02
Trauma, n (%)	34 (34.7)	15 (17.6)	0.36	0.74	0.39-1.40
Malignity, n (%)	3 (3.1)	13 (15.3)	0.11	1.73	0.88-3.39
Blood transfusion, n (%)	74 (75.5)	78 (91.8)	0.11	2.10	0.83-5.33
Hemodialysis/CRRT, n (%)	26 (26.5)	51 (60)	0.2	0.7	0.4-1.21
Acinetobacter baumannii, n (%)	38 (38.8)	52 (61.2)	0.01*	1.82	1.12-2.96

were compared to determine the predictors of mortality. Demographic characteristics including age and sex, APACHE II, SOFA and SAPS II scores at ICU admission, length of ICU stay, the median duration of mechanical ventilation prior to VAP onset were not statistically different between two groups. The proportion of patients with early and late-onset VAP were similar in both survivor and non-survivor groups (p=0.41). Among underlying diseases, the history of malignity was found to be at significantly higher rates in non-survivor group (p=0.003, OR:5.71, 95% CI:1.57-20.81). In terms of invasive procedures in ICU, the rates of blood transfusion and hemodialysis/continuous renal replacement therapy (CRRT) were more frequently applied in non-survivor group (p=0.03, OR:3.61, 95% CI:1.46-8.88, p<0.001, OR:4.1, 95% CI:2.22-7.75) Although APACHE II, SOFA and SAPS II scores at admission were not statistically different between each group, they were found to be higher in non-survivors on the day of VAP onset. When comparing survivor and non-survivors in terms of microorganisms causing VAP, only *Acinetobacter baumannii* isolates were found to be at a significantly higher rates in non-survivor group (p=0.01, OR:1.82, 95% CI:1.12-2.96). *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* infections were seen more commonly in non-survivor group, but the difference was not statistically significant. Variables that were found to be significant in univariate analysis were evaluated with Cox regression analysis to predict independent factors associated with the mortality of VAP. Only SOFA score on the day of VAP onset and *Acinetobacter baumannii* were found to be independently asociated with 30-day mortality. Results of univariate and multivariate analysis are shown in Tables 1 and 2.

In the univariate analysis, rate of patients hospitalized with trauma was seen significantly higher in survivor group (p=0.009, OR:0.4, 95% CI:0.2-0.8). However, according to the Cox regression analysis, trauma was not found to be an independent factor for the survival of VAP patients (p=0.36, OR:0.74, 95% CI:0.39-1.40).

DISCUSSION

Although there are several guidelines for preventing ventilator-associated pneumonia, VAP continues to be one of the most common hospital-acquired infec-

tions seen in ICUs. VAP rates vary between each hospital and each ICU and it has been stated at different rates in the studies. While the incidence of VAP was 10.8/1000 ventilator days in a study conducted in Thailand, it was found as 50.87/1000 ventilator days in another study in Argentina [14,15]. In our country, it was found to be 16.1/1000 ventilator days in the study of Engin et al, 8.98/1000 ventilator days in the study of Erbay et al, and 23.3 in the study of Uslu et al. [16-18]. In our study, the VIP incidence was found to be 12.5/1000 ventilator days. Even in the same unit, VAP incidence may change over time. Therefore, it is important to follow the rates with surveillance regularly, and taking measures when there is an increase in VAP rates.

In the studies, Gram-negative bacteria have been reported as the most common isolates in VAP patients. Among these, the most common bacteria are *Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Klebsiella pneumoniae* [19-28]. In our study, the microorganisms caused VAP were similar to those studies regarding their order of frequency (Figure 1). *Acinetobacter baumannii* strains were isolated in half of our patients and Pseudomonas aeruginosa strains were isolated in 20% of the patients.

One of the most important issue in nosocomial infections is the increasing antibiotic resistance over the years. Increasing rates of resistance against carbapenems, which were used in the treatment of MDR microorganisms, cause serious difficulties in the treatment of these patients and increase mortality and morbidity [29,30]. In our study, carbapenem resistance was observed in 42.6% of the patients. *Acinetobacter* spp. and *Klebsiella pneumoniae* isolates have been reported to develop resistance to most antibiotics at increasing rates over the years [22,27,31]. In the "European Antimicrobial Resistance Surveillance Network" (EARS-Net) 2017 report, carbapenem resistance among *Klebsiella pneumoniae* isolates were reported as 64.7% in Greece, 29.7% in Italy and 22.5% in Romania [32]. In Turkey, Candevir-Ulu et al. found carbapenem resistance to be 48% in the study conducted in ICUs in 2012 [33]. Akgül et al. reported that the carbapenem resistance against *K.pneumoniae* strains increased to 66.9% in 2014 [34]. In our study, although carbapenem resistance was 54.4% against *Acinetobacter* strains which were responsible for half of the VAP cases, it was higher (66.6%) against *Klebsiella pneumoniae* strains. As another finding, carbapenem resistance was found to be 40% in *E.coli* strains which were isolated in only 5 patients. With the widespread use of colistin for Gram-negative bacteria resistant to carbapenems, colistin resistance has also become a problem for these bacteria [35,36]. Koçak et al. found that 39.5% of 81 carbapenem-resistant *K.pneumoniae* isolates were also resistant to colistin [37]. In our study, 4 (25%) of 16 carbapenem-resistant Klebsiella pneumoniae strains were also found to be resistant to colistin.

VAP has the highest mortality rates among nosocomial infections. In our study, the 30-day mortality rate was found to be higher (46.4%). VAP mortality rates have been reported in studies varying between 14-70% [38,39]. In a study conducted in China, the 30-day mortality rate was 42.8%, in a Brazilian study 35%, in another study overall mortality in VAP patients was found to be 32.4 percent [40,6]. There are several factors affecting mortality in the patients with VAP. In our study, survivor and non-survivors were compared to evaluate the risk factors affecting 30-day mortality. Although there are several studies showing that older age has negative impact on survival, in our study no statistical difference was observed between two groups in terms of their mean ages. When the underlying diseases were evaluated, the rate of malignancy was found to be significantly higher in the non-survivor group. In two studies conducted in Turkey, the history of coronary artery disease was found to be independently associated with mortality in VAP patients [28,41]. In a study conducted in Thailand, history of malignancy was found to be associated with mortality, similar to our study [19]. Immunosuppression predisposes patients to infections by impairing the host defense. But et al. found that corticosteroid use and history of malignancy were found to be higher in non-survivor group, similar to our study [28]. Considering the effect of invasive procedures on mortality, blood transfusion and hemodialysis/CRRT were found to have a higher impact on mortality in the non-survivor group, but these variables lost their significance in multivariate analysis (Table 2).

When considering the univariate analysis, the proportion of patients with a history of trauma was found to be significantly higher in the survivor group (Table 1). Similarly, In a study performed in 2010, trauma and nontrauma groups were compared in VAP patients, and mortality was found to be lower in trauma patients [42]. In our data, when comparing the patients with and without trauma, it was seen that patients hospitalized due to trauma were significantly younger, with relatively fewer underlying diseases and with lower scores both on admission and the day of VAP onset. The low mortality in trauma patients was attributed to these reasons.

APACHE II, SOFA and SAPS II scale scores at admission were not statistically different between each group, but when looking at scores at the time of VAP onset, it was seen that all scores of these scales were significantly higher in the non-survivor group (Table 1). Studies have shown that severity of illness is important for the prognosis after infections [8,43,44]. High scores in our study are compatible with the literature. According to the results of multivariate analysis, the SOFA scores at the time of VAP onset was independently associated with mortality. In a study carried out by Inchai et al, SOFA and SAPS II scores at the time of VAP onset were found to be associated with mortality [19]. In a study in China, SOFA scores were independently associated with mortality in VAP patients, and in another study, APACHE II scores at the time of VAP onset were found to be a poor indicator for prognosis [40,45]. In our study, only the SOFA scores at the time of VAP onset and Acinetobacter baumannii were found to be independent predictors of 30-day mortality in VAP patients (Table 2). *Acinetobacter baumannii* strains isolated in VAP patients have been shown in many studies to increase the mortality, and our results were compatible with the literature in this respect [46,47].

The retrospective design and being a single-centre study are the major limitations of our study. Also our study did not investigate data about antimicrobial treatment, therefore we could not evaluate the effect of appropriateness of the antibiotherapy on mortality.

In conclusion, according to our results the most common cause of VAP was *Acinetobacter baumannii* isolated in half of the patients. Carbapenem resistance, one of the most important treatment challenges, was seen in more than half of *Acinetobacter baumannii* and *Klebsiella pneumoniae* isolates. Our study also has shown that VAP is associated with high mortality as well as high SOFA score on the day of VAP onset and *Acinetobacter baumannii* infections with poor outcome.

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REFERENCES

1. Čiginskienė A, Dambrauskienė A, Rello J, et al. Ventilator-Associated Pneumonia due to Drug-Resistant Acinetobacter baumannii: Risk Factors and Mortality Relation with Resistance Profiles, and Independent Predictors of In-Hospital Mortality. Medicina (Kaunas). 2019;55(2):49. https://doi.org/10.3390/medicina55020049
2. Ozgur E, Horasan E, Karaca K, et al. Ventilator-associated pneumonia due to extensive drug-resistant Acinetobacter baumannii: Risk factors, clinical features, and outcomes. Am J Infect Control. 2014;42(2):206-8. https://doi.org/10.1016/j.ajic.2013.09.003
3. Li YJ, Pan CZ, Fang CQ, et al. Pneumonia caused by extensive drug-resistant Acinetobacter baumannii among hospitalized patients: genetic relationships, risk factors and mortality. BMC Infect Dis. 2017;17(1):371. https://doi.org/10.1186/s12879-017-2471-0
4. Vazquez Guillamet C, Kollef MH. Acinetobacter Pneumonia: Improving Outcomes With Early Identification and Appropriate Therapy. Clin Infect Dis. 2018;67(9):1455-62. https://doi.org/10.1093/cid/ciy375
5. Kalil AC, Metersky ML, Klompas M, et al. Executive Summary: Management of Adults With Hospital-acquired and Ventilator-associated Pneumonia: 2016 Clinical Practice Guidelines by the Infectious Diseases Society of America and the American Thoracic Society. Clin Infect Dis. 2016;63(5):575-82. https://doi.org/10.1093/cid/ciw504
6. Huang Y, Jiao Y, Zhang J, et al. Microbial etiology and prognostic factors of ventilator- associated pneumonia: a multicenter retrospective study in Shanghai. Clin Infect Dis. 2018;67(suppl_2):146-52. https://doi.org/10.1093/cid/ciy686
7. Ding C, Zhang Y, Yang Z et al. Incidence, temporal trend and factors associated with ventilator-associated pneumonia in mainland China: A systematic review and

meta-analysis. BMC Infect Dis. 2017;17(1):468. https://doi.org/10.1186/s12879-017-2566-7
8. da Silveira F, Nedel WL, Cassol R et al. Acinetobacter etiology respiratory tract infections associated with mechanical ventilation: What impacts on the prognosis? A retrospective cohort study. J Crit Care. 2019;49: 124-8. https://doi.org/10.1016/j.jcrc.2018.10.034
9. Nseir S, Di Pompeo C, Pronnier P, et al. Nosocomial tracheobronchitis in mechanically ventilated patients: incidence, aetiology and outcome. Eur Respir J. 2002;20(6):1483-9. https://doi.org/10.1183/09031936.02.00012902
10. Torres A, Aznar R, Gatell JM, et al. Incidence, risk, and prognosis factors of nosocomial pneumonia in mechanically ventilated patients. Am Rev Respir Dis. 1990;142(3):523-8. https://doi.org/10.1164/ajrccm/142.3.523
11. Rello J, Ausina V, Ricart M, et al. Impact of previous antimicrobial therapy on the etiology and outcome of ventilator-associated pneumonia. Chest. 1993;104(4): 1230-5. https://doi.org/10.1378/chest.104.4.1230
12. Nseir S, Di Pompeo C, Soubrier S, et al. Impact of ventilator-associated pneumonia on outcome in patients with COPD. Chest. 2005;128(3):1650-6. https://doi.org/10.1378/chest.128.3.1650
13. European Centre for Disease Prevention and Control. European surveillance of healthcareassociated infections in intensive care units - HAI-Net ICU protocol, version 1.02. Stockholm: ECDC; 2015. Available from: https://www.ecdc.europa.eu/sites/default/files/media/en/publications/Publications/healthcare-associated-infections-HAI-ICU-protocol.pdf
14. Thongpiyapoom S, Narong MN, Suwalak N, et al. Device-associated infections and patterns of antimicrobial resistance in a medical-surgical intensive care unit in a university hospital in Thailand. J Med Assoc Thai. 2004;87(7):819-24. PMID: 15521239.
15. Rosenthal VD, Guzmán S, Crnich C. Device associated nosocomial infection rates in intensive care units of Argentina. Infect Control Hosp Epidemiol. 2004;25(3):251-5. https://doi.org/10.1086/502386
16. Ergin F, Kurt Azap Ö, Yapar G, et al. Başkent Üniversitesi Hastanesi'nde saptanan ventilatörle ilişkili pnömoniler: insidans, risk faktörleri, etken dağılımı ve antibiyotik direnç paternleri. Flora. 2004;9(2):119-24. Available from: http://www.floradergisi.org/getFileContent.aspx?op=html&ref_id=106&file_name=2004-9-2-119-124.htm&_pk=709c99b4-17fb-4d0c-868b-34-a3504d8102
17. Erbay RH, Yalçın AN, Zencir M, et al. Costs and risk factors for ventilator-associated pneumonia in a Turkish university hospital's intensive care unit: a case-control study. BMC Pulm Med. 2004;4:3. https://doi.org/10.1186/1471-2466-4-3
18. Uslu M, Öztürk DB, Kuşçu F, et al. Yoğun Bakım Ünitesinde Yatan Hastalarda Ventilatörle İlişkili Pnömoni Gelişmesine Etki Eden Risk Faktörleri. Klimik Dergisi. 2010;23(3):83-8. Available from: https://klimikdergisi.org/content/files/sayilar/6/buyuk/83-8.pdf
19. Inchai J, Pothirat C, Liwsrisakun C, et al. Ventilator-associated pneumonia: epidemiology and prognostic indicators of 30-day mortality. Jpn J Infect Dis. 2015;68(3):181-6. https://doi.org/10.7883/yoken.JJID.2014.282
20. Goel N, Chaudhary U, Aggarwal R, et al. Antibiotic sensitivity pattern of gram negative bacilli isolated from the lower respiratory tract of ventilated patients in the Intensive care unit. Indian J Crit Care Med. 2009;13(3): 148-51. https://doi.org/10.4103/0972-5229.58540
21. Palabıyık O, Öğütlü A, Toptaş Y. Yoğun bakım ünitesinde ventilatör ilişkili pnömoni ve etken mikroorganizmalar: iki yıllık retrospektif analiz. J Turk Soc Intens Care. 2016;14:80-5. https://doi.org/10.4274/tybdd.60490
22. Akın A, Esmaoğlu Çoruh A, Alp E, et al. Anestezi yoğun bakım ünitesinde beş yıl içerisinde gelişen nozokomiyal enfeksiyonlar ve antibiyotik direncinin değerlendirilmesi. Erciyes Med J 2011;33(1):7-16. Available from: https://www.journalagent.com/erciyesmedj/pdfs/EMJ_33_1_7_16.pdf
23. Tomak Y, Ertürk A, Şen A, et al. Anestezi yoğun bakım ünitesinde ventilatör ilişkili pnömoni hızları ve etken mikroorganizmaların dağılımı. ŞEEAH Tıp Bülteni. 2012;46(3):115-9. Available from: https://www.journalagent.com/sislietfaltip/pdfs/SETB_46_3_115_119.pdf
24. Göktaş U, Yaman G, Karahocagil MK, et al. Anestezi yoğun bakım ünitesinde hastane kaynaklı enfeksiyon etkenleri ve direnç profilinin değerlendirilmesi. J Turk Soc Intens Care. 2010;8(1):13-7. Available from: http://cms.galenos.com.tr/Uploads/Article_4235/13-17.pdf
25. Ertürk A, Çopur Çiçek A, Köksal E, et al. Yoğun bakım ünitesinde yatan hastaların çeşitli klinik örneklerinden izole edilen mikroorganizmalar ve antibiyotik duyarlılıkları. ANKEM Derg 2012;26(1):1-9. https://doi.org/10.5222/ankem.2012.001
26. Ak O, Batirel A, Ozer S, et al. Nosocomial infections and risk factors in the intensive care unit of a teaching and research hospital: a prospective cohort study. Med Sci Monit. 2011;17(5):PH29-34. https://doi.org/10.12659/MSM.881750
27. Sesli Çetin E, Kaya S, Pakbaş İ, et al. Yoğun bakım ünitelerinde yatan hastalardan izole edilen mikroorganizmalar ve antibiyotik duyarlılıkları. İnönü Üniversitesi Tıp Fakültesi Dergisi 2007;14:69-73. Available from: https://www.researchgate.net/profile/Selcuk_Kaya/publication/266464194
28. But A, Yetkin MA, Kanyılmaz D, et al. Analysis of epidemiology and risk factors for mortality in ventilator-associated pneumonia attacks in intensive care unit patients. Turk J Med Sci. 2017;47:812-6. https://doi.org/10.3906/sag-1601-38
29. Centers for Disease Control and Prevention (CDC). Guidance for control of infections with carbapenem-resistant or carbapenemase-producing Enterobacteriaceae in acute care facilities. MMWR Morb Mortal Wkly Rep. 2009;58:256-60. PMID: 19300408.
30. Schwaber MJ, Carmeli Y. Carbapenem-resistant Enterobacteriaceae: a potential threat. JAMA. 2008;300(24):2911-3. https://doi.org/10.1001/jama.2008.896
31. Senbayrak-Akcay S, Inan A, Cevan S, et al. Gram-negative bacilli causing infections in an intensive care unit of a tertiary care hospital in Istanbul, Turkey. J

Infect Dev Ctries. 2014;8(5):597-604. https://doi.org/10.3855/jidc.4277
32. European Centre for Disease Prevention and Control (ECDC). Surveillance of antimicrobial resistance in Europe Annual report of the European Antimicrobial Resistance Surveillance Network (EARS-Net) 2017. Available from: https://www.ecdc.europa.eu/sites/default/files/documents/AMR%202017_Cover%2BInner-web_v3.pdf
33. Candevir Ulu A, Kurtaran B, Inal AS, et al. Risk factors of carbapenem-resistant Klebsiella pneumoniae infection: a serious threat in ICUs. Med Sci Monit. 2015;21:219-24. https://doi.org/10.12659/MSM.892516
34. Akgul F, Bozkurt I, Sunbul M, et al. Risk factors and mortality in the carbapenem-resistant Klebsiella pneumoniae infection: case control study. Pathog Glob Health. 2016;110(7-8):321-5. https://doi.org/10.1080/20477724.2016.1254976
35. Rojas LJ, Salim M, Cober E, Richter SS, Perez F, Salata RA, et al. Colistin resistance in carbapenem-resistant Klebsiella pneumoniae: Laboratory detection and impact on mortality. Clin Infect Dis. 2017;64(6):711-8. https://doi.org/10.1093/cid/ciw805
36. Capone A, Giannella M, Fortini D, Giordano A, Meledandri M, Ballardini M, et al. High rate of colistin resistance among patients with carbapenem resistant Klebsiella pneumoniae infection accounts for an excess of mortality. Clin Microbiol Infect. 2013;19(1):E23-E30. https://doi.org/10.1111/1469-0691.12070
37. Koçak CÖ, Hazırolan G. Karbapeneme dirençli Klebsiella pneumoniae klinik izolatlarında kolistin direnci. Türk Mikrobiyoloji Cem Derg. 2019;49(1):17-23. https://doi.org/10.5222/TMCD.2019.017
38. Melsen WG, Rovers MM, Bonten MJ. Ventilator-associated pneumonia and mortality: a systematic review ofobservational studies. Crit Care Med. 2009;37(10):2709-18. https://doi.org/10.1097/CCM.0b013e3181ab8655
39. Gvozdenovi'c L, Kolarovi'c J, Sarkanovi'c-Luki'c M, et al. Incidence and outcome of ventilator-associated pneumonia (our experience). Braz J Infect Dis. 2012;16(6):599-600. https://doi.org/10.1016/j.bjid.2012.07.012
40. Feng DY, Zhou YQ, Zhou M, Zou XL, Wang YH, Zhang TT. Risk factors for mortality due to ventilator-associated pneumonia in a Chinese hospital: A Retrospective Study. Med Sci Monit. 2019;25:7660-5. https://doi.org/10.12659/MSM.916356
41. Ibrahim EH, Ward S, Sherman G, et al. A comparative analysis of patients with early-onset vs late-onset nosocomial pneumonia in the ICU setting. Chest. 2000;117(5):1434-42. https://doi.org/10.1378/chest.117.5.1434
42. Magret M, Amaya-Villar R, Garnacho J, et al. Ventilator-associated pneumonia in trauma patients is associated with lower mortality: results from EU-VAP study. J Trauma. 2010;69(4):849-54. https://doi.org/10.1097/TA.0b013e3181e4d7be
43. Inchai J, Pothirat C, Bumroongkit C, et al. Prognostic factors associated with mortality of drug-resistant Acinetobacter baumannii ventilator-associated pneumonia. J Intensive Care. 2015;3:9. https://doi.org/10.1186/s40560-015-0077-4
44. Karakuzu Z, Iscimen R, Akalin H, et al. Prognostic risk factors in ventilator-associated pneumonia. Med Sci Monit. 2018;24:1321-8. https://doi.org/10.12659/MSM.905919
45. Huang KT, Teng CC, Fang WF, et al. An early predictor of the outcome of patients with ventilator-associated pneumonia. Chang Gung Med J. 2010;33(3):274-82. Available from: http://cgmj.cgu.edu.tw/3303/330306.pdf
46. Siempos II, Vardakas KZ, Kyriakopoulos CE, et al. Predictors of mortality in adult patients with ventilator-associated pneumonia: a meta-analysis. Shock. 2010;33(6):590-601. https://doi.org/10.1097/SHK.0b013e3181cc0418
47. Chittawatanarat K, Jaipakdee W, Chotirosniramit N, et al. Microbiology, resistance patterns, and risk factors of mortality in ventilator-associated bacterial pneumonia in a Northern Thai tertiary-care university based general surgical intensive care unit. Infect Drug Resist. 2014;7:203-10. https://doi.org/10.2147/IDR.S67267


T.C. SAĞLIK BAKANLIĞI İL SAĞLIK MÜDÜRLÜĞÜ SAĞLIK BİLİMLERİ ÜNİVERSİTESİ
BAKIRKÖY DR. SADİ KONUK EĞİTİM VE ARAŞTIRMA HASTANESİ