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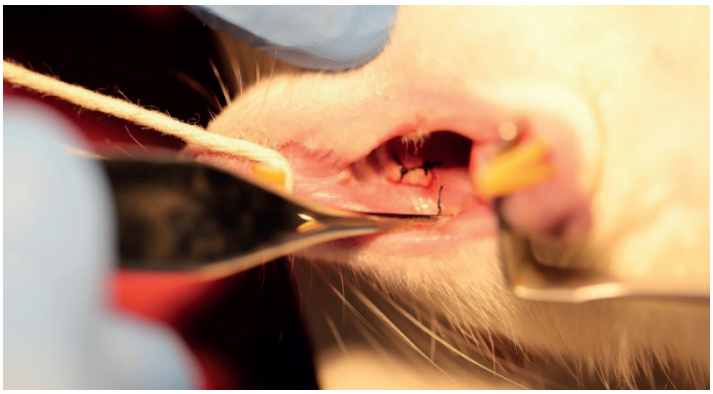

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From the Editor

Welcome reader to the *Journal of Advanced Studies in Health Sciences.* The June 2023 issue of our journal that you hold contains 14 articles.

The *Journal of Advanced Studies in Health Sciences* is one of Istanbul University's journals and is published triannually in February, June, and October according to international standards. Our journal started its publication life in 2018 and has been included in national and international indexes since 2021. The journal provides a wide range of services in the field of health and aims to enter the SCI - Science Citation Index Expanded. As such, only publications written in English will be accepted for our journal going forward. We await your research and will grow stronger with you. Our respect and thanks to the scientists who have contributed.

Prof. Dr. Zeynep Karakaş

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RESEARCH ARTICLE

CONTENTS

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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE
Journal of Advanced Research in Health Sciences / Sağlık Bilimlerinde İleri Araştırmalar Dergisi 2023
DOI: 10.26650/JARHS2023-1273011

GENETIC POLYMORPHISM OF APE1 ASP148GLU IS NOT ASSOCIATED WITH BLADDER CANCER RISK IN A TURKISH POPULATION

TÜRK POPÜLASYONUNDA APE1 ASP148GLU GEN POLİMORFİZMİNİN MESANE KANSERİ İLE İLİŞKİSİ BULUNMAMAKTADIR

Taghi AHMADİ RENDİ[1], Serhat KILINÇ[1], Selçuk ERDEM[1], Enes DEĞİRMENCİ[1], Öner ŞANLI[1], Canan KÜÇÜKGERGİN[1], Semra DOĞRU-ABBASOĞLU[1]

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ABSTRACT

Objective: The purpose of this investigative research was to investigate the potential impact of a single nucleotide polymorphism (Asp148Glu) within the APE1 gene on the development of bladder cancer (BCa) and spread, and to investigate the interaction of this polymorphism with cigarette smoking in BCa patients.

Materials and Methods: In a study of 256 participants, consisting of 136 healthy individuals and 120 patients with BCa, the APE1 Asp148Glu polymorphism was evaluated using "polymerase chain reaction restriction-fragment length polymorphism" analysis.

Results: There were no noteworthy difference variations observed in genotype distribution between the group of individuals with BCa and the control group with regards to the APE1 gene polymorphism. Furthermore, the APE1 gene polymorphism exhibited no correlation with the clinicopathological characteristics or smoking habits of individuals with BCa.

Conclusion: Based on our findings, it appears that the APE1 gene polymorphism, which plays a role in the BER pathway, does not appear to be a contributing factor in susceptibility to BCa in the Turkish population. In addition, the smoking habit may not modify BCa risk with respect to genetic variations in the APE1 gene.

Keywords: APE1, polymorphism, base excision repair, bladder cancer

ÖZ

Amaç: Çalışmamızda, APE1 (Asp148Glu) gen polimorfizmi ile mesane kanseri oluşumu ve gelişimi arasındaki ilişkiyi araştırmayı amaçladık. Ayrıca, mesane kanserinde sigara kullanımı ile APE 1 gen polimorfizmi arasındaki ilişkiyi değerlendirmeyi planladık.

Gereç ve yöntem: Çalışmamızda histopatolojik ve klinik açıdan mesane kanseri tanısı konan hastalar (n=120) ve sağlıklı normal kişiler (n=136) yer aldı. Çalışma grubundaki kişilerden elde edilen DNA'lardan Apürinik/Aprimidinik Endonükleaz 1 (APE1) gen polimorfizmi için polimeraz zincir reaksiyonu (PZR), sınırlayıcı enzim parça uzunluğu polimorfizmi (RFLP) teknikleri kullanıldı.

Bulgular: APE1 gen polimorfizminde genotip ve allel sıklığı incelendiğinde mesane kanseri ve kontrol grubu arasında anlamlı farklılık bulunmamaktadır. İlaveten, yüksek grade, ileri evre ve sigara kullanımı bakımından da incelendiğinde anlamlı bir faklılık bulunmamaktadır.

Sonuç: APE 1 gen polimorfizminin mesane kanseri oluşumunda ve gelişiminde bir risk içermediğini ayrıca, sigara kullanımının APE Asp148Glu gen polimorfizminde etkili olmadığını ileri sürebiliriz.

Anahtar Kelimeler: APE1, polimorfizm, baz kesip çıkarma onarımı, mesane kanseri

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INTRODUCTION

Bladder cancer (BCa) is the most commonly diagnosed tumor in the urinary tract and the fourth overall among all male neoplastic diseases (1, 2). Currently, about two million people in the world are struggling with this disease (3). Due to the efforts made in the field of cancer prevention and treatment, the death rate from BCa has decreased significantly over the past few years in many countries, especially in developing countries (4).

In the development of BCa, both environmental factors, including smoking, which is responsible for generating reactive oxygen species (ROS), and genetic factors are involved (5). Free oxygen radicals harm DNA and cause changes in the capacity of DNA repair systems, leading to the appearance of malignant tumors (6). To cope with oxidative DNA damage, the human body employs five distinct repairing DNA mechanisms: direct reversal, double-strand break repair mismatch repair, nucleotide excision repair (NER), and base excision repair (BER), (7, 9). The BER pathway that is in charge of making repairing the damage to DNA induced by oxidation and alkylation, that "apurinic/apyrimidinic endonuclease 1" (APE1) is a main enzyme in BER (7). Additionally, it participates in the redox control of transcription factors in cells and is commonly referred to as redox factor-1. (APE1/Ref-1) (8).

The APE1 gene is situated in the genomic region of chromosome 14q11.2–q12. The Asp148Glu polymorphism, which occurs due to T to G substitution at codon 148 in exon 5 of the APE1 gene, the outcome is the exchange of glutamate for an aspartate amino acid (10). Several reports found that many cancers, involving cancers such as prostate and colorectal cancer while other reports did not find any association with nasopharyngeal cancer or even showed a significant protective effect against BCa have been linked to Glu/Glu genotype of the APE1 Asp148Glu polymorphism as having an elevated risk (11, 13, 14). Therefore, the current research examined the potential impact of the APE1 Asp148Glu gene polymorphism on the development and advancement of BCa within a Turkish population.

MATERIALS and METHODS

Between 2012 and 2022, the Istanbul Faculty of Medicine, Urology Department conducted a study on 120 Turkish patients who had been diagnosed with BCa. A control group consisting of 136 people who were referred to routine medical examinations at the same facility and participants in the study had no prior history of cancer diagnosis of any type. The Ethics Committee, Istanbul Faculty of Medicine, granted ethical approval for the study, and each participant gave informed consent before participating (Date : 23/12/2022, No:23).

BCa was diagnosed histologically with samples received from patients through biopsy or surgery. The tumors were categorized based on their type, grade, and stage. Based on the 2004 WHO grading system for pathological bladder cancers, they were graded as low or high. 2002 TNM classification system was used to classify tumors as either low-stage or high-stage. BCa at a low stage (Ta/T1) is known as superficial BCa (SBC). BCa stages T2 to T4 are known as muscle-invasive BCa (MIBC).

A commercially accessible PCR template preparation kit (Roche Diagnostics, Mannheim, Germany)" was utilized to extract genomic DNA from white blood cells. The polymorphism of the APE1 Asp148Glu (rs 1130409) gene were genotyped using PCR-RFLP. The PCR reactions were carried through in "25 µl of 10 pmol APE1 primer, 0.3 mM dNTP, 2,5 mM $MgCl_{2,}$ 100 ng DNA, x10 PCR buffer (pH value: 8.8), and 1.25 U Taq polymerase (MBI Fermentas)".

The PCR cycling condition for APE1 was initial denaturation at 95 °C for a 2-minute period, 35 cycles of 30 sec at 95 °C, annealing at 52 °C for 45 sec and elongation at 72 °C for 45 sec, and a final extension step of 5 min at 72 °C. Following amplification, PCR products were subjected to restriction digestion using the FspBI enzyme (Thermo Scientific) (Hu et al. 2001). After completion, a 2% agarose gel treated with ethidium bromide was used to conduct gel electrophoresis, and to analyze the final products. Fragment patterns for the APE1 genotypes were AspAsp (164 bp), AspGlu (164,144, and 20 bp), and GluGlu (20 bp).

Statistical analyses

All statistical analyses conducted in this study were performed using SPSS version 21. The collected biochemical parameter data underwent statistical testing using either the Student's t-test for data with equal variances, or the 'Mann-Whitney U test' for data with unequal variances. To determine the difference in genetic distribution between the study group with BCa and the control group, Pearson's chi-square (v2) test was used. We used the Asp/Asp genotype as a reference due to it being the lowest-risk genotype. To assess the correlation between genotypes and tumor grade and stage, Pearson's chi-square test (x^2) was utilized in order to evaluate the total impact of the polymorphism. The statuses of age, sex, BMI, and smoking were determined using a logistic regression, which calculated

Table 1: The demographic and clinical characteristics of the control group and study group diagnosed with bladder cancer (mean±SD)

Parameters	Control group (n=136)	Patient group (n=120)	[a]p value
Age (years)	62.7±6.52	64.2±11.49	0.160
Female/male (%)	19.0/80.1	17.9/82.5	0.630
BMI (kg/m^2)	27.1±2.89	26.5±3.38	0.131
Smoking (%) (never/current)	61.0/39.0	33.3/66.7	0.000
Grade (1/2)		67/53	
Stage (invasive\ superficial)		96/24	

[a]ap: Value from Pearson`s χ^2 test to categorical variables and Mann Whitney-U to continuous variables

Table 2: Distributions of APE1Asp148Glu allele and genotype in controls and bladder cancer patients

	Control group n (%)	Patient group n (%)	p	OR[a](95% CI)
APE Asp148Glu				
AspAsp	93 (68.4)	86 (71.7)		Reference genotype.
AspGlu	40 (29.4)	30 (25.0)	0.767	0.91 (0.48-1.69)
GluGlu	3 (2.2)	4 (3.3)	0.339	1.86 (0.52-6.73)
AspGlu+GluGlu	43 (31.6)	34 (28.0)	0.924	0.97 (0.52-1.78)
Allele				
Asp	226 (83.0)	202 (84.1)		Reference allele.
Glu	46 (17.0)	38 (15.9)	0.742	2.05 (1.47-2.85)

[a]Odds ratios (OR) & 95% CI: Confidence intervals, adjusted for BMI: Age-sex and smoking status

adjusted odds ratios (aOR) and 95% "confidence intervals" (95% CI). To determine the effect size (W) with '2 degrees of freedom (2; 0.05), the NCSS 2000 statistical package (NCSS Inc; Kaysville UT)' was used, taking into account the sample size of the study. Based on these calculations, the study's power was determined to be 83%. The significance degree for the study was set at $p<0.05$, indicating that results with a p-value below this threshold were considered statistically significant.

RESULTS

There were no significant differences found in BMI, age, or sex when comparing the patients with the control group with BCa. However, more smokers were represented in the BCa patients as compared with the controls (p=0.000) (Table 1).

The APE1 Asp148Glu genotypes distribution in the control group was found to be coherent with the 'Hardy Weinberg equilibrium (HWE)' (p=0.587). The distributions of APE1 Asp148Glu genotypes among BCa patients were consistent with the HWE (p=0.496). The effect of APE1 Asp148Glu polymorphism on BCa risk is shown in Table 2. Regarding the controls, the genotype AspAsp was identified in 68.4%, the AspGlu genotype in 29.4%, and the GluGlu genotype in only 2.2% of individuals. The APE1 genotypes were observed in the bladder cancer patients, with 71.7% identified as AspAsp, 25.0% as Asp/Glu, and only 3.3% as GluGlu. There was no noteworthy correlation detected between the APE1 genotype/allele and the risk of BCa (Table 2).

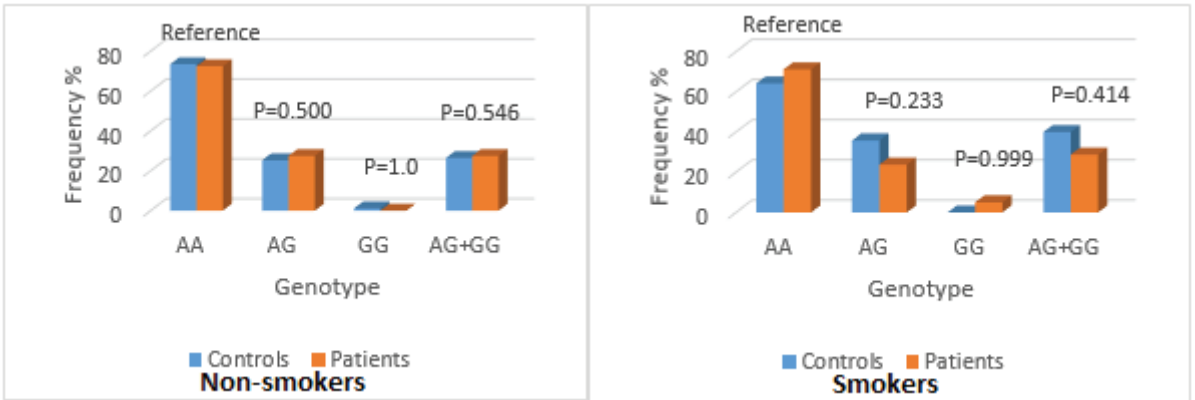

Figure 1: Distributions of APE1 Asp148Glu genotypes related to the smoking status

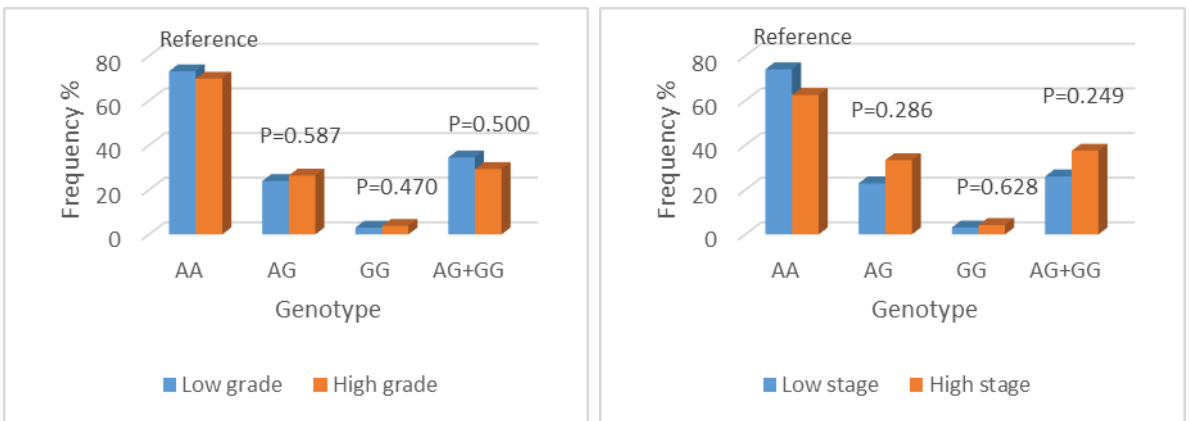

Figure 2: Distributions of APE1Asp148Glu genotypes according to the grade (Low/High) and stage (Low/High) of the disease

The potential dissimilarities in genotype distributions and allele frequencies were investigated between individuals who smoke and those who do not smoke, with respect to their vulnerability to bladder cancer. The APE1 Asp148Glu gene polymorphism was not found to be related within either smokers or non-smokers (Figure 1).

When classified based on BCa grade and stage, the distribution did not show any statistically significant differences in each APE1 genotype, as shown in Figure 2.

DISCUSSION

There are different biological mechanisms in the body as responses to repair DNA damage that preserve the totality of the genome (14). The decrease in capacity for DNA repair efficiency causes changes in the DNA damage-triggered biological response and, as a result, might result in the development of different malignant tumors (15).

Being under the influence of occupational carcinogenic factors is among the other important factors (16). Polycyclic aromatic hydrocarbons, arylamines, nitrosamines, and ROS in cigarettes have been shown to cause significant damage to the DNA molecular structure (17,18).

One of the mechanisms utilized for DNA repair is the BER pathway, which is repairing DNA damage brought on by alkylating agents, ionizing radiation, and oxidation (19). Its ability to function as an endonuclease and phosphodiesterase makes it possible for APE1 to assume a prominent role in repairing apurinic apyrimidinic sites (20).

Polymorphisms in genes responsible for DNA repair that cause changes in the effectiveness of the DNA repair system can be related to the tendency to manifest BCa (22). Of the DNA damage caused by ROS, 8-hydroxyguanine (8-OHG) is particularly mutagenic and results in G-C to T-A transversions during replication by DNA polymerases. (15). The main mechanism responsible for repairing 8-OHG is the short patch BER (15). It has been suggested that polymorphism of DNA repair genes is a risk factor for several cancers, including BCa (1, 23).

Polymorphisms in DNA repair genes cause changes in the amino acid sequence, cause damage to DNA repair capacity, and as a result, cancer appears (24). Various DNA damage is repaired in multiple pathways involving different proteins (25). Although the effect of DNA repair gene polymorphisms on BCa risk has been investigated in many studies, the relationship is not clear yet (1,3,7).

Numerous investigations have been conducted on the relationship between APE1 polymorphism and various cancers. (26). Hu et al. provided further insight into the biological significance of the APE1 codon 148 T-G transversion (Asp-Glu) polymorphism, which has been associated with lymphocyte mitotic delay in healthy individuals and increased sensitivity to ionizing radiation (22). Canbay et al. demonstrated this polymorphism has been linked to a higher incidence of gastric cancer (24). Peng et al. demonstrated APE1 genotypes have a relationship with lung cancer risk (18). The sole recognized common APE1 coding region variant that leads to a non-synonymous change is Asp148Glu. According to Ruchika et al. APE1 148 GG genotype was found to be linked to a protective effect against BCa in a North Indian population (20). Nonetheless, no discernible distinction existed at the allele level (8).

The APE1 Asp148Glu polymorphism did not demonstrate any correlation of significance with the risk of BCa in our study. Our findings are in line with previous research suggesting APE1 Asp 148 Glu polymorphism does not pose a risk for BCa (20, 21).

According to the results of our study data, the finding on gene-environment interaction showed no association between the APE1 Asp 148 Glu genotype and smoking status. Our data are consistent with some previous reports in BCa and prostate cancer (20, 27).

Based on the classification of the studied subjects in terms of disease stage at the time of testing, the APE1 Asp 148 Glu gene polymorphism did not indicate a noticeably higher risk for BCa in more advanced stages. In addition, the risk genotypes for the APE1 Asp 148 Glu polymorphism did not alter tumor grading among BCa patients. Sanyal et al. evaluated that there was no relation between the APE1 Asp 148 Glu gene polymorphism and pathological factors in BCa patients (21). In addition, Liu et al. reported that the APE1 variant allele was not associated with tumor grade and stage in BCa patients (28).

CONCLUSION

According to the results of our investigation, we cannot detect that an association exists between APE1 polymorphism and BCa. The current data from our research's findings demonstrate that APE1 Asp 148 Glu gene polymorphism may not affect the progression of BCa in the Turkish population.

Ethics Committee Approval: This study was approved by Istanbul Faculty of Medicine Clinical Research Ethics Committee (Date: 23.12.2022, 23).

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

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REFERENCES

1. Saginala K, Barsouk A, Aluru JS, Rawla P, Padala SA, Barsouk A. Epidemiology of bladder cancer. Med Sci (Basel) 2020;8(1):15.
2. Wong MC, Fung FDH, Leung C, Cheung WWL, Goggins WB, Ng ACF. The global epidemiology of bladder cancer: a joinpoint regression analysis of its incidence and mortality trends and projection. Sci Rep 2018;8:1129.
3. Antoni SFJ, Soerjomataram I, Znaor A, Jemal A, Bray F. Bladder cancer incidence and mortality: A global overview and recent trends. Eur Urol 2017;71(1):96-108.
4. Shah F, Logsdon D, Messmann RA, Fehrenbacher JC, Fishel ML, Kelley MR. Exploiting the Ref-1-APE1 node in cancer signaling and other diseases: from bench to clinic. NPJ Precis Oncol 2017;1:19.
5. Choi S, Shin JH, Lee YR, Joo HK, Song KH, Na YG, et al. Urinary APE1/Ref-1: A potential bladder cancer biomarker. Dis Markers 2016;2016:7276502.
6. Fishel ML, Xia H, McGeown J, McIlwain DW, Elbanna M, Craft AA, et al. Antitumor activity and mechanistic characterization of APE1/Ref-1 inhibitors in bladder cancer. Mol Cancer Ther 2019;18(11):1947-60.
7. Mir SM, Aliarab A, Goodarzi G, Shirzad M, Jafari SM, Qujeq D, et al. Melatonin: A smart molecule in the DNA repair system. Cell Biochem Func 2021;40(1):4-16.
8. Gangwar R, Ahirwar D, Mandhani A, Mittal RD. Influence of XPD and APE1 DNA repair gene polymorphism on bladder cancer susceptibility in North India. Urology 2009;73(3):675-80.
9. Benhamou S, Sarasin A. Variability in nucleotide excision repair and cancer risk: a review. Mutat Res 2000:462(2-3):149-58.
10. Huang HI, Chen CH, Wang SH, Wang LH, Lin YC. Effects of APE1 Asp148Glu polymorphisms on OPMD malignant transformation, and on susceptibility to and overall survival of oral cancer in Taiwan. Head Neck 2019:41(6):1557-64.
11. McIlwain DW, Fishel ML, Boos A, Kelley MR, Jerde TJ. APE1/Ref-1 redox-specific inhibition decreases survivin protein levels and induces cell cycle arrest in prostate cancer cells. Oncotarget 2018;9(13):10962-77.
12. Zhang S, Wang LA, Li Z, Peng Y, Cun YP, et al. APE1 polymorphisms are associated with colorectal cancer susceptibility in Chinese Hans. World J Gastroenterol 2014;20(26):8700-8.
13. Qing Li, Jian-Min Wang, Yu Peng, Shi-Heng Zhang, Tao Ren, Hao Luo, et al. Association of DNA Base-excision Repair XRCC1, OGG1 and APE1 gene polymorphisms with nasopharyngeal carcinoma susceptibility in a Chinese population. Asian Pac J Cancer Prev 2013;14(9):5145-51.
14. Narter KF. Analysis of XRCC3, hOGG1, XPD, XRCC1, XPG and APE1 Gene Polymorphisms in patients with bladder cancer. Istanbul University Institute of Health Sciences, PhD Thesis of Department of Molecular Medicine, 2008.
15. Karahalil B, Aygün Kocabaş N, Özçelik T. DNA repair gene polymorphisms and bladder cancer susceptibility in a Turkish population. Anticancer Res 2006;26(6C):4955-8.
16. Burch JD, Rohan TE, Howe GR, Risch HA, Hill GB, Steele R, Miller AB. Risk of bladder cancer by source and type of tobacco exposure: A case-control study. Int J Cancer 1989;44(4):622-8.
17. Kucukgergin C, Sanli O, Amasyalı AS, Tefik T, Seckin S. Genetic variants of MnSOD and GPX1 and susceptibility to bladder cancer in a Turkish population. Med Oncol 2012;29(3):1928-34.
18. Peng Y, Li Z, Zhang S, Xiong Y, Cun Y, Qian C, et al. Association of DNA base excision repair genes (OGG1, APE1 and XRCC1) polymorphisms with outcome to platinum-based chemotherapy in advanced nonsmall-cell lung cancer patients. Int J Cancer 2014;135(11):2687-97.
19. Ahirwar DK, Marthani A, Mittal RD. IL-8 -251 T>A polymorphism is associated with bladder cancer susceptibility and outcome after BCG immunotherapy in a Northern Indian cohort. Arch Med Res 2010;41(2):97-103.
20. Terry PD, Umbach DM, Taylor JA. APE1 genotype and risk of bladder cancer: Evidence for effect modification by smoking. Int J Cancer 2006;118(12):3170-3.
21. Sanyal S, Festa F, Sakano S, Zhang Z, Steineck G, Norming U, Wijkström H, Larsson P, Kumar R, Hemminki K. Polymorphisms in DNA repair and metabolic genes in bladder cancer. Carcinogenesis 2004;25(5):729-34.
22. Hu JJ, Smith TR, Miller MS, Mohrenweiser HW, Golden A, Case LD. Amino acid substitution variants of APE1 and XRCC1 genes associated with ionizing radiation sensitivity. Carcinogenesis 2001;22(6):917-22.
23. Gu D, Wang M, Zhang Z, Chen J. The DNA repair gene APE1 T1349G polymorphism and cancer risk: a meta-analysis of 27 case-control studies. Mutagenesis 2009;24(6):507-12.
24. Canbay E, Agachan B, Gulluoglu M, Isbir T, Balik E, Yamaner S, et al. Possible associations of APE1 polymorphism with susceptibility and HOGG1 polymorphism with prognosis in gastric cancer. Anticancer Res 2010;30(4):1359-64.
25. Agalliu I, Kwon EM, Salinas CA, Koopmeiners JS, Ostrander EA, Stanford JL. Genetic variation in DNA repair genes and prostate cancer risk: results from a population-based study. Cancer Causes Control 2010;21(2):289-300.
26. Wang M, Qin C, Zhu J, Yuan L, Fu G, Zhang Z, Yin C. Genetic variants of XRCC1, APE1, and ADPRT genes and risk of bladder cancer. DNA Cell Biol 2010;29(6):303-11.
27. Dhillon VS, Yeoh E, Fenech M. DNA repair gene polymorphisms and prostate cancer risk in South Australia results of a pilot study. Urol Oncol 2011;29(6):641-6.
28. Chuan Liu, Qinghua Yin, Lian Li, Ying-zhi, Zhuang Xuyu Zu, Yajie Wang. APE1 Asp148Glu gene polymorphism and bladder cancer risk: a meta-analysis. Mol Biol Rep 2013;40(1):171-6.

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COMPARISON OF FREQUENTIST AND BAYESIAN APPROACHES ON SAMPLE SIZE: METHODOLOGIC STUDY

İSTATİSTİKTE FREKANSÇI VE BAYESYEN YAKLAŞIMIN ÖRNEKLEM BÜYÜKLÜĞÜ ÜZERİNDEKİ ETKİLERİNİN KARŞILAŞTIRILMASI: METODOLOJİK ÇALIŞMA

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ABSTRACT

Objective: In the present study, we aimed to evaluate the effects of sample size on results of study by using frequentist and Bayesian approaches.
Material and Methods: The small sample consisted of 32 patients with ischemic heart disease (IHD) and 37 control subjects. In order to compare the statistical differences between small and large sample sizes, two samples were constituted. All the patients included in the study were male and between 40-50 years old. The large sample consisted of 355 IHD patients and 545 controls. Patients' biochemical variables including glucose, triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL), urea, creatinine, hemoglobin, hematocrit, (HCT), red cell distribution width (RDW), White blood cell (WBC), platelet (PLT), mean platelet volume (MPV), neutrophil (NEUT), lymphocyte (LYM) were recorded. Patients in the small and large samples were compared with both frequentist and Bayesian approaches.
Results: Except for glucose levels there were no statistical differences with respect to the biochemical variables of two groups in a small sample size when the variables were analyzed by the frequentist approach. Similarly, we did not find any differences between biochemical variables when the data were analyzed by the Bayesian approach. When the large sample size data were analyzed by the frequentist approach, glucose, TG, TC, HDL, LDL, urea, creatinine, hemoglobin, HCT, WBC, NEUT, LYMP levels were found to be statistically significantly different between patients who had IHD and the controls. Similarly, there were significant differences between two groups with respect to glucose, TG, TC, HDL, LDL, urea, creatinine, hemoglobin, HCT, WBC, NEUT, LYMP levels when the data analyzed by Bayesian approach.
Conclusion: Our study results suggested that there were no differences between the frequentist and Bayesian approach results when the sample size is large and the power of the study is high.
Key words: Frequentist, bayesian, sample size

ÖZ

Amaç: Bu çalışmada, frekantist ve Bayesyen yaklaşımlar kullanılarak örneklem büyüklüğünün araştırma sonuçları üzerindeki etkilerinin araştırılması amaçlanmıştır.
Gereç ve Yöntem: Çalışmamızda küçük ve büyük örneklem büyüklüğünde istatistiksel farklılıkları karşılaştırmak amacı ile küçük ve büyük olmak üzere iki örneklem oluşturulmuştur. Çalışmaya alınan tüm hastalar erkek ve 40 ile 50 yaş aralığındadır. Küçük örneklem için iskemik kalp hastalığı (İKH) olan 32, İKH olmayan 37 kişi çalışmaya dahil edilmiştir. Büyük örneklem için İKH olan 355, olmayan 545 kişi çalışmaya alınmıştır. Tüm hastaların glukoz, trigliserid (TG), total kolesterol (TKOL), yüksek yoğunluklu lipoprotein kolesterol (HDL), düşük yoğunluklu lipoprotein kolesterol (LDL), üre, kreatinin, hemoglobin, hematokrit (HCT), kırmızı kan hücresi dağılım genişliği (RDW), lökosit (WBC), trombosit (PLT), ortalama trombosit hacmi (MPV), nötrofil (NÖT), lenfosit (LYM) değerleri kaydedilmiştir. Küçük ve büyük örneklemler frekansçı ve Bayesyen yaklaşımla karşılaştırılımıştır.
Bulgular: Küçük örneklem büyüklüğünde frekantist yaklaşım ile yapılan analizde tüm biyokimyasal veriler İKH olan ve olmayan kişilerde karşılaştırılmış ve glukoz seviyeleri dışında diğer parametrelerde anlamlı fark saptanmamıştır. Yine grupların Bayesyen yaklaşımla yapılan karşılaştırmalarında parametreler arasında anlamlı istatistiksel fark elde edilmemiştir. Buna karşın büyük örneklem büyüklüğünde frekantist yaklaşım ile yapılan karşılaştırmalarda glukoz, TG, TKOL, HDL, LDL, üre, kreatinin, hemoglobin, HCT, WBC, NÖT ve LYM değerleri her iki grup arasında anlamlı olarak farklı çıkmıştır. Aynı şekilde Bayesyen yaklaşım ile yapılan karşılaştırmalarda glukoz, TG, TKOL, HDL, LDL , üre, kreatinin, hemoglobin, HCT, WBC, NÖT ve LYM değerleri iki grup arasında istatistiksel olarak anlamlı çıkmıştır.
Sonuç: Büyük örneklem büyüklüğünde ve yüksek bir güçte çalışmada verinin frekansçı ya da Bayesyen istatistik ile değerlenirilmesi açısından fark bulunmamamaktadır.
Anahtar Kelimeler: Frekantist, bayesyen, örneklem büyüklüğü

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INTRODUCTION

The main purpose of statistical interference is to make predictions about population based on the data that are derived from a sample. Population is defined as the entire group that we want to draw conclusions about (1).

The field of statistics, as a branch of science, has been influenced by different ideas during its development. These ideas have become evident over time and are now polarized as the frequentist and Bayesian approaches. Deductive and inductive methods have been adopted in frequentist and Bayesian approaches, respectively. In the frequentist approach, data are random, while parameters are unknown and fixed. In contrast, according to the Bayesian approach, data are fixed, and do not change after observations, whereas parameters are accepted as a random variable (1). Statistical hypothesis testing also differs between the two approaches. While deterministic rules are followed by the frequentist approach, the Bayesian methods are closer to a probability based interpretation. Bayesian statistics involves updating prior beliefs as more evidence becomes available (2). However, the frequentist statistics are interested in with whether an event (hypothesis) occurs or not. In this method, results of repeated experiments are examined under the same conditions and analysis of external information other than the sample data are not made.

Sample size estimation is one of the important steps in scientific studies. In statistics, the universe is the of set all experimental units, from which a sample is to be drawn. In order for the results of the study to be reliable, sample size should be sufficient in number and represent the universe appropriately. It is among one of the factors that directly affects the strength of a study. As the number of observations related to the research increases, the reliability of the data also increase (3). In large sample sizes, meaningless effects can be found to be statistically significant, whereas in small sample sizes these differences may not be detected. For these reasons, it is recommended to keep the sample size at the optimum level (4,5). Increasing the sample size reduces the standard error, resulting in more concentrated distributions around the mean (6).

Cardiovascular diseases account for a third of deaths worldwide (7). Among these diseases, the prevalence of ischemic heart disease (IHD) is the highest (9). Many studies have been conducted to compare the biochemical findings of IHD patients with normal subjects. As a result of these studies, various results have emerged. Leukocytes (WBC), which play a role in atherosclerosis pathogenesis, have been found as a prognostic factor for coronary artery disease (CAD) (9). It has been suggested that an increased number of leukocytes increases the risk of death due to IHD by 65% (10). Hyperlipidemia is a strong and modifiable risk factor for cardiovascular diseases. Patients with IHD have been shown to have higher levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and lower levels of high-density lipoprotein cholesterol (HDL-C) compared to healthy subjects (11). Lower levels of platelet (PLT) number and mean platelet volume (MPV) have been found in patients with acute coronary syndromes (12). However, the number of PLT did not differ between patients with chronic coronary syndromes and healthy subjects (13). Both high and low levels of hemoglobin (Hgb) concentrations have been associated with cardiovascular diseases and low levels of Hgb concentrations have prognostic value in patients with IHD (14).

In the present study, we aimed to compare the effects of frequentist and Bayesian approaches on sample size and to find whether any differences exist between two methods.

MATERIAL and METHOD

The present study compared the biochemical variables of IHD patients with healthy subjects. For comparison two sample sizes were constituted. The small sample size was composed of 32 consecutive patients with IHD and 37 consecutive healthy subjects who applied to our cardiology outpatient clinic between December 2021 and January 2022. The large sample size was composed of 355 consecutive patients with IHD and 545 consecutive healthy subjects who applied to our cardiology outpatient clinic between January 2021 and September 2022. Patients with chronic renal failure, hepatic diseases, thyroid function abnormalities or malignancy were excluded from the study. Ethical approval of the study was obtained from Bakırköy Dr. Sadi Konuk Training and Education Hospital Ethical Committee and the study was conducted in accordance with the Helsinki declaration (approval date: 04/10/2021, approval number: 2021-19). All patients gave written informed consent before study enrollment. Blood samples of the participants were drawn from the antecubital vein after 12-hour fasting. Patients' TC, HDL-C, LDL-C, triglyceride (TG), glucose, urea, creatinine and hemogram values were determined. Small and large sample sizes were compared both with frequentist and Bayesian approaches.

H_0 (null) and H_1 (alternative) hypotheses about the θ parameter were established and priori and posterior probabilities related to this parameter were calculated. The final decision was made by dividing the posterior distribution of the alternative hypothesis to posterior distribution of the null hypothesis.

if $\frac{P\,(H1\backslash Data)}{P\,(H0\backslash Data)} > 1$, then the H_1 hypothesis was selected,

if $\frac{P\,(H1\backslash Data)}{P\,(H0\backslash Data)} < 1$, then the H_0 hypothesis was selected.

Posterior distribution of the null hypothesis was calculated as follows;

$$P\,(H_0\backslash Data) = \frac{P\,(Data\backslash Ho)P(Ho)}{P\,(Data\backslash Ho)P(Ho)+P(Data\backslash H1)P\,(H1)}$$

Posterior distribution of the alternative hypothesis was calculated as follows;

$$P\ (H_1\backslash Data) = \frac{P\ (Data\backslash H1)P(H1)}{P\ (Data\backslash Ho)P(Ho)+P(Data\backslash H1)P\ (H1)}$$

The result of the division of two posterior distributions was: $\frac{P\ (Data\backslash H1)}{P\ (Data\backslash H0)}$. This ratio was called the Bayes factor. If the Bayes factor was between 1 and 3 or 3 and 10, then there was anecdotal or moderate evidence for the alternative hypothesis, respectively. If the Bayes factor was in between 10 and 30 or 30 and 100, then there was strong and very strong evidence for the alternative hypothesis, respectively. If the Bayes factor was greater than 100, then there was extreme evidence for the alternative hypothesis. Alternatively, if Bayes factor was in between 1/3 and 1/10, 1/10 and 1/30, 1/30 and 1/100, or less than 1/100, then there was anecdotal, moderate, strong and extreme evidence for thr null hypothesis respectively (15).

Statistical analysis

Normality testing of the data was made by the Kolmogorow-Smirnow test. Parametric and non-parametric data were expressed as mean±SD and median and interquartile range (25-75), respectively. A comparison of the two groups was made by the student's t test or Mann-Whitney U test. A p value of less than 0.05 was considered as significant. In order to make Bayesian comparisons, informative prior distributions were used. All of the statistical analyses were done by using IBM SPSS version 26 software (the Statistical Package for the Social Sciences).

RESULTS

Small sample size

Except for serum glucose levels, there were no differences between biochemical variables of the two groups when analyzed by frequentist methods. Serum glucose levels were found to be significantly higher in patients with IHD than that of controls (117.50 (96.50-160.00) mg/dl vs 102.20 (93.40-115.00) mg/dl, p=0.031, respectively). Table 1 shows the comparison of the two groups with frequentist approach.

Informative Bayesian t-test results showed no differences between the biochemical variables of IHD patients and controls. Values of Bayes factors were found to be between zero and one, indicating no significant difference. Since the results were not statistically significant, all of the 95% confidence intervals covered zero. Table 2 and Table 3 show informative Bayesian t test results and posterior distribution statistics, respectively.

Large sample size

According to the results of the frequentist methods, all biochemical variables except for red cell distribution width (RDW), PLT (platelet), and mean thrombocyte volume (MPV) were significantly different between two groups. Table 4 shows comparison results of the two groups according to the frequentist approach.

Informative Bayesian t-test results showed that levels of glucose, TG, TC, HDL-C, LDL-C, urea, creatinine, hematocrit (HCT), WBC, neutrophil (NEUT) and lymphocyte (LYMP) were

Table 1: Comparison of patients with IHD and controls by the frequentist approach (small sample size).

Parameter	Control	IHD	Z/t score	p
Glucose (mg/dL)	102.20 (93.40-115.00)	117.50 (96.50-160.00)	-2.154	**0.031**
Triglyceride (mg/dL)	169.75 (117.96-229.00)	141.00 (112.00- 303.00)	-1.294	0.196
TC (mg/dL)	190.091±33.672	189.648±58.088	0.039	0.969
HDL-C (mg/dL)	43.00 (41.80-47.80)	41.00 (36.32-51.50)	-1.071	0.284
LDL-C (mg/dL)	99.10 (88.30-132.68)	111.00 (62.52-152.60)	-0.102	0.919
Urea (mg/dL)	30.90 (26.05-34.275)	29.00 (26.25-36.00)	-0.614	0.539
Creatinine (mg/dL)	0.87 (0.825-0.940)	0.875 (0.765-0.98)	-0.175	0.861
Hemoglobin (g/dL)	15.45 (14.60-16.10)	14.95 (13.85-15.425)	-1.680	0.093
Hematocrit (%)	45.633±2.696	43.869±4.972	1.792	0.080
RDW (%)	13.125 (12.90-13.50)	13.10 (12.606-13.50)	-0.458	0.647
WBC (103/µL)	7.985±1.701	8.517±2.276	-1.108	0.272
Platelet (10e3/uL)	196.00 (20.895-287.00)	244.00 (166.27-289.25)	-0.963	0.336
MPV (fL)	10.081(9.90-10.30)	10.05 (9.225-10.425)	-0.441	0.659
NEUT (103/µL)	4.885 (4.205-4.885)	5.415 (3.89-6.21)	-1.280	0.200
LYMP (103/µL)	3.180 (1.180-4.02)	2.82 (0.77-4.67)	-1.280	0.200

TC: Total cholesterol, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol, RDW: Red cell distribution width, WBC: White blood cell, MPV: Mean platelet volume, NEUT: Neutrophil, LYMP: Lymphocyte.

Table 2: Results of informative Bayesian t-tests (small sample size).

Parameter	MD	PSD	Bayes factor	t score	df	p
Glucose (mg/dL)	22.834	13.184	0.882	1.732	67	0.088
Triglyceride (mg/dL)	-13.666	29.743	0.271	-0.459	67	0.647
TC (mg/dL)	-0.442	11.247	0.248	-0.039	67	0.969
HDL-C (mg/dL)	-0.465	2.475	0.252	-0.188	67	0.852
LDL-C (mg/dL)	3.418	10.186	0.260	0.336	67	0.738
Urea (mg/dL)	4.055	2.958	0.551	1.371	67	0.175
Creatinine (mg/dL)	0.032	0.142	0.254	0.227	67	0.821
Hemoglobin (g/dL)	-0.447	0.405	0.417	-1.104	67	0.273
Hematocrit (%)	-1.764	0.946	1.078	-1.866	67	0.066
RDW (%)	-0.268	0.388	0.304	-0.690	67	0.493
WBC (103/µL)	0.532	0.480	0.418	1.108	67	0.272
Platelet (10e3/uL)	32.793	26.998	0.465	1.215	67	0.229
MPV (fL)	-0.236	0.198	0.454	-1.192	67	0.237
NEUT (103/µL)	0.349	0.343	0.386	1.018	67	0.313
LYMP (103/µL)	-2.256	0.235	0.411	-1.089	67	0.280

MD: Mean difference, PSD: Pooled standard error difference, df: Degree of freedom, TC: Total cholesterol, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol, RDW: Red cell distribution width, WBC: White blood cell, MPV: Mean platelet volume, NEUT: Neutrophil, LYMP: Lymphocyte.

Table 3: Posterior distribution statistics (small sample size).

Parameter	Posterior			%95 CI	
	Mode	Median	Variance	Lower limit	Upper limit
Glucose (mg/dL)	23.124	23.124	173.918	-2.723	48.972
Triglyceride (mg/dL)	-12.226	-12.226	844.040	-69.167	44.716
TC (mg/dL)	-1.427	-1.427	129.644	-23.743	20.890
HDL-C (mg/dL)	-0.836	-0.836	6.130	-5.689	4.017
LDL-C (mg/dL)	2.609	2.609	105.487	-17.521	22.739
Urea (mg/dL)	3.992	3.992	9.129	-1.930	9.914
Creatinine (mg/dL)	0.095	0.095	0.016	-0.152	0.341
Hemoglobin (g/dL)	-0.447	-0.447	0.162	-1.235	0.342
Hematocrit (%)	-1.819	-1.819	0.941	-3.721	0.082
RDW (%)	-0.224	-0.224	0.150	-0.983	0.535
WBC (103/µL)	0.541	0.541	0.236	-0.411	1.494
Platelet (10e3/uL)	31.130	31.130	650.090	-18.843	81.102
MPV (fL)	-0.240	-0.240	0.038	-0.623	0.143
NEUT (103/µL)	0.367	0.367	0.121	-0.314	1.047
LYMP (103/µL)	-0.256	-0.256	1.347	-2.530	2.018

CI: Confidence interval, TC: Total cholesterol, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol, RDW: Red cell distribution width, WBC: White blood cell, MPV: Mean platelet volume, NEUT: Neutrophil, LYMP: Lymphocyte.

significantly different between the two groups. The Bayes factors were found to be extremely high which supported the alternative hypothesis. Similar to the frequentist approach, Bayesian methods also did not find any differences in RDW, PLT and MPV values between the two groups of subjects. Bayesian factors of these variables were found to be between

Table 4: Comparison of patients with IHD and controls by the frequentist approach (large sample size)

Parameter	Control	IHD	t score	p
Glucose (mg/dL)	100.75 (92.00-117.00)	111.75 (95.00-146.00)	-5.788	**<0.001**
Triglyceride (mg/dL)	155.25 (115.00-219.50)	185.00 (126.00-276.50)	-3.028	**0.003**
TC (mg/dL)	192.00 (172.50-220.125)	178.00 (146.50-207.50)	5.092	**<0.001**
HDL-C (mg/dL)	42.00 (37.00-47.50)	38.00 (34.00-44.00)	5.614	**<0.001**
LDL-C (mg/dL)	118.00 (99.00-141.50)	98.00 (72.81-122.75)	7.811	**<0.001**
Urea (mg/dL)	29.00 (25.00-34.00)	30.00 (25.00-35.00)	-2.385	**0.017**
Creatinine (mg/dL)	0.84 (0.76-0.94)	0.85 (0.76-0.95)	-2.387	**0.017**
Hemoglobin (g/dL)	15.20 (14.40-15.90)	14.80 (13.90-15.80)	2.927	**0.004**
Hematocrit (%)	44.70 (42.25-47.00)	43.95 (41.00-46.47)	2.998	**0.003**
RDW (%)	13.10 (12.60-13.50)	13.00 (12.60-13.70)	-0.389	0.698
WBC (103/µL)	7.92 (6.69-9.40)	8.75 (7.37-10.39)	-5.634	**<0.001**
Platelet (10e3/uL)	248.00 (212.50-290.00)	245.50 (209.25-290.50)	-1.181	0.238
MPV (fL)	10.10 (9.40-10.70)	10.10 (9.50-10.80)	-1.065	0.287
NEUT (103/µL)	4.74 (3.69-5.75)	5.20 (4.17-6.36)	-5.005	**<0.001**
LYMP (103/µL)	2.50 (1.96-3.03)	2.50 (2.09-3.04)	-2.186	**0.029**

TC: Total cholesterol, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol, RDW: Red cell distribution width, WBC: White blood cell, MPV: Mean platelet volume, NEUT: Neutrophil, LYMP: Lymphocyte.

zero and one. Table 5 and Table 6 show Bayesian t-test results and posterior distribution statistics of the two groups.

DISCUSSION

In many experimental studies, significance of scientific results should be supported by a p value which belongs to the frequentist paradigm. The frequentist approach consists of a combination of two approaches: the null hypothesis that was put forward by Fisher's inductive approach and Neyman and Pearson's deductive alternative hypothesis, and the concept of power (16, 17). According to the Fisher approach, the p-value is evaluated as the strength of the evidence

Table 5: Results of informative Bayesian t-tests (large sample size).

Parameter	MD	PSD	Bayes factor	t score	df	p
Glucose (mg/dL)	22.657	3.559	2.311e+7	6.366	898	**<0.001**
Triglyceride (mg/dL)	31.815	10.603	6.256	3.001	898	**0.003**
TC (mg/dL)	-19.091	3.750	22351.910	-5.092	898	**<0.001**
HDL-C (mg/dL)	-3.417	0.609	321857.523	-5.614	898	**<0.001**
LDL-C (mg/dL)	-21.231	2.634	2.105e+12	-8.062	898	**<0.001**
Urea (mg/dL)	1.769	0.697	1.801	2.540	898	**0.011**
Creatinine (mg/dL)	0.121	0.042	4.355	2.874	898	**0.004**
Hemoglobin (g/dL)	-0.315	0.104	6.540	-3.016	898	**0.003**
Hematocrit (%)	-0.853	0.271	9.689	-3.147	898	**0.002**
RDW (%)	0.040	0.103	0.082	0.389	898	0.698
WBC (103/µL)	0.915	1.156	1.228e+6	5.859	898	**<0.001**
Platelet (10e3/uL)	6.029	5.106	0.151	1.181	898	0.238
MPV (fL)	0.097	0.091	0.133	1.065	898	0.287
NEUT (103/µL)	0.639	0.121	54301.389	5.271	898	**<0.001**
LYMP (103/µL)	0.119	0.055	0.795	2.186	898	**0.029**

MD: Mean difference, PSD: Pooled standard error difference, df: Degree of freedom, TC: Total cholesterol, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol, RDW: Red cell distribution width, WBC:White blood cell, MPV, Mean platelet volume, NEUT: Neutrophil; LYMP: Lymphocyte.

against the null hypothesis. The closer the p-value gets to 0, the lower the probability that the null hypothesis is true (18). The biggest problem of the Fisher approach is that it does not give a critical p-value to reject the null hypothesis. Although the p-value is a quantitative measure against the null hypothesis, it does not provide an idea of how strong the evidence is (19). Neyman-Pearson solved this problem by putting forward a critical value.

The Bayesian approach criticizes the p-value, as it does not indicate the probability of truth of the null hypothesis and is incorrectly interpreted as the probability of the truth alternative hypothesis. However, there are also some limitations of the Bayes factor (20). It is not clear at what point the Bayesian factor should be accepted as a confirmation of one of the two hypotheses. Moreover, the Bayes-Factor is influenced by the priori distribution of effect size. In general, reaching a Bayes factor of 10 or greater is sufficient for early completion of a study- (15). The Bayesian factor depends on the t-statistic, the degree of freedom and the a priori distribution of the parameter. The Bayes factor and p value are interrelated to each other. Although the p value has the identical meaning for disparate samples, for the same t value, the Bayes factor differs with the changes of sample sizes. In small sample sizes, the Bayes factor allows us to obtain proof for the null hypothesis. In large sample sizes it allows for the detection of even small deviations. As the sample size increases, the statistical power of a study also increases.

In the present study we evaluated the effect of sample size on Bayesian and frequentist results. According to our results both Bayesian and frequentist approaches had higher power in order to detect small differences with larger sample sizes. We obtained higher values of Bayes factors in the larger sample size, indicating support for the alternative hypothesis relative to the null hypothesis that were not seen in the small sample size. Similarly, p values reached statistical significance with the higher sample size, which were found to be greater than 0.05 in the small sample size (except for glucose levels, p=0.031). One of the most important steps in the planning of scientific studies is to allocate resources in such a way that they have sufficient power to build statistical outcomes when a difference exists. With high statistical power, the p value is expected to be small and give the same information as the Bayes factor. High powered studies are associated with lower levels of type-II error rate. Therefore, analysis of high-powered studies can be applied to both frequentist and Bayesian statistics. Similar to the frequentist approach, the Bayesian approach can falsely support the null hypothesis in small sample sizes.

Kelter R. investigated the effect of sample sizes on Bayesian results. In that study, it was shown that an increase in sample size reduces the type II error rate to zero in both Bayesian and frequentist approaches (21). Bayesian inference required a higher number of sampling data for the same type II error rate compared to frequentist tests. In order to detect little deviations between the two samples, Bayesian inference needed higher sample sizes for the identical type II error

Table 6: Posterior distribution statistics (large sample size).

Parameter	Posterior			%95 CI	
	Mod	Median	Varyans	Lower limit	Upper limit
Glucose (mg/dL)	22.657	22.657	13.569	15.437	29.877
Triglyceride (mg/dL)	31.815	31.815	29.363	21.195	42.436
TC (mg/dL)	-19.091	-19.091	8.038	-24.647	-13.534
HDL-C (mg/dL)	-3.417	-3.417	0.384	-4.632	-2.202
LDL-C (mg/dL)	-21.231	-21.231	5.339	-25.760	-16.702
Urea (mg/dL)	1.770	1.770	0.625	0.221	3.318
Creatinine (mg/dL)	0.121	0.121	0.000	0.080	0.162
Hemoglobin (g/dL)	-0.315	-0.315	0.011	-0.525	-0.105
Hematocrit (%)	-0.853	-0.853	0.103	-1.482	-0.223
RDW (%)	0.040	0.040	0.012	-0.177	0.260
WBC (103/µL)	0.915	0.915	0.133	0.201	1.630
Platelet (10e3/uL)	6.029	6.029	16.596	-1.956	14.013
MPV (fL)	0.097	0.097	0.009	-0.090	0.283
NEUT (103/µL)	0.640	0.640	0.027	0.319	0.960
LYMP (103/µL)	0.119	0.119	0.003	0.013	0.225

CI: Confidence interval, TC: Total cholesterol, HDL-C: High density lipoprotein cholestero, LDL-C: Low density lipoprotein cholesterol, RDW: Red cell distribution width, WBC:White blood cell, MPV: Mean platelet volume, NEUT: Neutrophil, LYMP: Lymphocyte.

rate. For medium or large effect sizes, the situation was less problematic. It was stated that, for small sample sizes, it was necessary to conduct further research and evaluate the accuracy of Bayesian tests (21). Another simulation study which was also performed by Kelter R. showed that non-parametric Bayesian two-sample tests had lower type I error rate compared to the Mann-Whitney U test (22). In contrast, the strength of the Bayesian two-sample tests was found to be slightly lower than the frequentist methods. The ability of Bayesian tests to control type I and II error rates and detect an existing difference depends on the power of a priori modeling.

CONCLUSION

P value and Bayes factor should be interpreted correctly by the researcher. According to our results, both Bayesian and frequentist approaches depend on the proportion of sample errors, which depends on the sample size. Similar to the frequentist approach, the Bayesian approach had low accuracy for the acceptance of the null hypothesis in small sample sizes.

Ethics Committee Approval: This study was approved by Bakırköy Dr. Sadi Konuk Training and Education Hospital Ethical Committee (Date: 04.10.2021, No: 2021-19).

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REFERENCES

1. Rindskopf D. Overview of Bayesian statistics. Eval Rev 2020;44(4):225-37.
2. Beerenwinkel N, Siesbourg J. Probability, statistics, and computational science methods. Methods Mol Biol 2019;1910:33-70.
3. Sami W, Alrukban MO, Waqas T, Asad MR, Afzal K. Sample size determination in health research. J Ayub Med Coll Abbottabad 2018;30(2):308-11
4. Sacco W. Statistical power considerations in the use of cost-effectiveness analysis. Professional Psychology 1982;13(5):752-8.
5. Mazen AM, Graf LA, Kellog KE, Hemmasi M. Statistical power in contemporary management research. The Academy of Management Journal 1987;30(2):369-80.
6. Kim HY. Statistical notes for clinical researcher: Type I and type II errors in statistical decision. Restor Dent Endod 2015;40(3):249-52.
7. Mozaffarian D, Benjamin E, Go A, Arnett DK, Blaha MJ, Cushman M, et al. Heart disease and stroke statistics-2016 update. A report from the American Heart Association. Circulation 2016;133(4):e38-360.
8. Roth GA, Johnson C, Abajobir A, Abd-Allah F, Abera SF, Abyu G, et al. Global, regional, and national burden of cardiovascular diseases for 10 causes, 1990 to 2015. J Am Coll Cardiol 2017;70(1):1-25.
9. Sweetnam PM, Thomas HF, Yarnell JW, Baker IA, Elwood PC. Total and differential leukocyte counts as predictors of ischemic heart disease: The caerphilly and speedwell Studies. Am J Epidemiol 1997;145(5):416-21.
10. Weijenberg MP, Feskens EJ, Kromhout D. White blood cell count and the risk of coronary heart disease and all-cause mortality in elderly men. Arterioscler Thromb Vasc Biol 1996;16(4):499-503.
11. Gupta R, Gupta VP, Sarna M. Bhatnagar S, Thanvi J, Sharma V, et al. Prevalence of coronary heart disease and coronary risk factors in urban Indian population: Jaipur Heart Watch -2. Indian Heart J 2002;54(1):59-66.
12. Ranjith MP, Divya R, Metha VK, Krishnan MG, KamalRaj R, Kavishwar A. Significance of platelet volume indices and platelet count in ischaemic heart disease. J Clin Pathol 2009;62(9):830- 3.
13. Pizzulli L, Yang A, Martin JF, Lüderitz B. Changes in platelet size and count in unstable angina compared to stable angina or non-cardiac chest pain. Eur Heart J 1998;19(1):80-4.
14. Houghton DE, Koh I, Ellis A, Key NS, Douce DR, Howard G, et al. Hemoglobin levels and coronary heart disease risk by age, race, and sex in the reasons for geographic and racial differences in stroke study (REGARDS). Am J Hematol 2020;95(3):258-6.
15. Stefan AM, Gronau QF, Schönbrodt FD, Wagenmakers EJ. A tutorial on Bayes Factor Design Analysis using an informed prior. Behav Res Methods 2019;51(3):1042-58
16. Biau DJ, Jolles BM, Porcher R. P value and the theory of hypothesis testing: an explanation for new researchers. Clin Orthop Relat Res 2010;468(3):885-92
17. Pernet C. Null hypothesis significance testing: a short tutorial. F1000Res 2015;4:621.
18. Greenland S, Senn SJ, Rothman KJ, Carlin JB, Poole C, Goodman SN, et al. Statistical tests, P values, confidence intervals, and power: a guide to misinterpretations Eur J Epidemiol 2016;31(4):337-50.
19. Domenech RJ. The uncertainties of statistical “significance” Rev Med Chil 2018;146(10):1184-9.
20. Morey RD, Romeijn JW, Rouder JN. The philosophy of Bayes factors and the quantification of statistical evidence. J Math Psychol 2016;72:6-18.
21. Kelter R. Analysis of type I and II error rates of Bayesian and frequentist parametric and nonparametric two-sample hypothesis tests under preliminary assessment of normality. Computational Statistics 2021;36:1263-88.
22. Kelter R. Power analysis and type I and type II error rates of Bayesian nonparametric two-sample tests for location-shifts based on the Bayes factor under Cauchy priors. Computational Statistics Data Analysis 2022;165(21):107326.

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COMPARISON OF THE PERIOPERATIVE ANALGESIC OUTCOMES OF THORACIC PARAVERTEBRAL BLOCK VERSUS ERECTOR SPINAE PLANE BLOCK IN REDUCTION MAMMAPLASTY SURGERIES: A RETROSPECTIVE COHORT STUDY*

REDÜKSİYON MAMOPLASTİ AMELİYATLARINDA TORASİK PARAVERTEBRAL BLOK İLE EREKTOR SPİN PLAN BLOĞUNUN PERİOPERATİF ANALJEZİK SONUÇLARININ KARŞILAŞTIRILMASI: RETROSPEKTİF KOHORT ÇALIŞMA

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ABSTRACT

Objective: Thoracic paravertebral block (TPVB) and Erector spinae plane block (ESPB) are the most preferred regional analgesia techniques for breast surgeries. However, the literature is still lack of reliable evidence to prove superiority of one over another. We hypothesized that TPVB would provide better pain control for the acute postoperative period.
Material and Methods: In this retrospective cohort study, macromastia patients who underwent bilateral reduction mammaplasty surgery were grouped according to the regional technique performed prior to general anesthesia induction that are provided with 0.375% bupivacaine bilaterally (TPVB or ESPB). Presurgery (10th min, 20th min, and 30th min) and postoperative number of dermatomal sensory block, and postoperative pain scores were screened which referred to postoperative 0 minute,1st hour, 2nd hour, 4th hour, 6th hour, 12th hour, 24th hour and 48th hour examination. Intraoperative and postoperative analgesic administration, and comfort parameters such as time-to-first pain, nausea-vomiting (PONV) incidence, sleep duration, complications and patient/surgeon satisfaction scores were also investigated.
Results: Total 58 patients were screened. Pain scores were lower in TPVB group for the postoperative first 2 hours (P<0.05). TPVB blocked more dermatomes during postoperative 1st day (P<0.05) whereas postoperative tramadol consumption were similar with both blocks (P>0.05). On the other hand, postoperative 2nd day paracetamol consumption was less with TPVB (P=0.03). Time-to-first pain and sleep duration on the postoperative 1st day was shorter with ESPB (P<0.05).
Conclusions: Thoracic paravertebral block represents better analgesic features than erector spinae plane block for reduction mammaplasty. However, ESPB may still be considered to provide favorable analgesia.

Keywords: Thoracic paravertebral block, erector spinae plane block, macromastia, reduction mammoplasty, thoracic wall block, acute pain, nerve block, regional anesthesia

ÖZ

Amaç: Torasik paravertebral blok (TPVB) ve Erektor spina plan bloğu (ESPB) meme ameliyatlarında en çok tercih edilen rejyonal analjezi tekniklerindendir. Literatürde bu iki bloğun birbirine üstünlüğünü gösteren güvenilir kanıtlar kısıtlıdır. Hipotezimizi TPVB'nin akut postoperatif dönemde daha iyi analjezi sağlayacağı yönünde kurduk.
Gereç ve Yöntem: Bu retrospektif kohort çalışmada bilateral redüksiyon mamoplasti uygulanan hastalar genel anestezi öncesinde uygulanmış olan rejyonal tekniğe göre (bilateral TPVB veya ESPB, %0,375 bupivakain ile) gruplandırıldı. Ameliyat öncesi (Blok sonrası 10. dakika, 20. dakika ve 30. dakika) ve ameliyat sonrası 0. dakika, 1. saat, 2. saat, 4. saat, 6. saat, 12. saat, 24. saat ve 48. saatte duyu bloğu oluşmuş dermatom sayısı ve postoperatif ağrı skorları tarandı. İntraoperatif ve postoperatif verilen analjezik miktarı, ağrının ilk ortaya çıkışı süresi, bulantı-kusma (POBK) insidansı, uyku süresi, komplikasyonlar ve hasta/cerrah memnuniyet skorları gibi konfor parametreleri araştırıldı.
Bulgular: Toplam 58 hasta tarandı. Postoperatif ilk 2 saat ağrı skorları TPVB grubunda daha düşüktü (p<0,05). TPVB, postoperatif 1. günde daha fazla dermatomu bloke ederken (p<0,05), postoperatif tramadol tüketimi her iki blokta benzerdi (p>0,05). Postoperatif 2. gün parasetamol tüketimi ise TPVB ile daha azdı (p=0,03). Postoperatif 1. gün ilk ağrı süresi ve uyku süresi ESPB ile daha kısaydı (p<0,05).
Sonuç: Redüksiyon mamoplasti için torasik paravertebral blok, erektor spina plan bloğundan daha etkili analjezik özellikler göstermektedir. Bununla birlikte, ESPB yeterli analjezi sağlanmasında faydalıdır.

Anahtar kelimeler: Torasik paravertebral blok, erektor spina plan bloğu, makromasti, redüksiyon mamoplasti, torasik duvar bloğu, akut ağrı, sinir bloğu, rejyonal anestezi

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INTRODUCTION

Breast surgeries vary depending on the underlying pathology among which malignancy surgeries are more common. According to grade of the tumor and the lymphatic involvement, surgical indications may include a wide spectrum covering from simple excision to radical mastectomy with lymphatic dissection. This particular situation may affect proper examination of clinical pain and construct a homogenous patient group for regional anesthesia studies. On the other hand, bilateral reduction mammaplasty surgery is known to require large amounts for breast tissue extraction which causes severe acute pain postoperatively. Surgical techniques may differ according to the pedicle types or nipple grafting; but in the end, the patients face severe clinical pain related to the extracted tissue that is generally around two kilograms in total. Therefore, preemptive thoracic wall blocks are widely used to control postoperative pain and provide perioperative comfort as a part of multimodal analgesia.

Thoracic Paravertebral Block (TPVB) and Erector Spinae Plane Block (ESPB) are well-described regional techniques, mainly chosen for chronic pain predisposing operations such as thoracic and breast surgeries (1,2). TPVB is an advanced method which necessitates deep tissue puncture that targets the paravertebral space close to the pleura. On the other hand, ESPB is another method considered to be safer since the needle is proceeded in between erector spinae muscle and a bony structure (Transverse process) (3). The literature represents more clinical experience with TPVB; however, ESPB is equally popular with a claim to be safer (4). Yet, there is an ongoing debate regarding the superiority of one over another in terms of analgesic efficacy.

Both techniques are generally used as a part of multimodal analgesia under ultrasound (US) guidance. However, the question upon the efficacy and reliability of ESPB in comparison to TPVB still remains. Current study is designed to evaluate these regional techniques from the analgesic efficacy, analgesic consumption and perioperative comfort aspects. Our primary outcome was the numeric rating scales for pain (NRS) for the acute postoperative period.

Despite the well-investigated nature of the comparison of these two techniques, existing clinical trials include different types of breast surgeries. Here, in this study, we aimed to demonstrate our results in one single surgical indication which was reduction mammaplasty in macromastia patients. To our knowledge, the literature does not have any clinical trial for this specific issue which compares these blocks for the mentioned patient group.

MATERIALS and METHODS

Data colection and regulatory aspects

This retrospective cohort investigation was approved by the local ethics committee (Istanbul Faculty of Medicine Clinical Research Ethics Committee: Date: 16.10.2020, No:25). Informed consent was obtained from all the patients prior to surgery for scientific data presentation anonymously in the future. Data of total 61 macromastia patients who underwent bilateral reduction mammaplasty surgery between April 2017 and June 2019 in our tertiary university hospital were reviewed, but three patients were excluded due to the missing data. Among the 58 patients, 28 patients had received single injection US guided Erector Spinae Plane block while 30 had received single injection US guided Thoracic Paravertebral Block prior to macromastia surgery. The data screening was accomplished from the departmental written sources with regard to the intraoperative (operating room) and postoperative period (recovery room and ward). Our institutional standard anesthesiologic approach for bilateral reduction mammaplasty is described below.

Perioperative care and outcome measures

After the patients arrived to the operating room, standard monitoring (electrocardiography, pulse oximetry and non-invasive blood pressure monitoring) was applied to them, and mild sedation with 2 mg midazolam IV and 50 mcg fentanyl IV was provided. The patients were kept in the sitting position to perform thoracic wall blocks. After the skin disinfection with 10% povidone-iodine, a linear ultrasonography (USG) probe (Logiq, GE, USA, 4-12 hz) was placed on the level of T3 and shifted laterally to visualize T3 and T4 transverse processes and the pleura in between. An insulated peripheral block needle (50 mm, BBraun, Sonoplex, Melsungen, Germany) was advanced through the tissues in-plane towards the paravertebral space. Once the needle tip was inserted into the targeted area, the downward shifting of pleura was checked with 1 ml of saline injection, and 20 cc 0.375% bupivacaine was injected to provide TPVB, afterwards (Group TPVB). Similarly, ESPB was applied on the T3 level for the other group. Under linear USG probe visualization, the transverse process was identified to be approximately 2-3 cm lateral to the spinous process, and the needle was inserted out-of-plane until contacting the bone. Again, 1 ml saline was injected to the area to observe expansion of interfascial area between the erector spinae muscle and the transverse process. Followingly, 20 cc 0.375% bupivacaine was administered to perform ESPB (Group ESPB). Both procedures were performed bilaterally for each patient since the surgeries were both sided.

After performing the blocks, dermatomal distribution of the sensorial block was checked via pin-prick test on related dermatomes at 10^{th}, 20^{th} and 30^{th} minutes, and "number" of the dermatomes with a complete loss of sensation on the midclavicular line was recorded. After the sensory examination, general anesthesia was induced in the supine position with additional 2 mcg/kg fentanyl IV, 3 mg/kg propofol IV and 0.5 mg/kg rocuronium IV, and the hypnosis was maintained via sevoflurane inhalation. If an increase more than 20% in the heart rate or systolic blood pressure was observed, additional 50 mcg fentanyl was administered and recorded as "intraoperative additional fentanyl requirement". After the extubation, patients were transported to post-anesthesia care unit (PACU) where the dermatomal sensory examination was continued. Discharge to the ward was granted once the Aldrete score was nine or

more. The entrance to PACU was accepted as minute zero, and ongoing examinations were made at 1st,2nd,4th,6th,12th,24th, and 48th hours. All patients were asked to rate pain intensity on a standard numeric rating scale (NRS) ranging from 0 (no pain) to 10 (the worst imaginable pain) at the defined time points and this was recorded as our primary outcome.

Table 1: Patient demographics, surgical characteristics, block procedural time, durations of surgery and general anesthesia

	ESPB (n=28, 48.3%)	TPVB (n=30, 51.7%)	*P*
Age (year) (Median (min-max))	46 (24-61)	44 (25-60)	0.8[a]
BMI (kg/m²) (Mean±Std)	31±4.1	31.43±3.9	0.7[b]
ASA physical status (n, %)			
1	3, 10.7%	5, 16.7%	
2	22, 78.6%	23, 76.7%	0.7[c]
3	3, 10.7%	2, 6.7%	
Duration of block performance (min) (Median (min-max))	7 (4-12)	7 (4-15)	0.1[a]
Duration of anesthesia (min) (Mean±Std)	154.6±30.5	157.2±35.2	0.8[b]
Duration of surgery (min) (Median (min-max))	135 (90-200)	137.5 (85-220)	0.8[a]
Intraoperative fentanyl requirement (mcg) (Median (min-max))	0 (0-50)	0 (0-100)	0.1[a]
Breast reduction incision types (n, %)			
Wise pattern	25, 89.3%	26, 86.7%	1.0[d]
Circumvertical with short horizontal scar	3, 10.7%	4, 13.3%	
Breast reduction pedicle types (n, %)			
Superomedial pedicle	17, 60.7%	17, 56.7%	
Inferior pedicle	6, 21.4%	6, 20%	0.8[d]
Superior pedicle	4, 14.3%	4, 13.3%	
Free nipple grafts	1, 3.6%	3, 10%	
Weight of breast reduction (g) (Median (min-max))	732 (600-1020)	732 (580-1076)	0.9[a]

ESPB: Erector spinae plane block, TPVB: Thoracic paravertebral block, BMI: Body Mass Index, ASA: American Society of Anesthesiologists, a: Mann Whitney-U test, b: Student-t test, c: Pearson Chi-Square test, d: Fischer's Exact test

Rescue analgesic was administered once the NRS was more than 4 and recorded as "time-to-first pain". Accordingly, paracetamol 1 g IV was administered, and if the pain persisted in the following 1st hour, an additional tramadol 50 mg IV was also added. Intraoperative opioid requirement (fentanyl IV mcg), length of stay in PACU, amount of postoperative paracetamol (g) and tramadol (mg) requirement, procedural complications (hematoma, pneumothorax, local anesthetic toxicity etc), PONV incidence (%), sleep duration (hours), and patient/surgeon satisfaction were also recorded, which are presented here as secondary outcomes. Satisfaction scores were determined as follows: 0: Not satisfied, 1:Neutral, 2: Slightly satisfied, 3: Completely satisfied.

Statistical analysis

Based on an assumption of 25% difference in the postoperative 1st hour mean NRS scores, 27 patients were required per group for statistical evaluation (G*Power 3.1, Düsseldorf, Germany). Statistical analysis was completed via Statistical Package for the Social Sciences for Mac version 25 (IBM, New York, USA). Categorical data were evaluated with chi-square test. Intergroup analyses were made via student's t-test if the data were distributed homogenously, and Mann Whitney-U was chosen for the heterogenous data. Normally distributed data were expressed as mean±standard deviation, and heterogeneously distributed data were expressed as median (min-max).

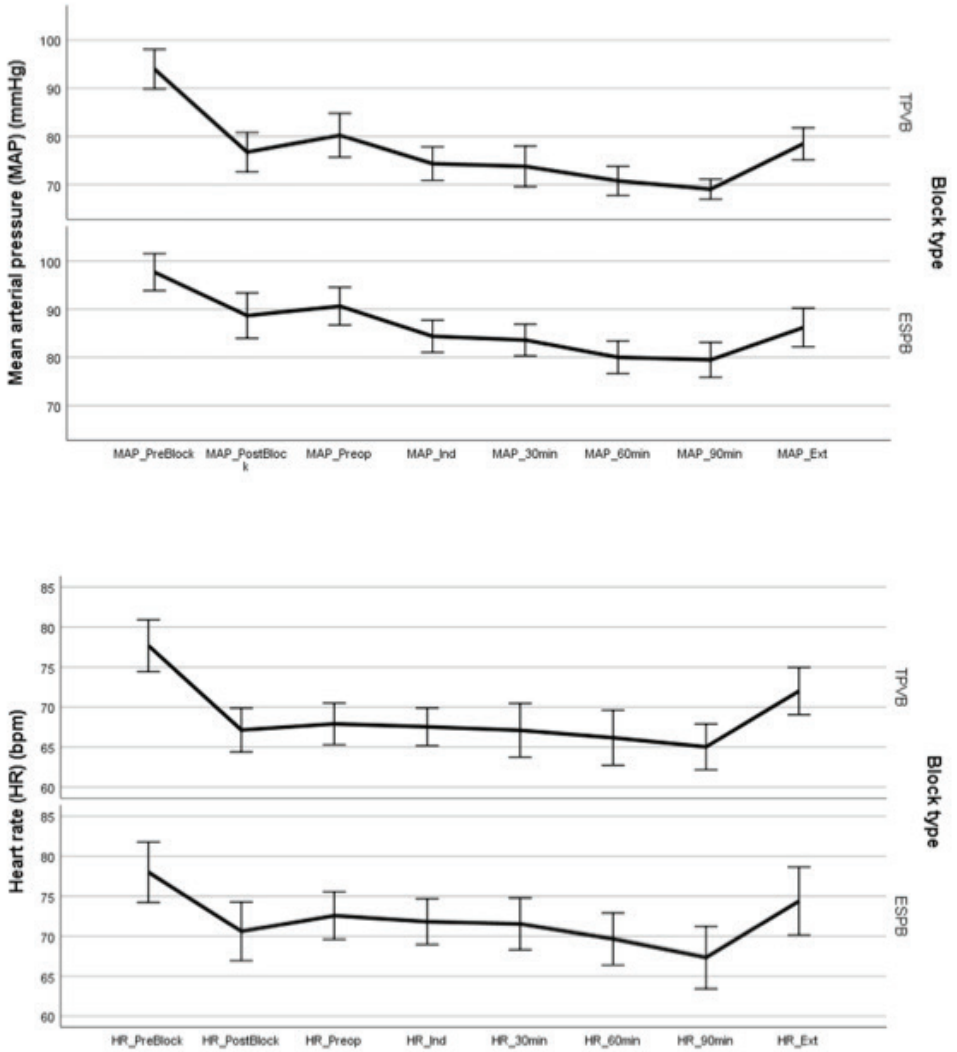
RESULTS

Age, ASA physical status, body mass index (BMI), surgery types, resected breast tissue mass, and duration of block performance, anesthesia, and surgery did not differ between the groups (*P*>0.05). Amount of administered intraoperative fentanyl IV was also similar (*P*>0.05). The data were summarized in Table 1. Also, intraoperative mean arterial pressure (MAP) and heart rate (HR) data were represented in Figure 1.

Presurgery pin-prick evaluation exhibited more dermatomal block distribution on the right and left midclavicular line in Group TPVB at 10th, 20th, and 30th minutes (Right: *P*=0.02, *P*=0.02, *P*=0.04; left: *P*=0.01, *P*=0.02, *P*=0.03) (Figure 2).

Postoperative NRS values were lower in Group TPVB than in Group ESPB for the 0th min, 1th and 2nd hours (Right: *P*= 0.01, *P*= 0.004, *P*= 0.02; Left: *P*= 0.02, *P*=0.005, *P*= 0.03) (Figure 3). However, postoperative pin-prick examination demonstrated more distinct differences. During the postoperative first 24 hours, TPVB group patients described more dermatomal coverage (*P*<0.05) (Figure 2).

Length of PACU stay, incidence of PONV, and postoperative tramadol consumption were not different between the groups (*P*>0.05). Time-to-first pain was shorter in Group ESPB (411.8±270.5 min vs 605±324.6 min, *P*<0.05). First day paracetamol consumption was also similar between the groups, but Group ESPB consumed more paracetamol on the postoperative 2nd day (1 (0-2) g vs 0 (0-2) g, *P*=0.03). Postoperative first day sleep duration was significantly more in group TPVB (6 (2-7) vs 6.25 (5-8), *P*=0.01). Both patients and surgeons had more satisfaction with the TPVB (*P*=0.04 for patients, and *P*=0.04 for surgeons) overall. The data are summarized in table 2.



MAP: Mean arterial pressure, TPVB: Thoracic paravertebral block, ESPB: Erector spinae plane block

Figure 1: Intraoperative hemodynamic variables.

According to our data screening; none of the possible complications (hematoma, pneumothorax or local anesthetic toxicity) were observed in any patients.

DISCUSSION

Our results revealed a better performance with thoracic paravertebral block in many aspects including perioperative pain control, total number of dermatomal coverage and postoperative comfort parameters. Among these, numerical rating scores as our primary outcome demonstrated a clinically better pain control for the acute postoperative period up to 2 hours with TPVB. However, this analgesic effect being balanced with ESPB after the 6th hour can be interpreted as "comparable" which should be further debated because "maximum" pain scores were higher on every time point with ESPB, which may indicate an inferiority with a larger sample size. Actually, this possibility was demonstrated by Swisher et al. with a relatively high pain scores and increased morphine consumption with ESPB in comparison to TPVB (5). Their results are based on intra-day pain spectrum of NRS which is different from our design that assess NRS values hourly. While examining the data "daily" may be a very good indicator of providing long-term analgesia, it doesn't make it possible to identify the certain time point when TPVB separates from ESPB. However, Gürkan et al. have not observed a different NRS between TPVB and ESPB at multiple time points. Of note, the primary outcome was postoperative morphine consumption in this specific trial (6).

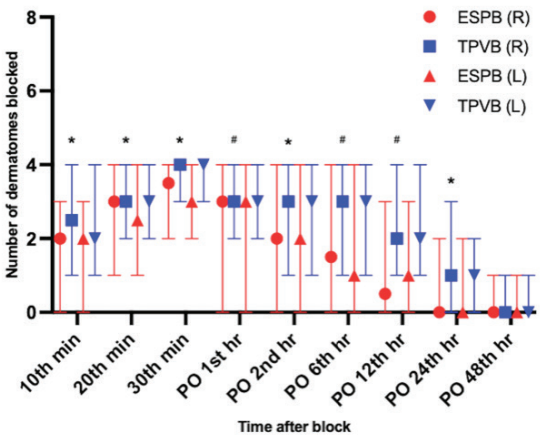


Figure 2: Number of dermatomes blocked at different time points after related thoracic wall block execution. Data are presented as median (min-max).
TPVB: Thoracic Paravertebral Block, ESPB: Erector Spinae Plane Block, R: Right, L: Left, PO: Postoperative, *: P<0.05 (valid for both right and left side), #: p<0.001 (valid for both right and left side)

There is an ongoing debate regarding the efficacy of ESPB which is under examination against TPVB for the recent years (7-10). ESPB gained excessive popularity due to its simplicity and "unexpected" effectiveness which is eventually questioned more and more by anesthetists. Despite the increasing number of randomized clinical trials for different types of surgeries, existing evidence is still low and arguable in the literature. For that, main reasons may be the limited participant numbers and changing study designs (single, bi-level or multi-level block techniques…), yet existing analyses show better features on behalf of TPVB (1, 11-13). One should note that ESPB is still more beneficial than "IV opioid only" analgesia regimen as the meta-analyses represent (14-16). Therefore, we believe ESPB should still be considered as a part of multimodal analgesia.

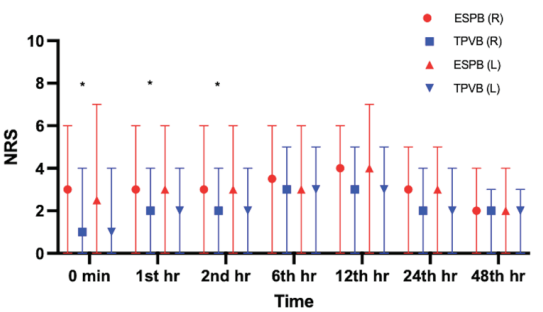


Figure 3: Postoperative Pain Numeric Rating Scale values at different time points. Data are presented as median (min-max).
NRS: Numeric Rating Scale, ESPB: Erector Spinae Plane Block, TPVB: Thoracic Paravertebral Block, R: Right, L: Left, *: P<0.05 (valid for both right and left side)

Table 2: Length of stay in postoperative care unit, postoperative time until first pain, postoperative analgesic consumption/the numbers of paracetamol and tramadol requirements, incidence of postoperative nausea and vomiting and duration of sleep on postoperative days 1 and 2, patient and surgeon satisfaction scores.

	ESPB (n=28, 48.3%)	TPVB (n=30, 51.7%)	*P*
Length of stay in postoperative care unit (min) (Mean±Std)	19.2±7.7	16.8±7.1	0.2[a]
Time to first pain (min) (Mean±Std)	411.8±270.5	605±324.6	**0.02**[a]
Incidence of PONV (n, %)			
Postoperative day1	5, 17.9%	6, 20%	0,8b
Postoperative day2	-	-	
Paracetamol consumption (g) (Median (min-max))			
Postoperative day1	2 (0-3)	1 (0-3)	0.1[c]
Postoperative day2	1 (0-2)	0 (0-2)	**0.03**[c]
Tramadol consumption (mg)			
Postoperative day1	50 (0-150)	50 (0-150)	0.2[c]
Postoperative day2	0 (0-100)	0 (0-50)	0.3[c]
Postoperative sleep duration (hour) (Median (min-max))			
Postoperative day1	6 (2-7)	6.25 (5-8)	**0.01**[c]
Postoperative day2	7 (4-8)	7 (6-8)	0.8[c]
Patient satisfaction (0-3) (Median (min-max))	3 (0-3)	3 (2-3)	**0.04**[c]
Surgeon satisfaction (0-3) (Median (min-max))	3 (2-3)	3 (2-3)	**0.04**[c]

ESPB: erector spinae plane block, TPVB: thoracic paravertebral block, PONV: Postoperative nause and vomiting, a: Student-t test, b: Pearson Chi-Square,, c: Mann Whitney-U test.

Another aspect of this dilemma is the anatomical implications and the spread of the local anesthetics (LA). Current trials are incapable of explaining the mechanism of ESPB. Cadaver studies exhibit epidural LA spread in at least 40% of the subjects with TPVB (17,18). On the other hand, ESPB is known not to cause epidural stain (18,19). This specific feature may arguably be the explanation of more and long-lasting dermatomal blockade coverage starting from the presurgical period until postoperative 24 hours with TPVB which is underlined in our results earlier. Yet, we do not have solid evidence.

Preemptive regional analgesia techniques are meant to provide a comfortable perioperative period for the patients. According

to our design, the "comfort" parameters indicate several entities such as time-to-first pain (NRS>4), length of stay in PACU, PONV incidence, analgesic consumption, and sleep duration. Our current results support TPVB as it delays "time-to-first pain". Although this specific parameter may be perceived subjectively by the patient, postoperative 1st day rescue analgesic consumption did not change among the groups. This result is similar with Zhao et al.'s and El Ghamry et al.'s studies in which one of them was even based on thoracic surgery (20,21). Generally speaking, both ESPB and TPVB represented similar analgesic features, but TPVB takes it slightly further with increased postoperative 1st day sleep duration and reduced postoperative 2nd day paracetamol consumption. Perhaps, these small differences should be interpreted in the light of patient/surgeon satisfaction, which was better on behalf of TPVB, meaning that small matters may lead to greater comfort. Evaluating pain density with such subjective classifications like NRS can cause confusion occasionally. Neither intraoperative/postoperative opioid administration nor NRS scores exhibited suggestive discrepancies between the two techniques. However, TPVB group patients declared more satisfaction with their perioperative process which is compatible with outcomes in several studies in the literature (13, 22, 23).

Clearly, ESPB is preferred due to its easy-to-perform and procedural properties (24). We believe appropriate techniques should be chosen based on the operating anesthetists' experience. Physicians should consider particular anatomic difficulties that harden paravertebral space US visualization such as obesity (25). In case of presence of a greater possibility of complication, TPVB may be avoided and can be replaced with ESPB which is obviously more beneficial than sole IV analgesics.

The retrospective nature of this study stands as a limitation which we aimed to overcome with our detailed data recording practice. Still, it would be an upside if the chronic pain was examined for long-term results which is lacking also. However, our study group has a rather specific and target-driven surgical indication (reduction mammaplasty only) that may benefit from the correct thoracic wall block choice. Considering most of the studies in the literature covering mastectomies with axillary incisions and lymph node extractions, our study group represents a well reflection for pain evaluation after thoracic wall blocks. Yet, well-designed randomized clinical trials comparing TPVB versus ESPB are quite sparse in the literature, and reliable data are still needed from this aspect. We believe our results provide an effective insight into this subject.

Thoracic paravertebral block provides better analgesia and postoperative comfort than erector spinae plane block for the acute postoperative period in reduction mammaplasty surgeries. However, ESPB still provides efficient analgesia, and since the procedural difficulty of paravertebral block represents a solid handicap, ESPB may be chosen to avoid possible complications of paravertebral block.

Ethics Committee Approval: This study was approved by Istanbul Faculty of Medicine Clinical Research Ethics Committee (Date: 16.10.2020, No: 25).

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REFERENCES

1. Saadawi M, Layera S, Aliste J, Bravo D, Leurcharusmee P, Tran Q. Erector spinae plane block: A narrative review with systematic analysis of the evidence pertaining to clinical indications and alternative truncal blocks. J Clin Anesth 2021;68:110063.
2. Chin KJ. Thoracic wall blocks: From paravertebral to retrolaminar to serratus to erector spinae and back again - A review of evidence. Best Pract Res Clin Anaesthesiol 2019;33(1):67-77.
3. Helander EM, Webb MP, Kendrick J, Montet T, Kaye AJ, Cornett EM, et al. PECS, serratus plane, erector spinae, and paravertebral blocks: A comprehensive review. Best Pract Res Clin Anaesthesiol 2019;33(4):573-81.
4. Chin KJ, Versyck B, Pawa A. Ultrasound-guided fascial plane blocks of the chest wall: a state-of-the-art review. Anaesthesia 2021;76 Suppl 1:110-26.
5. Swisher MW, Wallace AM, Sztain JF, Said ET, Khatibi B, Abanobi M, et al. Erector spinae plane versus paravertebral nerve blocks for postoperative analgesia after breast surgery: a randomized clinical trial. Reg Anesth Pain Med 2020;45(4):260-6.
6. Gurkan Y, Aksu C, Kus A, Yorukoglu UH. Erector spinae plane block and thoracic paravertebral block for breast surgery compared to IV-morphine: A randomized controlled trial. J Clin Anesth 2020;59:84-8.
7. Hussain N, Brull R, Noble J, Weaver T, Essandoh M, McCartney CJ, et al. Statistically significant but clinically unimportant: a systematic review and meta-analysis of the analgesic benefits of erector spinae plane block following breast cancer surgery. Reg Anesth Pain Med 2021;46(1):3-12.
8. Barrington MJ. Erector spinae plane block: did low p values overstate the evidence against the null hypothesis and distract us from clinically unimportant effects? Reg Anesth Pain Med 2021;46(1):1-2.
9. Hung KC, Liao SW, Sun CK. Comparable analgesic efficacy between erector spinae plane and thoracic paravertebral blocks for breast and thoracic surgeries? J Clin Anesth 2021;71:110200.
10. Chin KJ. Erector spinae plane and paravertebral blocks have similar opioid-sparing effects following breast surgery. Reg Anesth Pain Med 2020;45(11):940.
11. Turhan O, Sivrikoz N, Sungur Z, Duman S, Ozkan B, Senturk

M. Thoracic paravertebral block achieves better pain control than erector spinae plane block and intercostal nerve block in thoracoscopic surgery: a randomized study. J Cardiothorac Vasc Anesth 2021;35(10):2920-7.

12. Jacobs A, Lemoine A, Joshi GP, Van de Velde M, Bonnet F, collaborators PWG. PROSPECT guideline for oncological breast surgery: a systematic review and procedure-specific postoperative pain management recommendations. Anaesthesia 2020;75(5):664-73.
13. Zhao Y, Tao Y, Zheng S, Cai N, Cheng L, Xie H, et al. Effects of erector spinae plane block and retrolaminar block on analgesia for multiple rib fractures: a randomized, double-blinded clinical trial. Braz J Anesthesiol 2022;72(1):115-21.
14. Zhang Y, Liu T, Zhou Y, Yu Y, Chen G. Analgesic efficacy and safety of erector spinae plane block in breast cancer surgery: a systematic review and meta-analysis. BMC Anesthesiol 2021;21(1):59.
15. Huang W, Wang W, Xie W, Chen Z, Liu Y. Erector spinae plane block for postoperative analgesia in breast and thoracic surgery: A systematic review and meta-analysis. J Clin Anesth 2020;66:109900.
16. Leong RW, Tan ESJ, Wong SN, Tan KH, Liu CW. Efficacy of erector spinae plane block for analgesia in breast surgery: a systematic review and meta-analysis. Anaesthesia 2021;76(3):404-13.
17. Cowie B, McGlade D, Ivanusic J, Barrington MJ. Ultrasound-guided thoracic paravertebral blockade: a cadaveric study. Anesth Analg 2010;110(6):1735-9.
18. Nielsen MV, Moriggl B, Hoermann R, Nielsen TD, Bendtsen TF, Borglum J. Are single-injection erector spinae plane block and multiple-injection costotransverse block equivalent to thoracic paravertebral block? Acta Anaesthesiol Scand 2019;63(9):1231-8.
19. Bonvicini D, Boscolo-Berto R, De Cassai A, Negrello M, Macchi V, Tiberio I, et al. Anatomical basis of erector spinae plane block: a dissection and histotopographic pilot study. J Anesth 2021;35(1):102-11.
20. Zhao H, Xin L, Feng Y. The effect of preoperative erector spinae plane vs. paravertebral blocks on patient-controlled oxycodone consumption after video-assisted thoracic surgery: A prospective randomized, blinded, non-inferiority study. J Clin Anesth 2020;62:109737.
21. El Ghamry MR, Amer AF. Role of erector spinae plane block versus paravertebral block in pain control after modified radical mastectomy. A prospective randomised trial. Indian J Anaesth 2019;63(12):1008-14.
22. Boughey JC, Goravanchi F, Parris RN, Kee SS, Kowalski AM, Frenzel JC, et al. Prospective randomized trial of paravertebral block for patients undergoing breast cancer surgery. Am J Surg 2009;198(5):720-5.
23. Fahy AS, Jakub JW, Dy BM, Eldin NS, Harmsen S, Sviggum H, et al. Paravertebral blocks in patients undergoing mastectomy with or without immediate reconstruction provides improved pain control and decreased postoperative nausea and vomiting. Ann Surg Oncol 2014;21(10):3284-9.
24. Moustafa MA, Alabd AS, Ahmed AMM, Deghidy EA. Erector spinae versus paravertebral plane blocks in modified radical mastectomy: Randomised comparative study of the technique success rate among novice anaesthesiologists. Indian J Anaesth 2020;64(1):49-54.
25. Brodsky JB, Mariano ER. Regional anaesthesia in the obese patient: lost landmarks and evolving ultrasound guidance. Best Pract Res Clin Anaesthesiol 2011;25(1):61-72.

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COMPARISON OF THE IMMUNOMODULATORY PROPERTIES OF THE CORD BLOOD AND WHARTON'S JELLY DERIVED MESENCHYMAL STEM CELLS

KORDON KANI VE WHARTON JELİ KAYNAKLI MEZENKİMAL KÖK HÜCRELERİN İMMUNOMODÜLATÖR ÖZELLİKLERİNİN KARŞILAŞTIRILMASI

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ABSTRACT

Objective: Mesenchymal stem cells (MSCs) are promising as a treatment option for many immune-related diseases due to their immune regulatory properties. Wharton's Jelly (WJ-MSC) and cord blood (CB-MSC) have recently received more attention than the other MSC sources. In this study, it was aimed to investigate the difference in the mechanisms of the immunological effects of WJ-MSCs and CB-MSCs.
Material and Methods: The intracellular cytokine levels of peripheral blood mononuclear cells (PBMC) and CD4+ T cells before and after MSC co-culture (Interleukin-4 (IL-4), Interferon-γ (IFN-γ), and Interleukin-17 (IL-17)) were determined by flow cytometry. At the same time, tumor growth factor (TGF)-β, IL-4, IL-17, IFN-γ supernatant cytokine levels were measured by ELISA. In the study, incubation times of 24 hours and 72 hours were applied with co-culture MSC/PBMC ratios of 1/5 and 1/10.
Results: Our data showed that WJ-MSCs and CB-MSCs have different morphological features, proliferation capacities, proliferation times and immunomodulating abilities. One of the cytokines of IFN-γ decreased significantly at both 1/5 and 1/10 ratios in the cell at 24 hours and increased significantly at 72 hours after CB-MSC/PBMC co-culture compared to the level after WJ-MSC/PBMC co-culture ($p<0.05$). Unlike for IL-17 cytokine, the intracellular level decreased significantly in the CB-MSC group only at 72 hours compared to the WJ-MSC group. For IL-4, the 1/10 ratio in the CB-MSC group decreased significantly at 24 hours, while the intracellular level was increased in all other groups. After CB-MSC/PBMC co-culture, TGF-β supernatant level decreased by 1/5 in 24 hours and increased in 72 hours. On the contrary, TGF-β supernatant level increased in the CB-MSC group at 24 hours and decreased significantly at 72 hours in 1/10 ratios ($p<0.05$).
Conclusion: CB-MSCs and WJ-MSCs show different immunomodulatory properties. Based on these findings, it can be said that the use of WJ-MSCs is more advantageous in terms of cell therapies due to the different isolation, proliferation capacity and immunomodulatory properties of CB-MSCs.
Keywords: Cord blood, Cord tissue, MSCs, Immunomodulation

ÖZ

Amaç: Mezenkimal kök hücreler (MKH'ler), immün düzenleyici özellikleri nedeniyle bir çok immün sistem ilişkili hastalık için tedavi seçeneği olarak umut vaad etmektedir. Wharton Jeli (WJ-MKH) ve kordon kanı (KK-MKH), diğer MKH kaynaklarına göre son dönemlerde daha çok ilgi görmektedir. Çalışmada, WJ-MKH'ler ve KK-MKH'lerin immünolojik etkilerinin mekanizmalarındaki farkı araştırmak amaçlanmıştır.
Gereç ve Yöntem: Periferik kan mononükler hücreler (PKMH) ve MKH ko-kültür öncesi ve sonrası CD4+ T hücrelerin hücre içi sitokin seviyeleri (Interlökin-4 (IL-4), Interferon-γ (IFN-γ), and Interlökin-17 (IL-17)) flow sitometri ile tespit edildi. Aynı zamanda Tümör büyüme faktörü (TGF)-β, IL-4, IL-17, IFN-γ süpernatant sitokin seviyeleri ELISA ile ölçüldü. Çalışmada, ko-kültür MKH/PKMH 1/5 ve 1/10 oranlarında, 24 saat ve 72 saat inkübasyon süreleri uygulandı.
Bulgular: Verilerimiz WJ-MKH'lerin ve KK-MKH'lerin farklı morfolojik özellikleri, çoğalma kapasiteleri, çoğalma süreleri ve immünomodülasyon yetenekleri olduğunu gösterdi. IFN-γ, KK-MKH/PKMH ko-kültür sonrası WJ-MKH/PKMH ko-kültürü sonrasına göre hücre içerisinde hem 1/5 hem 1/10 oranlarında 24. saatlerde anlamlı derecede azalırken, 72. saatlerde anlamlı derecede arttı ($p<0,05$). IL-17 sitokinin için farklı olarak sadece 72. saatte KK-MKH grubunda WJ-MKH grubuna göre anlamlı derecede hücre içi seviyesi düştü. IL-4 için KK-MKH grubunda 1/10 oran 24 saatte hücre içi seviyesi anlamlı derecede düşerken diğer tüm gruplarda artış gösterdi. KK-MKH/PKMH ko-kültür sonrası TGF-β supernatant seviyesi 1/5 oranında 24 saatte azalırken 72 saatte artış gösterdi. 1/10 oranlarında ise tam tersi 24. saatte KK-MKH grubunda artarken 72 saatlerde anlamlı derecede azaldı ($p<0,05$).
Sonuç: KK-MKH'ler ve WJ-MKH'ler, farklı immünmodülatör özellikler göstermektedir. Bu bulgular, KK-MKH'lerin izolasyonun, çoğalma kapasitesinin ve immünmodülatör özelliklerinin farklı olması sebebiyle hücre tedavileri açısından WJ-MKH'lerin kullanılmasının daha avantajlı olduğu söylenebilir.
Anahtar Kelimeler: Kordon kanı, Kordon dokusu, mezenkimal kök hücre, PKMH, ko-kültür, immunmodülasyon

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INTRODUCTION

Mesenchymal stem cells (MSCs) are the multipotent stromal cells that have the ability to differentiate into different cell types such as osteoblasts, chondrocytes, and adipocytes (1).

MSCs have the ability to trans-differentiate into various germ layers such as ectoderm, mesoderm and endoderm and are the undifferentiated cells with high proliferation and differentiation potential that are found in various tissues such as bone marrow, adipose tissue, cord blood, wharton's jelly. Conventionally, MSCs have been isolated from bone marrow, and bone marrow-derived MSCs (BM- MSCs) have been identified as an important candidate for cell therapy (2). However, their clinical application is limited because the invasive procedures in obtaining, and the decrease of their proliferation and differentiation capacities with age, their use in treatment of patients with hereditary diseases are difficult (3, 4). Therefore, other adult and fetal tissues including the adipose tissue, umbilical cord blood, umbilical cords, amniotic fluid and placental tissue have been investigated as the alternative stem cell sources (5-7).

Mesenchymal stem cells are the cells of interest due to their proliferation, differentiation and immunomodulatory properties. MSCs showing low expression of major histocompatibility complex (MHC) class II and costimulatory molecules can be considered as immune privileged cells. MSCs can alter immune system cell function by inhibiting the T-cell, B-cell, and natural killer (NK) cell proliferation and through directing monocytes and dendritic cells to an immature state (8, 9).

Although the exact mechanisms underlying the immunomodulation of the mesenchymal stem cells are still not fully understood, a number of soluble factors involved in the process have been identified. Its immunosuppressive effects are mainly revealed with the production of the soluble factors such as indolamine 2,3-dioxygenase (IDO), converting growth factor-β, human leukocyte antigen (HLA) -G and hepatocyte growth factor (10-13). While the soluble factors are the primary mechanism by which MSCs exert immunosuppressive effects, the direct cell-cell contact is also a contributing factor (14). Cell-to-cell contact between CD3+ T cells and MSCs can induce the CD4+CD25+ regulatory T cells. Researchers reported that it significantly reduces the inducing capacity of CD3+ T cell proliferation and secretion of the inflammatory factor, and has the ability to induce the Th2 responses (15).

Various studies emphasize that the cord-derived MSCs are preferable over other MSC sources due to their immunomodulatory properties. In particular, the potential of the cord-derived MSCs was emphasized to be higher as an alternative to other MSC sources. However, the cord-derived MSCs were not compared in detail, both for their proliferation capacity and their potential to affect the immune system, as cord blood and cord tissues. Therefore, we compared the similarities and differences between WJ-MSCs and CB-MSCs in our study. In particular, we aimed to compare the cord blood and cord tissue MSC isolation and culture conditions, and the immunomodulatory effects of these two MSC sources through measuring the cytokine levels secreted after co-cultures with PBMC (1, 2).

MATERIAL and METHODS

This study was approved by Istanbul Faculty of Medicine Ethics Committee (Date:11.11.2022, No: 20). The human cord blood and tissue samples used in the study were provided with the official permission after the informed consent forms were obtained from pregnant women who had no chronic disease and a healthy pregnancy process from the T.C. Ministry of Health Istanbul Bakırköy Dr.Sadi Konuk Training and Research Hospital, Obstetrics Clinic.

Mesenchymal stem cell isolation and culture

After the cesarean delivery, the blood transfer from the umbilical cord to the heparin tube was performed by an authorized clinician. For each sample, an average of 70-80 cc (8 cc heparin tubes) of blood samples were obtained by the physician in charge of the maternity ward. The blood and tissue brought to the laboratory promptly were processed in a sterile cabin. Cord blood samples were isolated by the Ficoll paque gradient method under the sterile cabin within 24 hours. Cells were planted on 25 cm^2 flasks, and 1% pen/strep complex, 10% FBS, DMEM medium was incubated at 37^0 C, 5% CO2. 24 hours after being planted, the medium was refreshed to remove other mononuclear cells in the cord blood. The cells were removed by trypsinization when the cells were 80-90% confluent, and were prepared for characterization.

The WJ-MSCs were isolated using mechanical fragmentation method in sterile cabine in the laboratory within 24 hours. Tissue pieces were washed with the transfer and shaking process into 4 separate 50 cc falcons into pre-prepared antibiotic complex (Streptomycin, Penicillin, Amphotericin B), and the last falcon was incubated for 1 hour at 37degrees on the shaker. The tissue pieces were placed carefully and regularly into 6 wells. 4 ml DMEM containing 10% FBS was incubated at 37^o C,5% CO2. Sufficiently proliferated cells were passaged on 75 cm^2 flask. The cells were trypsinizated when the cells were 80-90% confluent, and were prepared for characterization.

Characterization of mesenchymal stem cells

The cells were trypsinizated and washed once with PBS (centrifuge at 2000 rpm for 10 min). They were tested in accordance with the Human MSC Characterization Kit (BD- AB_2869404) protocol and analyzed by reading on a flow cytometry device.

Peripheral bood mononuclear cells isolation

Peripheral blood taken from a healthy person in approximately 20-30 cc into heparin tubes was processed. The peripheral blood mononuclear cells were isolated with Ficoll paque gradient method.

The appropriate dose and period of the phytohemaglotunine (PHA) demonstrated for cytokine release from PBMCs were identified as 10µg/ml PHA, and 72 hours for IL-4, IFNγ and IL-17 cytokine release using the flow cytometry methods for intracellular cytokine identification, and ELISA for cytokine release

from supernatant after the preliminary study.

Co-Culture of Mesenchymal Stem Cells, Wharton's Jelly Mesenchymal Stem Cells and Peripheral Blood Mononuclear Cells

The mesenchymal stem cells were characterizated by flow cytometry up to the 3rd passage. The PBMCs were co-cultured in different doses (1/5, and 1/10) for 24 h and 72h with both MSCs sources (Figure 1).

The intracellular cytokine was performed using the Human Th1/Th2/Th17 Phenotype Kit (BD - AB_2869360) flow cytometry, and the supernatant was performed using the Human IFN-gamma ELISA Kit (Diaclone-950.000.192); Human TGF-β ELISA Kit (Abbkine-Ket6030); Human IL-4 ELISA Kit (Diaclone-950.020.192); Human IL-17A ELISA Kit (Diaclone-850.940.192)) was used to perform the Elisa method. The intracellular cytokine levels were evaluated as the % values. The supernatant cytokine levels were calculated and evaluated in pg/ml unit by taking absorbance values from the ELISA plate reader.

Statistical analysis

The mean, standard deviation, median lowest, highest, frequency and ratio values were used in the descriptive statistics of the data. The distribution of the variables was measured by the Kolmogorov-Smirnov test. The Mann-Whitney U test was used for the analysis of quantitative independent data. The Statistical Package for the Social Sciences (SPSS) 28.0 program was used in the analyses. $p<0.05$ was considered statistically significant.

RESULTS

Mesenchymal stem cells express CD73, CD90, CD105 and CD44 from plastic adherent and specific surface markers and can differentiate into adipocytes, chondrocytes, osteoblasts, in vitro environment (1).

In our study, the adherent properties of the mesenchymal stem cells were examined under a microscope after mesenchymal stem cells were isolated and cultured (Figure 2). CB-MSCs began to adhere and reproduce in the first 7 days. Wharton's Jelly mesenchymal stem cells, on the other hand, separated from the tissue in a mean of 10 days and began to adhere and reproduce. Although cord blood-derived MSCs adhered and reproduced faster, we found in our study that Wharton's jelly MSCs preserved their morphology up to P7 and have an increased capacity to become confluent compared to CB-MSCs. The comparison for the surface markers showed that the percentages of CD73, CD90, CD105 and CD44 in MSCs obtained from both sources were over 95%.

With the comparison of the intracellular IFN-γ and IL-17 levels in terms of WJ-MSC and CB-MSC, a significant increase was detected at 24h both in 1/5 and 1/10 ratios in the group which was included WJ-MSC. However, at 72h, IFN-γ was significantly less, while IL-17 levels were higher. The comparison of the supernatant IFN-γ levels showed, on the contrary, that the level was significantly smaller at 24h in both 1/5 and 1/10 ratios in the group which was included WJ-MSC compared with the level in CB-MSC group. However, the level was higher at 72h ($p<0.0001$). The IL-17 supernatant level changes at the 1/5 ratio which was added WJ-MSC were significantly lower both at 24h and 72h. The level was significantly lower at 1/10 ratios ($p<0.0001$).

The comparison of the intracellular IL-4 levels in terms of WJ-MSC and CB-MSC showed that there was a significant decrease in the 1/5 ratio at 24h and 72h, and at the ratio of 1/10 at 72h in the group which was included WJ-MSC. However, the ratio of 1/10 was significantly higher at 24h. The comparison of the supernatant IL-4 levels showed that the 1/5 ratio at 24h, and the ratio of 1/10 at 72 hours decreased in the group with WJ-MSC and the ratio of 1/5 at 72h, and the ratio of 1/10 at 24h significantly increased ($p<0.0001$). The TGF-β supernatant level in the group which was added WJ-MSC, the ratio of 1/5 at 24h, and the ratio of 1/10 at 72h had significantly increased, however it had significantly decreased in other groups ($p<0.0001$) (Table 1-4).

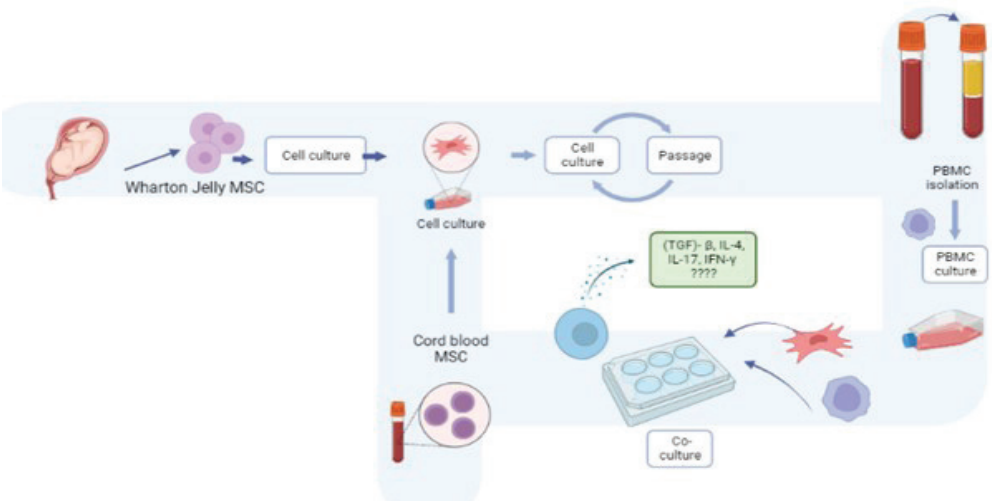

Figure 1: The experimental plan flow schema of the cord derived MSC and PBSC isolation and cultures

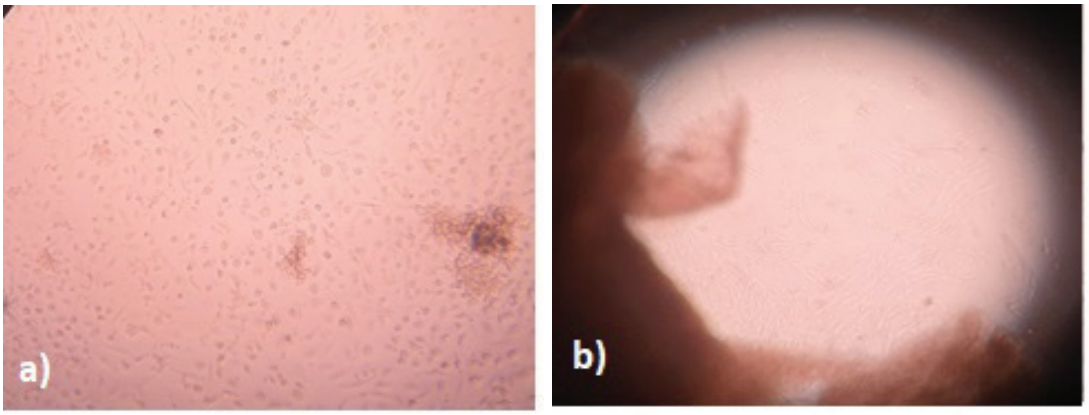

Figure 2: The microscopic images of mesenchymal stem cell in the first 7 days a) Cord blood derived MSC b) Wharton's Jelly derived MSC (Images were taken with 40x magnification on Motic AE21 inverted microscope)

Table 1: Comparison of the cytokine changes of groups which were added 1/5 ratio WJ-MSC, and CB-MSC for 24 hours

		WJ-MSC/STI-PBMC 1/5-24h		CB-MSC/STI-PBMC 1/5-24h		*p*
		avg.±sd	**Median**	**avg.±sd**	**Median**	
Intracellular	**IL-17**	5.47±0.06	5.48	2.32±0.15	2.24	***0.000***
	IFN-γ	11.62±0.02	11.62	7.96±0.06	7.99	***0.000***
	IL-4	8.94±0.01	8.94	10.22±0.01	10.22	***0.000***
Supernatant	**TGF-β**	167.86±0.11	167.80	12.03±0.06	12.02	***0.000***
	IFN-γ	104.20±0.21	104.28	136.18±0.16	136.24	***0.000***
	IL-4	0.02±0.00	0.02	2.35±0.03	2.36	***0.000***
	IL-17	11.02±0.02	11.02	17.04±0.02	17.04	***0.000***

IL-4: Interleukin-4, IFN-γ: Interferon-γ, IL-17: Interleukin-17, TGF-β: Tumor growth factor, MSC: Mesenchymal stem cells, WJ: Wharton's Jelly, STI: Stimule, PBMC: Peripheral blood mononuclear cells, CB: Cord blood, avg.: Average, SD: Standard deviation, h: Hour

Table 2: Comparison of the cytokine changes of groups which were added 1/5 ratio WJ-MSC, and CB-MSC for 72 hours

		WJ-MSC/STI-PBMC 1/5-72h		CB-MSC/STI-PBMC 1/5-72h		*p*
		avg.±sd	**Median**	**avg.±sd**	**Median**	
Intracellular	**IL-17**	2.05±0.02	2.05	14.54±0.03	14.53	***0.000***
	IFN-γ	1.32±0.03	1.31	31.05±0.01	31.04	***0.000***
	IL-4	0.94±0.02	0.95	7.13±0.03	7.13	***0.000***
Supernatant	**TGF-β**	0.69±0.01	0.69	6.62±0.43	6.51	***0.000***
	IFN-γ	90.88±0.30	90.96	63.35±46.75	90.31	***0.000***
	IL-4	0.31±0.00	0.31	0.18±0.00	0.18	***0.000***
	IL-17	11.04±0.02	11.04	19.30±0.04	19.31	***0.000***

IL-4: Interleukin-4, IFN-γ: Interferon-γ, IL-17: Interleukin-17, TGF-β: Tumor growth factor, MSC: Mesenchymal stem cells, WJ: Wharton's Jelly, STI: Stimule, PBMC: Peripheral blood mononuclear cells, CB: Cord blood, AVG: Average, SD: Standard deviation, h: Hour

Table 3: Comparison of the cytokine changes of groups which were added 1/10 ratio WJ-MSC, and CB-MSC for 24 hours

		WJ-MSC/STI-PBMC 1/10-24h		CB-MSC/STI-PBMC 1/10-24h		*p*
		avg.±sd	Median	avg.±sd	Median	
Intracellular	IL-17	16.93±0.02	16.93	6.17±0.21	6.10	***0.000***
	IFN-γ	10.24±0.02	10.24	5.33±0.03	5.34	***0.000***
	IL-4	6.96±0.01	6.96	5.80±0.02	5.80	***0.000***
Supernatant	TGF-β	1.36±0.36	1.30	23.40±0.35	23.37	***0.000***
	IFN-γ	94.02±0.11	94.04	126.77±0.22	126.79	***0.000***
	IL-4	2.24±0.03	2.25	0.65±0.03	0.64	***0.000***
	IL-17	19.94±0.14	19.87	17.92±0.07	17.92	***0.000***

IL-4: Interleukin-4, IFN-γ: Interferon-γ, IL-17: Interleukin-17, TGF-β: Tumor Growth Factor, MSC: Mesenchymal stem cells, WJ: Wharton's Jelly, STI: Stimule, PBMC: Peripheral blood mononuclear cells, CB: Cord blood, avg.: Average, SD: Standard deviation, h: Hour

Table 4: Comparison of the cytokine changes of groups which were added 1/10 ratio WJ-MSC, and CB-MSC for 72 hours

		WJ-MSC/STI-PBMC 1/10-72h		CB-MSC/STI-PBMC 1/10-72h		*p*
		avg.±sd	Median	avg.±sd	Median	
Intracellular	IL-17	12.37±0.03	12.36	8.67±0.02	8.68	***0.000***
	IFN-γ	13.4±0.06	13.50	27.15±0.05	27.16	***0.000***
	IL-4	2.15±0.05	2.15	6.46±0.04	6.46	***0.000***
Supernatant	TGF-β	5.28±0.03	5.28	0.93±0.12	1.00	***0.000***
	IFN-γ	103.28±0.48	103.38	89.19±0.04	89.21	***0.000***
	IL-4	0.27±0.00	0.27	0.76±0.01	0.77	***0.000***
	IL-17	16.50±0.00	16.50	13.86±0.01	13.87	***0.000***

IL-4: Interleukin-4, IFN-γ: Interferon-γ, IL-17: Interleukin-17, TGF-β: Tumor growth factor, MSC: Mesenchymal stem cells, WJ: Wharton's Jelly, STI: Stimule, PBMC: Peripheral blood mononuclear cells, CB: Cord blood, avg.: Average, SD: Standard deviation, h: Hour

DISCUSSION

Mesenchymal stem cells can be isolated from various sources including peripheral blood, adipose tissue, bone marrow, umbilical cord blood, and umbilical cord tissue. Various studies have shown that adipose-derived MSCs have the most colony-forming ability, and that bone marrow and adipose tissue are more successful than cord compared to those that can be best isolated. Although the bone marrow is a suitable source for obtaining MSC, the fact that it is invasive and that the differentiation potentials decrease with age, raises the issue of obtaining MSC from different sources. Cord blood and cord tissue are considered as the suitable cells for cell replacement therapy due to their non-invasive nature, having the same morphological, characteristics features, low immunogenicity, high proliferation and differentiation potential (5). MSCs are candidates as a suitable treatment option for immune system-related diseases such as autoimmune diseases, especially due to their immunomodulatory effects. It acts with some factors secreted by the immune system cells in the regulation of the immune response by MSCs (15).

In light of these data, we aimed to compare two different sources of MSCs by investigating the effects of MSCs derived from cord blood and cord tissue on isolation, culture stages and immune system in our project.

Our results showed that CB-MSCs and WJ-MSCs share similar phenotypic properties and immunomodulatory capacities. The comparison of the obtaining and isolation of cord blood and Wharton's Jelly showed that cord blood isolation and replication procedures were easier. At the same time, we observed that cord blood MSCs have a faster proliferation capacity (average 7 days). For the isolation of WJ-MSC, longer and more careful procedures to protect against any infection were required for isolation from the cord tissue which was taken into the container after childbirth. We found with the investigation of MSCs under microscopy after tissue fragmentation and subsequent culture procedures that MSCs started reproduction in a mean of 10 days. Although CB seems to be more advantageous than WJ MSCs in terms of isolation and proliferation capacities, WJ-MSCs reach up to 90% in terms of their confluent capacity, however, CB-MSCs reaching up to 80% in the 1st passage and

their capacity to become confluent were highly lower with the increase of the passage count. Similarly, as the number of passages increased, the CB-MSCs morphologically changed. The comparison of the two different sources in terms of characterization showed no significant difference between the percentage values of CD73, CD90, CD44 and CD105. Studies have shown that MSCs obtained from three different sources were negative for hematopoetic stem cell markers and positive to similar degrees in terms of expression of CD73, CD90, CD105 and CD44 markers (16, 17).

The immunomodulatory potential of the mesenchymal stem cells has been demonstrated on T cells, which plays a key role in the immune system and on the cytokines secreted from these cells (18-19). Studies have suggested that adipose-derived MSCs exhibit stronger immunomodulatory effects compared with the effects of bone marrow-derived MSCs, which suggests that adipose-derived MSCs would be a better alternative for immunomodulatory therapy. On the other hand, it has been suggested that CB-MSC and WJ-MSCs have a minimal risk of initiating an allogeneic immune response when administered in vivo. In addition to the ease of collection, this will make cord-derived MSCs as the suitable therapeutic candidates (20).

Mesenchymal stem cells do not always have an immunosuppressive effect, and they can also have a pro-inflammatory effect depending on the microenvironment in which they are located. Stimulation with low levels of IFNy and TNF, can confer immunostimulating properties on MSCs. Some clinical studies show that a certain level of inflammation is necessary to induce immunosuppressive properties in MSCs, and the lessons learned from these studies should be used to guide therapeutic methodologies (21, 22). The MSCs obtained in our study were not stimulated by a stimulating factor. We predict that more clear results can be obtained by adding stimuli to the MSC culture.

In general, the studies show relationship between MSCs and immune system from the adipose tissue and bone marrow sources. In this present study, the relationship between WJ and CB as the source of MSC and immune system was evaluated. It has given a different perspective to the literature. The effect of different MSC sources on the immune system in the study was evaluated in different combinations as 1/5 and 1/10, evaluated as 24 hours and 72 hours. The separate evaluation of these parameters, analysis and comparison have given a unique value to this study. The comparison of WJ-MSC and CB-MSC showed that the intracellular and supernatant cytokine levels differed in terms of affecting the immune system. The secretion of intracellular proinflammatory cytokines were suppressed in the WJ-MSC added experimental group particularly at 72h compared with the level in CB-MSC, and the level of anti-inflammatory cytokines were found to be significantly higher. In the studies conducted so far, bone marrow, adipose tissue and cord tissue have been compared as a source of mesenchymal stem cell. The comparison of cord blood and cord tissue provides new data to the literature together with this study. Considering the limitations of our study, we suggest that more clear and understandable results will be obtained with a more detailed study of intracellular cytokine expression pathways, with the addition of any stimulus when replicating MSC, and with the presence of cytokines secreted from MSC as well as with the inclusion of cytokines secreted from PBMC into the study.

Ethics Committee Approval: This study was approved by Istanbul Faculty of Medicine Clinical Research Ethics Committee (Date:11.11.2022, No: 20).

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REFERENCES

1. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006;8(4):315-7.
2. Le Blanc K, Rasmusson I, Sundberg B, Götherström C, Hassan M, Uzunel M, et al. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. Lancet 2004;363(9419):1439-41.
3. Atala A. Recent developments in tissue engineering and regenerative medicine. Curr Opin Pediatr 2006;18(2):167-71.
4. Stenderup K, Justesen J, Clausen C, Kassem M. Aging is associated with decreased maximal life span and accelerated senescence of bone marrow stromal cells. Bone 2003;33(6):919-26.
5. Kern S, Eichler H, Stoeve J, Klüter H, Bieback K. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. Stem Cells 2006;24(5):1294-301.
6. Zheng YB, Gao ZL, Xie C, Zhu HP, Peng L, Chen JH, et al. Characterization and hepatogenic differentiation of mesenchymal stem cells from human amniotic fluid and human bone marrow: a comparative study. Cell Biol Int 2008;32(11):1439-48.
7. Chen L, Tredget EE, Liu C, Wu Y. Analysis of allogenicity of mesenchymal stem cells in engraftment and wound healing in mice. PLoS One 2009;4:e7119. doi: 10.1371/journal.pone.0007119
8. De Miguel MP, Fuentes-Julian S, Blazquez-Martinez A, Pascual CY, Aller MA, Arias J, et al. Immuno-suppressive properties of mesenchymal stem cells: Advances and applications. Curr Mol Med 2012;12(5):574-91.
9. Uccelli A, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. Nat. Rev Immunol 2008;8(9):726-36.
10. Krampera M, Cosmi L, Angeli R, Pasini A, Liotta F, Andreini A, et al. Role for interferon-gamma in the immunomodulatory activity of human bone marrow mesenchymal stem cells. Stem Cells 2006;24(2):386-98.
11. Kögler G, Radke TF, Lefort A, Sensken S, Fischer J, Sorg RV, et al.

Cytokine production and hematopoiesis supporting activity of cord blood-derived unrestricted somatic stem cells. Exp Hematol 2005;33(5):573-83.

12. Selmani Z, Naji A, Zidi I, Favier B, Gaiffe E, Obert L, et al. Human leukocyte antigen-G5 secretion by human mesenchymal stem cells is required to suppress T lymphocyte and natural killer function and to induce CD4+CD25highFOXP3+ regulatory T cells. Stem Cells 2008;26(1):212-22.
13. Di Nicola M, Carlo-Stella C, Magni M, Milanesi M, Longoni PD, Matteucci P, et al. Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. Blood 2002;99(10):3838-43.
14. Krampera M, Glennie S, Dyson J, Scott D, Laylor R, Simpson E, Dazzi F. Bone marrow mesenchymal stem cells inhibit the response of naive and memory antigen-specific T cells to their cognate peptide. Blood 2003;101(9):3722-9.
15. Di Ianni M, Del Papa B, De Ioanni M, Moretti L, Bonifacio E, Cecchini D, et al. Mesenchymal cells recruit and regulate T regulatory cells. Exp Hematol 2008;36(3):309-18.
16. Bieback K, Kern S, Klüter H, Eichler H. Critical parameters for the isolation of mesenchymal stem cells from umbilical cord blood. Stem Cells 2004;22(4):625-34.
17. Zeddou M, Briquet A, Relic B, Josse C, Malaise MG, Gothot A, et al. The umbilical cord matrix is a better source of mesenchymal stem cells (MSC) than the umbilical cord blood. Cell Biol Int 2010;34(7):693-701.
18. Galipeau J, Sensébé L. Mesenchymal stromal cells: clinical challenges and therapeutic opportunities. Cell Stem Cell 2018;22(6):824-33.
19. Weiss ARR, Dahlke MH. Immunomodulation by mesenchymal stem cells (MSCs): Mechanisms of action of living, apoptotic, and dead mscs. Front Immunol 2019;10:1191.
20. Kim JH, Jo CH, Kim HR, Hwang YI. Comparison of immunological characteristics of mesenchymal stem cells from the periodontal ligament, umbilical cord, and adipose tissue. Stem Cells Int 2018;8429042.
21. Inoue S, Popp FC, Koehl GE, Piso P, Schlitt HJ, Geissler EK, et al. Immunomodulatory effects of mesenchymal stem cells in a rat organ transplant model. Transplantation 2006;81(11):1589-95.
22. Sudres M, Norol F, Trenado A, Gregoire S, Charlotte F, Levacher B, et al. Bone marrow mesenchymal stem cells suppress lymphocyte proliferation in vitro but fail to prevent graft-versus-host disease in mice. J Immunol 2006;176(12):7761-7.

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IS WHAT WE KNOW ABOUT POSTERIOR REVERSIBLE ENCEPHALOPATHY SYNDROME (PRES) TRUE?

POSTERİOR REVERSİBL ENSEFALOPATİ SENDROMU (PRES) HAKKINDA BİLDİKLERİMİZ DOĞRU MU?

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ABSTRACT

Objective: The aim of this study is to evaluate the risk factors and clinical course in cases of posterior reversible encephalopathy syndrome (PRES).
Material and Methods: In this study, we retrospectively reviewed the data of pregnant or puerperal women diagnosed with PRES in the tertiary center emergency obstetrics outpatient clinic and intensive care unit between 2017 and 2022. All patients were evaluated by obstetrics, neurology, ophthalmology, radiology, and intensive care physicians, and blood tests and imaging were performed in the same center. Application complaints, laboratory values, imaging methods, comorbidities, mode of delivery, and postpartum period were evaluated for each patient.
Results: In five years, a total of seven patients were diagnosed with PRES based on imaging methods and clinical findings. One of these patients had PRES twice, three years apart. Six of them presented with eclampsia. One patient was diagnosed with PRES postpartum in the first week, while other patients were diagnosed at pregnancy. Four patients had blurred vision, two patients had blindness, and one patient had no visual complaints. Three of the patients had mood changes (one patient confused, two patients agitated). One of the patients had diabetes mellitus (DM), which was known and treated with oral antidiabetics. One patient was under follow-up and treatment because of hypertension (HT) that started before pregnancy and three patients were under follow-up due to hypertension that started during pregnancy. There was no known additional disease in one patient. The delivery week of the patients was between 28 and 34 weeks of gestation. Pathological laboratory values were most frequently seen in LDH, albumin, and protein values. Every patient was discharged with outpatient follow-up. Epilepsy continues in one patient, HT in two patients, and isolated nephropathy in one patient after PRES.
Conclusion: PRES should be considered especially in pregnant women with neurological symptoms including visual impairment and headache. Clinical suspicion and neuroimaging are required for the diagnosis of PRES.
Key words: PRES, eclampsia, blindness, visual changes

ÖZ

Amaç: Bu çalışmanın amacı posterior reversible ensefalopati sendromunun (PRES) risk faktörlerini ve klinik gidişatı değerlendirmektir.
Gereç ve Yöntem: Bu çalışmada 2017-2022 yılları arasında tersiyer bir sağlık merkezinin acil kadın doğum polikliniği ve yoğun bakım ünitesinde PRES tanısı almış gebe veya lohusalara ait veriler retrospektif olarak incelendi. Tüm hastalar kadın doğum, nöroloji, göz, radyoloji ve yoğun bakım hekimleri tarafından değerlendirildi, kan tahlilleri ve görüntülemeleri aynı sağlık kuruluşunda yapıldı. Her hastanın başvuru şikâyetleri, laboratuvar değerleri, görüntüleme yöntemleri, eşlik eden hastalıklar, doğum şekli ve doğum sonrası dönemi ile ilgili verileri kaydedildi.
Bulgular: Beş yılda görüntüleme yöntemleri ve klinik bulgulara göre toplam yedi hastaya PRES tanısı kondu. Bu hastalardan birinde üç yıl arayla iki kez PRES gelişmişti. Altı hasta eklampsi ile başvurdu. Bir hastaya postpartum ilk haftada, diğer hastalara gebelikte tanı kondu. Hastalardan 42'sinde bulanık görme, 2'sinde körlük varken 1 hastada görme şikâyeti yoktu. Hastaların 3 tanesinde yeni başlayan mood değişiklikleri (bir hastada konfüzyon, iki hastada ajite) vardı. Ek hastalık sorgulamasında hastalardan birinde bilinen diabetes mellitus (DM) vardı oral antidiyabetik kullanıyordu. Bir hasta gebelik öncesi başlayan hipertansiyon (HT), üç hasta gebelikte başlayan hipertansiyon nedeniyle takip ve tedavi altındaydı. Bir hastada bilinen ek hastalık yoktu. Hastaların doğum haftası 28-34 gebelik haftaları arasındaydı. En sık LDH, albümin ve protein değerlerinde anormal kan düzeyi saptanmıştı. Tüm hastalar ayaktan taburcu edildi. PRES sonrası bir hastada epilepsi, iki hastada HT ve bir hastada izole nefropati varlığı devam etmişti.
Sonuçlar: PRES özellikle görme bozukluğu ve baş ağrısı gibi nörolojik semptomları olan gebe ve lohusalarda akla gelmelidir ve tanı için nörogörüntüleme gereklidir.
Anahtar Kelimeler: PRES, eklampsi, körlük, görme bozukluğu

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INTRODUCTION

Posterior reversible encephalopathy syndrome (PRES) is a clinical radiological diagnosis that is accompanied by neurological complaints most commonly affecting the posterior cerebral area, and symmetrical vasogenic edema in the parieto-occipital region, classically seen on cerebral CT and MRI imaging. This was first described in 1996. PRES is also known as acute hypertensive encephalopathy or reversible posterior leukoencephalopathy. Acute HT causes different cortical changes in MRI than in chronic HT patients. As the cases reported in the literature increased, it was observed that the posterior frontal and temporal lobes and even the cerebellum were involved. In the syndrome, which is initially known as reversible, clinical complaints are usually temporary, but there is also literature showing an increased risk of stroke in these patients in the long term (1,2).

The most common neurological complaints include visual disturbances, headaches, convulsions, and confusion.

The pathogenesis of PRES is not clear, but hypertension, preeclampsia/eclampsia, renal failure, autoimmune diseases, and the use of cytotoxic agents are risky for PRES. It is more common in women than men (3). Preeclampsia or eclampsia are present in 7-20% of PRES cases (4). According to one opinion, PRES is a part of the pathogenesis of eclampsia (5). In the acute phase of the disease, neurological complaints (vision loss (sometimes even Anton's syndrome), visual aura, hemianopia, visual hallucinations, headache, cognitive changes such as drowsiness, confusion, agitation, coma, convulsions, nausea, regional muscle weakness, sensory disturbances, speech deterioration) are seen (6). In the fundoscopic examination of the eclamptic patient, papilledema, flame-shaped hemorrhage, and exudation can be seen (7).

In the treatment of PRES, the risk of complications and seizures can be reduced with agent-directed treatment and supportive ICU treatments.

With this study, we wanted to discuss the presentation, risk factors, and clinical course in PRES cases.

MATERIAL and METHODS

The data of the patients were obtained from the tertiary health center Gynecology and Obstetrics Clinic and intensive care unit records with the approval of the ethics committee dated 10/11/2022, numbered 953.

Age, gravida, comorbidities (DM, thyroid diseases, HT, kidney diseases), symptoms, relapse status, mood changes, obstetric history, complications, arterial blood pressure at admission, neurology/ophthalmology/radiology, and reanimation unit study records of the patients were examined and information was recorded.

The diagnosis of PRES was made with the joint evaluations of obstetrics, radiology, and neurology.

MRI examinations were performed on either a Siemens Amira 1.5 Tesla or Philips Ingenia 1.5 Tesla (All MRI studies included T2-weighted, T2 FLAIR, DWI, and SWI).

Albumin, total protein, liver function tests (Ast, Alt, Ldh), RDW, and blood sugar results were compared in the blood when the application was made with clinical complaints from the laboratory values. In cases of repeated PRES, the values in both cases were recorded separately.

RESULTS

There were seven patients in total, one of whom was a patient who had PRES for the second time, three years apart.

While one patient had PRES in the previous pregnancy, there was no PRES in the next pregnancy.

The patients were between the ages of 21 and 33. Two patients were nulliparous, and four were multiparous.

Of the six patients, one was from Turkmenistan, one was Moroccan, one was Syrian, and three were Turkish.

One patient had PRES postpartum during week 1 and the others had PRES during pregnancy.

Only one patient had known HT before pregnancy and was using regular medication. HT was detected for the first time in three patients when they were pregnant or presented with seizures.

In the case of recurrent PRES, the first PRES developed after delivery, and the second PRES developed before delivery. She did not have any known systemic disease in her pregnancy, which was her first PRES. In the second attack, she had HT that continued after the first PRES. In the recurrent PRES case, blurred vision developed in the first attack, and blindness developed in the second attack.

All patients complained of headaches.

One patient had PRES in her first pregnancy, and her pregnancy was uneventful one year later.

Of the seven patients, two had blindness and three had blurred vision. One patient had only convulsions without vision problems.

The patients delivered at between 28 and 34 weeks of gestation. All babies were discharged without any problem after the neonatal intensive care process.

All seven patients had generalized tonic-clonic seizures, but there was no seizure in the second attack of the recurrent PRES case.

Agitation developed in two patients and confusion in one patient. There was no mood change in the other patients.

One patient had gestational DM. Her blood sugar was normal at the time of admission, and it never increased afterward (Table 1).

Table 1: Demographic-clinical characteristics of the patients

	Age-Gestational week	Comorbidity	COVID	Gravida	TA (mmHg)	Onset time	Headache	Eclampsia	Visual disturbance	Mood changes	Complication	Recurrence
Case 1	27 y, 33 gw	GHT	None	G3	180/110	At birth	+	+	Blurred vision	None	Ongoing HT	First
Case 2	30 y, 33 gw	HT	None	G4	130/80	At birth	+	–	Blindness	None	Ongoing proteinuria	Second
Case 3	31 y, 30 gw	GHT, obesity	None	G4	140/70	At birth	+	+	Blurred vision	Confusion	For 6 months HT	None
Case 4	25 y, 32 gw	GHT	None	G3	160/100	At postpartum 1st week	+	+	Blurred vision	None	For 6 months HT	None
Case 5	30 y, 30 gw	None	None	G3	150/70	At birth	–	+	None	Agitation	Epilepsy	None
Case 6	21 y, 28 gw	GHT	None	G1	180/110	At birth	+	+	Blurred vision	None	1 month proteinuria	None
Case 7	33 y, 34 gw	GDM	None	G1	137/80	At birth	+	+	Blindness	Agitation	None	None

GHT: Gestational hypertension, GDM: Gestational Diabetes Mellitus, HT: Hypertension

The patient whose albumin and protein levels were extremely low at admission, and who received DIC treatment in the ICU, had cerebral infarction areas and epilepsy that started after a PRES attack and has been continuing for five years.

All patients were delivered with SCA due to either eclampsia or severe preeclampsia, and all clinical complaints regressed within one to two days after delivery.

In biochemical tests, every patient had elevated LDH and low albumin and total protein. There were elevated liver function tests in some patients. RDW was normal in all the patients.

The nephrology follow-up of one patient continues due to proteinuria.

HT, which required six months of medication, continued in two of the patients (Table 2).

DISCUSSION

With our study results, we determined that PRES is a recurring syndrome with permanent neurological complications in the long term, and we think that the diagnosis of PRES can be made more frequently if cranial imaging is performed in every pregnant woman with neurological findings.

Despite the large population and high number of births in the tertiary health center where the study was conducted, the fact that only seven patients (one case being recurrent) were diagnosed with PRES in five years may be due to the fact that the diagnosis could not be made. According to the study by Chao et al. in the literature, the majority of patients with eclampsia actually have PRES, but when the patient has convulsions, the diagnosis can be made less frequently than necessary because the diagnosis is made with neurological imaging and evaluations. Especially in women with severe preeclampsia and headache, if MRI is performed, it can be detected at a higher rate (8).

PRES may start secondarily to many pathologies such as hyperperfusion and arterial extravasation, vasogenic edema and hypoperfusion, endothelial dysfunction, fluid overload, and metabolic changes. The main problem in preeclampsia and eclampsia is thought to be related to endothelial damage. Supporting this theory is the increase in the secretion of substances such as fibrinonectin, TPA, thrombomodulin, endothelin1, and VWF that can damage the endothelium in preeclampsia, and high LDH and erythrocyte morphology changes indicating endothelial damage (9). In our study, LDH values were high in all the patients, and RDW was within physiological values in all the patients.

Albumin and total protein levels were low in all patients. There are results in the literature showing that low albumin levels in adults may be a risk factor for PRES (10). AST, ALT was elevated in only two patients. Blood glucose levels were variable as viewed from the spot. Hemoglobin, urea, and creatinine levels were also normal in the patients. However, we think that the laboratory values of the patients did not deteriorate much because they were delivered close to the onset of symptoms.

In healthy people, there is a mechanism that protects the brain from systemic blood pressure changes. However, in cases such as sudden increased blood pressure or excessive fluctuation, autoregulation may be impaired, and the blood-brain barrier becomes disrupted with an increase in systemic arterial blood pressure, with blood flow in the brain increasing and arterial extravasation occurring in the cerebral parenchyma (11,12).

Table 2: Laboratory results of the patients

	Albumin-total protein (3.5-5.2)-(6.6-8.3) gr/dl	LDH (1-248) U/L	AST-ALT (1-35) U/L	RDW (11-16) %	Blood sugar (74-106) mm/dl
Case 1	2.35-5.05	1771	113-33	14.3	119
Case 2	3.27-6.22	264	20-12	13.3	129
Case 3	3.18-5.47	419	25-11	12.5	144
Case 4	3.3-5.98	291	21-14	15.6	80
Case 5	0.6-3.1	574	24-11	13.3	119
Case 6	2.18-5.56	383	26-12	12.5	92
Case 7	2.46-4.67	357	143-122	14.1	88

LDH: Lactate dehydrogenase, AST: Aspartate aminotransferase, ALT: Alanin aminotransferase, RDW: Red blood cell distribution width

Although increased perfusion is seen more frequently on MRI in PRES cases, hypoperfusion may be seen in some patients because in some cases, disruption of autoregulation causes hypoperfusion and cytotoxic edema and cerebral local infarcts. Cerebral infarcts may also occur due to vasogenic edema and compression of the edema. This allows vasoconstriction findings to be seen on MRI. In one of our patients, who developed permanent epilepsy complications, edematous areas due to PRES as well as cerebral infarct areas were observed.

PRES is most common in posterior cerebral arterial circulation areas (parieto-occipital region) with weaker sympathetic innervation, which are less tolerant of edema and vascular changes that occur following the destruction of the blood-brain barrier. Therefore, vasogenic edema and related sensory changes, seizures, and vision loss are the most common complaints (13).

MRI is superior to CT in imaging (14). However, in an MRI performed very close to the onset of symptoms, the diagnosis may not be made because the signs of vasogenic edema are not fully established yet. In addition, the possibility of the disappearance of symptoms and radiological findings until the patient is delivered and stabilized due to concomitant obstetric emergencies (eclampsia, severe preeclampsia, fetal distress, etc.) may also cause the diagnosis to be missed. PRES symptoms usually disappear very quickly, even with a reduction in blood pressure and mild supportive care, and the patient may not have had a control radiological evaluation after the initial MRI, which is normal. Another problem that should not be forgotten is the inadequacy of the radiological method or team. MRI findings may not be as typical, or the sensitivity of the device used may be weak. ASL (arterial spin labeling)-based perfusion is a new marker for the diagnosis of PRES, even in the absence of vasogenic edema (15). We diagnosed all seven patients with MRI, but since we did not have ASL in our hospital, we were able to use a 1.5 Tesla MRI.

Cerebral MRI findings in PRES are symmetrical and reversible in almost every patient. All of our patients had involvement of the parieto-occipital region. There was also involvement in the frontal area in two patients and at the basal ganglia and pons level in one patient. We believe that a small number of patients were diagnosed because MRI findings may not be established in the early period and the findings quickly disappear in the late period.

Although it is known that the COVID-19 infection, which started in 2019 and led to a pandemic, may increase the frequency of PRES through a cytokine storm, vascular permeability changes, dysregulation in the renin-angiotensin system, and the drugs used (hydroxychloroquine and tocilizumab), we found that the patients at least did not have a diagnosed COVID-19 infection (16).

Visual disturbances are seen in 26-67% of patients and cortical blindness in 8-33% in PRES (17). They described blindness in two of the patients and blurred or impaired vision in four. There was no visual impairment in the patient who later developed neurological complications.

PRES is typically associated with severe hypertension, but it may also occur with rapid increases in blood pressure in patients with endothelial damage and in patients with only mildly elevated blood pressure (18). PRES may also develop in normotensive or hypotensive patients (19). TA was high in all of our patients.

The literature warns that having PRES may cause ischemic brain damage, permanent neurological deficit, relapse, or death (20,21). Permanent brain damage, recurrence, or death may not be present in PRES that are intervened in a timely and correct manner (22). In cases where adequate and rapid treatment is not performed, epilepsy becomes permanent most frequently (23). In fact, permanent neurological damage is related to ischemia and/or intracranial hemorrhage. In such cases, the mortality rate is 15% (24,25). AntiPL and thrombophilia scans are clean, but MRI results suggestive of cerebral infarction are detected in our PRES cases, which have convulsions despite still using antiepileptic drugs.

PRES is a rare clinical syndrome that can occur for reasons other than pregnancy. Clinical complaints usually resolve rapidly after PRES and there are no sequelae. One of the patients in the study has epileptic seizures that started and continued after PRES. Two patients had HT for six months, and one patient had persistent proteinuria. Headache and visual disturbances in

every patient resolved completely in one to two days.

In the literature, the rate of PRES being reversible is between 35% and 100%. In reversible cases, sometimes there is a complete recovery, and sometimes there is a partial recovery (26). Among patients with a follow-up CT or MRI, 49% to 75% have a resolution of the initial abnormalities within five days to seventeen months (27).

The most critical treatment is the regulation of blood pressure. However, an aggressive TA reduction is not recommended because it decreases cerebral perfusion. It is recommended to keep it at systolic 130-150/diastolic 80-100 mmHg. It would be correct to avoid nimodipine, diazoxide, and ketanserine in the treatment of HT. It is suspected that nifedipine may increase angioedema. Nicardipine and labetalol are suitable. No additional treatment is recommended for convulsions only if status epilepticus is present (28).

The most important limitation of the study is the small number of patients and the absence of late-term and control MRI.

According to the results of our study, PRES should be considered in pregnant women with neurological findings including headache and visual impairment, especially if they have preeclampsia/eclampsia. Because PRES is a clinical entity that can recur and cause permanent neurological deficits, MRI should be used more liberally to diagnose it and start treatment as early as possible.

Ethics Committee Approval: This study was approved by Istanbul Medipol University Non-Interventional Clinical Research Ethics Committee (Date: 10.11.2022, No: 953).

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REFERENCES

1. Parauda SC, Zhang C, Salehi Omran S, Schweitzer AD, Murthy SB, Merkler AE,et al. Risk of stroke after posterior reversible encephalopathy syndrome. Stroke 2022;53(11):3313-9.
2. Hinchey J, Chaves C, Appignani B, Breen J, Pao L, Wang A.,et al. A reversible posterior leukoencephalopathy syndrome. N Engl J Med 1996;22;334(8):494-500.
3. Bartynski WS. Posterior reversible encephalopathy syndrome, part 1: fundamental imaging and clinical features. AJNR Am J Neuroradiol 2008;29(6):1036-42.
4. Tek ŞÇ, Uyar AŞ, Çakıcı Z, İnal MT, Memiş D, Tekataç A, et al. Posterior reversible ensefalopati sendromu: iki olgunun sunumu. Turk J Intensive Care 2019;17:44-8.
5. Martin JN, Jr Brewer JM, Blake PG, Owens MY, LaMarca B. PP137. Posterior reversible encephalopathy syndrome (PRES) is a constant component of eclampsia. Pregnancy Hypertens 2012;2(3):314.
6. Triplett JD, Kutlubaev MA, Kermode AG, Hardy T. Posterior reversible encephalopathy syndrome (PRES): diagnosis and management. Pract Neurol 2022;22(3):183-9.
7. Stott VL, Hurrell MA, Anderson TJ. Reversible posterior leukoencephalopathy syndrome: a misnomer reviewed. Intern Med J 2005;35(2):83-90.
8. Chao AS, Chen YL, Chang YL, Chao A, Su, SY, Wang TH. Severe pre-eclamptic women with headache: is posterior reversible encephalopathy syndrome an associated concurrent finding? BMC Pregnancy Childbirth 2020;1;20(1):336.
9. Schwartz RB, Feske SK, Polak JF, DeGirolami U, Iaia A, Beckner KM, et al. Preeclampsia-eclampsia: clinical and neuroradiographic correlates and insights into the pathogenesis of hypertensive encephalopathy. Radiology 2000;217(2):371-6.
10. Furtado A, Hsu A, La Colla L, Zuccoli G. Arterial blood pressure but not serum albumin concentration correlates with ADC ratio values in pediatric posterior reversible encephalopathy syndrome. Neuroradiology 2015;57(7):721-8.
11. Strandgaard S, Paulson OB. Cerebral autoregulation. Stroke 1984;15(3):413-6.
12. Rabinstein AA, Mandrekar J, Merrell R, Kozak OS, Durosaro O, Fugate JE. Blood pressure fluctuations in posterior reversible encephalopathy syndrome. J Stroke Cerebrovasc Dis 2012;21(4):254-8.
13. Akgün N, Karaman M, Başyiğit S, Yılmaz H, Özcan AA. Posterior reversible ensefalopati: 2 olgu sunumu. İstanbul Tıp Dergisi 2010;11(2): 82-5.
14. Doelken M, Lanz S, Rennert J, Alibek S, Richter G, Doerfler A. Differentiation of cytotoxic and vasogenic edema in a patient with reversible posterior leukoencephalopathy syndrome using diffusion-weighted MRI. Diagn Interv Radiol 2007;13(3):125-8.
15. Fazeli S, Noorbakhsh A, Imbesi SG, Bolar DS. Cerebral perfusion in posterior reversible encephalopathy syndrome measured with arterial spin labeling MRI. Neuroimage Clin 2022;35:103017.
16. Donaldson L, Margolin E. Posterior Reversible Encephalopathy Syndrome in Acute COVID19 Pneumonia. Can J Neurol Sci 2022;49(6):791-2.
17. Vandenbossche G, Maquet J, Vroonen P, Lambert G, Nisolle M, Kridelka F, et al. A reversible posterior leucoencephalopathy syndrome including blindness caused by preeclampsia. Facts Views Vis Obgyn 2016;8(3):173-7.
18. Eastabrook G, Brown M, Sargent I. The origins and end-organ consequence of pre-eclampsia. Best Pract Res Clin Obstet Gynaecol 2011;25(4):435-47.
19. Granata G, Greco A, Iannella G, Granata M, Manno A, Savastano E, Magliulo G. Posterior reversible encephalopathy syndrome--Insight into pathogenesis, clinical variants and treatment approaches. Autoimmun Rev 2015;14(9):830-6.
20. Ferraldeschi M, Tari Capone F, Di Lisi F, Patella R, Ceschim V, Cao M, et al. When a pregnancy required a neurological consultation: a case report. Clin Ter 2012;163(6):487-90.
21. Vandenbossche G, Maquet J, Vroonen P, Lambert G, Nisolle M, Kridelka F, et al. A reversible posterior leucoencephalopathy syndrome including blindness caused by preeclampsia. Facts Views

Vis Obgyn 2016;8(3):173-7.

22. Pizon AF, Wolfson AB. Postpartum focal neurologic deficits: posterior leukoencephalopathy syndrome. J Emerg Med 2005;29(2):163-6.
23. Burnett MM, Hess CP, Roberts JP, Bass NM, Douglas VC, Josephson SA. Presentation of reversible posterior leukoencephalopathy syndrome in patients on calcineurin inhibitors. Clin Neurol Neurosurg 2010;112(10):886-91.
24. Servillo G, Bifulco F, De Robertis E, Piazza O, Striano P, Tortora F, et al. Posterior reversible encephalopathy syndrome in intensive care medicine. Intensive Care Med 2007;33(2):230-6.
25. Lee VH, Wijdicks EF, Manno EM, Rabinstein AA. Clinical spectrum of reversible posterior leukoencephalopathy syndrome. Arch Neurol 2008;65(2):205-10.
26. Hinchey J, Chaves C, Appignani B, Breen J, Pao L, Wang A, et al. A reversible posterior leukoencephalopathy syndrome. N Engl J Med 1996;334(8):494-500.
27. Casey SO, Sampaio RC, Michel E, Truwit C.L. Posterior reversible encephalopathy syndrome: utility of fluid-attenuated inversion recovery MR imaging in the detection of cortical and subcortical lesions. AJNR Am J Neuroradiol 2000;21(7):1199-206.
28. Vandenbossche G, Maquet J, Vroonen P, Lambert G, Nisolle M, Kridelka F, et al. A reversible posterior leucoencephalopathy syndrome including blindness caused by preeclampsia. Facts Views Vis Obgyn 2016;8(3):173-7.

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EVALUATION OF FLEXURAL STRENGTH OF DIFFERENT RESTORATIVE MATERIALS

FARKLI RESTORASYON MATERYALLERİNİN EĞİLME DAYANIMLARININ KARŞILAŞTIRILMASI

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ABSTRACT

Objective: The aim of this in vitro study was to compare the biaxial flexural strengths of permanent restorative materials produced by additive manufacturing and milling after thermal aging.

Material and methods: For this study, three study groups were formed by producing disc-shaped specimens with a 3D printer (Form3, Formlabs) using permanent crown material (Permanent Crown, Somerville, MA) and two different resin-containing materials (Brilliant Crios,Coltene/Whaledent; Cerasmart, GC Europe) by the milling method (n=10). The entire production process was carried out in accordance with the manufacturer's instructions. All specimens were then polished under water with silicon carbide papers. The specimens were then subjected to thermal aging (5-55°C, 5000 cycles). After aging, biaxial flexural strength values of all specimens were measured with a universal testing machine. The obtained data were evaluated with the one-way ANOVA test and post-hoc TUKEY test (α=0.05).

Results: According to the data obtained, no significant difference was found between the groups produced by the milling method (p=0.878). While no difference was found between the group produced by additive manufacturing and the Cerasmart group (p=0.110), it was observed that the flexural strength was significantly lower than the Brilliant Crios group (p=0.040).

Conclusion: As a result of this in vitro study, the lowest biaxial flexural strength after thermal aging among the groups was observed in the group produced with additive manufacturing.

Key words: Additive manufacturing, biaxial flexural strength, CAD/CAM

ÖZ

Amaç: Bu in vitro çalışmanın amacı termal yaşlandırma sonrası eklemeli üretim ve kazıma yöntemi ile üretilen daimi restoratif materyallerin biaksiyal eğilme dayanımlarını karşılaştırmaktır.

Gereç ve Yöntem: Bu çalışma için 3D yazıcı (Form3, Formlabs) ile daimi kron materyali kullanılarak (Permanent Crown, Somerville, MA) ve kazıma yöntemi ile iki farklı rezin içerikli materyal (Brilliant Crios (Coltene/Whaledent; Cerasmart (GC Europe) ile 10 mm×2 mm boyutlarında disk şeklinde örnekler üretilerek üç çalışma grubu oluşturuldu (n=10). Üretim aşamaları üretici talimatlarına uygun şekilde gerçekleştirildi. Daha sonra tüm örnekler su altında silikon karbid kâğıtlarla zımparalandı. Ardından örnekler termal yaşlandırmaya (5-55°C, 5000 döngü) tabi tutuldu. Yaşlandırma sonrası tüm örneklerin universal test cihazı ile biaksiyal eğilme dayanımı değerleri ölçüldü. Elde edilen veriler Tek yönlü ANOVA testi ve post-hoc TUKEY testi ile değerlendirildi. (α=0,05)

Bulgular: Elde edilen verilere göre kazıma yöntemi ile üretilen gruplar arasında anlamlı fark bulunmamıştır. (p=0,878) Eklemeli üretimle üretilen grubun Cerasmart grubuyla arasında fark bulunmazken (p=0,110) Brillant Crios grubundan anlamlı ölçüde düşük eğilme dayanımı gösterdiği görülmüştür. (p=0,040)

Sonuçlar: Elde edilen sonuçlara göre gruplar arasında termal yaşlandırma sonrası en düşük biaksiyal eğilme dayanımı eklemeli üretimle üretilen grupta görülmüştür.

Anahtar Kelimeler: Eklemeli üretim, biaksiyal eğilme dayanımı, CAD/CAM

INTRODUCTION

As digital dentistry continues to develop day by day, it offers innovations in both additive and subtractive systems. Esthetic concerns and the requirements for rapid and predictable restorations have increased the trend towards chairside procedures (1). While many restorations can be produced by saving time through intraoral scanners and milling methods, patients can also have insights about the final restoration thanks to digital designs (2, 3). Through CAD/CAM systems, a wide variety of restorations have been produced using subtractive methods for many years, using blocks or discs (4). Subtractive or milling

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methods today have a wide range of materials and offer many successful alternatives to expectations (5). After the appropriate material is selected, a previously designed restoration can be produced by means of a milling device (6). However, despite its many advantages, material wastage is inevitable and microreproducibility is questioned on the inner surfaces of the restorations due to factors related to the milling device, and the material geometry affects the success (7, 8).

Additive manufacturing methods are now known by different names such as 3D manufacturing and rapid prototyping (9). This method, which has been used in metal-supported restorations under the name of selective laser sintering for years in dentistry, allows the production of almost all types of restorations with the production of tooth-like colored materials and the development of devices using different Technologies (10). These developments in additive systems contribute to the adaptation to the full digital workflow of prosthetic applications (11). In the literature, different layering systems have been introduced for this technology. SLA and DLP systems, which often use the vat polymerization method, are widely used in dentistry (12). In this system, a powder or liquid material is polymerized and solidified to create 3-dimensional objects (13). Multiple simultaneous productions are possible, and material wastage is greatly reduced as the remaining material can be used repeatedly (14). Production speed, type of material, and restoration properties may vary depending on the capacity of the 3D printer (15). Using these technologies, surgical guides, dental models, maxillofacial prostheses, occlusal splints, and prosthetic infrastructures can be produced (11).

While only temporary crown materials were available for 3D printers until recent years, permanent crown materials are now also available in dental markets (16). However, the variety in these relatively new materials is limited. In addition, information about the mechanical properties of these materials is quite insufficient. Therefore, the aim of this in vitro study is to compare the flexural strength of permanent restorative materials produced by additive manufacturing and milling after thermal aging. The hypothesis of the study is that there would be no difference between the flexural strengths of the materials after thermal aging.

MATERIAL and METHOD

In the study, three study groups were used for different materials, 1 printed and 2 milled (n=10). An STL file was created for the disc-shaped specimens designed with a diameter of 10 mm and a thickness of 2 mm in accordance with ISO standards. The STL data prepared for the Printed group were transferred to the nesting software compatible with the 3D printer. The printing direction was determined as horizontal and after the supports were placed, the data was transferred to the 3D printer (Form 3; Formlabs) with SLA technology. The printed specimens were first immersed in the bath tank (Form Wash; Formlabs) for 3 minutes with 99% isopropyl alcohol (IPA). Then, it was cured at 60°C for 20 minutes in the curing device (Formcure; Formlabs) in accordance with the manufacturer's instructions, and the curing process was repeated after the supports were removed.

Two different resin-based restorative materials (Brilliant Crios [Coltene/Whaledent]; Cerasmart [GC Europe]) were used for the Milled groups. The discs were produced with the same STL data by using CAD/CAM blocks through the milling device. All produced specimens were polished under water with silicon carbide papers. Then, the specimens were subjected to 5000 cycles of thermal aging with a 30-second dwell time (5-55ºC). After aging, the biaxial flexural strength test of the specimens was performed on a Universal test device.

$$\frac{\sigma = -0{,}25\ N\ (X - Y)}{b^2}$$

$$X = (1 + v)\ In\ \left(\frac{r_2}{r_3}\right)^2 + [(1 - v)/2]\ \left(\frac{r_2}{r_3}\right)^2$$

$$Y = (1 + v)\left[1 + In\ \left(\frac{r_1}{r_3}\right)^2\right] + (1 - v)\left(\frac{r_1}{r_3}\right)^2;$$

σ flexural strength (MPa), N fracture load (N), v value Poisson ratio (=0.3), r1 radius of support circle (mm), r2 radius of loaded area (mm), r3 specimen radius (mm), b the thickness of the specimen (mm).

The distribution of the obtained data was evaluated with the Saphiro-Wilk normality test. Then, one-way ANOVA and the Tukey Posthoc test were applied. All analyses were performed using statistical software.

RESULTS

According to the results of one-way analysis of variance, there was a significant difference between the groups (df:2, F:3.758, p=0.036). According to the data obtained, there was no significant difference between the groups produced by the milling method (p=0.878). While there was no difference between the additive manufacturing group and the Cerasmart group (p=0.110) it was observed that the flexural strength was significantly lower than the Brillant Crios group (p=0.040). Table 1 presents the descriptive statistics of flexural strength values. The highest flexural strength was seen in the BC group (313.49 MPa), followed by Cerasmart (305.92 MPa). The lowest flexural strength belongs to the printed specimens (273.41 MPa).

DISCUSSION

Nowadays, resin-containing materials are preferred for permanent restorations as well as popular materials such as glass ceramics and zirconia (17). Subtractive and additive manufacturing technologies are developing rapidly and allow the long-term use of resin-containing materials. Resin materials produced for 3D printers using additive manufacturing technologies have been introduced mostly for interim restorations. However, there have been resin materials introduced by some companies to the dental market for permanent restorations in

Table 1: Mean ± standard deviation biaxial flexural strength (BFS in MPa) values

Material	BFS
BC	313.49 (287.49-339.49)[a]
CE	305.92 (277.60-334.24)[a]
Printed	273.41 (254.06-292.76)[b]

*Different superscript letters indicate significant differences ($p<0.05$)

recent years. Knowledge of permanent 3D printed resins, which are relatively new compared to interim ones, is rather limited. Therefore, in our study, the flexural strength of the 3D printed material, which is defined as permanent resin, was compared with the milled resin material with two different contents. As a result of the experiments, there was a significant difference between the BFS values after thermal aging of the permanent crown materials produced with different production techniques, thus the hypothesis of the study was rejected.

In the present study, while the highest fracture strength belonged to the BC group, no significant difference was observed between CE, another milled material. The nonsignificant difference in flexural strength of the materials can be attributed to the relatively similar contents of the materials. The filler content and particle sizes of both materials are similar (18). In a previous study, the fracture strength of permanent crowns, including Cerasmart and Brillant Crios materials, was evaluated (19). The researchers reported that although the BC group had the highest fracture strength, it was not statistically different from the CE group, which is consistent with the results of the presented study. However, the 3D group used in the same study showed similar fracture strength to BC, in contrast to this study (19). This difference may be due to different specimen thicknesses and shapes, the content of the 3D resin materials used and the test conditions. In another study testing the fracture strength of 3D permanent crown material, the researchers also evaluated 3 different millable materials, including BC and CE blocks. As a result of the study in which all specimens were produced in the form of implant supported crowns, no difference was found between the experimental groups in terms of fracture strength (16). In another study in which permanent 3D printed material was produced as implant-supported screw-retained crowns, researchers tested the fracture strength of anterior and premolar crowns (20). Researchers have reported that crowns produced by subtractive manufacturing have higher fracture strength than those produced by additive manufacturing. However, in the aforementioned study, unlike this study, a 3D printer with DLP technology was used instead of SLA.

The composition of resin materials affects water absorption rates, and mechanical and physical properties. Details of the chemical composition of the materials used are required for more accurate comparisons (21). The content of the Permanent Crown (Formlabs) material used in present study has not yet been disclosed by the manufacturer. Although there are a few studies using 3D printed permanent resins in the literature, a different brand of restoration material was used in this study. Therefore, it is not possible to make a comparison in terms of material content.

There are many parameters that affect the mechanical properties of materials in production with 3D printers (22). Factors such as printing direction, layer thickness, and the postpolymerization process have been evaluated in many studies and it has been reported that 3D printed interim materials have effects on flexural resistance (22-27). However, the results presented in previous studies belong to interim materials. In the present study, only the manufacturer's instructions were followed and no changes were made to the parameters. Evaluation of different parameters in further studies will provide more detailed information. Moreover, this study has some limitations. It has been reported that glaze application improves the mechanical and optical properties of the materials before the clinical use of resin-containing materials (18, 28). However, the glaze process was not applied to the specimens in this study. In addition, because only a group of printed materials was used and the content of this material has not been disclosed by the producer yet, the inability to interpret the results in terms of content can be shown as another limitation. In future studies, evaluating 3D printed resins from different companies together, applying thermomechanical aging procedures, and examining the effects of different parameters will contribute to the addition of more detailed information in the literature. Within the limitations of this current study, it was concluded that permanent resin materials produced by additive manufacturing offered lower biaxial flexural strength after thermal aging than those produced by the milling method.

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REFERENCES

1. Alghazzawi TF. Advancements in CAD/CAM technology: Options for practical implementation. J Prosthodont Res 2016;60(2):72-84.
2. Lee WS, Lee DH, Lee KB. Evaluation of internal fit of interim crown fabricated with CAD/CAM milling and 3D printing system. J Adv Prosthodont 2017;9(4):265-70.
3. Proussaefs P. A Novel Technique for Immediate Loading Single Root Form Implants with an Interim CAD/CAM Milled Screw-Retained Crown. J Oral Implantol 2016;42(4):327-32.
4. Sulaiman TA. Materials in digital dentistry-A review. J Esthet Restor Dent 2020;32(2):171-81.

5. Spitznagel FA, Boldt J, Gierthmuehlen PC. CAD/CAM Ceramic Restorative Materials for Natural Teeth. J Dent Res 2018;97(10):1082-91.
6. Peng CC, Chung KH, Ramos V, Jr. Assessment of the adaptation of interim crowns using different measurement techniques. J Prosthodont 2020;29(1):87-93.
7. Torabi K, Farjood E, Hamedani S. Rapid prototyping technologies and their applications in prosthodontics, a review of literature. J Dent (Shiraz) 2015;16(1):1-9.
8. Bayarsaikhan E, Lim JH, Shin SH, Park KH, Park YB, Lee JH, et al. Effects of postcuring temperature on the mechanical properties and biocompatibility of three-dimensional printed dental resin material. Polymers (Basel) 2021;13(8):1180.
9. Alammar A, Kois JC, Revilla-Leon M, Att W. Additive manufacturing technologies: current status and future perspectives. J Prosthodont 2022;31(S1):4-12.
10. Schweiger J, Edelhoff D, Guth JF. 3D printing in digital prosthetic dentistry: an overview of recent developments in additive manufacturing. J Clin Med 2021;10(9):2010.
11. Kessler A, Hickel R, Reymus M. 3D printing in dentistry-state of the art. Oper Dent 2020;45(1):30-40.
12. Hata K, Ikeda H, Nagamatsu Y, Masaki C, Hosokawa R, Shimizu H. Development of dental poly(methyl methacrylate)-based resin for stereolithography additive manufacturing. polymers (Basel) 2021;13(24):4435.
13. Unkovskiy A, Schmidt F, Beuer F, Li P, Spintzyk S, Kraemer Fernandez P. Stereolithography vs. direct light processing for rapid manufacturing of complete denture bases: an in vitro accuracy analysis. J Clin Med 2021;10(5):1070.
14. Piedra-Cascon W, Krishnamurthy VR, Att W, Revilla-Leon M. 3D printing parameters, supporting structures, slicing, and post-processing procedures of vat-polymerization additive manufacturing technologies: A narrative review. J Dent 2021;109:103630.
15. Della Bona A, Cantelli V, Britto VT, Collares KF, Stansbury JW. 3D printing restorative materials using a stereolithographic technique: a systematic review. Dent Mater 2021;37(2):336-50.
16. Donmez MB, Okutan Y. Marginal gap and fracture resistance of implant-supported 3D-printed definitive composite crowns: An in vitro study. J Dent 2022;124:104216.
17. Jovanovic M, Zivic M, Milosavljevic M. A potential application of materials based on a polymer and CAD/CAM composite resins in prosthetic dentistry. J Prosthodont Res 2021;65(2):137-47.
18. Matzinger M, Hahnel S, Preis V, Rosentritt M. Polishing effects and wear performance of chairside CAD/CAM materials. Clin Oral Investig 2019;23(2):725-37.
19. Zimmermann M, Ender A, Egli G, Ozcan M, Mehl A. Fracture load of CAD/CAM-fabricated and 3D-printed composite crowns as a function of material thickness. Clin Oral Investig 2019;23(6):2777-84.
20. Martin-Ortega N, Sallorenzo A, Casajus J, Cervera A, Revilla-Leon M, Gomez-Polo M. Fracture resistance of additive manufactured and milled implant-supported interim crowns. J Prosthet Dent 2022;127(2):267-74.
21. Revilla-Leon M, Meyers MJ, Zandinejad A, Ozcan M. A review on chemical composition, mechanical properties, and manufacturing work flow of additively manufactured current polymers for interim dental restorations. J Esthet Restor Dent 2019;31(1):51-7.
22. Nold J, Wesemann C, Rieg L, Binder L, Witkowski S, Spies BC, et al. Does printing orientation matter? in-vitro fracture strength of temporary fixed dental prostheses after a 1-year simulation in the artificial mouth. Materials (Basel) 2021;14(2):259.
23. Alharbi N, Osman R, Wismeijer D. Effects of build direction on the mechanical properties of 3D-printed complete coverage interim dental restorations. J Prosthet Dent 2016;115(6):760-7.
24. Diken Turksayar AA, Donmez MB, Olcay EO, Demirel M, Demir E. Effect of printing orientation on the fracture strength of additively manufactured 3-unit interim fixed dental prostheses after aging. J Dent 2022;124:104155.
25. Alshamrani AA, Raju R, Ellakwa A. Effect of printing layer thickness and postprinting conditions on the flexural strength and hardness of a 3D-printed resin. Biomed Res Int 2022;2022:8353137.
26. Scherer M, Al-Haj Husain N, Barmak AB, Kois JC, Ozcan M, Revilla-Leon M. Influence of the layer thickness on the flexural strength of aged and nonaged additively manufactured interim dental material. J Prosthodont 2022:1-6. doi: 10.1111/jopr.13582.
27. Scherer MD, Barmak BA, Ozcan M, Revilla-Leon M. Influence of postpolymerization methods and artificial aging procedures on the fracture resistance and flexural strength of a vat-polymerized interim dental material. J Prosthet Dent 2022;128(5):1085-93.
28. Yao Q, Morton D, Eckert GJ, Lin WS. The effect of surface treatments on the color stability of CAD-CAM interim fixed dental prostheses. J Prosthet Dent 2021;126(2):248-53.

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THE EFFECT OF SYSTEMIC ZOLEDRONIC ACID ON THE HEALING POTENTIAL OF RATS WITH EXPERIMENTAL PERIODONTITIS

SİSTEMİK OLARAK KULLANILAN ZOLEDRONİK ASİTİN, DENEYSEL PERİODONTİTİS OLUŞTURULAN SIÇANLARDA İYİLEŞME POTANSİYELİ ÜZERİNE ETKİSİNİN İNCELENMESİ

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ABSTRACT

Objective: In this study, our goal was to observe the effect of zoledronic acid histopathologically, which is used without removing the periodontitis agent, on the healing potential of the tissue after the experimental periodontitis formation in an animal model.

Material and Method: 30 adult male Sprague-Dawley rats were divided into 2 groups as bisphosphonate users and non-users. At the beginning of the experiment, all rats were put under anesthesia, and 5.0 silk sutures were placed around their right upper first molars. No suture was placed around the left upper first molar teeth. It was waited for 3 weeks after the placement of the sutures. After experimental periodontitis was observed in the animals on the 21st day, 7.5uq/kg zoledronic acid was injected intramuscularly for 6 weeks in the animals in the experimental group. After intramuscular drug administration once a week for 6 weeks, weekly weight monitoring was performed on days 0, 7, 14, 21, 28, and 35 and noted in the experimental group rats.

At the end of 6 weeks, the sutures were removed under the anesthesia from the experimental group animals whose last drug injections were completed and the control group animals that were administered 0.9% saline on the same days. A recovery period of two weeks was expected after which all animals were sacrificed.

Results: Zoledronic acid was used in the histological evaluation results, and experimental inflammation, necrosis, periodontal space and epithelial proliferation in the group with periodontitis statistically significant $p<0.05$ was found to be higher.

Conclusion: When evaluated clinically, positive effects on wound healing were observed in rats treated with bisphosphonate-derived drugs by treating existing periodontitis prior to drug administration.

Key words: Zoledronic acid, rat, experimental periodontitis, osteonecrosis, histology

ÖZ

Amaç: Bu çalışmada amacımız hayvan modelinde deneysel periodontitis oluşumundan sonra, periodontitis etkeni kaldırılmadan kullandırılan zoledronik asidin, periodontitis etkeni ortadan kaldırıldıktan sonra dokunun iyileşme potansiyeli üzerindeki etkisini histopatolojik olarak gözlemlemeyi hedeflemektir.

Gereç ve Yöntem: Sprague-Dawley cinsi 30 adet yetişkin erkek sıçan bifosfanat kullanan ve kullanmayan olarak 2 gruba ayrılmıştır. Deney başlangıcında tüm sıçanlara, anestezi altında, sağ üst 1.molar dişlerinin etrafına 5.0 ipek dikiş yerleştirilmiştir. Sol üst 1. molar dişlere ise herhangi bir uygulama yapılmamıştır. Dikişlerin yerleştirilmesinden sonra 3 hafta beklenilmiştir. 21. günde hayvanlarda deneysel periodontitis gözlendikten sonra deney grubundaki hayvanlara kas içi 6 hafta boyunca 7,5uq/kg zoledronik asit enjekte edilmiştir. 0., 7., 14., 21., 28., ve 35.günlerde; 6 hafta boyunca, haftada bir, kas içi ilaç verilmesinden sonra deney grubu sıçanlarında, haftalık ağırlık takibi yapılmıştır ve not edilmiştir. Son ilaç enjeksiyonları tamamlanan deney grubu hayvanlarının ve aynı günlerde %0,9 serum fizyolojik uygulanan kontrol grubu hayvanlarının 6 hafta sonunda, anestezi altında yerleştirilen dikişleri kaldırılmıştır. İki haftalık iyileşme süresi için beklenilmiş ve bu iki hafta içinde de deney grubundaki hayvanlara zoledronik asit enjekte edilmiştir.

Bulgular: Histolojik değerlendirme sonuçlarında zoledronik asit kullanılmış ve deneysel periodontitis oluşturulan grupta iltihap, nekroz, periodontal aralık ve epitel proliferasyonu istatiksel anlamlı $p<0,05$ olarak daha fazla bulunmuştur

Sonuç: Klinik açıdan değerlendirildiğinde, bifosfanat türev ilaç kullandırılmış sıçanlarda, ilaç kullanımı öncesinde, var olan periodontitisin tedavi edilmesinin yara iyileşmesi üzerinde olumlu etkileri gözlenmiştir.

Anahtar Kelimeler: Zoledronik asit, sıçan, deneysel periodontitis, osteonekroz, histoloji

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INTRODUCTION

Periodontitis is a chronic infectious disease characterized by inflammation of the tissues supporting the teeth, resulting in loss of periodontal support tissues; loss of attachment and significant bone loss due to the accumulation of chronic plaque and calculus in its pathogenesis (1-3). Bisphosphonates are chemical compounds characterized by phosphorus-carbon-phosphorus structure (4). Nitrogenous bisphosphonates show their antiresorptive effects via the mevalonate pathway. Inhibition of farnesyl pyrophosphate synthesis leads to inhibition of the mevalonate pathway end product, geranylgeranyl pyrophosphate (5,6). Although the effect of bisphosphonates on bone tissue has been clearly demonstrated in scientific studies today, studies on the effect on the oral mucosa are limited (7,8). The limited number of studies conducted are not sufficient to determine the safety, efficacy and usage doses of bisphosphonates. It has been shown in studies that this drug, which acts on the bone tissue, also affects the jaw bones and periodontal tissues. Today, there are studies showing that the use of bisphosphonates increases the risk of osteonecrosis in the jaw bones (9,10). However, studies on the effects of bisphosphonates on periodontal tissue are insufficient. It is clear that more work needs to be done on this subject.

Zoledronic acid is a strong, new generation nitrogen-containing imidazole cyclic side-chain heterocyclic nitrogen-containing bisphosphonate. The difference from other bisphosphonates is the presence of the second nitrogen atom in the ring structure. Zoledronic acid (ZA) has the highest binding ability to bone among bisphosphonates; it has antitumor activity that affects apoptosis, tumor cell growth, adhesion, invasion and angiogenesis (11). Although there is knowledge about the effect of bisphosphonates on healthy bone tissue in scientific studies today, its possible effects on periodontal tissues in the presence of chronic periodontal inflammation and when this inflammation is eliminated constitute a new field of study (12,13).

Therefore, the aim of this study is to histopathologically evaluate the effect of zoledronic acid on the healing potential of periodontal tissues after removal of the periodontitis agent in an experimental periodontitis model.

MATERIAL and METHOD

Ethics committee approval

For the animal subjects to be used in the experiments, T.C. Istanbul University, Aziz Sancar Experimental Medicine Research Institute, Animal Experiments Local Ethics Committee's Presidency approval was obtained on 25.09.2020 with the number 2020/26.

The sample number of the study was calculated with the program named G*Power 3.1.9.2. Based on the results of Vaycan's study and considering the distribution of histological findings, the sample size for each group was made with a minimum of n=15 animals, a total of 60 cases, and the power of the test was 98.8% according to the PostHoc power analysis result (14).

Procedures

In our study, a total of 30 male rats, Sprague-Dawley, 15 in control and 15 in experimental groups, were used. Periodontitis was induced by placing suture on the right molar teeth of the rats,but the left side was never touched. Therefore, 2 different groups were created in each rat, and there are 4 different groups in total. According to the condition of the periodontium in the right upper jaw and left upper jaw of the 15 rats in the control group; a control group of 15 rats with healthy periodontium and a control group of 15 rats with periodontitis were formed. The same situation is valid for rats in the experimental group.

Experimental animals are housed in polycarbonate transparent cages at room temperature of 21 degrees Celsius, humidity of 60%, with 2 rats in each cage. Each cage contains libitum pellets and fresh, clean tap water.

At the beginning of the experiment, all rats were ligated with 5.0 silk sutures around the right upper first molars under general anesthesia. Clinical signs of periodontitis in animals were evaluated by measuring bleeding on probing on day 21 after placement of the sutures. Animals in the experimental group were injected intramuscularly with 7.5uq/kg zoledronic acid for 6 weeks. The animals in the control group were injected with 0.9% saline for 6 weeks.

Following the administration of drugs and saline for 6 weeks, the sutures around the first molar teeth were removed under anesthesia, and it was waited for 2 weeks for healing. Zoledronic acid continued to be given to the experimental group for the expected time for this improvement. The control group continued to be given physiological saline.

All animals were then sacrificed by cervical dislocation. In the cervical dislocation method, the spinal cord is severed, and the connection between the brain and vital organs is lost.

This sacrification method was preferred due to the fact that it does not cause chemical substance residues in the tissues and forms rapid death, and with the approval of the ethics committee.

After sacrification, the upper jaws were resected, and the removed parts were kept in 10% buffered formalin solution for 2 weeks. After fixation, it was decalcified in 20% sodium citrate and 50% formic acid solution.

After this process, the hard palate was removed from the vestibule surfaces of the molar teeth from all jaws. Sagittal sections passing through the middle were taken, and dissection of the parts was made. Vestibule faces 3 microns obtained from paraffin blocks prepared by laying on the cross-sectional

Table 1: The flow followed during the experiment

Day	Experiment	Control
1	Silk stitch binding upper right 1.molar	Silk stitch binding (upper right 1.molar)
21	ZA injection	SF injection
28	ZA injection	SF injection
35	ZA injection	SF injection
42	ZA injection	SF injection
56	ZA injection	SF injection
63	ZA injection Removal of stitches	SF injection Removal of stitches
70	ZA injection	SF injection
77	ZA injection	SF injection
84	Sacrification	Sacrification

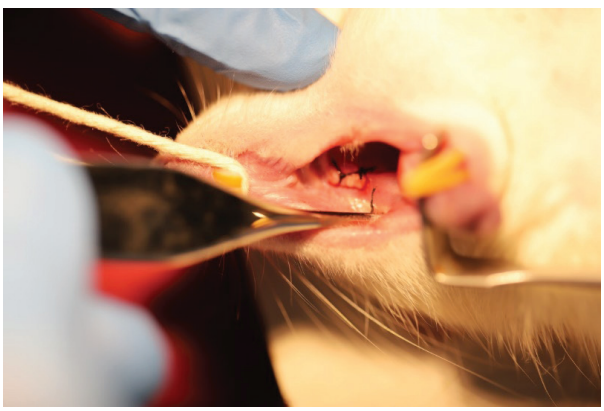

Figure 1: Placement of sutures in the interproximal areas of the upper jaw of rats

Statistical analysis

Data were analyzed with IBM SPSS V23. Conformity to the normal distribution was examined using the Shapiro-Wilk Test. In the data that did not confirm to the normal distribution, comparisons between groups were made with the Kruskal Wallis Test, and multiple comparisons were examined with the Dunn Test.

In categorical data, comparisons between groups were made with the Pearson Chi-Square test, and multiple comparisons were examined with the Bonferroni-corrected Z test. For categorical data, results were presented as frequency (percentage). Significance level was accepted as $p<0.050$.

RESULTS

Histological evaluation

Histological images and statistical results obtained from the experimental animals are below. When the results were evaluated histologically, inflammation, necrosis increase in periodontal space and epithelial proliferation were observed more in the groups with experimental periodontitis where zoledronic acid was used, and it was statistically significant ($p<0.05$) (Figures 2, 3, 4, 5).

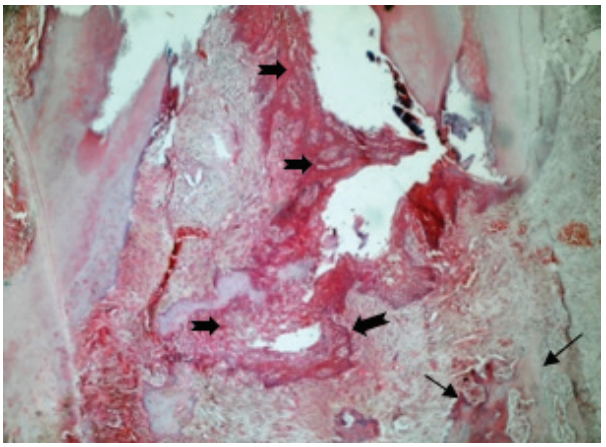

Figure 2: Necrotic bone fragments are seen in the histopathological image of the EEP (experimental group in experimental periodontitis) group (H&EX100).
Thick arrows: stratified squamous epithelium, thin arrows: necrotic bone

In the experimental animals, necrotic bone was found in one or more areas in 46.7 percent of the first molar teeth that were given zoledronic acid and ligated (Table 3). In the presence of experimental periodontitis, epithelial proliferation in subjects using zoledronic acid was found to be statistically significant compared to healthy periodontal groups (Table 2).

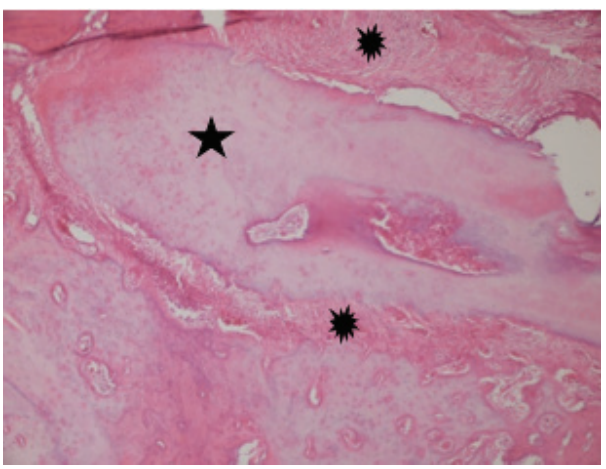

Figure 3: In the histopathological image of the EHP (experimental group in healthy periodontium group), tooth roots and periodontal tissues with normal physiological appearance are seen (H&EX100. Star: tooth's root, asterisk: periodontal space).
No necrotic bone was found around the first molar roots of the subjects in this group. No epithelial proliferation was observed around the roots of the first molar tooth.
Star: Tooth's root, thick arrows: Alveolar bone, asterisk: Periodontal space

No necrotic bone was found in any of the subjects in this group. There is no inflammatory infiltration in this group (Tables 1, 3).

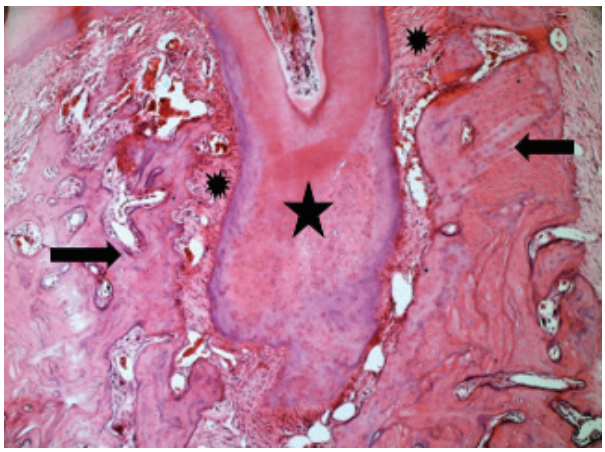

Figure 4: In the histopathological image of the CHP (control group healthy periodontium) group, natural tooth roots and healthy periodontal tissues are seen around them(H&EX100). Thin arrows: cell epithelium, star: Tooth's root

The presence of necrotic bone was not detected in the experimental animals in this group. In this group, inflammatory infiltration is 60 percent. This shows that periodontal inflammation developed significantly in ligatured teeth (Table 2).

Statistics

A statistically significant difference was found between the distributions of inflammation between the groups (p<0.001).

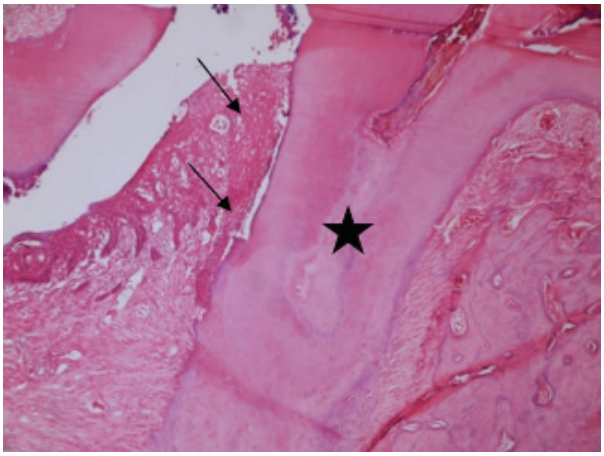

Figure 5: Histopathological image of CDP (control group experimental periodontitis) group (H&EX100)

Epithelial proliferation distributions between the groups showed statistically significant differences (p<0.001). A statistically significant difference was found between the distributions of necrosis status between the groups (p=0.001). No necrosis was found in the CDP, CHP, and EHP groups (Table 4). The median value of the periodontal space in the CHP group was lower than the median value of the EEP group (Table 5). A statistically significant difference was found between the median value of the periodontal space in the CHP group and the median value of the periodontal space in the EEP group (p<0.001).

Table 2: Histopathology results- inflammation

	CEP	CHP	EEP	EHP	Total	p
Inflammation						
None	6 (40)a	15 (100)b	5 (33.3)a	15 (100)b	41 (68.3)	
Mild	9 (60)a	0 (0)b	8 (53.3)a	0 (0)b	17 (28.3)	**<0.001***
Moderate	0 (0)a	0 (0)a	2 (13.3)a	0 (0)a	2 (3.3)	

*Pearson Chi-Square Test; a-b: No difference between groups with the same letter in each line, frequency (percent) EEP: Experimental group in experimental periodontitis group, EHP: Experimental group in healthy periodontium group, CHP: Control group healthy periodontium, CDP: Control group experimental periodontitis

Table 3: Histopathology results- epithelial cell proliferation

	CEP	CHP	EEP	EHP	Total	p
Epithelial proliferation						
None	11 (73.3)abc	15 (100)c	5 (33.3)b	15 (100)ac	46 (76.7)	
Mild	4 (26.7)a	0 (0)a	5 (33.3)a	0 (0)a	9 (15)	**<0.001***
Moderate	0 (0)a	0 (0)a	2 (13.3)a	0 (0)a	2 (3.3)	
Severe	0 (0)a	0 (0)a	3 (20)a	0 (0)a	3 (5)	

*Pearson Chi-Square Test, a-c: There is no difference between the distributions of groups with the same letter, frequency (percentage)
EEP: Experimental group in experimental periodontitis group, EHP: Experimental group in healthy periodontium group, CHP: Control group healthy periodontium, CDP: Control group experimental periodontitis

Table 4: Histopathology results- Osteonecrosis

	CEP	CHP	EEP	EHP	Total	p
Osteonecrosis						
None	15 (100)a	15 (100)a	8 (53.3)b	15 (100)a	53 (88.3)	**0.001***
Mild	0 (0)a	0 (0)a	6 (40)b	0 (0)a	6 (10)	
Moderate	0 (0)a	0 (0)a	1 (6.7)a	0 (0)a	1 (1.7)	

* Pearson Chi-Square Test, a-b: No difference between groups with the same letter in each line, frequency (percent); EEP: Experimental group in experimental periodontitis) group, EHP: Experimental group in healthy periodontium group, CHP: control group healthy periodontium, CDP: Control group experimental periodontitis

Table 5: Histopathology results- periodontal space group

	n	Average	Standard deviation	Median	Minimum	Maximum	p
CEP	15	0.1227	0.0212	0.13	0.09	0.16	**<0. 001***
CHP	15	0.07	0.0169	0.07	0.04	0.1	
EEP	15	1.0227	3.31348	0.17	0.14	13	
EHP	15	0.0953	0.1125	0.07	0.05	0.5	
Total	60	0.3277	1.66508	0.095	0.04	13	

*Kruskal Wallis Test, EEP: Experimental group in experimental periodontitis group, EHP: Experimental group in healthy periodontium group, CHP: Control group healthy periodontium, CDP: Control group experimental periodontitis

DISCUSSION

The evaluation of the findings is from a histopathological point of view. With this method, we aimed to examine the effect of using zoledronic acid on the healing of periodontal tissues when the factor causing the periodontal problem is removed.

In other words, the medical situation applied in our study is to evaluate the effect of ZA treatment on the periodontal tissue of the patients whose periodontal health is not in good condition, but who received zoledronic acid due to medical need, when they regain their periodontal health in this process. In this way, we aimed to obtain new strategies for the prevention and treatment of the condition that causes alveolar bone loss in periodontal diseases.

The most important effect of bisphosphonate is to prevent bone resorption by inhibiting osteoclast activity. The decrease in osteoclast activity causes a change in the osteoclast-osteoblast interaction. Considering this feature of bisphosphonates, the extent to which it will control alveolar bone destruction due to periodontal diseases can form the basis of a new field of study (15).

Dental trauma procedures, such as tooth extraction, are serious risk factors for drug-induced osteonecrosis. More than fifty percent of patients with osteonecrosis of their jaws have a history of tooth extraction. It is known that tooth extraction increases the risk of developing osteonecrosis 33 times (16,17). However, it can be thought that the spontaneous development of drug-induced jaw necrosis is related to the presence of periodontal or periapical infection in the mouth.

The most well-known side effect of bisphosphonates is necrosis of the jaws. Reported bisphosphonate-related chin necrosis emerged after the use of infused, nitrogen-containing drugs such as zoledronic acid or pamidronate (18). Bacterial infection and inflammation are present in the lesions revealed in the bronchi.

Oral mucosa has a unique and complex structure in the human body. Unlike other parts of the body, it is very close to the bone underneath. Fat and muscle tissues in the mouth serve as an insulator between the cells of the oral mucosa and the bisphosphonate-rich bone that is treated with bisphosphonates. Areas with a tendency to BRONJ in the mouth are areas with thin oral mucosa, such as the crests of the torus, maxilla, and mandible. These anatomical areas are formations specific to the oral environment. The occurrence of these necrosis, which is a side effect of bisphosphonates, especially in the oral mucosa, shows that the cells here have an important place in explaining the formation of BRONJ. It is a matter of debate whether BRONJ originates from the cells of the oral mucosa or from the underlying bone (19, 20).

Unlike the results of our study, in the study of Li et al., in 2 of 15 animals in the periodontally healthy group, in which zoledronic acid was used, and silk sutures were not placed; the presence of necrotic bone, characterized by large empty lacunae and loss of mineral density in the osteocytes, was observed (21). This difference is remarkable and can be thought to be related to technical sensitivity and sample size.

According to the results of our study, in the group with periodontal destruction and ZA (EEP), bone necrosis was detected at a rate of 46.7% around the alveolar bone and root. Bone necrosis was not observed in the experimental (EHP) and control groups (CHP), which were periodontally healthy, and in the control groups (CEP) with periodontal destruction.

The presence of necrotic bone was significantly higher in the experimental group given this ZA and periodontal destruction compared to the other groups.

When the experimental group experimental periodontitis (EEP) and the experimental group healthy periodontium (EHP) groups were compared, the presence of inflammation was not present in EHP. According to the findings of our study, no inflammation was found in either group as a result of the histopathological results obtained from the healthy periodontium group (CHP) that did not use ZA and the healthy periodontium group (EHP) that used ZA. As a result of these histopathological findings, it can be thought that ZA alone does not potentiate the inflammatory effect. By inhibiting the mevalonate pathway ZA, which is one of the nitrogenous bisphosphonates, they disrupt the cytoskeletal structure of the osteoclast, thereby inhibiting bone resorption.

In the study of Aghaloo et al., the role of progressive periodontal disease due to the stimulation of osteonecrosis of the jaw due to bisphosphonate use was examined by microtomography and histopathological method on ligatured rats (22).

Aghaloo et al.'s drug-induced inflammation hypothesis of osteonecrosis of the jaw parallels with the presence of inflammation and necrosis in the EEP group in our study.

According to the results of the study by Aghaloo et al., out of 19 animals with experimental periodontitis given ZA, 4 had exposed bone surfaces. Unlike our study, experimental periodontitis was created, and necrotic areas characterized by empty lacunae were found in 1 of 19 animals in the control group that did not receive ZA.

CONCLUSION

In our study, zoledronic acid was used in subjects with existing periodontitis, and its effects on alveolar bone destruction and necrosis were investigated. In the histopathological findings in our study; in the presence of periodontal disease, necrotic bone was present when we gave zoledronic acid, but in the absence of periodontal disease, no necrotic bone finding was found despite giving zoledronic acid.

These findings show that there is bone necrosis due to bisphosphonate use in the presence of periodontal disease, which is the precursor of periodontal destruction. More studies are needed to evaluate the possibility of bone necrosis caused by systemic use of bisphosphonates in cases where periodontal health is preserved. In addition, new immunohistochemical studies are needed to explain the effect of zoledronic acid on proliferation and differentiation in the bone formation mechanism. The healing potential of zoledronic acid on the tissue can be evaluated more effectively by changing the dose, the frequency of administration, the period of use, and by creating more study groups.

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REFERENCES

1. Page RC, Shroeder HE. Pathogenesis of inflammatory periodontal disease. A summary of current work. Lab Invest 1976;34(3):235-49.
2. Seymour GJ, Taylor JJ. Shouts and whispers: an introduction to immunoregulation in periodontal disease. J Clin Periodontol 2004;35(3):9-13.
3. Leira Y, Iglesias-Rey R, Gomez-Lado N, Aguiar P , Sobrino T, D'Aiuto F et al. Periodontitis and vascular inflammatory biomarkers: an experimental in vivo study in rats. Odotonlogy 2020;108(2):202-12.
4. Rusell RGG. Bisphonates from bench to bedside. Ann NY Acad Sci 2006;1068:367-401.
5. Fleisch H. Development of bisphosphonates. Breast Cancer Res 2002;4(1):30-4.
6. Russell RGG, Watts NB, Ebetino FH, Rogers MJ. Mechanisms of action of bisphosphonates: Similarities and differences and their potential influence on clinical efficacy. Osteoporos Int 2008;(19):733-59.
7. Cheng A, Mavrokokki T, Carter G, Stein B, Fazzalari N, Wilson D, et al. The dental implications of bisphosphpnates and bone disease. Aust Dent J 2005;50(4 Suppl 2):S4-13.
8. Russell RGG. Bisphosphonates: The first 40 years. Bone 2011;49(1):2-19.
9. Marx RE, Cillo JE, Ulloa JJ. Oral bishosphonate-induced osteonecrosis: risk factors, prediction of risk using serum CTX testing, prevention and treatment. J Oral Maxillofac Surg 2009;(67):2-12.
10. Russell RGG. Bishosphonates: Mode of action and pharmocology. Am Acad J Pediatr 2007;119 Suppl 2:150-62.
11. Chen T, Berenson J, Vescio R, Swift R, Gilchick A, Goodin S et al. Pharmacokinetics and pharmacodynamics of zoledronic acid in cancer patients with bone metastases. J Clin Pharmacol 2002;42(11):1228-36
12. Nobuyuki H, Hiraga T, Williams PJ, Niewolna M, Shimizu N, Mundy GR, et al. The bisphosphonate zoledronic acid inhibits metastases to bone and liver with suppression of osteopontin production in mouse mammary tumor. J Bone Miner Res 2001;16(Suppl 1):S191.
13. Senaratne SG, Mansi JL, Colston KW. The bisphosphonate zoledronic acid impairs membrane localisation and induce cytochrome c release in breast cancer cells. Br J Cancer 2002;86(9):1479-86.
14. Vaycan HS. Sistemik kullanılan zoledronik asitin var olan

deneysel periodontitis üzerine etkisinin histomorfometrik olarak incelenmesi. İstanbul Üniversitesi Sağlık Bilimleri Enstitüsü. Doktora Tezi. İstanbul 2020.

15. Ficarra G, Beninati F, Rubino I, Vannucchi A, Longo, Tonelli P. GOsteonecrosis of the jaws in periodontal patients with a history of bisphosphonates treatment J Clin Periodontol 2005;32(11):1123-8.
16. Saad F, Brown JE, Van Poznak C, Ibrahim T, Stemmer SM, Stopeck AT et al. Incidence, risk factors, and outcomes of osteonecrosis of the jaw: integrated analysis from three blinded active-controlled phase III trials in cancer patients with bone metastases. Ann Oncol 2012;(23):1341-7.
17. Vahtsevanos K, Kyrgidis A, Verrou E, Katodritou E, Triaridis S, Andreadis CG et al. Longitudinal cohort study of risk factors in cancer patients of bisphosphonate-related osteonecrosis of the jaw. J Clin Oncol 2009;(27):5356-62
18. Sonis ST, Watkins BA, Lyng GD, Lerman MA, Anderson KC. Bony changes in the jaws of rats treated with zoledronic acid and dexamethasone before dental extractions mimic bisphosphonate-related osteonecrosis in cancer patients Oral Oncol 2009;45(2):164-72.
19. Kim RH, Lee RS, Williams D, Bae S, Woo J, Lieberman M et.al. Bisphosphonates induce senescence in normal human oral keratinocytes. J Dent Res 2011;90(6):810-6.
20. Landesberg R, Cozin M, Cremers S, Woo V, Kousteni S, Sinha S et al. Inhibition of oral mucosal cell wound healing by bisphosphonates. J Maxillofac Surg 2008;66(5):839-47.
21. Li CL, Lu WW, Seneviratne CJ, Leung WK, Zwahlen RA, Zheng LW et al. Role of periodontal disease in bisphosphonates-related osteonecrosis of the jaws in ovariectomized rats. Clin Oral Implants Res 2016;27(1):1-6.
22. Aghaloo TL, Kang B, Sung EC, Shoff M, Ronconi M, Gotcher JE et al. Periodontal disease and bisphosphonates induce osteonecrosis of the jaws in the rat. J Bone Miner Res 2011;26(8):1871-81

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IN VITRO STUDY OF FRACTAL ANALYSIS OF OSTEOTOMIES PERFORMED WITH DIFFERENT DESIGN IMPLANT DRILLS IN LOW DENSITY BONE BLOCKS

DÜŞÜK YOĞUNLUKLU KEMİK BLOKLARINDA FARKLI TASARIMA SAHİP İMPLANT FREZLERİ İLE GERÇEKLEŞTİRİLEN OSTEOTOMİLERİN FRAKTAL ANALİZLERİNİN VİTRO İNCELENMESİ

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ABSTRACT

Objective: Alternative osteotomy techniques were introduced instead of the traditional drilling procedures of dental implant placement. One of these techniques is bone densification through a special design of drills. To study the effect of densification burs on low bone quality in comparison with conventional drilling procedures, the trabecular bone changes will be determined quantitively using fractal analysis (FA).

Material and Methods: A control group (PKD) with the standard osteotomy burs and a test group (PV) with the osseodensification burs were used. In each group, 10 dental implants (4.2x10mm) were placed. A three dimensional (3D) image was obtained from the blocks on a Micro Computed Tomography (micro-CT) device. Fractal analysis was performed, and the results were analyzed by nonparametric tests ($p<0.05$).

Results: According to the results, the FA value of apical and lateral areas from the PV group (1.292, 1.251 respectively) were significantly higher than the control group ($p<0.01$). When apical and lateral FA values of the PV group were compared, the apical values were found to be significantly higher than the lateral ones ($p<0.01$).

Conclusion: In terms of fractal analysis, the bone densification effect of the burs in the PV group around the implants, compared to the PKD group, apical and lateral values, were found to be higher and have been found to provide better trabecular compression. FA may be useful for preoperative and non-invasive assessment of bone quality at implant sites.

Keywords: Osseodensification, fractal analysis, micro-CT, artificial bone block

ÖZ

Amaç: Dental implant cerrahisinde; osteotomi bölgelerindeki kemik partiküllerini dışarı çıkarma prensibi ile çalışan geleneksel yaklaşımlara ek olarak; partikülleri osteotomi alanında tutarak yoğunlaştırılmasını sağlayan frezleme teknikleri geliştirilmiştir. Bu çalışma ile, düşük kemik yoğunluğuna sahip yapay bloklar kullanılarak; iki farklı frez tasarımı ile oluşturulan osteotomilerde; trabeküler kemik değişikliklerinin kantitatif olarak belirlenmesinde fraktal analizin (FA) etkinliği araştırılmıştır.

Gereç ve Yöntem: Standart osteotomi frezleri ile bir kontrol grubu (PKD) osseodensifikasyon frezleri ile bir test grubu (PV) oluşturulmuştur. Osteotomiler 2x2x2cm ebatlarındaki yapay kemik bloklarında gerçekleştirilmiştir ve her gruba 10 adet dental implant (4,2x10mm) yerleştirilmiştir. Blokların, Mikro Bilgisayarlı Tomografi (mikro-BT) cihazında elde edilen kesitlerinin mezial ve lateral bölgelerinden ölçümler gerçekleştirilmiş, kutu sayma algoritması ile ImageJ programı kullanılarak FA yapılmıştır. Veriler varyans analizi ile değerlendirilmiştir ($p<0,05$).

Bulgular: Elde edilen bulgulara göre PV grubunda apikal ve lateral alanlarda FA değerleri (sırasıyla 1,292, 1,251), PKD grubuna göre (1,258, 1,233) istatistiksel olarak anlamlı bulunmuştur ($p<0,01$). PV grubunun apikal ve lateral FA değerleri karşılaştırıldığında ise apikal değerler laterale göre anlamlı derecede yüksek bulunmuştur ($p<0,01$).

Sonuçlar: Fraktal analiz değerleri açısından; osseodensifikasyon frezlerinin implant etrafındaki kemik yoğunlaştırma etkisi, geleneksel grubuna göre, hem apikalde hem de lateralde yüksek bulunmuş ve daha etkin trabeküler sıkıştırma sağladığı tespit edilmiştir. FA; implant bölgelerindeki kemik kalitesinin, preoprative ve noninvaziv değerlendirilmesinde, faydalı bir yöntem olarak düşünülmektedir.

Anahtar Kelimeler: Osseodensifikasyon, fraktal analiz, mikro-BT, yapay kemik bloğu

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INTRODUCTION

Nowadays, dental implant sockets are prepared by conventional burs. These burs are surrounded by flutes that cut through the bone with their tapered ends and accumulate hard tissues as the drill progresses through the bone (1). These bone chips are preserved as an autogenous bone graft that can be used to compensate for bone volume deficiencies that occur during implant surgery (2).

Although traditional osteotomies are obtained by drilling out the bone, some approaches attempt to increase primary stability in low-bone density by using a counterclockwise (CCW) applied drilling process that results in the densification of the osteotomy site wall by tapering the bur geometry and placing the flutes at different angles (3-6).

Another clinical approach recommended for low-bone density is an "undersize" drilling procedure (7). The main purpose of this modified preparation technique is to reduce the size of the final osteotomy compared to the implant diameter (8). Sahalabi et al., in their animal studies, investigate the effect of different osteotomy techniques on primary stability in trabecular bone; they concluded that undersize prepared osteotomies improved implant fixation according to the standard protocol (9).

50%-90% of trabecular bone has a high porosity and many fractal-like structures (10). There is a correlation between the fractal dimension and the complexity of the structure, which is expressed numerically with FA. For determining the interior trabecular structure of bone, the FA was used; this analysis was easy to use, non-invasive, and offers objective data (11).

FA is a mathematical technique used to evaluate the amount and complexity of bone structure (13). FA is a reliable method for evaluating the shape and structure of the alveolar bone as claimed by published studies (14, 21). Nevertheless, FA results are affected by the Region of Interest (ROI) selection parameters such as shape, size, and sample area (22). It was also noted that the inaccurate positive or negative results in the ROI area might result in differences in FA values (25). Additionally, FA is highly reliable to distinguish osteoporotic and non-osteoporotic cases (24). A decrease in FA values caused by a bone loss in osteoporotic patients was observed in some studies (12,13,22).

The box-counting method, which forms the basis of fractal analysis, is a method used to examine bone morphology. A scale with boxes is placed over the trabecular structure to be sized. Boxes containing trabecular bone are counted in grids created from boxes with sizes ranging from 2-64 pixels (13).

A high FA value indicates that the bone structure is more complex and the spaces in the bone are less, while a low FA value means that the bone has more lacuna (13-15).

In the determination of bone structure, there are also studies suggesting that the combined use of FA and CT is beneficial (16). In CT scans, it is possible to examine the internal structure of bone tissue and the internal adaptation of materials to surfaces without destroying them (33).

Bone microarchitecture can be studied in great detail with high-resolution micro-CT in laboratory environments (31,32). Micro-CT is a tool that provides 3D imaging at very high resolution on a small scale. It has been proposed as a standard imaging tool for many applications, such as tissue engineering, dentistry, and research on the mineral density of hard tissues and bone growth. The 3D trabecular architecture projects a roughly two-dimensional pattern on plain radiographs, and the FA can then be calculated on these projected patterns and used to describe the spatial arrangement of the trabecular bone (31). However, the high costs of micro-CT, the time required for scanning and reconstruction, computer expertise requirements, and lack of usage knowledge are disadvantages (17). Recent studies have shown a good correlation between micro-CT morphology and fractal dimension (18).

In our study, FA and micro-CT were used to determine the bone densification properties of Osseo densification burs in comparison to standard conventional milling techniques in D4 artificial bone blocks, which mimic trabecular bone in vitro and exhibit isotropic fractal properties.

MATERIALS and METHODS

Study group and sample size

A total of 20 implants were inserted into each of the manufactured artificial bone blocks that mimic D4 bone with two different drilling procedures. Considering similar studies, the sample size was determined by G Power analysis (G Power, Dusseldorf, Germany).

Osteotomy procedure, artificial bone block supply and implant placement

A control group (PKD) with standard osteotomy burs (Figure 1) was formed. A test group (PV) was formed with osteotomy burs displaying osseodensification (Figure 2). Standard burs (Implant Direct, CA, USA) in the normal protocol, undersize preparation (soft bone protocol), 800rpm, clockwise (1.6 mm pilot, 2.3 mm and 3.4 mm drills) and Densah burs (Versah, Jackson, MI, USA) in osseodensification protocol, 800rpm, counterclockwise (2.0 mm pilot, 2.3 mm and 3.3 mm multi-channel burs) were applied. 10 implants (4.2 mmx10 mm) were placed in each group (Table 1).

ASTM standard and F-1839 reference artificial polyurethane blocks (Pacific Research Laboratories Inc, WA, USA) were preferred to represent D4 bone, mimicking trabecular bone. Homogeneous bone blocks produced from cellular rigid polyurethane foam offer an alternative test environment similar to human cancellous bone (35). The blocks were divided into homogeneous pieces 2x2x2 cm in size.

After the appropriate osteotomy areas were created in the artificial bone blocks with the drilling procedures, the placement of the implants was carried out. A total of 20 dental implants (4.2x10 mm) were manufactured from polyetheretherketone (PEEK) material (Uysal Medical, Istanbul, TURKEY) to obtain a clear measurement of bone-implant contacts in high-resolution computed tomography imaging (Figure 3).

Micro-CT imaging and fractal analysis of digital images

The trabecular bone around dental implants has been evaluated utilizing FA on cone-beam computed tomography (CBCT) images. The sections were examined with Micro CT imaging through an X-ray tube of 90–150 kVA, a filter and collimator, a computer-controlled electric motor, a CCD camera for converting X-ray image data, and an image intensifier apparatus. Ultra-high-resolution images were obtained with artificial bone blocks placed on a rotating platform with an X-ray tube (17). Consequently, the cross-section of the implant surrounded by bone was obtained for all 20 implants.

For each implant, the middle slice of the implant illustrating its center was extracted from the CBCT radiograph. Measurements were made on two areas determined from the mesial and lateral regions of this section. The first ROI was assigned by creating a rectangular box along the whole lateral (16x64 pixel) part of the implant length and the second ROI was a square box apical (32x32 pixel) part of the implant, involving the bone intimately surrounding the implant without any threads included, which may distort the results.

All the radiographic samples were inserted into ImageJ software (National Institutes of Health, Bethesda, MD; http://rsb.info.nih.gov/nih-image) for evaluation. The box-counting FA was computed using an algorithm featured in ImageJ as described in a previous study (19). In this procedure, several grids of reduced size (box size) were placed on the ROI and the number of boxes containing pixels was counted for each grid (12). The sections were subjected to various image processes and a skeletonized image was obtained (Figure 4). The mean gray level, trabecular area, perimeter, and the number of terminal points were measured from the transformed image.

Statistical analysis

All analyses were performed using a custom software program (SPSS 11.5.0, SPSS, Chicago, IL, USA). A Bartlett test was per-

Figure 1: Standard osteotomy Bur Kit

Figure 2: Osseodensification Bur Kit

Table 1: Experimental design and drill protocols

		1.DRILL (Pilot drill)	2. DRILL	3.DRILL (Soft bone protocol)	IMPLANT (Diameter)
Control group (PKD)	**Standard Osteotomy Burs** (Undersize Preparation) (Implant Direct)	1.7mm	2.3/2.0mmD	3.4/2.8mmD	4.2mm X 10mm
		Clockwise	Clockwise	Clockwise	
Test group (PV)	**Densah Burs** (Versah)	1.7mm	2.3 Apical: 1.8 mmD Coronal: 2.8 mmD	3.3 Apical: 2.8 mmD Coronal: 3.8mmD	4.2mm X 10mm
		Clockwise	Counter-Clockwise	Counter-Clockwise	

PKD: Control group, PV: Test group

Figure 3: PEEK implant and artificial bone block with d4 bone characteristics

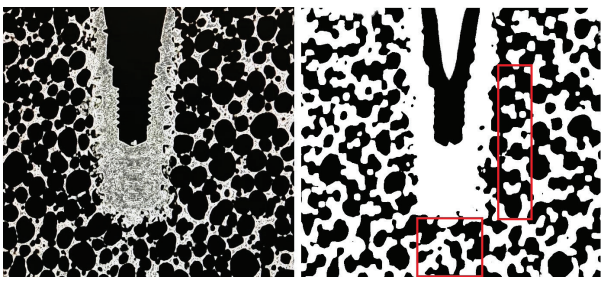

Figure 4: Image processing steps of micro-CT slices and region of interest (ROI) selection for fractal analysis

formed to check the suitability of the data set for analysis. A one-way ANOVA test was performed to determine whether there was a significant difference in the comparisons between the groups. The P value was accepted as 0.05. Tukey's multiple comparison test was used for pairwise comparisons.

RESULTS

As a result of the Anova test, a statistically significant difference was found between all groups ($p<0.01$) (Figure 5). In binary comparisons, the groups with a significant relationship are as follows: PKD apical and PKD lateral ($p<0.01$); PKD apical and PV apical ($p<0.01$); PKD lateral and PV apical ($p<0.01$); PKD lateral and PV lateral ($p<0.01$); PV apikal and PV lateral ($p<0.01$). However, no significant relationship was found between PKD apical and PV lateral ($p=0.0004$).

According to the results, apical and lateral ROI (1.292 and 1.251, respectively) taken from the PV group were found to be statistically significant and higher in terms of FA values (1.258, 1.233) compared to the control group ($p<0.01$).

The highest fractal analysis value was observed at the apical part of the osteotomy PV made with the osseodensification bur (1.292). The lowest fractal analysis value was found in the

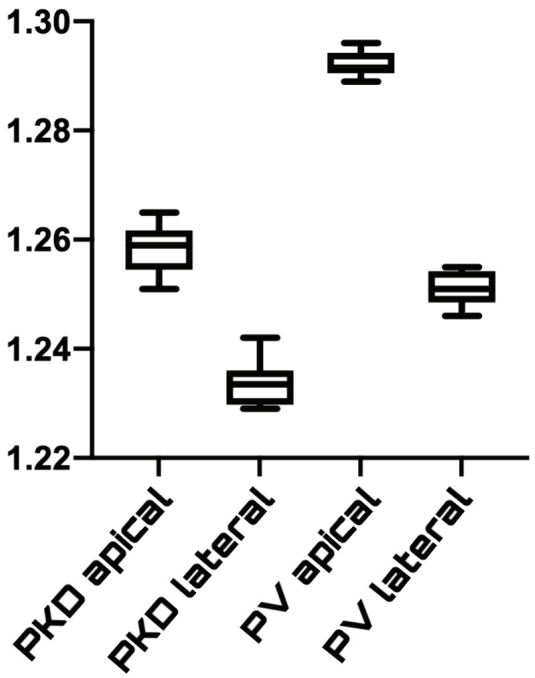

Figure 5: Box-whisker pilot plot showing median, min, max values between groups in terms of FA value PKD: Control group, PV: Test group

lateral ROI of the osteotomy PKD performed with the conventional bur (1.233).

Comparing the apical FA values of the PV and PKD groups, bone condensation in the apical part of the peri-implant bone by osseodensification bur showed a significantly higher value than the conventional technique ($p<0.01$).

Comparing the lateral FA values of the PV and PKD groups, bone condensation in the lateral part of the peri-implant bone by osseodensification bur showed a significantly higher value than the conventional technique ($p<0.01$).

In terms of fractal analysis, the bone densification effect of the burs in the PV group around the peri implant bone, compared to the PKD group, showed higher values both apically and laterally, and has been found to provide better trabecular compression in bone.

DISCUSSION

In this study, FA was used to evaluate the bone pattern surrounding the peek implants placed inside polyurethane blocks following two different osteotomy protocols.

The homogeneity and the horizontal isotropic pattern and the vertical anisotropic fractal pattern of the bone substitute blocks used in the present study make the results and evaluation more accurate regarding the FA values compared with other human or animal experimental models; this makes it possible to mea-

sure the fractal analysis independent from the locational differences of natural human bone (mandible, maxilla and/or frontal and posterior areas), and various negative factors that cannot be standardized due to physiological diversity that are encountered during bone growth or turnover period (15). In addition, the location of the trabecula in space changed under the effect of functional forces and loading, which negatively affect FA (25).

It is thought that osseodensification positively affects the implant primary stability in the apical area, thus making bone augmentation redundant in individuals with low bone density. Compared to conventional techniques, osseodensification increases the bone volume percentage surrounding the implants placed in a low-density bone (5).

In a similar study, 20 clockwise (control) and counterclockwise (test) osteotomy areas were prepared on polyurethane blocks like type iv bone microstructure, and tomography and Image J software were used to evaluate and measure the bone density. Compared to clockwise bur usage, counter-clockwise bur usage was densified and altered the microstructure of apical areas in the osteotomy site (p=0.026). But the researchers noted that it was due to the direction the bur was used in rather than the design of the osseodensification bur (29).

Delgado-Ruiz et al. revealed in their animal study that clockwise bur usage increased the bone density in lateral walls and caused higher bone density in the apical area as inspected by CT imaging (30). Their findings are consistent with the present study in terms of bone density in the apical, with disparate ranges attributed to the viscoelastic nature of polyurethane blocks used in the present study.

Due to Densah's osseodensification bur having bone density requirement in both lateral and apical aspects, the ROIs chosen to be analyzed in our study were determined to have a standard pixel size of 32x32 in apical and 16x64 in lateral. Our findings confirmed that Densah burs resulted in an increase in bone density, as already found in previous studies (3,4,5). In a human prospective study by Zeytinoğlu et al., 3 different ROIs of non-standard sizes from different areas (mesial, distal, and apical) were determined to evaluate the changes in surrounding trabecular bones in 198 implants. The results of their fractal analysis are consistent with our findings (apical:1.202; mesial:1.224) (27).

Based on studies that indicate that fractal analysis might be applied to CBCT images in order to estimate bone quality (16, 22). Corpas et al. evaluated the peri-implant bone tissue using fractal analysis on conventional intraoral, CBCT, and histological images after 3 months following the implant replacement. While the bone mass measurements are correlated in all methods, it was noted that the FA method cannot detect any histological changes, so it was correlated not with histological results but with bone density. No significant correlations were detected between fractal analysis on CBCT, intra-oral radiography, and histology (34).

Lee et al. concluded that osteointegration was successful due to the increase in the fractal dimension of the bone surrounding the implant and noted that the result of their analysis revealed a correlation between bone density and FA values (23).

Sansare et al. applied preoperative and post-osseointegration fractal analyses on ROIs determined from the apical area of the 50 implants. There was an increase in bone microstructure and significantly higher post-operative FA values were observed compared to pre-operative values (26).

Due to the low-density bone protocol, an undersized osteotomy was preferred in our study and micro-CT imaging was performed for the evaluation of the fractal analysis of the bone substitute block. Similarly, in another study that performed fractal analysis on the immediate implant with standard burs and undersized preparations, a circular ROI (10.7 mm2) around each implant was chosen for post-operative CBCT imaging. The researchers performed two FA measurements, one on the day of operation and another six months later, and indicated that undersized preparation might have a positive effect on bone healing, due to a 3% increase in the FA value measured after 6 months compared to the pre-operative value (28).

The increase in fractal dimension in both conventional and CBCT radiography compared to previous studies indicates a higher amount of bone mineralization (13, 18). As a result, the increasing bone structure measured by fractal analysis seems consistent with the significant increase in the quality of the bone surrounding the implant.

CONCLUSIONS

In terms of fractal analysis, in the bone densification effect of the burs in the PV group around the implants, compared to the PKD group, apical and lateral values were found to be higher, and this has been found to provide better trabecular compression. Fractal analysis (FA) may be useful for preoperative and non-invasive assessment of bone quality at artificial bone blocks.

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REFERENCES

1. Boustany CM, Reed H, Cunningham G, Richards M, Kanawati A. Effect of a modified stepped osteotomy on the primary stability of dental implants in low-density bone: a cadaver study. Int J Oral Maxillofac Implants 2015;30(1):48-55.
2. El-Kholey KE, Elkomy A. does the drilling technique for implant site preparation enhance implant success in low-density bone? a systematic review. Implant Dent 2019;28(5):500-9.
3. Huwais S, Meyer EG. A novel osseous densification approach in implant osteotomy preparation to increase biomechanical primary stability, bone mineral density, and bone-to-implant contact. Int J Oral Maxillofac Implants 2017;32(1):27-36.
4. Lahens B, Neiva R, Tovar N, Alifarag AM, Jimbo R, Bonfante EA, et al. Biomechanical and histologic basis of osseodensification drilling for endosteal implant placement in low density bone. An experimental study in sheep. J Mech Behav Biomed Mater 2016;63:56-65.
5. Trisi P, Berardini M, Falco A, Podaliri Vulpiani M. New Osseodensification Implant Site Preparation Method to increase bone density in low-density bone: In Vivo Evaluation in Sheep. Implant Dent 2016;25(1):24-31.
6. Trisi P, Perfetti G, Baldoni E, Berardi D, Colagiovanni M, Scogna G. Implant micromotion is related to peak insertion torque and bone density. Clin Oral Implants Res 2009;20(5):467-71.
7. Ostman PO, Hellman M, Wendelhag I, Sennerby L. Resonance frequency analysis measurements of implants at placement surgery. Int J Prosthodont 2006;19(1):77-84.
8. Skalak R, Zhao Y. Interaction of force-fitting and surface roughness of implants. Clin Implant Dent Relat Res 2000;2(4):219-24.
9. Shalabi MM, Wolke JG, de Ruijter AJ, Jansen JA. A mechanical evaluation of implants placed with different surgical techniques into the trabecular bone of goats. J Oral Implantol 2007;33(2):51-8.
10. Groen WM, Diloksumpan P, van Weeren PR, Levato R, Malda J. From intricate to integrated: Biofabrication of articulating joints. J Orthop Res 2017;35(10):2089-97.
11. Taşsöker M, Özcan S, Güleç M. Tıpta ve diş hekimliğinde fraktal analiz. EÜ DişHek Fak Derg 2019;40(1):17-31.
12. Updike SX, Nowzari H. Fractal analysis of dental radiographs to detect periodontitis-induced trabecular changes. J Periodontal Res 2008;43(6):658-64.
13. Southard TE, Southard KA, Jakobsen JR, Hillis SL, Najim CA. Fractal dimension in radiographic analysis of alveolar process bone. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1996;82(5):569-76.
14. Bollen AM, Taguchi A, Hujoel PP, Hollender LG. Fractal dimension on dental radiographs. Dentomaxillofac Radiol 2001;30(5):270-5.
15. Sánchez I, Uzcátegui G. Fractals in dentistry. J Dent 2011;39(4):273-92.
16. Hua Y, Nackaerts O, Duyck J, Maes F, Jacobs R. Bone quality assessment based on cone beam computed tomography imaging. Clin Oral Implants Res 2009;20(8):767-71.
17. Ghavami-Lahiji M, Davalloo RT, Tajziehchi G, Shams P. Micro-computed tomography in preventive and restorative dental research: A review. Imaging Sci Dent 2021;51(4):341-50.
18. Guggenbuhl P, Bodic F, Hamel L, Baslé MF, Chappard D. Texture analysis of X-ray radiographs of iliac bone is correlated with bone micro-CT. Osteoporos Int 2006;17(3):447-54.
19. Veltri M, Ferrari M, Balleri P. Correlation of radiographic fractal analysis with implant insertion torque in a rabbit trabecular bone model. Int J Oral Maxillofac Implants 2011;26(1):108-14.
20. Hetaimish BM. Sawbones laboratory in orthopedic surgical training. Saudi Med J 2016;37(4):348-53.
21. Tosoni GM, Lurie AG, Cowan AE, Burleson JA. Pixel intensity and fractal analyses: detecting osteoporosis in perimenopausal and postmenopausal women by using digital panoramic images. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;102(2):235-41.
22. Shrout MK, Farley BA, Patt SM, Potter BJ, Hildebolt CF, Pilgram TK, et al. The effect of region of interest variations on morphologic operations data and gray-level values extracted from digitized dental radiographs. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1999;88(5):636-9.
23. Lee DH, Ku Y, Rhyu IC, Hong JU, Lee CW, Heo MS, et al. A clinical study of alveolar bone quality using the fractal dimension and the implant stability quotient. J Periodontal Implant Sci 2010;40(1):19-24.
24. Kavitha MS, An SY, An CH, Huh KH, Yi WJ, Heo MS, et al. Texture analysis of mandibular cortical bone on digital dental panoramic radiographs for the diagnosis of osteoporosis in Korean women. Oral Surg Oral Med Oral Pathol Oral Radiol 2015;119(3):346-56.
25. Baksi BG, Fidler A. Fractal analysis of periapical bone from lossy compressed radiographs: a comparison of two lossy compression methods. J Digit Imaging 2011;24(6):993-8.
26. Sansare K, Singh D, Karjodkar F. Changes in the fractal dimension on pre- and post-implant panoramic radiographs. Oral Radiology 2011;28(1):15-23.
27. Zeytinoğlu M, İlhan B, Dündar N, Boyacioğlu H. Fractal analysis for the assessment of trabecular peri-implant alveolar bone using panoramic radiographs. Clin Oral Investig 2015;19(2):519-24.
28. González-Martín O, Lee EA, Veltri M. CBCT fractal dimension changes at the apex of immediate implants placed using undersized drilling. Clin Oral Implants Res 2012;23(8):954-7.
29. Delgado-Ruiz R, Mahdian M, Benezha I, Romanos G. Counterclockwise drilling with different tapered drills condenses the implant bed-an optical coherence tomography in vitro study. Medicina (Kaunas) 2021;57(9):940.
30. Delgado-Ruiz R, Gold J, Somohano Marquez T, Romanos G. Under-drilling versus hybrid osseodensification technique: differences in implant primary stability and bone density of the implant bed walls. Materials (Basel) 2020;13(2):390.
31. Link TM, Majumdar S. Current diagnostic techniques in the evaluation of bone architecture. Curr Osteoporos Rep 2004;2(2):47-52.
32. van der Linden JC, Weinans H. Effects of microarchitecture on bone strength. Curr Osteoporos Rep 2007;5(2):56-61.
33. Reznikov N, Bilton M, Lari L, Stevens MM, Kröger R. Fractal-like hierarchical organization of bone begins at the nanoscale. Science 2018;360(6388):eaao2189. doi: 10.1126/science.aao2189.
34. Corpas LS, Jacobs R, QuirynenM HY, Naert I, Duyck J. Periimplant bone tissue assessment by comparing the outcome of intraoral radiograph and cone beam computed tomography analyses to the histological standard. Clin Oral Implants Res 2011;22(5):492-9.
35. Devlin H, Horner K, Ledgerton D. A comparison of maxillary and mandibular bone mineral densities. J Prosthet Dent 1998;79(3):323-7.

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EVALUATION OF RARE MAXILLARY SINUS PNEUMATIZATIONS WITH CBCT AND ITS RELATIONSHIP WITH PATHOLOGY

NADİR OLARAK GÖRÜLEN MAKSİLLER SİNÜS PNÖMATİZASYONLARININ KONİK IŞINLI BİLGİSAYARLI TOMOGRAFİ (KIBT) İLE RETROSPEKTİF OLARAK DEĞERLENDİRİLMESİ VE PATOLOJİ İLE İLİŞKİSİ

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ABSTRACT

Objective: This study aims to evaluate maxillary sinus pneumatizations with Cone-beam Computed Tomography (CBCT) and correlate sinus pneumatizations with pathology.
Material and Method: Maxillary sinus pneumatization can be listed as frontal process pneumatization (FPP), zygomatic process pneumatization (ZPP), nasal cavity pneumatizations (NCP), palatal process pneumatizations (PPP), orbital process pneumatization (OPP), palatal pneumatization to sphenomaxillary plate (SPP), pneumatization caused by tooth loss, and alveolar bone pneumatization (ABP).
Istanbul University Faculty of Dentistry Department of Oral and Maxillofacial Radiology Volumetric Tomography images of patients entering the image region of the maxillary sinus were examined between December 2015 and December 2017 in this study.
The images were examined with the software called OnDemand 3D™ (Cybermed, California, USA). CBCT device is Scanora 3Dx brand CBCT (Scanora® 3Dx, Soredex, Tuusula, Finland). It was subjected to appropriate statistical analysis retrospectively using CBCT images.
Results: Our study has revealed that some pathologies such as mucosal thickening, polypoidal mucosal thickening, partial opacification, total opacification, and effusion are due to maxillary sinus pneumatization. Statistically significant differences were found between ABP, PPP, and ZPP with mucosal thickenings ($p<0.05$).
Mucosal thickening is the most common which is seen with alveolar bone pneumatization statistically (36.5% on the right, 38% on the left).
In addition, a significant correlation was found between ABP and total opacification on the left side ($p=0.001$).
Conclusion: CBCT is the most appropriate imaging method for imaging the pneumatization of the maxillary sinus. Knowing maxillary sinus pneumatizations is of great importance in terms of dentistry in order to prevent complications that may occur during and after surgical operations. Our study will make an important contribution to the dentistry literature in order to define rare maxillary sinus pneumatizations and to explain the relationship of these pneumatizations with pathology.
Key words: Pneumatization, CBCT, maxillary sinus

ÖZ

Amaç: Bu çalışmanın amacı, Konik Işınlı Bilgisayarlı Tomografi (KIBT) ile maksiller sinüs pönomatizasyonlarının değerlendirilmesi ve pnömatizasyonlarının sinüs patolojileri ile ilişkilendirilmesidir.
Gereç ve Yöntem: Maksiller sinüs pnömatizasyonlarını; frontal proses pnömatizasyonları, zigomatik proses pnömatizasyonları, palatal proses pnömatizasyonları, orbital proses pnömatizasyonları, palatinalden sfenomaksiller plakaya pnömatizasyonlar diş kaybının neden olduğu pnömatizasyonlar ve alveolar kemiğe pnömatizasyon olarak sıralayabiliriz. Bu çalışmada İstanbul Üniversitesi Diş Hekimliği Fakültesi Ağız Diş Çene Radyolojisi Anabilim Dalında 2015-2017 yılları arasında alınan, maksiller sinüsün görüntüleme alanına girdiği KIBT görüntüleri incelenmiştir. Scanora 3Dx marka KIBT cihazı (Scanora® 3Dx, Soredex, Tuusula, Finland) ile alınan görüntüler, OnDemand 3D™ (Cybermed, California, USA) adlı yazılım ile incelenmiştir. Retrospektif olarak değerlendirilen görüntüler uygun istatistiksel analize tabi tutulmuştur.
Bulgular: Çalışmamız, mukozal kalınlaşma, polipoidal mukozal kalınlaşma, parsiyel opasifikasyon, total opasifikasyon ve efüzyon gibi bazı patolojilerin maksiller sinüs pnömatizasyonlarına bağlı olduğunu ortaya çıkarmıştır. İstatiksel olarak en çok karşılaşılan alveolar kemik pnömatizasyonlarında mukozal kalınlaşmadır (%36,5 sağda ve %38 solda).
Alveolar kemik pnömatizasyonu, palatal proses pnömatizasyonu ve zigomatik kemik pnömatizasyonu'nun mukozal kalınlaşmalar ile arasında istatiksel olarak anlamlı farklılıklar bulunmuştur ($p<0,05$). Ayrıca sol tarafta alveolar kemik pnömatizasyonu ile total opasifikasyon arasında anlamlı ilişki bulunmuştur ($p=0,001$).
Sonuç: Maksiller sinüs ile ilgili pnömatizasyonlarını görüntülemede en uygun görüntüleme yöntemi KIBT' tır. Cerrahi operasyonlar sırasında ve sonrasında oluşabilecek komplikasyonları önlemek amacıyla maksiller sinüs pnömatizasyonlarının bilinmesi diş hekimliği açısından büyük önem arz etmektedir.
Bizim çalışmamız; nadir görülen maksiller sinüs pnömatizasyonlarını tanımlamak ve bu pnömatizayonların patoloji ile ilişkisi açıklamak amacıyla diş hekimliği literatürüne önemli katkı sağlayacaktır.
Anahtar Kelimeler: Pnömatizasyon, KIBT, maksiller sinüs

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INTRODUCTION

The maxillary sinus is the largest paranasal sinus, and the first to form (1, 2). The maxillary sinus volume at birth is 6-8 ml (2). Maxillary sinus size is approximately 34x33x23 mm in adults, and its volume is 14.75 ml (3). The anterior/posterior part is the maximum volume (2). The maxillary sinus volume decreases after growth reaches its maximum (4). As the minerals in the bone matrix of the body are lost, this also affects the volume of the maxillary sinus. Maxillary sinus dimensions are affected by anatomical variations. The volume of the maxillary sinus is significant in the planning of surgical treatment (5).

The maxillary sinus resembles a pyramid in shape. The base of this pyramid is formed by the lateral wall of the nasal cavity; the apex is towards the zygomatic process and the anterior wall is associated with the fossa canina. The maxillary sinus is adjacent to the nasal cavity medially and the zygoma laterally (1, 3).

Maxillary sinus pneumatization is a physiological process. Pneumatization begins in the ethmoid sinuses, then in the maxillary sinus, sphenoid sinuses, and frontal sinuses. Although the pneumatization of the paranasal sinuses is different on the right and left, it can also vary from person to person (6).

The sinus with the most pneumatization is the frontal sinus, followed by the ethmoid and sphenoid sinuses, respectively (7). Maxillary sinus pneumatization is rare. Pneumatization causes increased sinus volume (8). The maxillary sinus pneumatization begins in the third week of pregnancy and continues to be pneumatized after birth (4, 7). In the first three years, growth is rapid, and change is slower between ages 3 and 7 (4). Between 7-12 years, growth accelerates again and slows further into adulthood (4). Pneumatization of the maxillary sinus ends after the third molars erupt at age 20 (8, 9).

The panoramic radiograph gives limited information about maxillary sinus pneumatization. CBCT (cone beam computed tomography) is a required imaging method in dentistry (10). CBCT plays an essential role in diagnosing anatomical variations (11). CBCT is a gold standard and significant imaging method. (10). Sinus pneumatization is best evaluated with CBCT (8).

This study aims to evaluate maxillary sinus pneumatizations with CBCT and to associate these pneumatizations with pathology. Rare pneumatizations will contribute to the dentistry literature, and their relationship with pathology will enable dentists to obtain information before surgical operations.

MATERIAL and METHODS

Approval for this study was obtained by the Istanbul University Faculty of Dentistry Clinical Research Ethics Committee (Date/File no:28.08.2018/72). The study was carried out by the Helsinki Declaration of Human Rights guidelines at every stage. Patients admitted to the Department of Oral and Maxillofacial Radiology at Istanbul University Faculty of Dentistry between 2015 and 2017 for any reason were examined, and 4158 patients who entered the field of imaging of the maxillary sinus were included in the study. These Fields of View (FOV) were 8x10,14x16,24x16 mm. Data were evaluated by using technical properties of 60-90 kVp, 4-10mA, 18-34s, and 0.2 mm slice thickness in the coronal, axial, and sagittal planes. Patients were excluded from the study in the presence of a history of maxillofacial trauma or surgery, any syndrome, and artifacts that reduce image quality. A total of 621 patients (1242 maxillary sinuses), aged 18-51, over 51 years old were evaluated retrospectively with CBCT images. Patient images were obtained with a Scanora 3Dx brand CBCT device (Scanora® 3Dx, Soredex, Tuusula, Finland). Images were analyzed using the device's original program OnDemand 3D™ (Cybermed, California, USA), and a medical monitor.

Radiological evaluation was performed by an oral and maxillofacial radiologist and four investigators. CBCT images were examined in axial, sagittal, and coronal sections, and maxillary sinus pneumatization was detected. Maxillary sinus pathologies, mucosal thickening, polypoidal mucosal thickening, partial opacity, total opacity, and effusion were examined in 5 categories.

Data were analyzed using the Statistical Package for and Social Sciences (SPSS) program. Chi-square tests (Pearson Chi-square, Yates Chi-square, and Fisher Exact Test) were used to compare categorical variables. A p-value of <0.05 was considered statistically significant in all analyses.

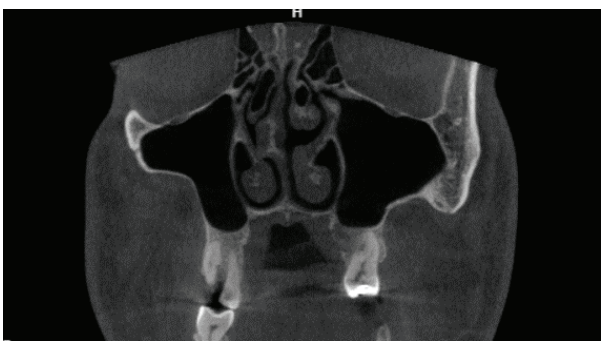

Figure 1: Frontal process pneumatization

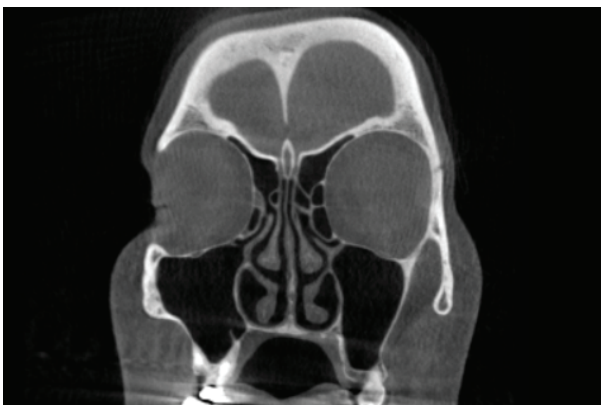

Figure 2: Zygomatic process pneumatization

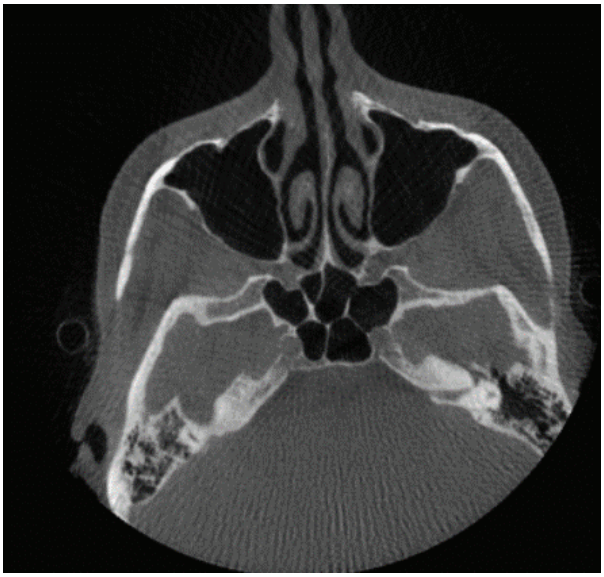

Figure 3: Zygomatic process pneumatization

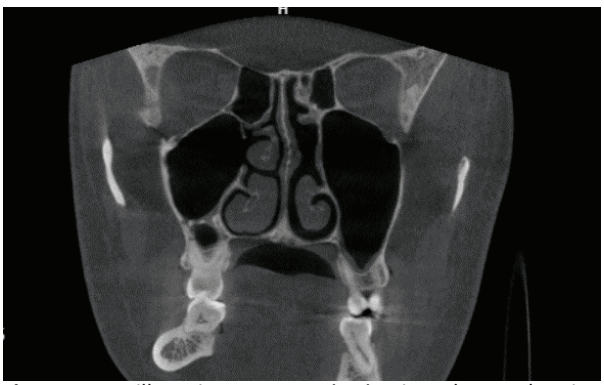

Figure 4: Maxillary sinus pneumatization into the nasal cavity

Maxillary sinus pneumatization is classified as:

-Frontal process pneumatization: This type of pneumatization is pneumatization towards the frontal recess (Figure 1). It is an extremely rare pneumatization (12). This is a pneumatization to the superomedial side.

Zygomatic process pneumatization: This type of pneumatization pneumatizes the malar bone, and this type of pneumatization pneumatizes laterally (Figure 2,3) (7).

- Maxillary sinus pneumatization into the nasal cavity: The most common anatomical variation is concha bullosa in the middle turbinate. These are called concha bullosa or bullous concha (13). Some variations can cause nasal congestion or facial pain. Nasal turbinate variations can be easily diagnosed with CBCT (14). This type of pneumatization is rare, and the maxillary sinus is pneumatized into the nasal cavity (Figure 4).

- Pneumatization to the orbit: This type of pneumatization is of two types, wavy tapered and vertical (Figure 5) (12).

- Pneumatization from palatal to sphenomaxillary plate: This type of pneumatization connects the maxillary sinus to the sphenoid sinus via the septum (15). Sphenomaxillary plate pneumatization is essential to avoid orbital complications during maxillary sinus surgery (12).

- Palatal pneumatization: This type of pneumatization pneumatizes the hard palate towards the midline (Figure 6) (12).

- Pneumatizations caused by tooth loss (inferior pneumatization, alveolar): After tooth extraction, alveolar bone size reduction is observed. This causes insufficient bone volume, and dental implant placement becomes difficult due to reduced bone volume. Bone resorption is observed after tooth extraction due to maxillary sinus pneumatization (16). Pneumatization with tooth loss may require various clinical treatments (e.g., sinus lift, bone graft applications, use of oral implants) (5, 15).

The rate and degree of alveolar pneumatization are affected by some conditions. These situations are:

1. The cortical bone layer of the root may break during tooth extraction, and the maxillary sinus may become pneumatized.

2. A significant defect occurs in the alveolar bone, unlike other teeth, after molar extraction (Figure 7) (7,8).

Rare pneumatizations will contribute to the dentistry literature, and their relationship with pathology will enable dentists to obtain information before surgical operations.

RESULTS

A total of 621 patients (1242 maxillary sinuses) aged 18-51, over 51 years old, were evaluated retrospectively with CBCT images.

Maxillary sinus pneumatization can be listed as frontal process pneumatization (FPP), zygomatic process pneumatization (ZPP), nasal cavity pneumatization (NCP), palatal process pneumatizations (PPP), orbital process pneumatization (OPP), palatal pneumatization to sphenomaxillary plate (SPP), pneumatization caused by tooth loss, and alveolar bone pneumatization (ABP).

It has been revealed that some pathologies such as mucosal thickening, polypoidal mucosal thickening, partial opacification, total opacification, and effusion are due to maxillary sinus pneumatization.

Table 1 shows the pneumatizations and pneumatizations associated with right and left mucosal thickening, polypoidal mucosal thickening, partial opacification, total opacification, and effusion.

As seen in Table 1, there is a significant relationship between ABP and mucosal thickening ($p=0.006$) and polypoidal thickening ($p=0.008$) on the right side ($p<0.05$), but there is

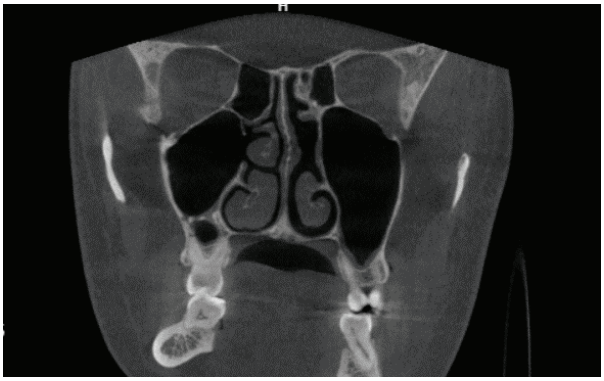

Figure 5: Pneumatization to the orbit (Orbital)

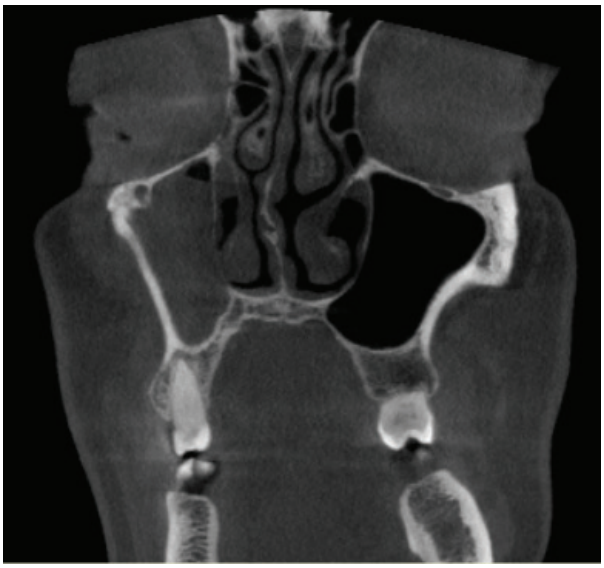

Figure 6: Pneumatization in the palatal

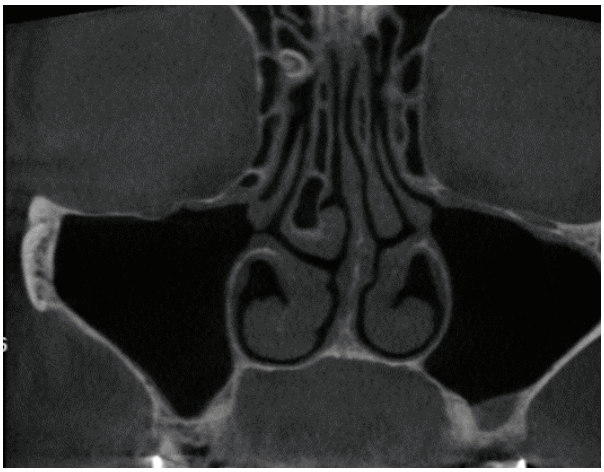

Figure 7: Alveolar pneumatization

a substantial relationship between ABP and only mucosal thickening (p=0.016) on the left side (p<0.05). This relationship tells us that ABP affects maxillary sinus pathology in both dimensions of thickening.

There is also a significant relationship between PPP and polypoidal mucosal thickening (p=0.008) on the right side (p<0.05). This relationship tells us that PPP affects maxillary sinus pathology only at the extent of partial mucosal thickening.

There is also a significant relationship between ZPP and mucosal thickening (p=0.032) on the right side (p<0.05). This relationship shows that ZPP affects maxillary sinus pathology in one dimension called mucosal thickening.

On the left side, ABP and total opacification have an important relationship (p=0.001, p<0.05). This means that total opacification affects maxillary sinus pathology. Also, there is a significant relationship between ABP and mucosal thickening (p=0.016) on the left side (p<0.05).

Table 2 shows the distribution of alveolar pneumatization by age groups.

According to the chi-square test, right and left alveolar pneumatizations show significant differences according to age groups (p<0.05). From this analysis, it can be determined that alveolar pneumatization increases with increasing age.

Kalavagunta and Reddy detected 8% of maxillary sinus pneumatization in the UK population (3.5% male and 4,5 female) (8,12).

200 CBCT were detected, and 16 patients had pneumatizations in their study.

DISCUSSION

Pneumatization begins in the ethmoid sinuses, then in the maxillary sinus, sphenoid sinuses, and frontal sinuses. Although the pneumatization of the paranasal sinuses is different on the right and left, it can also vary from person to person (6).

The cause of pneumatization is not fully understood. Possible causes are heredity, craniofacial configuration, growth hormones, bone density, sinus surgery, sinus air pressure, and age-related process. (7, 17). Another theory is trauma that precedes maxillary sinus pneumatization (18).

If the patient is asymptomatic and the pneumatization does not affect other structures, it is called hypersinus (5). If the patient has symptomatic and local pressure symptoms and the pneumatization occurs to other structures, it is called pneumosinus (4). The general appearance of the patient is a facial deformity. Other possible manifestations are cheek pain with pressure, nasal congestion, and sinusitis (18). These deformities require surgical operation.

The aim is to evaluate maxillary sinus pneumatizations with CBCT and to correlate the pneumatizations with any associated pathology. The pneumatizations examined will contribute to the dentistry literature and enable dentists to obtain information about their relationship with pathology before surgical operations.

Table 1: Pneumatization types and the relationship with maxillary sinus pathologies

		No finding			Mucosal thickening			Polypoidal mucosal thickening			Partial opacification			Total opacification			Effusion			Total
		Absent	Present	P value	Absent	Present	P value	Absent	Present	P value	Absent	Present	P value	Absent	Present	P value	Absent	Present	P value	
		n (%)	n (%)		n (%)	n (%)		n (%)	n (%)		n (%)	n (%)		n (%)	n (%)		n (%)	n (%)		
Right	ABP	76 (66.1)	39 (33.9)	0.987	73 (63.5)	42 (36.5)	0.006	80 (69.6)	35 (30.4)	0.008	108 (93.9)	7 (6.1)	0.271*	114 (99.1)	1 (0.9)	0.698**	110 (95.7)	5 (4.3)	1*	115
	PPP	9 (90.0)	1 (10.0)	0.201*	6 (60.0)	4 (40.0)	0.849*	4 (40)	6 (60)	0.008**	10 (100)	0 (0)	1**	9 (90)	1 (10)	0.151**	10 (100)	0 (0)	1**	10
	ZPP	11 (47.8)	12 (52.2)	0.06	17 (73.9)	6 (26.1)	0.032	17 (73.9)	6 (26.1)	0.751*	23 (100)	0 (0)	1**	23 (100)	0 (0)	1**	22 (95.7)	1 (4.3)	1**	23
	FRP	5 (62.5)	3 (37.5)	1**	4 (50)	4 (50)	1*	7 (87.5)	1 (12.5)	1**	8 (100)	0 (0)	1**	8 (100)	0 (0)	1**	8 (100)	0 (0)	1**	8
	OPP	0	0	_	0	0	_	0	0	_	0	0	_	0	0	_	0	0	_	0
	SPP	0	0	_	0	0	_	0	0	_	0	0	_	0	0	_	0	0	_	0
	NCP	2 (66.7)	1 (33.3)	1**	2 (66.7)	1 (33.3)	1**	2 (66.7)	1 (33.3)	0.512**	3 (100)	0 (0)	1**	3 (100)	0 (0)	1**	3 (100)	0 (0)	1**	3
Left	ABP	73 (60.3)	48 (39.7)	0.153	75 (62)	46 (38)	0.016	95 (78.5)	26 (21.5)	0.347	114 (94.2)	7 (5.8)	0.225*	116 (95.9)	5 (4.1)	0.001**	113 (93.4)	8 (6.6)	0.138*	121
	PPP	25 (83.3)	5 (16.7)	0.039	13 (43.3)	17 (56.7)	0.32	20 (66.7)	10 (33.3)	0.267	29 (96.7)	1 (3.3)	1**	30 (100)	0 (0)	1**	27 (90)	3 (10)	0.104**	30
	ZPP	11 (57.9)	8 (42.1)	0.457	10 (52.6)	9 (47.4)	0.968	17 (89.5)	2 (10.5)	0.233*	18 (94.7)	1 (5.3)	0.501**	19 (100)	0 (0)	1**	19 (100)	0 (0)	1**	19
	FRP	10 (62.5)	6 (37.5)	0.774	9 (56.3)	7 (43.8)	0.741	11 (68.8)	5 (31.3)	0.755*	16 (100)	0 (0)	1**	16 (100)	0 (0)	1**	16 (100)	0 (0)	1**	16
	OPP	3 (75)	1 (25)	1**	2 (50)	2 (50)	1**	3 (75)	1 (25)	1**	4 (100)	0 (0)	1**	4 (100)	0 (0)	1**	4 (100)	0 (0)	1**	4
	SPP	0	0	_	0	0	_	0	0	_	0	0	_	0	0	_	0	0	_	0
	NCP	2 (40)	3 (60)	0.344**	4 (80)	1 (20)	0.375**	4 (80)	1 (20)	1**	5 (100)	0 (0)	1**	5 (100)	0 (0)	1**	5 (100)	0 (0)	1**	5

* Yates Chi-square; ** Fisher's exact test, ABP: Alveolar bone pneumatization, PPP: Palatal process pneumatization, ZPP: Zygomatic process pneumatization, FRP: Frontal process pneumatization, OPP: Orbital process pneumatization, SPP: Palatal pneumatization to sphenomaxillary plate, NCT: Nasal cavity process pneumatizations

Table 2: Distribution of alveolar pneumatization according to age

		Alveolar right		Total	p	Alveolar left		Total	p
		-	+			-	+		
Age Groups	18-30	180	31	211	0.012*	176	35	211	0.044*
	19-50	182	35	217		180	37	217	
	51+	144	49	193		144	49	193	
Total		506	115	621		500	121	621	

*Chi-square test

Preoperative evaluation is critical in evaluating maxillary sinus variations and detecting pathological problems (10).

This study provides information about different pneumatizations. CBCT is a gold standard that contributes to different pneumatizations. In this study, CBCT was used to identify pneumatizations. These pneumatizations are frontal process pneumatizations, zygomatic process pneumatizations, orbital pneumatizations, pneumatizations from palatal to sphenomaxillary plate, and pneumatizations caused by tooth loss (pneumatizations to alveolar bone). The present research made the following contributions:

- broadened our knowledge between pneumatizations and pathologies.
- provided data on rare pneumatizations

The prevalence of pneumatization in Turkey is 27.7%.

In the literature, the overall pneumatization in the world is 8% to 83.2%.

Schuh et al. reported the rate of alveolar pneumatization as 50% and as 100% by Lana et al. Shaidi et al. found alveolar pneumatization at a rate of 57.5% (10). With CBCT, it is possible to detect anatomical variations and perform more successful surgical applications.

Ketenci et al. found 244 alveolar pneumatizations in 300 patients in the CBCT evaluation in their study (8). In their assessment, mucosal thickening was stated as 63%. In this research, it was determined as 42% on the right and 46% on the left. Ketenci et al. achieved a higher result than this study.

Lana et al. 83.2% reported alveolar pneumatization in the Brazilian population (19). In 2003, Kalavagunta and Reddy detected 8% of maxillary sinus pneumatization in the UK population (8, 12). In 2015, Göçmen et al. reported that maxillary sinus pneumatization was 27.7% in the Marmara region of Turkey (9). Alveolar pneumatization increases with age (8). The results of the authors' study are similar to this finding.

The rate of mucosal thickening was stated as 48.8% and reported the rate of mucosal thickening as 27.5% (8). In this study, the incidence of mucosal thickening was 36.5%. The results of this study are similar to these two studies.

Yang et al. reported 8 cases of maxillary sinus pneumatization into the nasal cavity (20). Timurlenk et al. reported the ninth case (21). In this study, maxillary sinus pneumatization occurred in 4 nasal cavities.

Kalavagunta et al. reported sphenomaxillary plaque pneumatization as 3%, frontal process pneumatization 0.5%, and pneumatization to the orbit as 6% (12). This study found 1 pneumatization to the orbit and 9 pneumatizations to the frontal process.

CONCLUSION

CBCT best detects pneumatizations of the maxillary sinus. Maxillary sinus pneumatizations and pathologies should be diagnosed and understood to prevent complications that may occur during and after surgical operations. Rare maxillary sinus pneumatizations will make a significant contribution to the dentistry literature. Maxillary sinus pneumatizations are observed due to some pathologies such as mucosal thickening, polypoidal mucosal thickening, partial opacification, total opacification, and effusion. The most common is mucosal thickening with alveolar bone pneumatizations.

To prevent complications that may occur during and after surgical operations, it is necessary to know the pneumatizations of the maxillary sinus. Pathologies with maxillary sinus pneumatizations will contribute to the dentistry literature.

Ethics Committee Approval: This study was approved by Istanbul University Faculty of Dentistry Clinical Research Ethics Committee (Date: 28.08.2018, No: 72).

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REFERENCES

1. Iwanaga J, Wilson C, Lachkar S, Tomaszewski KA, Walocha JA, Tubbs RS. Clinical anatomy of the maxillary sinus: application to sinus

floor augmentation. Anat Cell Biol 2019;52(1):17-24.
2. Scuderi AJ, Harnsberger HR, Boyer RS. Pneumatization of the paranasal sinuses: normal features of importance to the accurate interpretation of CT scans and MR images. AJR Am J Roentgenol 1993;160(5):1101-4.
3. Demir K. Polipozis Tanılı Hastalarda Endonazal Anatomik Varyasyonların Görülme Sıklığının Tespiti ve Toplum ile Karşılaştırılması (dissertation). İstanbul: İstanbul Eğitim ve Araştırma Hastanesi K.B.B. Kliniği. 2006.
4. Lawson W, Patel ZM, Lin FY. The development and pathologic processes that influence maxillary sinus pneumatization. Anat Rec (Hoboken) 2008;291(11):1554-63.
5. Anbiaee N, Khodabakhsh R, Bagherpour A. Relationship between Anatomical Variations of Sinonasal Area and Maxillary Sinus Pneumatization. Iran J Otorhinolaryngol 2019;31(105):229-34.
6. Al-Taei JA. Computed tomographic measurement of maxillary sinus volume and dimension in correlation to the age and gender (comparative study among individuals with dentate and edentulous maxilla). J BaghColl Dent 2013;25(1):87-93.
7. Sharan A, Madjar D. Maxillary sinus pneumatization following extractions: a radiographic study. Int J Oral Maxillofac Implants 2008;23(1):48-56.
8. Ketenci F, Yalçın Yeler D, Koraltan M, Ünal Y. Maksiller sinüste alveolar pnömatizasyon ve ilişkili faktörlerin panoramik ve kıbt görüntüleme yöntemleri ile değerlendirilmesi. 7tepe Klinik 2019;15(3):339-44.
9. Göçmen G, Borahan MO, Aktop S, Dumlu A, Pekiner FN, Göker K. Effect of septal deviation, concha bullosa and Haller's cell on maxillary sinus's inferior pneumatization; a retrospective study. Open Dent J 2015;9:282-6.
10. Shahidi S, Zamiri B, Momeni Danaei S, Salehi S, Hamedani S. Evaluation of anatomic variations in maxillary sinus with the aid of cone beam computed tomography (CBCT) in a population in south of Iran. J Dent (Shiraz) 2016;17(1):7-15.
11. De Miranda CM, de Miranda Maranhão CP, Arraes FM, Padilha IG, De Farias LD, de Araujo Jatobá, MS. Anatomical variations of paranasal sinuses at multislice computed tomography: what to look for. Radiol Bras 2011;44(4):256-62.
12. Kalavagunta S, Reddy KT. Extensive maxillary sinus pneumatization. Rhinology 2003;41(2):113-7.
13. Rusu MC, Sava CJ, Stoenescu MD. Bilateral pneumatization of middle and superior nasal turbinates. Romanian Journal of Military Medicine 2019;123(3):196-201.
14. Ozcan KM, Selcuk A, Ozcan I, Akdogan O, Dere H. Anatomical variations of nasal turbinates. J Craniofac Surg 2008;19(6):1678-82.
15. Selcuk A, Ozcan KM, Akdogan O, Bilal N, Dere H. Variations of maxillary sinus and accompanying anatomical and pathological structures. J Craniofac Surg 2008;19(1):159-64.
16. Lombardi T, Bernardello F, Berton F, Porrelli D, Rapani A, Camurri Piloni A, et al. Efficacy of alveolar ridge preservation after maxillary molar extraction in reducing crestal bone resorption and sinus pneumatization: a multicenter prospective case-control study. Biomed Res Int 2018;2018: 9352130. doi: 10.1155/2018/9352130.
17. Cavalcanti MC, Guirado TE, Sapata VM, Costa C, Pannuti CM, Jung RE, César Neto JB. Maxillary sinus floor pneumatization and alveolar ridge resorption after tooth loss: a cross-sectional study. Braz Oral Res 2018;32(e64):1-10.
18. Nayak DR, Pujary K, Ramaswamy B, Mahesh SG, Muddaiah D. Anterior pneumatization of the maxillary sinus - Presenting as a facial swelling. Indian J Otolaryngol Head Neck Surg 2007;59(3):277-9.
19. Pelinsari Lana J, Moura Rodrigues Carneiro P, de Carvalho Machado V, Eduardo Alencar de Souza P, Ricardo Manzi F, Campolina Rebello Horta M. Anatomic variations and lesions of the maxillary sinus detected in cone beam computed tomography for dental implants. Clin Oral Implants Res 2012;23(12):1398-403.
20. Yang BT, Chong VFH, Wang ZC, Xian JF, Chen QH. CT appearance of pneumatized inferior turbinate. Clin Radiol 2008;63(8):901-5.
21. Timurlenk E, Eğilmez OK, Uzun L, Acar G, Kafkaslı Ç. Common cavity of pneumatized inferior turbinate and maxillary sinus: A case report. CausePedia 2014;3:1-5.

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USE OF CONE-BEAM COMPUTED TOMOGRAPHY IN PEDIATRIC PATIENTS: A RETROSPECTIVE OBSERVATIONAL STUDY

PEDİATRİK HASTALARDA KONİK IŞINLI BİLGİSAYARLI TOMOGRAFİ KULLANIMI: RETROSPEKTİF GÖZLEMSEL BİR ÇALIŞMA

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ABSTRACT

Objectives: This study aimed to determine and classify the reasons for cone-beam computed tomography (CBCT) imaging requests in the Oral and Maxillofacial Radiology Department, Istanbul University Faculty of Dentistry, and to examine which reasons more commonly require CBCT imaging.

Material and methods: From the local CBCT database, 956 pediatric patients gave their consent. Subsequently, age, gender, the reason for referral, a field of view (FOV), and referral departments were collected. Patients were grouped according to age distribution as 1-6 years, 7-12 years, and 13-17 years. Descriptive and comparative statistical analysis was performed.

Results: When CBCT referral reasons were analyzed, some of the most common requests were bone pathology (26.25%), impacted teeth (19.87%), dental anomalies (14.12%), cleft lip, and palate (13.91%), respectively. When referral reasons are evaluated according to age groups, dentoalveolar trauma (p=0.049), impacted teeth (p=0.000), dental anomalies, surgical applications (p=0.021), bone pathology (p=0.004), cleft lip and palate statistically significant differences were found between the (p=0.000) indications and age groups (p=0.000).

Conclusion: This study can guide dental practitioners in referring pediatric patients for three-dimensional imaging.

Keywords: Cone-beam computed tomography, field of view, indication, pediatric dentistry, radiation effects

ÖZ

Amaç: Bu çalışmada diş hekimliği fakültemizde konik ışınlı bilgisayarlı tomografi (KIBT) görüntüleme isteklerinin nedenlerinin belirlenmesi, sınıflandırılması ve hangi nedenlerin daha sık KIBT görüntüleme gerektirdiğinin incelenmesi amaçlanmıştır.

Gereç ve Yöntem: KIBT veri tabanından 956 pediatrik hasta incelenmiştir. Daha sonra yaş, cinsiyet, sevk nedeni, görüş alanı (FOV) ve sevk departmanları toplanmıştır. Hastalar yaş dağılımına göre 1-6 yaş, 7-12 yaş ve 13-17 yaş olarak gruplandırılmıştır. Tanımlayıcı ve karşılaştırmalı istatistiksel analiz yapılmıştır.

Bulgular: KIBT sevk nedenleri incelendiğinde en sık başvurulan istekler sırasıyla kemik patolojisi (%26,25), gömülü dişler (%19,87), diş anomalileri (%14,12), yarık dudak ve damak (%13,91) idi. Sevk nedenleri yaş gruplarına göre değerlendirildiğinde, dentoalveolar travma (p=0,049), gömülü dişler (p=0,000), diş anomalileri (p=0,000), cerrahi uygulamalar (p=0,021), kemik patolojisi (p=0,004), dudak damak yarığı, endikasyonlar ve yaş grupları arasında (p=0,000) istatistiksel olarak anlamlı fark bulundu.

Sonuç: Bu çalışma, diş hekimlerine üç boyutlu görüntüleme için çocuk hastaları sevk etmede rehberlik edebilir.

Keywords: Konik ışınlı bilgisayarlı tomografi, FOV, endikasyon, pediatrik diş hekimliği, radyasyonun etkileri

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INTRODUCTION

Cone-beam computed tomography (CBCT) is a three-dimensional imaging modality widely used in Dentistry to diagnose bone tissue in the maxillofacial region (1). The first CBCT was invented by British scientist Godfrey Housfield in 1967, developed from computerized tomography (2). Later, the first prototype was produced for radiotherapy and, in 1982, for angiography (2, 3). CBCT was developed independently and simultaneously by Arai et al. in Japan and by Mozzo et al. in Italy in the late 90s (4).

CBCT consists of an X-ray source and a detector rotating with this source; contrary to computerized tomography, not a fan-shaped but a conical-shaped radiation beam is sent through the region of interest (ROI). These projections are converted into a three-dimensional image (in axial, coronal, and sagittal planes, respectively) (4). Cone-beam imaging is used in dentistry to view high-contrast objects such as teeth and bone, as opposed to tasks that require soft tissue separations.

CBCT, based on a cone-shaped X-ray beam centered on a two-dimensional detector, can be used to diagnose pediatric dental clinic conditions involving impacted and supernumerary teeth (5-7). CBCT has many advantages: image accuracy, rapid scan time, reduced image artifact, reduced radiation dosage, and X-ray beam limitation (8). In addition; it provides less imaging time, easy data transfer, and less scattered radiation in comparison to computed tomography (CT) (6, 9).

Considering that children are more susceptible to the risks of ionizing radiation, every effort should be made to minimize the radiation burden while maintaining sufficient diagnostic yield (10, 11). The American Academy of Pediatric Dentistry (AAPD) recommends that CBCT use in pediatric dentistry be considered when conventional radiographs are inadequate for diagnosis and treatment planning and when the potential benefits outweigh the risk of additional radiation dose. It also emphasizes that it should not be routinely prescribed for diagnostic or screening purposes without clinical indications (12).

In children, CBCT images have been used to evaluate impacted teeth, airway analysis, and for periodontal, endodontic, and orthodontic purposes (13). According to the current guidelines of the AAPD, CBCT can be used for assessing the periapical pathosis in endodontics, oral pathology, anomalies in the developing dentition (e.g., impacted, ectopic, or supernumerary teeth), oral maxillofacial surgery (e.g., cleft palate), dental and facial trauma, and orthodontic and surgical preparation for orthognathic surgery (12). Nevertheless, there is limited evidence about the appropriate use of CBCT in children and adolescents. Still, guides on this subject are essential to reduce radiation risks in this age group. The SEDENTEXCT guidelines allow for several recommendations for usage. However, there are no unambiguous guidelines for pediatric dentistry (14). Although, the European DIMITRA project (dentomaxillofacial paediatric imaging: an investigation toward low-dose radiation induced risks) was focused on the pediatric field (15). The following CBCT suggestions in pediatric patients are mentioned in the position statement: -Impacted and supplementary teeth, -Dentoalveolar trauma, -Orofacial clefts, -Dental anomalies, -Bone pathology -Cone-beam-CT-based surgical planning of autotransplantation, -Syndromes. On the other hand, there are deficiencies in the DIMITRA position statement published in 2018: suggestions such as -TMJ/condylar abnormalities, -facial asymmetry, -surgical applications, -endodontics, -orthodontics. These suggestions should be added to or expanded upon.

This study aimed to develop evidence-based research on using CBCT in pediatric dentistry, including referral criteria. This retrospective study evaluated the referral reasons for CBCT in pediatric individuals.

MATERIALS and METHODS

The institutional review board approved the study protocol of the local clinic (Date: 10.01.2019, No: 78).

We performed a retrospective analysis of 956 patients under the age of 18 years who underwent a CBCT scan in our clinic between December 2015 and 2018 for three years.

All scans were taken with the CBCT-unit Soredex SCANORA®3Dx (Tuusula, Finland) and were stored in the OnDemand 3D Project Viewer Cybermed Inc. (California, USA) database. The CBCT device we used to have 8 different FOV (field-of-view) options; 50x50mm, 50x100mm, 80x100mm, 80x160mm, 140x100mm, 140x165mm, 180x165mm and 240x165mm. All parents signed a letter of consent permitting to use of data for research purposes before CBCT scans were taken. When there were multiple CBCTs per patient, only the first CBCT was included. Our study did not include low-image quality images and motion or metal artifacts. Figure 1 illustrated the study selection.

We evaluated the patients according to age, gender, the reason for referrals, Field of View (FOV), and referral departments: dentomaxillofacial radiology, oral and maxillofacial surgery, endodontics, periodontology, orthodontics, pedodontics, prosthodontics, and restorative dentistry. Patients were grouped according to age distribution as 1-6 years, 7-12 years, and 13-17 years.

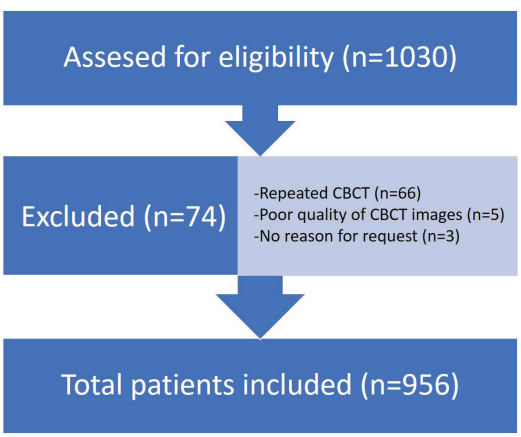

Figure 1: Flowchart

Based on the reason for referrals, the references for the SEDENTEXCT guideline were categorized under the following headings: Dent alveolar trauma, Facial trauma, Craniofacial anomalies and syndromes, Facial asymmetry, Dental anomalies, Surgical applications, TMJ/condylar abnormalities, Bone pathology, Cleft lip and palate, Endodontics, Orthodontics, Other.

Statistical analysis was performed for descriptive and comparative statistics using IBM® SPSS 25.0 (SPSS Inc, Chicago, IL, USA). The Pearson chi-square test was used for the statistical evaluation of categorical variables, and a p-value below 0.05 was considered statistically significant.

RESULTS

In the present study, 956 patients (451 females, 505 males) with a mean age of 13.19±3.281 (range 1-17) were examined. When the reasons for CBCT requests were analyzed, some of the most common requests in 956 patients were bone pathology (26.25%), impacted teeth (19.87%), dental anomalies (14.12%), cleft lip and palate (13.91%), and the distribution of all requests by gender is shown in the Table 1.

Table 1: Clinical indications for the cone-beam computed tomography (CBCT) examinations in the study

Indications	Females		Males		Total		p
	n	%	n	%	n	%	
Dentoalveolar trauma	10	2.21	16	3.16	26	2.71	0.482**
Facial trauma	19	4.21	22	4.35	41	4.28	1**
Craniofacial anomalies and syndromes	11	2.43	9	1.78	20	2.09	0.630**
Facial asymmetry	7	1.55	3	0.59	10	1.04	0.205***
Impacted teeth	102	22.61	88	1.74	190	19.87	**0.045***
Dental anomalies	48	10.64	87	17.22	135	14.12	**0.004***
Surgical applications	18	3.99	12	2.37	30	3.13	0.214**
TMJ/condylar abnormalities	8	1.77	10	1.98	18	1.88	1**
Bone pathology	119	26.38	132	26.13	251	26.25	0.931*
Cleft lip and palate	51	11.30	82	16.23	133	1.39	**0.028***
Endodontics	4	0.88	1	0.19	5	0.52	0.194***
Orthodontics	14	3.10	9	1.78	23	2.40	0.263**
Other	40	8.86	34	6.73	74	7.74	0.217*
Total	451		505		956	100	

*Pearson chi-square test, **Yates chi-square test, ***Fisher exact test

Patients were grouped according to age distribution as 1-6 years (n=36), 7-12 years (n=317), and 13-17 years (n=603). When the CBCT referral reasons are evaluated according to age groups, dentoalveolar trauma (p=0.049), impacted teeth (p=0.000), dental anomalies (p=0.000), surgical applications (p=0.021), bone pathology (p=0.004), cleft lip and palate (p=0.000), statistically significant differences were found between indications and age groups. The results are shown in Table 2. In addition, dental anomalies (22.22%) and bone pathology (22.22%) were the most common reasons for requests in the 1-6 age group. In the 7-12 age group, the most common reason for the request is dental anomalies, with a rate of 26.49%. The most common referral reason in the 13-17 age group is bone pathology with 29.85%.

When the FOV ranges of the CBCT device used according to age were examined, it was seen that the highest rate of images (43.1%) was obtained in all age groups with a FOV range of 50x100mm, and the FOV distribution is shown in Figure 2.

When the regions of interest (ROI) were evaluated, it was seen that the images were taken from the maxilla region at the highest rate in all age groups (1-6, 7-12, 13-17) (58%, 53%, 49%, respectively). While the images taken from the mandible region at the ages of 1-6 and 7-12 are at the lowest rate (11%, 21%, respectively), the images taken from the double chin in the 13-17 age group are less common (18.6%) compared to other regions in this age group (Figure 3).

When the departments that referral reasons for CBCT are examined, the departments that request CBCT the most in the 1-6 age group, respectively; Pedodontics Department (58.33%), Surgery Department (27.77%), Orthodontics (13.88%). In the 7-12 age group, Pedodontics Department (55.20%), Surgery Department (29.33%), Orthodontic Department (15.45%). In the 13-17 age group, the Department of Surgery (33.49%), Pedodontics (30.34%), Dentomaxillofacial Radiology (21.72%), and others are shown in detail in Figure 4.

DISCUSSION

There are a limited number of studies on the use of CBCT in pediatric patients, and these studies are based on different indications and in various age groups. The present study evaluated the

Table 2: Distribution of CBCT indications by age group

Indications	Group 1	Group 2	Group 3		p
	n (%)	n (%)	n (%)	Total (%)	
Dentoalveolar trauma	3 (8.33)	5 (1.57)	18 (2.98)	26 (2.71)	**0.049**
Facial trauma	4 (11.11)	16 (5.04)	21 (3.48)	41 (4.28)	0.065
Craniofacial anomalies and syndromes	2 (5.55)	4 (1.26)	14 (2.32)	20 (2.09)	0.189
Facial asymmetry	0 (0)	2 (0.63)	8 (1.32)	10 (1.04)	0.505
Impacted teeth	5 (13.88)	41 (12.93)	144 (23.88)	190 (19.87)	**0.000**
Dental anomalies	8 (22.22)	84 (26.49)	43 (7.13)	135 (14.12)	**0.000**
Surgical applications	1 (2.77)	3 (0.94)	26 (4.31)	30 (3.13)	**0.021**
TMJ/condylar abnormalities	1 (2.77)	7 (2.20)	10 (1.65)	18 (1.88)	0.778
Bone pathology	8 (22.22)	63 (19.87)	180 (29.85)	251 (26.25)	**0.004**
Cleft lip and palate	2 (5.55)	68 (21.45)	63 (10.44)	133 (13.91)	**0.000**
Endodontics	0 (0)	1 (0.31)	4 (0.66)	5 (0.52)	0.712
Orthodontics	0 (0)	4 (1.26)	19 (3.15)	23 (2.40)	0.130
Other	2 (5.55)	19 (5.99)	53 (8.78)	74 (7.74)	0.283
Total	36	317	603	956	

Group 1: Age 1–6 years, group 2: Age 7–12 years, group 3: Age 13–17 years, CBCT: Cone beam computed tomography

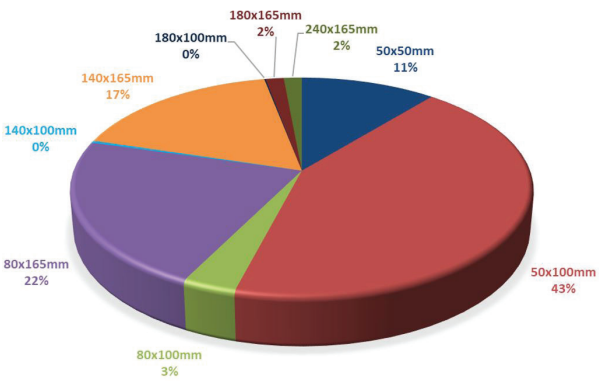

Figure 2: Distribution of the FOVs by number (n=956)

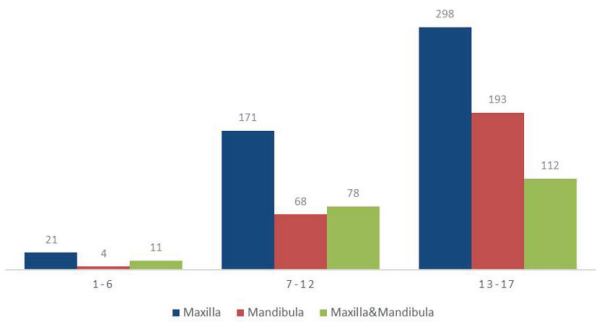

Figure 3: Distribution of the dentomaxillofacial areas by age groups

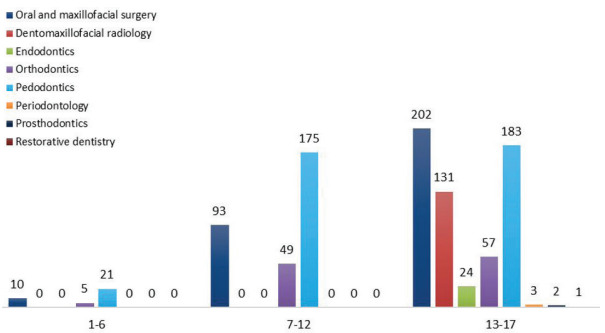

Figure 4: Distribution of Departments Requesting CBCT by Age

referral reasons for CBCT in pediatric patients at a higher rate (956 CBCT) than other studies (4, 13, 16-18). The present study focused on the basis for CBCT referrals according to the guidelines SEDENTEXCT and DIMITRA for clinical use in pediatric patients in a Turkish subpopulation (14, 15). In the DIMITRA position statement, we found that although it was intended to determine the CBCT indications in the pediatric patient group, there was no category for some indications (15). For this reason, we have categorized the request reasons based on SEDENTEXCT. In our study, different from the DIMITRA position statement, the following were added: Facial trauma, Facial asymmetry, Surgical applications, TMJ/condylar abnormalities, Endodontics, and Orthodontics.

There are limited resources in the literature investigating the referral reasons for CBCT in the pediatric population. Some of the indications reported among the causes of CBCT imaging

in pediatric patients in the literature are: an eruption of the dentition, viewing local resorptions associated with unerupted teeth, and examining the severity of facial traumas (19). In the study of Isman et al., the most common CBCT referral reason was; malocclusion and dentofacial anomalies with the highest rate in primary and permanent dentition, and impacted tooth localization in mixed dentition were the most common reasons for the indication (13). In the study of Van Acker et al., the most common CBCT referral reason was developing dentition-localized (4). Unlike other studies, bone pathology was the most common reason for CBCT referrals. In the present study, patients with bone pathology were mainly diagnosed with deeply carious teeth and radicular cysts caused by lesions in the related teeth. We think that the higher rate of bone pathology due to dental infectious conditions is the most common reason for the request in our study. At the same time, it is reported in the literature that caries and caries-related lesions are seen more frequently in the Turkish population (20). Compared to other studies, we can explain the higher incidence of bone pathology requests in our research in this way.

In the present study, we evaluated the referral reasons for CBCT patients under 18 years old. In similar studies in the literature, the mean age ranged from 8.3 to 13.42, from high to low, respectively; Yiğit et al., Isman et al., the present study, Hidalgo et al., Van Acker et al., Gümrü et al., Suzuki et al., mean age respectively; 14.32, 13.42, 13.19, 13.1, 12.35, 11.15, 8.3. (4, 13, 16, 17, 18, 21). In any way, this is induced by differences in referral reasons and racial differences. In the study of Isman et al. the most common indication for CBCT was malocclusion and dentomaxillofacial anomalies in the primary and permanent dentition age groups, whereas the localisation of impacted teeth was the most common indication in the mixed dentition age group (13). In the study of Van Acker et al. the most CBCT request (36%) was the developing dentition-localized which consists for the greatest part typically for second transitional period and the permanent dentition (4). In the study of Yigit et al. CBCT request in the 12- to 18-year age group is mostly impacted teeth (21). In the present study, similar to the Isman et al., CBCT indication in the 1- to 6-year age group and the 7- to 12-year age group were mostly dental anomalies (13). However, in our study, unlike the literature (4, 13, 21), the bone pathology was the most common indication in the 13- to 17-year age group.

Fundamental principles and guidelines for the use of CBCT include: 1) use appropriate image size or field of view, 2) assess radiation dose risk, 3) minimize patient radiation exposure, and 4) maintain professional competence in performing and interpreting CBCT studies (12). Published research on the pediatric use of CBCT mentions that a smaller field of view (FOV) in the pediatric population may meet the prescribing physician's or dentist's needs. The smaller the FOV used, the less effective the patient receives. When referring a patient to CBCT screening, the dentist should provide the CBCT practitioner with adequate clinical information (19). Van Acker et al. most commonly used a small FOV size of 50x55 mm at 81.5% (4). In the present study, it was observed that a maximum FOV of 50x100 mm was used with a rate of 43.1%, and it can be associated with referral reasons.

Contrary to the benefits of CBCT imaging, the radiation dose is higher than a single conventional periapical or panoramic radiograph (13). With this in mind, CBCT should be justified before imaging, as with any radiographic examination. The potential benefits of CBCT must outweigh the harms associated with exposure to ionizing radiation. A radiological examination should be performed with ALARA (As Low as Reasonably Achievable), a safety principle designed to minimize radiation doses and release radioactive materials (3). Many reasons can be counted among the reasons for the need for CBCT imaging, and the classification and standardization of these reasons will benefit physicians who will request CBCT. Our study is among the evidence that will contribute to developing existing guidelines on this subject. In the present study, the chi-square test revealed the relations between two categorical variables. The chi-squared test applied an approximation assuming the sample is large, while the Fisher's exact test and Yates's correction for continuity were used to provide a more conservative result for contingency tables with small cell counts. The limited number of samples and the evaluation of the archive belonging to a single center can be counted among the limitations of our study.

CONCLUSION

In the present study, it was revealed that the most common indication for CBCT was bone pathology and secondly impacted teeth. Research on the use of CBCT in dentistry in children is limited. Since the CBCT device contains ionizing radiation, it is important to master the use of CBCT in children in appropriate indications. Therefore, there is a need for detailed guidelines on the use of CBCT in the pediatric field.

Ethics Committee Approval: This study was approved by Istanbul University, Faculty of Dentistry Clinical Research Ethics Committee (Date: 10.01.2019, No: 78).

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REFERENCES

1. Costa EDD, Queiroz PM, Santaella GM, Capelozza ALA, Ambrosano

GMB, Freitas DQ. Influence of scan mode (partial/full rotations) and FOV size in the formation of artefacts in cone beam CT. Dentomaxillofac Radiol 2019;48(4):20180340.
2. Shukla S, Chug A, Afrashtehfar KI. Role of cone beam computed tomography in diagnosis and treatment planning in dentistry: an update. J Int Soc Prev Community Dent 2017;7(Suppl 3):S125-36.
3. Rehani M, Gupta R, Bartling S, Sharp G, Pauwels R, Berris T, et al. ICRP publication 129: Radiological protection in cone beam computed tomography (CBCT). Ann ICRP 2015;44(1):9-127.
4. Van Acker JW, Martens LC, Aps JK. Cone-beam computed tomography in pediatric dentistry, a retrospective observational study. Clin Oral Invest 2016;20(5):1003-10.
5. De Vos W, Casselman J, Swennen G. Cone-beam computerized tomography (CBCT) imaging of the oral and maxillofacial region: a systematic review of the literature. Int J Oral Maxillofac Surg 2009;38(6):609-25.
6. Katheria BC, Kau CH, Tate R, Chen J-W, English J, Bouquot J. Effectiveness of impacted and supernumerary tooth diagnosis from traditional radiography versus cone beam computed tomography. Pediatr Dent 2010;32(4):304-9.
7. Nematolahi H, Abadi H, Mohammadzade Z, Ghadim MS. The use of cone beam computed tomography (CBCT) to determine supernumerary and impacted teeth position in pediatric patients: A case report. J Dent Res Dent Clin Dent Prospects 2013;7(1):47-50.
8. Mehta V, Ahmad N. Cone beamed computed tomography in pediatric dentistry: Concepts revisited. J Oral Biol Craniofac Res 2020;10(2):210-11.
9. Korbmacher H, Kahl-Nieke B, Schöllchen M, Heiland M. Value of two cone-beam computed tomography systems from an orthodontic point of view. J Orofac Orthop 2007;68(4):278-89.
10. Little MP, Wakeford R, Tawn EJ, Bouffler SD, De Gonzalez AB. Risks associated with low doses and low dose rates of ionizing radiation: why linearity may be (almost) the best we can do. Radiology 2009;251(1):6-12.
11. Theodorakou C, Walker A, Horner K, Pauwels R, Bogaerts R, Jacobs Dds R, et al. Estimation of paediatric organ and effective doses from dental cone beam CT using anthropomorphic phantoms. Br J Radiol 2012;85(1010):153-60.
12. American Academy of Pediatric Dentistry. Guideline on prescribing dental radiographs for infants, children, adolescents, and persons with special health care needs. Pediatr Dent 2012;34(5):189-91.
13. İşman Ö, Yılmaz HH, Aktan AM, Yılmaz B. Indications for cone beam computed tomography in children and young patients in a Turkish subpopulation. Int J Paediatr Dent 2017;27(3):183-90.
14. European Commission, Directorate-General for Energy, Cone beam CT for dental and maxillofacial radiology: evidence-based guidelines. Publications Office; 2012. Available from: doi/10.2768/21874
15. Oenning AC, Jacobs R, Pauwels R, Stratis A, Hedesiu M, Salmon B. Cone-beam CT in paediatric dentistry: DIMITRA project position statement. Pediatr Radiol 2018;48(3):308-16.
16. Gümrü B, Guldali M, Tarcin B, Idman E, Peker S. Evaluation of cone beam computed tomography referral profile: Retrospective study in a Turkish paediatric subpopulation. Eur J Paediatr Dent 2021;22(2):66-70.
17. Hidalgo-Rivas JA, Theodorakou C, Carmichael F, Murray B, Payne M, Horner K. Use of cone beam CT in children and young people in three United Kingdom dental hospitals. Int J Paediatr Dent 2014;24(5):336-48.
18. Suzuki H, Fujimaki S, Chigono T, Yamamura M, Sakabe R, Sakabe J, et al. Survey on the using limited area cone beam CT in pediatric dentistry. Japan J Pediatr Dent 2006;44(4):609-16.
19. Yepes JF, Booe MR, Sanders BJ, Jones JE, Ehrlich Y, Ludlow JB, et al. Pediatric phantom dosimetry of Kodak 9000 cone-beam computed tomography. Pediatr Dent 2017;39(3):229-32.
20. Eronat N, Koparal E. Dental caries prevalence, dietary habits, tooth-brushing, and mother's education in 500 urban Turkish children. J Marmara Univ Dent Fac 1997;2(4):599-604.
21. Yiğit T, Yüksel HT, Evirgen Ş, Kaçmaz I, Türkmenoğlu A. Evaluation of use of cone beam computed tomography in paediatric patients: A cross-sectional study. Int J Paediatr Dent 2023;00:1-9.

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PSYCHOLOGICAL RESILIENCE AND SPIRITUAL WELL-BEING OF UNDERGRADUATE STUDENTS IN A PEDIATRIC NURSING COURSE

ÇOCUK SAĞLIĞI VE HASTALIKLARI HEMŞİRELİĞİ DERSİNİ ALMIŞ ÖĞRENCİLERDE PSİKOLOJİK DAYANIKLILIK VE SPİRİTÜEL İYİLİK HALİ

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ABSTRACT

Objective: This study was carried out to determine the psychological resilience and spiritual well-being levels of undergraduate students in a pediatric nursing course and the relationship between their psychological resilience and spiritual well-being levels.

Materials and Methods: The study was implemented in a cross-sectional descriptive design between October 27 and November 18, 2022. The study was conducted with 131 undergraduate students in a pediatric nursing course using an online questionnaire. Data were collected using the online demographic form, the Psychological Resilience Scale for Adults, and the Spiritual Well-Being Scale.

Results: Most of the students (92.4%) were between the ages of 21-24 and 77% of them were female students. It was determined that female students' spiritual well-being scale averages were higher, and socioeconomic status affected both psychological resilience and spiritual well-being. Most of the students (71.7%) stated that the pediatrics course increased their love for children. A moderate positive relationship was found between the students' psychological resilience level and spiritual well-being. In particular, a statistically significant relationship was found between the resilience scale's self-perception and social resources sub-dimensions and the Spiritual Well-Being level ($p<0.05$).

Conclusion: The study emphasizes that there is a relationship between the level of resilience and the level of spiritual well-being in nursing students who have taken pediatric courses. In this study, it is recommended that the education and clinical practices of the child health and diseases nursing course should be arranged in a way that strengthens the resilience and spiritual well-being of the students. There is a need for program arrangements that will increase resilience and spiritual well-being in the education and care practices of nursing students.

Keywords: Nursing students, resilience, spirituality

ÖZ

Amaç: Bu çalışma, çocuk sağlığı ve hastalıkları hemşireliği dersini almış hemşirelik öğrencilerinin psikolojik dayanıklılık ve spiritüel iyi oluşluk düzeylerini ve birbirleriyle olan ilişkilerini belirlemek amacıyla yapılmıştır.

Gereç ve Yöntemler: 27 Ekim-18 Kasım 2022 tarihleri arasında kesitsel tanımlayıcı desende yürütülmüştür. Çalışma 131 çocuk sağlığı ve hastalıkları hemşireliği dersini almış hemşirelik öğrencileriyle çevrimiçi anket kullanılarak yapılmıştır. Verilerin toplanmasında online demografik form, Yetişkinler için Psikolojik Dayanıklılık Ölçeği ve Spiritüel İyi Oluşluk Ölçeği kullanılmıştır.

Bulgular: Öğrencilerin çoğu (%92,4) 21-24 yaş arasında ve %77'si kadın öğrencidir. Kadın öğrencilerin spiritüel iyi oluşluk ölçek puan ortalamalarının daha yüksek olduğu, sosyoekonomik durumun hem psikolojik dayanıklılığı hem de spiritüel iyi oluşluğu etkilediği belirlenmiştir. Öğrencilerin çoğu (%71,7) pediatri dersinin çocuklara olan sevgilerini arttırdığını belirtmiştir. Öğrencilerin psikolojik dayanıklılık düzeyi ile spiritüel iyi oluşluk düzeyi arasında pozitif yönde orta düzey ilişki tespit edilmiştir. Özellikle psikolojik dayanıklılık ölçeği kendilik algısı ve sosyal kaynaklar alt boyutları ile Spiritüel İyi Oluşluk düzeyi arasında istatistiksel olarak anlamlı ilişki bulunmuştur ($p<0,05$).

Sonuç: Bu çalışma, çocuk sağlığı ve hastalıkları hemşireliği dersini almış hemşirelik öğrencilerinde dayanıklılık düzeyi ile spiritüel iyi oluşluk düzeyi arasında ilişki olduğunu vurgulamaktadır. Bu çalışma ile çocuk sağlığı ve hastalıkları hemşireliği dersinin eğitim ve klinik uygulamalarının öğrencilerin dayanıklılık ve spiritüel iyi oluşluklarını güçlendirecek şekilde düzenlemesi gerektiği öngörülmektedir. Hemşirelik öğrencilerinin eğitim ve bakım uygulamalarında dayanıklılık ve spiritüel iyi oluşluğu arttıracak program düzenlemelerine gereksinim bulunmaktadır.

Anahtar Kelimeler: Hemşirelik öğrencileri, dayanıklılık, spiritüalite

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INTRODUCTION

Nursing education can be a stressful experience for students (1,2). Nursing students experience stress due to clinical practice in nursing education. In addition, the fear of being infected during the pandemic, increased academic workload, and ineffective distance education methods can cause stress and anxiety (3,4). Moreover, factors such as studying and exam anxiety, caring for patients, lack of clinical competence, feeling unprepared for practice, and fear of making mistakes are among the sources that cause stress (5,6). In particular, nursing students may experience anxiety during the Child Health and Diseases Nursing course (7, 8). In the clinical practice of pediatric nursing courses, students experience high levels of anxiety during their clinical experience with children and are anxious while giving care (7, 9). In the literature, the reasons that students experience anxiety during their pediatric internships are communication with the child, pediatric drug administration, and fear of harm (10, 11). All these sources of stress can affect both the health status and academic performance of nursing students (2, 12).

Resilience is described as the ability to cope with difficulties and includes how an individual learns to become stronger from experience (13, 14). Resilience is a positive adaptation that improves an individual's ability to adapt to adversity (13). Nursing students need resilience to overcome stressors and to be ready to take on their nursing roles after graduation (12, 15). In an integrative review on resilience in nursing education, it was reported that resilience is important in nursing education (16). Studies have reported that resilience increases the learning experience and has positive effects on academic performance and long-term professional practice (16). The resilience levels of nursing students differ between countries. A moderate level of resilience was identified in Nigeria, while a high level of resilience was reported in Spain (17,18). It is emphasized that psychological health is better in nursing students who have higher levels of resilience.

Well-being is an individualized and multidimensional construct that includes physical, mental, and psychosocial dimensions (2). Spiritual well-being is described as the individual's positive feelings and behaviors regarding his/her relationships with himself/herself and others, inner peace, positive attitudes, satisfaction, love, respect, and meaning in life. Spiritual well-being contributes to the development of coping methods (19). Resilience is important in nursing students and is reported to have a positive effect on their well-being (2).

It is thought that the well-being of individuals who use resilience and coping strategies will be better. However, it is important to evaluate the level of psychological resilience in nursing students faced with stress due to their education and working conditions and its relationship with spiritual health, which includes coping strategies. This is even more important in pediatric clinical practices, where stress can be experienced intensely. If the relationship between resilience and spiritual well-being levels in nursing students is determined, integration into nursing education, practice, and management can be achieved. In addition, looking at the literature, it is seen that only resilience or spiritual well-being is evaluated in nursing students. Only one study evaluated the resilience and spiritual well-being of all general health workers (20-24). A study examining resilience and spiritual well-being in nursing students taking pediatrics courses could not be found in the literature. For this reason, it is thought that this study will fill an important gap in the literature in terms of determining both spiritual well-being and resilience for nursing senior students who are in pediatric clinical practice. Therefore, the purpose of the study is to evaluate the psychological resilience and spiritual well-being levels of senior nursing students in pediatric nursing clinical practice.

Research questions

1. What is the psychological resilience level of undergraduate students in the pediatric nursing course?

2. What is the spiritual well-being level of undergraduate students in the pediatric nursing course?

3. What is the relationship between psychological resilience and spiritual well-being in undergraduate students in the pediatric nursing course?

MATERIAL and METHODS

Design

This study was administered as a cross-sectional study to evaluate the relationship between psychological resilience and spiritual well-being levels of senior nursing students. Helsinki Declaration principles and the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) checklist were used to guide this study (see Supplementary file).

Sample and setting

Between 07 April 2022 and 30 June 2022, undergraduate students in the pediatric nursing course of a university in Turkey were included in the study. The sample consisted of 163 nursing students studying at a state university. The study aimed to reach the entire universe by not choosing a sample. A hundred and thirty-one students who met the criteria of the study and filled out the forms completely formed the sample. Nursing students in the study received training in clinical practice for the first four days of the week for a semester.

Participants

The inclusion criteria of this study were as follows: senior nursing students, having internet access, and volunteering to participate in this research. The exclusion criteria of this study were as follows: receiving psychiatric treatment and substance abuse.

Procedures

At the beginning of the online survey, the participants were informed about the aim of this study, and they were given the choice of whether to volunteer or not. The Faculty of Health Sciences institutional permission (number: E-73351307-605.01-2200033465) was obtained for the study. Ethical approvals were obtained from University Ethical Committee (26.10.2022/

protocol number: 2022/387). All responses were obtained with informed consent.

Data collection
The following scales were used to collect data: a) the online demographics form created by the researchers, b) the Psychological Resilience Scale for Adults, and c) the Spiritual Well-Being Scale.

Online demographics form
Data collection was performed through an online demographics form. The form consists of two parts. In the first part of the form, there were questions such as the sociodemographic characteristics (age, gender, grade point average, etc.) of the students, and in the second part, questions such as the difficulties experienced in the pediatric internship and the effect of the pediatrics course on the state of liking children. The online demographics form was created by the authors based on information in the literature (2, 12).

Psychological resilience scale for adults (PRSA)
The Resilience scale for Adults was developed by Friborg et al. and includes six the sub-dimensions of "self-perception," "perception of the future," "structural style," social competence," "family cohesion," and "social resources" (25). The scale was adapted to Turkish culture by Basım and Çetin (26).

The scale is a 5-point Likert scale composed of 33 items and six sub-dimensions. In scoring the scale, the guidelines determined by the researchers who developed the scale were followed. When scores on the scale increased and resilience was desired to increase, then from left to right, the answer boxes were evaluated as 1, 2, 3, 4, and 5. If the scores decreased, and resilience was desired to increase, then the answer boxes were evaluated as 5, 4, 3, 2, and 1. If this opinion is taken into account, in the scale, questions 1-3-4-8-11-12-13-14-15-16-23-24-25-27-31-33 are specified as questions that should be reverse coded. This principle was taken into account in the evaluation of the scale. The score to be taken from the scale varies between 33 and 165. In the context of the reliability analysis of the scale, Cronbach's alpha reliability coefficient values ranged from 0.68 to 0.79 for the sub-dimensions. The total Cronbach's alpha value of the scale was calculated as 0.86. The scale does not have a cut-off score. In the evaluation of the scale, it indicates that as the scores increase, psychological resilience increases, and as the scores decrease, psychological resilience decreases.

Spiritual well-being scale (SWBS)
The SWBS was developed by Ekşi and Kardaş (27). This scale was applied to individuals between the ages of 16-54. The items of the scale were written by scanning the relevant literature, and making use of other scales. Opinions were taken from 17 experts who had done studies on the subject. The items were finalized by taking the expert opinions into account. The scale consisted of 29 items and 3 sub-scales (anomie, harmony with nature, and transcendence). The scale is a 5-point Likert-type scale. The Cronbach's alpha value of the scale was found to be 0.886 (27).

Data analysis
Statistical Package for Social Sciences, version 22.0, for Windows (SPSS) was used in the analysis of the data. The Kolmogorov-Smirnov test was used for the normal distribution of the data. The comparison of the non-normally distributed variables was performed using the Mann-Whitney U Test and Kruskal-Wallis Test.

The comparison of the non-normally distributed variables was performed Correlation analysis, called Spearman, was used to investigate the relationship between the Psychological Resilience Scale for Adults and the Spiritual Well-Being Scale. Regression analysis was used for the variables predicting the Spiritual Well-Being Scale. Statistical significance was accepted as $p<.05$.

RESULTS

Descriptive descriptive characteristics of students and comparison of these characteristics with scale scores
Most of the participants (92.4%) were between 21 and 24 years old, with a mean age of 22.56±1.37, and female (77.1%). Most of the students (68.7%) live in dormitories. 34% of nursing students have been diagnosed with COVID-19 and 47% have experienced quarantine. Most of the students (82.4%) defined their economic status as medium level. Most of the students (59%) stated that they want to work in pediatric services after graduation. Having taken the pediatrics course increased most students' love of children (71.7%). When the problems encountered by the students in the pediatric services were examined, it was reported that they most commonly thought that they would harm the children (90%), and that they had difficulties communicating with the children. The mean scores on the PRSA of the students were 116.31±13.6, and the mean score on the SWBS was 114.73±16.37 (Table 1).

It was concluded that there was a relationship between the gender of the students and the spiritual well-being scale score ($U= 1093.000$; $p<0.05$), and the spiritual scale score average for women was higher. It has been determined that the socioeconomic status of the students had an impact on both the resilience scale score and the spiritual well-being scale score. It was determined that there was no relationship between the other descriptive characteristics of the students and the distributions of scale scores (Table 2).

Relationship between resilience and spiritual well-being
As seen in Table 3, it is observed that there is a moderately significant positive correlation between students' resilience and their spiritual well-being. We assessed the association between the resilience scale sub-group and SWBS. Perception of self and social resources were associated with SWBS. No significant relationship was found between the structural style, perception of the future, family cohesion, and social competence sub-dimension of the PRSA and the SWBS ($p<0.001$) (Table 4).

DISCUSSION

The study aimed to evaluate the psychological resilience and spiritual well-being levels of undergraduate students in a pediatric nursing course. It is concluded that there is a relationship

Table 1: Demographic characteristics of students (n=131)

Characteristics	M±SD	Min-Max
Age	22.56±1.37	21-28
Grade average	2.94±0.31	1.86-3.70
Resilience Scale for Adults		
Structural style	14.12±2.06	9-19
Perception of future	14.74±3.58	6-20
Family cohesion	19.53±2.73	13-30
Perception of self	21.32±4.67	8-29
Social competence	21.04±3.86	9-30
Social resources	25.54±4.31	11-33
PRSA Total score	116.31±13.6	86-142
SWBS-Subscales		
Transcendence subscale	61.10±11.45	16-75
Harmony with nature subscale	30.77±4.12	21-35
Anomie subscale	22.84±5.71	8-35
SWBS Total Score	114.73±16.37	58-144
	n	**%**
Age		
21-24	121	92.4
25-28	10	7.6
Gender		
Female	101	77.1
Male	30	22.9
High school education status		
Anatolian high school	95	72.5
Health vocational high school	25	19.1
General high school	8	6.1
Science high school	3	2.3
Place of stay in education period		
Dormitory	90	68.7
At home (with friends)	18	13.7
At home (with family)	16	12.2
At home (alone)	7	5.3
Chronic illness		
No	115	87.8
Yes	16	12.2
COVID-19 diagnosis before		
No	86	65.6
Yes	45	34.4
Quarantine status		
No	69	52.7
Yes	62	47.3
Experiencing important life event in 6 months		
No	103	78.6
Health problems in family member/s	17	13
Loss of relative	6	4.6
Accident	5	3.8
Socioeconomic status		
Middle	108	82.4
Low	21	16.0
High	2	1.5
Work in the pediatric service after graduation		
Yes, I would like to	78	59.5
No, I don't want to	53	40.5
The effect of pediatrics course on liking children		
Increased	94	71.75
Decreased	6	4.5
Has not changed	31	23.6
Difficulties experienced in pediatric internship[c]		
Difficulty communicating with the child and/or their relatives	64	48.9
Students think that they will harm the child	119	90.8
Thoughts of harming the child by misapplication during care and/or treatment	61	46.6
Not having enough knowledge about the care and/or application to be made to the child	40	30.5

[a]: Mann-Whitney U test, b: Kruskall-Wallis test, [c]: Multiple options were ticked, SD: Standart deviation

between the gender of the students and their spiritual well-being; spiritual well-being is higher in female students. Consistent with our study, Aydın et al.'s study also reported that female nursing students had higher psychological well-being than males (28). This may be related to the high number of female students in both studies. It has been determined that there is a positive relationship between the socioeconomic status of the students and their spiritual well-being and resilience levels. Another study on the factors affecting resilience in nursing students revealed that economic status was an important factor (29). The literature on the subject emphasizes that financial difficulties are one of the environmental risk factors that negatively affect mental health. The research concluded that economic self-sufficiency is an important concept that affects psychological resilience and spiritual well-being (30). Most of the students in our study want to work in pediatric services after graduation. Similarly, to our study, 38% of the students in Top's study and 80% in Bektaş's study want to work in a pediatric service (31, 32). It is thought that this situation is related to the increase in the level of love for children among students who take pediatric courses.

Although there is no universal definition of resilience, it is defined in the literature as adapting to difficulties, maintaining balance, strength in coping with problems, and growing developmentally (33, 34). After caring for adult patients in nursing education, the pediatrics course, which requires a different approach due to its developmental characteristics, can be a challenging process for students. For this reason, it is emphasized that the difficulties encountered in the pediatric nursing internship and the adaptation process can affect the resilien-

Table 2: Comparison of students' descriptive characteristics and PRSA and SWBS score distributions

		Psychological Resilience Scale for Adults				Spiritual Well-Being Scale			
	n	**M (SD)**	**Min-Max**	**Test**	**P**	**M (SD)**	**Min-Max**	**Test**	**p**
Gender									
Female	101	116.59 (13.99)	86-141	1414.500 [a]	0.582	116.29 (16.89)	58-144	1093.000[a]	0.02
Male	30	115.37 (12.81)	94-142			109.47 (13.41)	87-133		
Socioeconomic status									
Middle	108	117.18 (12.89)	90-141	6.017[b]	0.04	116.41 (15.46)	82-144	5.996[b]	0.05
Low	21	110.43 (15.94)	86-138			107.71 (18.84)	58-140		
High	2	131.50 (14.84)	121-141			97.50 (14.84)	87-108		
Place of stay in education period									
Dormitory	90	115.07 (13.54)	87-140	7.575 [b]	0.05	115.13 (15.95)	58-144	3.914 [b]	0.27
At home (with friends)	18	117.61 (12.38)	94-141			108.50 (16.86)	82-139		
At home (with family)	16	115.88 (14.13)	86-134			116.81 (18.19)	82-138		
At home (alone)	7	130.00 (12.68)	106-142			120.71 (15.09)	101-140		
COVID-19 diagnosis before									
No	86	115.59 (12.96)	86-141	1779.000 [a]	0.44	113.12 (16.07)	58-140	1621.000	0.12
Yes	45	117.69 (15.04)	87-142			117.80 (16.68)	85-144		
Quarantine status									
No	69	114.62 (13.06)	86-141	1828.000[a]	0.15	112.13 (16.58)	58-140	1768.000v	0.08
Yes	62	118.19 (14.22)	87-142			117.61 (15.76)	84-144		
The effect of pediatrics course on liking children									
Increased	94	116.24 (14.00)	86-142	0.273[b]	0.88	114.43 (15.89)	82-144	1954	0.37
Decreased	6	119.00 (12.42)	102-134			110.83 (9.36)	96-121		
Has not changed	31	116.00 (13.30)	95-141			116.39 (18.91)	58-140		

[a]Mann-Whitney U test, [b]Kruskal Wallis H test

Table 3: Comparison of the relationship between the Psychological Resilience Scale for Adults and the Spiritual Well-Being Scale

	n	**r***	**p**
PRSA-SWBS	131	.50	0.00

* Spearman Correlation Analysis, PRSA: Psychological Resilience Scale for **Adults**, WBS: Spiritual Well-Being Scale

ce and well-being of the students (11). In our study, however, no significant relationship was found between the difficulties in resilience and spiritual well-being that were experienced in pediatric clinics. In Mutlu's study, it was reported that the challenging experiences in pediatric internships affect students' well-being, self-efficacy, and resilience (11). It is thought that this situation may be related to the differences in the education program. For this reason, it is important to organize prog-

Table 4: Association of the Resilience scale sub-groups and Spiritual Well-Being Scale

	B	**SE**	**β**	**t**	**p**
Structural style	0.25	0.64	0.03	0.38	0.69
Perception of future	0.87	0.50	0.19	1.74	0.08
Family cohesion	0.51	0.47	0.08	1.08	0.28
Perception of self	0.99	0.38	0.28	2.60	0.01
Social competence	-0.11	0.38	-0.02	0.30	0.75
Social resources	0.70	0.31	0.18	2.23	0.02
	R_2=0.254				

B: Beta coefficient, SE: Standard error, β: Standardized beta coefficient, R2: Coefficient of determination

rams that will increase resilience and well-being for students who take the course of pediatric nursing to be ready for an internship.

Our study revealed that there was a moderately positive relationship between resilience and spiritual well-being. Chow et al. reported a moderately positive correlation between resilience and well-being in a study of 678 nursing students in Hong Kong (12). Similarly, Chiang et al. emphasized in their study that spiritual health and resilience are interrelated (13). The results of these studies are similar to our study. Resilient individuals can cope with negative situations by buffering them with positive ones. This type of emotion regulation is associated with well-being (35). The education programs for pediatric nursing students who are faced with various stressors should include elements that strengthen their resilience and well-being and improve their positive thinking skills.

Our study, which examined the effect of self-perception and social resources sub-dimensions of the PRSA on the SWBS score, determined that the model was significant ($p<0.05$). It can be said that as individuals' self-perception and the support they receive from social resources increase, their well-being levels are also positively affected. Studies on nurses in the literature (36, 37) have concluded that self-perception and social resources are associated with well-being, which supports our study. It is thought that the programs that will improve the self-perception of nursing students will increase their well-being and resilience. It is thought that having knowledge about the situations that affect the well-being and resilience of pediatric nursing students will play a guiding role in nursing education, practice, care, and accreditation. One of the strengths of the study is that the majority of the sample was reached, and the participation rate of the students was high. Moreover, the online survey, which made it easy to reach students, is one of the strengths of the study.

There are limitations regarding the use of questionnaires based on participants' self-reports and the cross-sectional research design used in the study. The data analysis was carried out according to the cross-sectional research design. Therefore, it can be difficult to establish temporal and causal relationships between variables. Additionally, the fact that the majority of the study sample was female had a major impact on the study outcomes, which is another limitation of the study. In addition, one of the limitations of the study is that the study was conducted only in the relevant school of a state university. Therefore, the study results cannot be generalized.

CONCLUSIONS

In this study, it was revealed that there was a moderate positive relationship between spiritual well-being and psychological resilience. It was concluded that there was a significant relationship when the effect of the spiritual well-being scale score and the resilience scale self-perception and social resources sub-dimension scores were evaluated together. It is thought that initiatives that improve self-perception and increase social resource support in nursing programs will positively affect students' resilience and spiritual well-being. Also, in terms of determining the effects of child health courses and clinical internships, it is thought that the results of this study will be beneficial for the review of pediatrics courses. Accordingly, it is recommended to implement initiatives that will increase the resilience and spiritual well-being of nursing students in nursing education and care practices. In this context, it is recommended to apply training programs that will increase students' ability to cope with stress and to adapt, and increase resilience.

Ethics Committee Approval: This study was approved by Bolu Abant Izzet Baysal University, Human Research Ethics Committee in Social Sciences (Date: 26.10.2022, No: 2022/387).

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REFERENCES

1. Onan N, Karaca S, Unsal Barlas G. Evaluation of a stress coping course for psychological resilience among a group of university nursing students. Perspect Psychiatr Care 2019;55(2):233-8.
2. Spurr S, Walker K, Squires V, Redl N. Examining nursing students' wellness and resilience: An exploratory study. Nurse Educ Pract 2021;51:1-9.
3. Fernández IAM, Carreño Moreno S, Chaparro Díaz L, Gallegos-Torres RM, Medina Fernández JA, Hernández Martínez EK. Fear, stress, and knowledge regarding COVID-19 in nursing students and recent graduates in Mexico. Invest Educ Enferm 2021;39(1):e05. doi: 10.17533/udea.iee.v39n1e05.
4. Labrague LJ. Specific coping styles and its relationship with psychological distress, anxiety, mental health, and psychological well-being among student nurses during the second wave of the COVID-19 pandemic. Perspect Psychiatr Care 2022;58(4):2707-14.
5. Brown J, McDonald M, Besse C, Manson P, McDonald R, Rohatinsky N, et al. Nursing Students' Academic Success Factors: A Quantitative Cross-sectional Study. Nurse Educ 2021;46(2): E23-7.
6. Keener TA, Hall K, Wang K, Hulsey T, Piamjariyakul U. Quality of Life, Resilience, and Related Factors of Nursing Students During the COVID-19 Pandemic. Nurse Educ 2021;46(3):143-8.
7. Kubin L, Wilson CE. Effects of community volunteer children on student pediatric assessment behaviors. Clin Simul Nurs 2017;13(7):303-8.
8. Lassche M, Al-Qaaydeh S, Macintosh CI, Black, M. Identifying changes in comfort and worry among pediatric nursing students following clinical rotations. J Pediatr Nurs 2013;28(1):48-54.

9. Al-Qaaydeh S, Lassche M, Macintosh CI. Exploratory factor analysis of the pediatric nursing student clinical comfort and worry assessment tool. J Pediatr Nurs 2012;27(9):39-43.
10. Arslan S, Şener DK, Cangür Ş. Validity and reliability of clinical comfort and worry assessment tool of pediatric nursing student. Düzce Üniversitesi Sağlık Bilimleri Enstitüsü Dergisi 2018;8(2):61-6.
11. Mutlu B, Azak M, Kalaycı S. Çocuk sağlığı ve hastalıkları hemşireliği dersini almış öğrencilerin klinik rahatlık ve endişe durumu ile öz yeterlilik algısı arasındaki ilişkinin incelenmesi. JSR 2020;15(26):4293-315.
12. Chow KM, Tang WKF, Chan WHC, Sit WHJ, Choi KC, Chan S. Resilience and well-being of university nursing students in Hong Kong: a cross-sectional study. BMC Med Educ 2018;18(1):13.
13. Chiang YC, Lee HC, Chu TL, Wu CL, Hsiao YC. The relationship between spiritual health, health-promoting behaviors, depression and resilience: A longitudinal study of new nurses. Nurse Educ Pract 2021;56:103219.
14. Thomas LJ, Revell SH. Resilience in nursing students: An integrative review. Nurse Educ Today 2016;36:457-62.
15. García-Izquierdoa M, Ríos-Risquez M I, Carrillo-García C, Sabuco-Tebar EDLÁ. The moderating role of resilience in the relationship between academic burnout and the perception of psychological health in nursing students. Educ Psychol 2018;38(8):1068-79.
16. Reyes AT, Andrusyszyn MA, Iwasiw C, Babenko-Mould Y. Resilience in nursing education: an integrative review. J Nurs Educ 2015;54(8):438-44.
17. Aloba O, Olabisi P, Aloba T. The 10-item connor-davidson resilience scale: factorial structure, reliability, validity, and correlates among student nurses in southwestern Nigeria. J Am Psychiatr Nurses Assoc 2016;22(1):43-51.
18. PRíos-Risquez MI, García-Izquierdo M, Sabuco-Tebar EDL, Carrillo-Garcia C, Martinez-Roche ME. An exploratory study of the relationship between resilience, academic burnout and psychological health in nursing students. Contemp Nurse 2016;52(4):430-9.
19. Fabbris JL, Mesquita AC, Caldeira S, Carvalho, ECD. Anxiety and Spiritual well-being in nursing students: a cross-sectional study. J Holist Nurs 2017;35(3):261-70.
20. Areshtanab HN, Rezvani R, Ebrahimi H, Hosseinzadeh M. Moral intelligence and its relationship to resilience among nursing students in Iran. J Psychiatr Nurs 2022;13(1):9-14.
21. Jafarianamiri SR, Qalehsari MQ, Zabihi A. Investigating the professional identity and resilience in nursing students during the COVID-19 pandemic. J Educ Health Promot 2022;11(1):151-6.
22. Tomsovic E, Bond L, Wyant D. Spiritual Well-Being and Use of spiritual care interventions of undergraduate nursing students. J Christ Nurs 2022;39(1):15-21
23. Yun SY. Convergent relationship between spiritual well-being, spiritual nursing competence, and attitude toward death of nursing students. J Korea Convergence Society 2022:13(3):371-8.
24. Rogers M, Windle A, Wu L, Taylor V, Bale, C. Emotional well-being, spiritual well-being and resilience of advanced clinical practitioners in the United Kingdom during COVID-19: an exploratory mixed method study. J Nurs Manag 2022;30(4):883-91.
25. Friborg O, Barlaug D, Martinussen M, Rosenvinge JH, Hjemdal O. Resilience in Relation to personality and intelligence. Int J Methods Psychiatr Res 2005;14(1):29-42.
26. Basım HN, Çetin F. Yetişkinler için psikolojik dayanıklılık ölçeği'nin güvenilirlik ve geçerlilik çalışması. Türk Psikiyatri Dergisi 2011;22(2):104-14.
27. Ekşi H, Kardaş S. Spiritual well-being: Scale development and validation. SPC 2017;2(1):73-88.
28. Aydin A, Kahraman N, Hiçdurmaz D. Determining the Perceived social support and psychological well-being levels of nursing students. J Psychiatr Nurs 2017;8(1):40-7.
29. Güngörmüş K, Okanlı A, Kocabeyoğlu T. Hemşirelik öğrencilerinin psikolojik dayanıklılıkları ve etkileyen faktörler. Psikiyatri Hemşireliği Dergisi 2015;6(1):9-14.
30. Antunes A, Frasquilho D, Zózimo JR, Silva M, Cardoso G, Ferrão J, et al. Exploring socioeconomic and mental health trajectories during times of economic recession: A qualitative study with primary health care users and professionals. J Ment Health 2020;29(5):597-604.
31. Bektaş M, Ayar D, Bektaş İ, Selekoğlu Y, Kudubeş AA, Altan SS. Hemşirelik öğrencilerinin çocuk sevme durumlarını etkileyen faktörlerin belirlenmesi. Pediatr Research 2015;2(1):37-41.
32. Top FÜ. Pediatri hemşirelik öğrencilerin çocuk sevme düzeyleri ile duygusal emek davranışları, uygulama performansları arasındaki ilişki. Gümüşhane Üniversitesi Sağlık Bilimleri Dergisi 2021;10(4):809-18.
33. Aburn G, Gott M, Hoare K. What is resilience? An integrative review of the empirical literature. J Adv Nurs 2016;72(5):980-1000.
34. Waterworth S, Grace, AM. Resilience and burnout in pediatric nurses in a tertiary children's hospital. MCN Am J Matern Child Nurs 2021;46(3):168-73.
35. Pradhan RK, Kumar U. Emotion, Emotion, well-being, and resilience: theoretical perspectives and practical applications. USA: CRC Press; 2021.
36. Cevizci O, Müezzin EE. Sağlık çalışanlarında psikolojik belirtilerin ve psikolojik dayanıklılığın incelenmesi. Kıbrıs Türk Psikiyatri ve Psikoloji Dergisi 2019;1(3):166-72.
37. Yeşilot SB, İnel A, Dündar Y. Bölge Bilge ruh sağlığı ve hastalıkları hastanesinde çalışan hemşirelerin başa çıkma tarzları, psikolojik dayanıklılık ve ruhsal durumlarının değerlendirilmesi. Adnan Menderes Üniversitesi Sağlık Bilimleri Fakültesi Dergisi 2021;5(2):275-87.

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EVALUATING THE RELATIONSHIP BETWEEN TENDENCIES OF ORTHOREXIA NERVOSA AND BODY DISSATISFACTION AMONG ADULTS

YETİŞKİNLERDE ORTOREKSİYA NERVOZA EĞİLİMLERİ İLE BEDEN MEMNUNİYETSİZLİĞİ ARASINDAKİ İLİŞKİNİN DEĞERLENDİRİLMESİ

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ABSTRACT

Objective: The aim of the study is to evaluate the relationship between the tendencies of orthorexia nervosa and body dissatisfaction among adults.

Material and Methods: The study was conducted on 239 voluntary individuals who were aged between 19 and 64 years and residing in Gaziantep. While the ORTO-11 scale was used to assess the orthorexic tendencies of the participants, the body shape questionnaire (BSQ-34) was employed to assess their body dissatisfaction. Also the general characteristics, anthropometric measurements were collected. In ORTO-11 scale, 25 points and below were considered as an orthorexic tendency. In the BSQ-34, a score between 80-110 points was classified as slight dissatisfaction, a score between 111-140 points as moderate dissatisfaction, and a score above 140 points as severe dissatisfaction.

Results: ORTO-11 and BSQ-34 mean scores of the participants were 29.54±5.98 and 83.20±45.46, respectively. 27.6% of the participants had orthorexic tendencies and 40.6% had dissatisfaction (slight: 10.9%, moderate: 13.4%, severe: 16.3%). There was no statistically significant difference in the mean BSQ-34 scores of the participants based on the orthorexic tendency classification. Furthermore, according to the body dissatisfaction classification, there was no statistically significant difference between the participants' mean ORTO-11 scores. However, the participants with severe dissatisfaction had significantly higher mean body weight and mean body mass index than those with no dissatisfaction.

Conclusion: This study revealed that there was no correlation between tendency of orthorexia nervosa and body dissatisfaction. Further studies need to be conducted to verify the results.

Keywords: Orthorexia nervosa, body dissatisfaction, adult

ÖZ

Amaç: Bu çalışma, yetişkin bireylerin ortoreksiya nervoza eğilimleri ile beden memnuniyetsizlikleri arasındaki ilişkiyi değerlendirmek amacıyla planlanmıştır.

Gereç ve Yöntemler: Çalışma, Gaziantep'te yaşayan, 19-64 yaş arası 239 gönüllü birey üzerinde yürütülmüştür. Katılımcıların ortorektik eğilimlerini değerlendirmek için ORTO-11, beden memnuniyetsizliklerini değerlendirmek için beden şekli ölçeği (BSQ-34) kullanılmıştır. Ayrıca katılımcıların demografik özellikleri sorgulanmış ve antropometrik ölçümleri alınmıştır. ORTO-11 ölçeğinde 25 puan ve altı ortorektik eğilim olarak değerlendirilmiştir. Beden şekli ölçeğinde 80-110 puan hafif beden memnuniyetsizliğini, 111-140 puan orta derecedeki beden memnuniyetsizliğini ve 140 puan üstü ciddi beden memnuniyetsizliği olarak sınıflandırılmıştır.

Bulgular: Çalışmaya katılan bireylerin ORTO-11 ve BSQ-34 puan ortalamaları sırasıyla; 29,54±5,98 ve 83,20±45,46'dır. Çalışmaya katılan bireylerin %27,6'sında ortorektik eğilim ve %40,6'sında beden memnuniyetsizliği (%10,9 hafif düzey, %13,4'ü orta düzey, %16,3'ü ciddi düzey) olduğu saptanmıştır. Katılımcıların ortorektik eğilimlerine göre BSQ-34 ölçek puanlarında anlamlı fark saptanmamıştır ($p>0,05$). Ayrıca, bireylerin beden memnuniyetsizliği sınıflamasına göre ORTO-11 ölçek puanlarında anlamlı farklılık göstermemiştir. Ciddi düzey beden memnuniyetsizliği olan bireylerin vücut ağırlığı ve beden kütle indeksi beden memnuniyetsizliği olmayan bireylerden daha fazladır ($p<0,05$).

Sonuç: Bu çalışma, ortoreksiya nervoza eğilimi ile beden memnuniyetsizliği arasında bir ilişki olmadığını göstermektedir. Bu bulguları doğrulamak için daha fazla araştırma yapılması gerekmektedir.

Anahtar Kelimeler: Ortoreksiya nevroza, beden memnuniyetsizliği, yetişkin

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INTRODUCTION

The desire to consume healthy food is not a pathological condition. However, when this desire becomes an obsessive situation, it can increase the anxiety and stress level of individuals and in turn become a pathological condition (1). Orthorexia nervosa refers to an obsession with healthy eating behaviours (2). This obsession is caused by the desire to optimise one's own health and well-being. Orthorexic individuals eliminate the foods they perceive as unhealthy and impure from their daily diets (3). They also pay attention to the preparation and cooking methods as well as the production, processing and packaging steps of foods (1). They avoid consuming foods containing carcinogenic substances, additives, dyes or hormones, and high amounts of salt, sugar, and unhealthy fat (3). Orthorexia nervosa is not officially recognised as an eating disorder in DSM-5 or ICD-10. In the literature, there is an ongoing discussion regarding whether or not orthorexia nervosa is classified as an eating disorder, obsessive compulsive disorder, or a mental disorder (4-5). Some researchers have suggested that orthorexia nervosa may be a precursor to an eating disorder. Changes occurring in the eating habits of the individuals may cause impaired body image (6). Body dissatisfaction refers to the negative thoughts and feelings of individuals about their body and is usually caused by the inconsistency between the way individuals perceive their body and the ideal body shape they perceive (7). Body dissatisfaction, impaired body image, and weakness obsession take place in the centre of clinically diagnosed eating disorders (anorexia nervosa, bulimia nervosa, binge eating disorder, other specified feeding and eating disorders) (8). However, there are a limited number of studies in the literature on how non-clinical disorders such as orthorexia nervosa affect body satisfaction (3, 5, 9-12) and the results of the studies are contradictory. Therefore, the aim of the study is to examine the correlation between the tendencies of orthorexia nervosa and body dissatisfaction among adults.

MATERIAL and METHODS

Sample of the study

The study was conducted between May and July 2022 with 239 participants (117 women, 122 men) aged 19 to 64 who lived in Gaziantep and did not have psychiatric or chronic diseases. Approval of Gaziantep Islam Science and Technology University Non-invasive Clinical Trials Ethics Committee was obtained (Date:26.04.2022, No:101.16.04). The principles of the Declaration of Helsinki were followed to conduct the study. Participants were informed about the study in detail and they read and signed the informed consent form.

Data collection tool

General characteristics (age, gender, marital status, and period of education), orthorexic tendencies, and body dissatisfaction of participants were collected by using the questionnaire and face-to-face interview method. Also, body weight, height, and body mass index were measured by a trained dietician according to proper methods (13). Body mass index (BMI; kg/m^2) was calculated and classified according to the World Health Organization recommendations (14).

Orto-11 scale

The ORTO-11 scale was used to determine the obsession of the participants regarding healthy diet. The 10-item scale developed by Bratman et al. was revised by Donini et al. and became the ORTO-15 scale (3, 15). Turkish validity and reliability of the scale was conducted by Arusoğlu et al. and adapted as ORTO-11 (2). Items 1, 2, 3, 4, 5, 7, 8, 9, 10, and 11 of the scale are rated between 4 points and 1 point (4: always, 3: frequently, 2: sometimes, and 1: never). The sixth question is reversely scored. Higher scores signify that the participants have less risk of orthorexia nervosa. A cut-off point method was used for evaluation of the scale. The cut-off point of the study was determined as 25 points in the 25% percentile, and 25 points or less were evaluated as orthorexic tendency.

Body shape questionnaire (BSQ-34)

Opinions of the participants regarding body dissatisfaction were obtained by using the Body Shape Questionnaire (BSQ). The one-dimensional questionnaire was developed by Cooper et al. and consisted of 34 items (16). The items are rated on a 6-point Likert scale (1: never, 2: rare, 3: sometimes, 4: often, 5: very often, and 6: always). Minimum and maximum scores of the scale are 34 and 204 points, respectively. BSQ was reorganised into two categories: no dissatisfaction – those classified as free from body dissatisfaction, and presence of dissatisfaction - those who were classified as having some level of body dissatisfaction (slight, moderate or severe). Body dissatisfaction was classified as no dissatisfaction in those with less than 80 points, slight dissatisfaction between 80 and 110 points, moderate dissatisfaction between 110 and 140 points, and severe dissatisfaction in those with more than 140 points. Its Turkish validity and reliability study was conducted by Akdemir et al. (17).

Statistical analysis

The SPSS 22.0 (SPSS Inc., Chicago, IL, USA) programme was employed to evaluate the data and to prepare the tables. Graphs were created using GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA). Quantitative variables obtained by measurement were expressed as mean ($\bar{X}$), standard deviation (SD), and maximum and minimum values; whereas categorical variables were expressed as number (n) and percentage (%). Continuous variables were compared using the independent sample t-test for two independent groups or a one-way analysis of variance (ANOVA) with the Tukey post hoc test for more than two groups. The chi-square test was used for the analysis of categorical variables. Pearson's correlation test calculates correlation when normality conditions are met. Spearman's correlation test is used when these criteria aren't met. The significance level was set at $p< 0.05$.

RESULTS

Two hundred and thirty-nine adult participants (48.9% men, 52.1% women) with a mean age of 32.39±11.57 years were included in the study. The ORTO-11 mean score of the participants was 29.54±5.98 and their BSQ-34 mean score was 83.30±45.46.

Orthorexia tendency was found in 27.6% of the participants, and body dissatisfaction in 40.6% (Table 1).

Table 1: General characteristics of the participants

Descriptive characteristics	Total (n=239)	
	X±SD	Min-Max
Age (years)	32.39±11.57	19-63
Body weight (kg)	66.93±14.59	46.50-106.50
BMI (kg/m²)	24.21±4.68	17.20-33.91
Period of education (years)	12.29±3.08	2-18
ORTO-11	29.54±5.98	19-42
BSQ-34	83.30±45.46	38-204
	n	%
Gender		
Men	117	48.9
Women	122	52.1
Marital Status		
Married	139	58.2
Single	100	41.8
BMI classification		
Underweight (BMI < 18.5)	46	19.2
Normal weight (18.5 ≤ BMI ≤ 24.9)	79	33.1
Pre-obese (25.0 ≤ BMI ≤ 29.9)	61	25.5
Obese (BMI ≥ 30.0)	53	22.2
Risk estimates for ORTO-11		
Orthorexic tendency	66	27.6
Without orthorexic tendency	173	72.4
Risk estimates for BSQ-34		
No dissatisfaction	142	59.4
Dissatisfaction	95	40.6
Slight dissatisfaction	26	10.9
Moderate dissatisfaction	32	13.4
Severe Dissatisfaction	39	16.3

X: Mean, SD: Standard deviation, n: Number of participants, %: Percentage of participants, Min-Max: minimum and maximum values, BMI: Body mass index, BSQ-34: Body shape questionnaire

BSQ-34 mean score of the participants with orthorexic tendency was 84.53±50.53, and BSQ-34 mean score of those without orthorexic tendency was 82.93±43.47 (p>0.05). Orthorexic tendency were detected in 32.5% of men participants and 23.0% of women participants (p>0.05). In addition, no significant difference was found in terms of the presence of orthorexic tendencies in the participants regarding, age, period of education, body weight, BMI and marital status (p>0.05) (Table 2).

The mean ORTO-11 score of those with no dissatisfaction was 29.80±6.47, the mean ORTO-11 score of those with slight dissatisfaction, moderate dissatisfaction, and severe dissatisfaction was 29.62±5.84, 29.34±3.39 and 28.72±5.98, respectively (p>0.05). The mean age of individuals with moderate dissatisfaction is lower than those with slight dissatisfaction or individuals with no dissatisfaction (p<0.05). The mean body weight of the participants with severe dissatisfaction was significantly higher than those with no dissatisfaction and slight dissatisfaction (p<0.001) (Table 3).

Table 2: General characteristics of the participants according to the tendency of orthorexia nervosa

	Classification of tendency of orthorexia nervosa		p
	Orthorexic tendency (n: 66)	Without orthorexic tendency (n: 173)	
	X±SD	X±SD	
Age (years)	34.60±12.65	31.54±11.06	0.068*
Period of education (Years)	12.60±2.98	12.17±3.12	0.334*
Body weight (kg)	68.57±14.88	66.31±14.47	0.285*
BMI (kg/m²)	24.24±4.44	24.20±4.78	0.757*
ORTO-11	22.46±2.24	32.24±4.59	**<0.001***
BSQ-34	84.53±50.53	82.93±43.47	0.811*
	n(%)	n(%)	
Gender			
Men	38 (32.5)	79 (67.5)	0.112**
Women	28 (23.0)	94 (77.0)	
Marital Status			
Married	32 (34.0)	107 (66.0)	0.053**
Single	34 (23.0)	66 (77.0)	

X: Mean, SD: Standard deviation, n: Number of participants, %: Percentage of participants, BMI: Body mass index, BSQ-34: Body shape questionnaire, *Independent sample t-test, **Chi-Square test, **Bold values are for p<0.05**

In Figure 1, the BMI of the participants are presented according to the body dissatisfaction classification. The mean BMI for participants who had no dissatisfaction, slight, moderate, or severe body dissatisfaction was 22.92±4.21 kg/m², 24.60±4.82 kg/m², 26.17±4.89 kg/m², and 27.29±4.24 kg/m², respectively. Also, it was found that participants who had no dissatisfaction had a lower mean BMI than the participants who had moderate and severe dissatisfaction (p<0.001). Furthermore, a significant positive correlation was found in this study between the BSQ-34 score and body weight and BMI (r:0.029, p<0.001, r:0.31, p<0.001, respectively). There was no significant correlation between ORTO-11 and BSQ-34 scores (r:-0.10, p:0.13).

DISCUSSION

Orthorexia nervosa is characterized by an obsession with healthy eating and restrictive eating behaviors. It significantly affects a person's psychological and physical health (18-19). Some researchers have stated that the underlying reason of

Table 3: General characteristics of the participants according to the body dissatisfaction classification

	Body Dissatisfaction classification				
	No Dissatisfaction	Dissatisfaction			
		Slight	Moderate	Severe	p
	X±SD	X±SD	X±SD	X±SD	
Age (years)	33.04±12.17[a]	36.27±12.07 [a]	26.28±9.63[b]	32.49±8.66[ab]	**0.006**[*]
Period of education (Years)	12.38±3.2	12.23±2.55	12.5±3.42	11.85±2.77	0.783[*]
Body weight (kg)	63.63±13.14 [a]	66.48±15.79 [a]	69.69 ± 13.67[ab]	77.52±14.35[b]	**<0.001**[*]
ORTO-11	29.80±6.48	29.62±5.84	29.34±3.39	28.72±5.98	0.792[*]
BSQ-34	51.15±11.24[a]	88.23±9.14[b]	123±7.90[c]	164.54±19.27[d]	**<0.001**[*]
	n (%)	n (%)	n (%)	n (%)	
Gender					
Men	63 (53.8)	16 (13.7)	17 (14.5)	21 (17.9)	0.329[**]
Women	79 (64.8)	10 (8.2)	15 (12.3)	18 (14.8)	
Marital status					
Married	59 (59.0)	13 (13.0)	11 (11.0)	17 (17.0)	0.683[**]
Single	83 (59.7)	13 (9.4)	21 (15.1)	22 (15.8)	

X: Mean, SD: standard deviation, n: Number of participants,%: Percentage of participants BMI: Body mass index, BSQ-34: Body shape questionnaire, [*] One-way ANOVA was used for the test of differences and Tukey's post-hoc test. Different lower letters in the same column indicate a statistical difference among the groups, [**]Chi-Square test, Bold values are for p<0.05

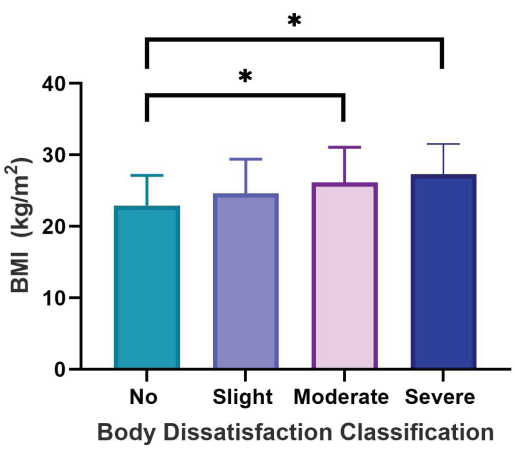

Figure 1: Distribution of BMI according to body dissatisfaction classification. Bar graphs representing BMI (kg/m2) according to body dissatisfaction classification. Data are expressed as mean±SD, and the results were analyzed using one-way ANOVA followed by post hoc analysis. *p<0.05 was considered statistically significant.

Healthy eating obsession of the individuals with orthorexia nervosa may be the concern about body appearance (20-21). However, there is little research on this subject in the literature. Therefore, more is needed. In the current study, the correlation between orthorexic tendency and body satisfaction was investigated in adults.

In a study conducted by Bagcı Bosi et al. using ORTO-15 scale in a sample including doctors, the prevalence of orthorexic tendency was found as 45.5% (22). Another study on adults doing sports via the ORTO-11 scale, they determined that the rate of the individuals showing orthorexic tendency was 29.9% (6). In this study, the ORTO-11 scale was used to evaluate the orthorexic tendency of individuals, and the rate of individuals with orthorexic tendency was found to be 26.4%, and this rate is similar to the rate reported in a previous study conducted in Turkey using the ORTO-11 scale (6). With regards to body dissatisfaction, studies have found that between 19% and 51.3% of people have body dissatisfaction (23). In this study, 40.6% of participants showed body dissatisfaction.

When BMIs of the individuals were evaluated according to their orthorexic tendencies, similar to the previous studies, no significant difference was found between the orthorexic tendencies and BMI of the participants in the current study (11, 24-25). Excessive concentration of people with the orthorexic tendency on the quality of food does not mean control of the amount of food consumed (26). In the literature, it is stated that BMI is an important determinant of body dissatisfaction (27). In their study, Karr et al. determined that there was a positive correlation between the BMI and body dissatisfaction of athletes. In other words, as the BMI of the athletes increased, their body dissatisfaction also increased (28). In the present study, body weight and mean BMI of the participants with severe dissatisfaction were statistically significantly higher than the values of those with no dissatisfaction. A positive significant correlation was found between BSQ-34 scores and body weight and BMI. As the body weight or BMI of the participants increased, BSQ-34 score also increased. That is, body dissatisfaction increased. There are conflicting results in the studies examining the cor-

relation between orthorexic tendency and body dissatisfaction (3, 5, 9-12). In another study, Bratman's orthorexia test and Sociocultural attitudes toward appearance questionnaire were employed and fit participants with orthorexic tendencies were found to be concerned about body dissatisfaction (9). In a study conducted with Chinese elders, Dusseldorf Orthorexia Scale and body dissatisfaction subscale of the Eating Disorder Inventory were used and as a result, orthorexic symptoms were significantly and negatively correlated with body dissatisfaction (5). There are also opinions in the literature that orthorexic individuals do not have body dissatisfaction. Because the main goals of orthorexic individuals are not to lose weight but to eat healthy (3). A study conducted on young adults reported that body image was not a predictor for orthorexic tendency (12). In their study, Plitcha et al., determined that there was no significant difference between body dissatisfaction classifications of the participants according to their orthorexic tendencies (11). In their study, Morais Freire et al. evaluated orthorexic tendency by using ORTO-15 scale and body dissatisfaction by using BSQ-34 scale, and found no significant correlation between ORTO-15 and BSQ-34 (10). In the current study, there was no statistically significant difference between the ORTO-11 scores of the individuals in terms of the body dissatisfaction classification, and their BSQ-34 mean scores in terms of the orthorexic tendency classification. In addition, no correlation was found between BSQ-34 and ORTO-11 scores ($p>0.05$). The results of the study are compatible with the results of previous studies (10-12). It can be asserted that the reason why individuals seek healthy food in order to reach better health standards is not body dissatisfaction and there is no direct correlation between orthorexic tendency and body satisfaction (29).

Even though there are many studies in the literature regarding orthorexia nervosa or body dissatisfaction, there are a limited number of studies that evaluate the correlation between body dissatisfaction and tendency of orthorexia nervosa. This study's findings guide future research. This is the first study in Turkey that evaluates the correlation between tendency of orthorexia nervosa and body dissatisfaction by using the BSQ-34 scale. In addition, the scales used in this study have been validated in the Turkish population. Despite its strengths and contribution to the literature, this study has limitations. First, because this is a cross-sectional study, causal conclusions about orthorexic tendencies and body dissatisfaction cannot be drawn. A longitudinal study may reveal the cause-and-effect relationship between orthorexia and body dissatisfaction. Second, the study had few participants, so its results may be limited.

CONCLUSION

It was concluded that body dissatisfaction cannot be considered a determining factor for tendency of orthorexia nervosa. Further studies are needed on this subject.

Ethics Committee Approval: This study was approved by Gaziantep Islam Science and Technology University Non-invasive Clinical Trials Ethics Committee (Date: 26.04.2022, No: 101.16.04).

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REFERENCES

1. Koven NS, Abry AW. The clinical basis of orthorexia nervosa: emerging perspectives. Neuropsychiatr Dis Treat 2015;18(11):385-94.
2. Arusoğlu G, Kabakçi E, Köksal G, Merdol TK. Orthorexia Nervosa and Adaptation of ORTO-11 into Turkish. Turk Psikiyatri Derg 2008;19(3):283-91.
3. Bratman S, Knight D. Health food junkies: overcoming the obsession with healthful eating. New York:Broadway Books; 2000. p.1-242.
4. Cena H, Barthels F, Cuzzolaro M, Bratman S, Brytek-Matera A, Dunn T, et al. Definition and diagnostic criteria for orthorexia nervosa: a narrative review of the literature. Eat Weight Disord 2019;24(2):209-46.
5. He J, Zhao Y, Zhang H, Lin Z. Orthorexia nervosa is associated with positive body image and life satisfaction in Chinese elderly: Evidence for a positive psychology perspective. Int J Eat Disord 2021;54(2):212-21.
6. Baysal I, Kızıltan G. The Determination of the Relationship between Food Neophobia and Orthorexia Nervosa Tendencies and Nutritional Status of Individuals who Engage in Sports. Bes Diy Derg 2020;48(3):48-55.
7. Melzer K, Kayser B, Saris WH, Pichard C. Effects of physical activity on food intake. Clin Nutr 2005;24(6):885-95.
8. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 5th ed. Arlington: American Psychiatric Publishing; 2013.
9. Eriksson L, Baigi A, Marklund B, Lindgren E-C. Social physique anxiety and sociocultural attitudes toward appearance impact on orthorexia test in fitness participants. Scand J Med Sci Sports 2008;18(3):389-94.
10. Freire GLM, da Silva Paulo JR, da Silva AA, Batista RPR, Alves JFN, do Nascimento Junior JRA. Body dissatisfaction, addiction to exercise and risk behaviour for eating disorders among exercise practitioners. J Eat Disord 2020;8(1):1-9.
11. Plichta M, Jezewska-Zychowicz M, Gębski J. Orthorexic tendency in Polish students: exploring association with dietary patterns, body satisfaction and weight. Nutrients 2019;11(1):100.
12. Topçu F, Aricak OT. The Effect of Perfectionism and Body Perception on Orthorexia Among Young Adults. JCBPR 2019;8(3)170-8.
13. Lee RD, David C, editors. Nutritional assessment. New York: McGraw-Hill; 2010.
14. World Health Organization 2020. (cited 2022 July 26). Available from: URL: http://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi.
15. Donini LM, Marsili D, Graziani MP, Imbriale M, Cannella C. Orthorexia nervosa: validation of a diagnosis questionnaire. Eat Weight Disord 2005;10(2):e28-e32. doi: 10.1007/BF03327537
16. Cooper PJ, Taylor MJ, Cooper Z, Fairbum CG. The development and validation of the Body Shape Questionnaire. Int J Eat Disord 1987;6(4):485-94.

17. Akdemir A, Inandi T, Akbas D, Karaoglan Kahilogullari A, Eren M, Canpolat BI. Validity and reliability of a Turkish version of the Body Shape Questionnaire among female high school students: Preliminary examination. Eur Eat Disord Rev 2012;20(1):e114-e5. doi: 10.1002/erv.1106
18. Greetfeld M, Hessler-Kaufmann JB, Brandl B, Skurk T, Holzapfel C, Quadflieg N, et al. Orthorexic tendencies in the general population: association with demographic data, psychiatric symptoms, and utilization of mental health services. Eat Weight Disord 2021;26(5):1511-9.
19. Haddad C, Obeid S, Akel M, Honein K, Akiki M, Azar J, et al. Correlates of orthorexia nervosa among a representative sample of the Lebanese population. Eat Weight Disord 2019;24(3):481-93.
20. Brytek-Matera A, Gramaglia C, Gambaro E, Delicato C, Zeppegno P. The psychopathology of body image in orthorexia nervosa. J Psychopathol 2018;24:133-40.
21. Featherstone M. The body in consumer culture. In: Featherstone M, Hepworth M, Turner SB, editors. The Body: Social Process And Cultural Theory. London: Sage Publications; 1991.p.170-97.
22. Bosi ATB, Camur D, Güler C. Prevalence of orthorexia nervosa in resident medical doctors in the faculty of medicine (Ankara, Turkey). Appetite 2007;49(3):661-6.
23. Kapoor A, Upadhyay MK, Saini NK. Prevalence, patterns, and determinants of body image dissatisfaction among female undergraduate students of University of Delhi. J Family Med Prim Care 2022;11(5):2002-7.
24. Cinosi E, Matarazzo I, Marini S, Acciavatti T, Lupi M, Corbo M, et al. Prevalence of orthorexia nervosa in a population of young Italian adults. Eur Psychiatry 2015;30(S1):1330. 25.
Sanlier N, Yassibas E, Bilici S, Sahin G, Celik B. Does the rise in eating disorders lead to increasing risk of orthorexia nervosa? Correlations with gender, education, and body mass index. Ecol Food Nutr 2016;55(3):266-78.
26. Brytek-Matera A. Orthorexia nervosa–an eating disorder, obsessive-compulsive disorder or disturbed eating habit. Arch Psychiatry Psychother 2012;1(1):55-60.
27. Swami V, Steadman L, Tovée MJ. A comparison of body size ideals, body dissatisfaction, and media influence between female track athletes, martial artists, and non-athletes. Psychol Sport Exerc 2009;10(6):609-14.
28. Karr TM, Davidson D, Bryant FB, Balague G, Bohnert AM. Sport type and interpersonal and intrapersonal predictors of body dissatisfaction in high school female sport participants. Body Image 2013;10(2):210-9.
29. Coelho GC, Troglio GM, Hammes L, GalvÃ TD, Cyrino LAR. As consequências físicas, psíquicas e sociais em indivíduos com ortorexia nervosa. RBONE 2016;10(57):160-8.

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ADVANCE CARE PLANNING IN ADULT AND PEDIATRIC CANCER PATIENTS: A REVIEW

YETİŞKİN VE PEDİATRİK KANSERLİ HASTALARDA İLERİ BAKIM PLANLAMASI: GÖZDEN GEÇİRME

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ABSTRACT

Advance care planning (ACP) aims to support patients regardless of age or stage of health to understand and share personal values, goals in life, and future medical care preferences. This process which may be started at any time should be reviewed at periodical intervals depending on individuals' medical conditions. According to previous research, end-of-life care is a source of deep concern for many people who want to ensure active involvement in decision-making processes. Compared to other patient groups, individuals suffering from advanced cancer may have different levels of willingness regarding being a part of ACP discussions, as evidence shows. The barriers to ACP, the fear surrounding ACP, cultural differences, and previous health experiences have generated differences of opinion on the use and implementation of ACP. Across the world, palliative care principles and advance care planning are developing in the field of Medical Oncology. This review aimed to present the most important features of ACP in adult and pediatric cancer patients and to incorporate this concept to Turkish literature.

Keywords: Advance care planning, adult cancer, pediatric cancer

ÖZ

İleri bakım planlaması (İBP) herhangi bir yaştaki veya sağlıklarının farklı aşamalarındaki yetişkinlerin kişisel değerlerini, yaşam hedeflerini ve gelecekteki tıbbi bakımla ilgili tercihlerini anlama ve paylaşma konusunda destekleyen bir süreçtir. Bu süreç herhangi bir zamanda başlayabilir ve periyodik olarak tekrar gözden geçirilebilir fakat bir kişinin tıbbi durumu her değiştiğinde İBP' ye yeniden odaklanılmalıdır. Daha önceki araştırmalar, birçok insanın yaşamlarının sonunda bakımla ilgili endişe ve isteklerinin olduğunu ve karar alma süreçlerine aktif olarak katılmak istediklerini ortaya koymuştur. İleri evre kanser tanısı olan bireylerin İBP tartışmalarına katılma istek ve arzularının diğer hasta gruplarına göre farklılık gösterdiğine dair kanıtlar mevcuttur. İBP kullanımı ve uygulanmasında hâla görüş farklılıkları bulunmaktadır. Bu görüş farklılıklarının nedenleri arasında İBP' nin önündeki engellerin, İBP'yi çevreleyen korkunun, kültürler arası farklılıkların ve önceki sağlık deneyimlerinin etkisi olabilmektedir. Dünya' da Tıbbi Onkoloji alanında, palyatif bakım ilkeleri ve ileri bakım planlaması için giderek artan bir literatür mevcuttur. Bu derlemenin amacı; Erişkin ve çocuk kanser hastalarında İBP' nin göze çarpan özelliklerini aydınlatmak ve bu kavramı Türk literatürüne kazandırmaktır.

Anahtar Kelimeler: İleri bakım planlaması, yetişkin kanser, pediatrik kanser

INTRODUCTION

Advance care planning (ACP) is designed to define patients' future medical and end-of-life care based on their values, wishes, and preferences from the beginning of their illness to the end of life (1). ACP was first defined by Joan Teno et al. As "a communication process that aims to ensure the consistency of clinical care with the care preferences of patients" (2). Parallel to this definition, a consensus stated the goal of ACP in 2017 ACP as "giving individuals the opportunity to determine their own values, to think deeply about what serious illness means and what consequences it has, to detail their goals and preferences in regards to the future medical treatment and care they would like to receive, and to discuss all options with their respective families" (3,4).

ACP addresses individuals as a whole and focuses on physical, psychological, social, and spiritual concerns. It has been shown that advance care planning improves the quality of life (5). In addition, ACP encourages individuals to select a representative for themselves and to record any preferences by regularly reviewing them (3,6). However, care personnel should have the

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capacity to undertake the necessary steps so that ACP can provide the individuals with the opportunity to plan their future care and support, including medical treatment (7).

While not everyone in the community may be willing to make a prospective care plan, ACP will be useful for specific groups. For example, prolongation of hospitalization due to chronic diseases affects the decision-making ability of individuals by causing mood changes (7). ACP is more commonly used in Europe and America for cancer patients, in addition for patients with other serious diseases such as chronic obstructive pulmonary disease (COPD), human immunodeficiency virus (HIV), and amyotrophic lateral sclerosis (ALS) (8, 9).

Today, the concept of ACP has been expanded to include more patient groups as well as healthy individuals (3, 10). ACP is expected to reduce concerns about the future and improve patients' and their relatives' quality of life (11). Giving patients a sense of control and a peace of mind, and ensuring that patients can talk about end-of-life issues with family and friends are among the reported ACP benefits (8). Ideally, these discussions take place with the individual's health care representative and clinician along with clinical team members. These discussions should be recorded and updated when the need arises to ensure flexible decision making in regard to patient's current medical condition (12). Current guidelines recommend that specialist physicians initiate advance care planning discussions (9, 13). However, little is known about the preferences of general population about the individuals with whom they would like to discuss end-of-life care choices and with whom they can really involve in these discussions (14).

Despite the positive evidence for the effectiveness of ACP, clinical practice does not really entail such discussions between patients and healthcare professionals to take place at the desired level (15,16). This may be partly due to barriers related to patients (8, 17). For example, patients reported reluctance to take part in ACP discussions due to their fear of facing death, the worry about placing an unnecessary burden on their families, and the feeling that it would be impossible to plan for the future (18) (Table 1). It is stated that patients' lack of knowledge about ACP may cause inadequate use of ACP (19). Barriers faced by physicians include lack of knowledge in handling discussions about ACP, not knowing the appropriate time for discussion, the belief that patients should initiate the discussion, the fear of losing hope and revealing unmet needs (20). Another study cited the barriers to ACP as: 1) the perception that ACP discussions are overwhelming or stressful for the patient, 2) the wish to represent the care plan and its course positively, 3) the concern that it may create difficulties in accepting poor prognosis, and 4) the complexity surrounding patients' understanding about the complications of life-prolonging procedures (21, 22). However, the angles of the ACP discussions that were difficult and unpleasant at the beginning may later be considered beneficial. Starting ACP at very early stages can generate concern and anxiety (9). A systematic review demonstrated that the ACP process is more beneficial and positive for the patients depending on the readiness of the patient (8).

Another review presented that ACP training provided to healthcare professionals positively affected their knowledge, attitudes, and skills and increased their communication skills in discussing the decisions about end-of-life. Adequate training and experience will help doctors and nurses in addressing patients' and their families' needs and preferences regarding their care (23). It is imperative that healthcare professionals create the required time and venue for ACP in patients' social and healthcare settings to ensure active and meaningful involvement of patients (9).

Advance care planning in adult cancer patients

With the help of ACP, patients will be able to reflect on and share their personal values, life goals, and preferences regarding their prospective medical treatment and care (4). ACP reduces the burden on doctors and family members by minimizing the rate of using undesired treatments at the end of patients' lives. Although ACP is an encouraging and favorable approach to increase the quality of life in advance cancer patients, there is inadequate evidence backing its effectiveness for this patient group (11, 24). The cancer patients' responses to ACP and their values and needs have been reported to be different compared to other patient populations (9). Among the patients with advance cancer, the individuals who are close to death may be more open to early end-of-life conversations (25). On the other hand, a study conducted with cancer patients in 6 countries showed that while patients who participated in ACP discussions received specialist palliative care support more frequently, ACP discussions had no effect on their life quality, coping mechanisms or taking part in processes related to decision-making (24).

Table 1: Barriers to ACP

Factors related to patients	Factors related to health care professionals	Factors related to the system
Insufficient information about health status	Hesitations to discuss possible future complications with patients, especially when they seem well	Focusing on medical treatment in general
Unpredictable course of the disease and difficult prognosis	Fear of taking away the feeling of hope from the patient	Lack of coordinated and structured approach to ACP
Hesitation about considering/discussing treatment choices	Time barrier	Uncertainty in the literature about ACP initiation
Expectations that doctors should initiate ACP	Difficulties in finding the right moment to start ACP	Limited resources

ACP: Advance care planning

Advance care planning for pediatric cancer patients

Advance care planning is widely advocated to increase the participation of patients and family members in areas related to comprehending the values, preferences, and care goals of the patients regardless of prognosis and the course of the disease (26, 27). ACP requires a communication process that aims to timely coordinate prospective medical care and treatment with the patients' values and preferences throughout the disease (27).

Cancer diagnosis affects the individual and the family for a long time to come (28). Since the treatment process is highly complex, clinicians, parents, and children regularly encounter difficult decisions and discussions about not only the current care and treatment options, but also about the future ones as well (29). ACP is strongly recommended for children and adolescents by international guidelines and medical societies (26). However, research on pediatric ACP is highly limited, and little is known about how families respond to this concept (30). In particular, pediatric ACP lacks the professional perspective (31). The literature on ACP in the field of pediatrics primarily focuses on the intensive care setting and oncology population (32). In these populations, discussions of ACP are often driven by the imminent expectation of death, the need for explanation regarding resuscitation practices, the situations where curative treatments have failed, and where the focus is palliative care.

There are many barriers to ACP discussions such as unrealistic expectations and differences between how the parent and the clinician understand and approach the prognosis (33, 34). The clinicians reported that they were uncomfortable with ACP due to the fear of losing hope, the uncertainty of prognosis, and not knowing the right time to address these problems (33). Research has shown that many clinicians knowingly avoid these discussions not to destroy or damage patients' feelings of hope, even when the individual is in the advanced stages of the disease (35, 36). In addition, a lack of communication about living with the disease among individuals with cancer, parents, and healthcare professionals has been consistently reported by various studies (37-39). The physician must first understand the child's and family's perspective on the illness and its effect on their lives. Beliefs, values, hopes, and fears shape their perspectives and must be understood to guide them throughout the process (40).

When pediatric cancer patients are excluded from treatment discussions and decisions, they may have difficulties in coping with their disease (41). Palliative care with ACP is an evidence-based standard of care in pediatric cancer (42). The importance of communication on the application of ACP with pediatric cancer patients is indicated in several studies (29,43,44). According to studies, pediatric cancer patients and their families wish to receive direct, empathetic, and frequent communication, even when the disease is progressive (44, 45).

Children and adolescents with cancer may desire information and the ability to take part in decision making to identify their care plan, to choose and refuse treatment, and to decide how they will be remembered after their death (46, 47). Discussions related to care and ACP will ensure that patients will make sense of and course their hopes, fears, and care preferences more securely (47). Studies have shown that ACP discussions do not harm the patient in regards to anxiety (46, 48). A study conducted with children with cancer and their families compared randomly selected families for pediatric advance care planning with the families in the control group and reported that care giving was evaluated more positively and stated that ACP discussion experiences were valuable (30).

The best interests of the child should be kept in mind when considering the decisions regarding end-of-life care. ACP will support pediatricians in their efforts to engage in sensitive, timely, and honest discussions so that the wishes of families at the end of their child's life can be facilitated (49). It is important to consider the cultural differences in the society and the desires and views of the family during ACP discussions.

CONCLUSION

ACP is associated with positive outcomes and should be encouraged regardless of the limitations and critical issues surrounding the concept. Many healthcare organizations are seeking strategies to integrate ACP into their regular practices. A comprehensive approach beyond a single setting and a single discipline is necessary for ACP to be regarded as a continuous conversational process across time and settings (50). Since ACP may generate fear and distress, it appears to carry both benefits and risks, in social, psychological, and emotional realms which may affect patients, family members, and healthcare professionals in different ways. The end-of-life behaviors and choices of patients, their loved ones, and caregivers are strongly influenced by the organizational culture, as well as by their earlier experiences with the treatment setting or death. Therefore, ACP is not only about patients' choices, it is the outcome of a complicated and ever-changing reciprocity between patients and caregivers. The level of joint decision-making desired by individuals should be determined. Open and honest discussions should be initiated at the earliest opportunity. Health professionals have a duty to plan advance care so that severely ill patients can be provided with care that meets their individual needs. In this context, facilitating patient autonomy is both complicated and controversial. In Turkiye, ACP is not yet implemented in institutions due to uncertainties and legal reasons. More research is needed to raise awareness related to ACP, to present the ethical framework for ACP, and to grasp the philosophical approach of healthcare professionals, cancer patients, and caregivers towards ACP.

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REFERENCES

1. Sedini C, Biotto M, Crespi Bel'skij LM, Moroni Grandini RE, Cesari M. Advance care planning and advance directives: an overview of the main critical issues. Aging Clin Exp Res 2022;34(2):325-30.
2. Teno JM, Nelson HL, Lynn J. Advance care planning. Priorities for ethical and empirical research. Hast Cent Rep 1994;24(6):S32-6.
3. Rietjens JAC, Sudore RL, Connolly M, van Delden JJ, Drickamer MA, Droger M, et al. European association for palliative care definition and recommendations for advance care planning: an international consensus supported by the european association for palliative care. Lancet Oncol 2017;18(9):543-51.
4. Sudore RL, Lum HD, You JJ, Hanson LC, Meier DE, Pantilat SZ, et al. Defining advance care planning for adults: a consensus definition from a Multidisciplinary Delphi Panel. J Pain Symptom Manage 2017;53(5):821-32.
5. Owen L, Steel A. Advance care planning: what do patients want? Br J Hosp Med 2019;80(5):263-7.
6. Sudore RL, Boscardin J, Feuz MA, McMahan RD, Katen MT, Barnes DE. Effect of the PREPARE Website vs an Easy-to-Read Advance Directive on Advance Care Planning Documentation and Engagement Among Veterans: A Randomized Clinical Trial. JAMA 2017;177(8):1102-9.
7. National Institute for Health and Care Excellence. Advance care planning. University of Nottingham 2019- Jan (cited 2023 Jan 13): 1(1). Available from:file:///C:/Users/90506/Downloads/advance-care-planningquick-guide.pdf.
8. Zwakman M, Jabbarian LJ, van Delden J, van der Heide A, Korfage IJ, Pollock K, et al. Advance care planning: A systematic review about experiences of patients with a life-threatening or life-limiting illness. Palliative Medicine 2018;32(8):1305-21.
9. Johnson S, Butow P, Kerridge I, Tattersall M. Advance care planning for cancer patients: a systematic review of perceptions and experiences of patients, families, and healthcare providers. Psychooncology 2016;25(4):362-86.
10. Fleuren N, Depla MFIA, Janssen DJA, Huisman M, Hertogh CMPM. Underlying goals of advance care planning (ACP): a qualitative analysis of the literature. BMC Palliat Care 2020;19(1):27.
11. Brinkman-Stoppelenburg A, Rietjens JA, Van Der Heide A. The effects of advance care planning on end-of-life care: a systematic review. Palliat Med 2014;28(8):1000-25.
12. Committee on Approaching Death: Addressing Key End of Life Issues; Institute of Medicine. Dying in America: Improving Quality and Honoring Individual Preferences Near the End of Life. Washington (DC): National Academies Press (US); March 19, 2015.p.624-6242.
13. Houben CHM, Spruit MA, Groenen MTJ, Wouters EFM, Janssen DJA. Efficacy of advance care planning: a systematic review and meta-analysis. J Am Med Dir Assoc 2014;15(7):477-89.
14. Busa C, Pozsgai E, Zeller J, Csikos A. Who should talk with patients about their end-of-life care wishes? A nationwide survey of the Hungarian population. Scand J Prim Health Care 2022;40(1):157-64.
15. Jabbarian LJ, Zwakman M, van der Heide A, Kars MC, Janssen DJA, van Delden JJ, et al. Advance care planning for patients with chronic respiratory diseases: a systematic review of preferences and practices. Thorax 2018;73(3):222-30.
16. Rietjens J, Korfage I, Taubert M. Advance care planning: the future. BMJ Support Palliat Care 2021;11(1):89-91.
17. Mullick A, Martin J, Sallnow L. An introduction to advance care planning in practice. BMJ 2013;347(3):60-4.
18. Simon J, Porterfield P, Bouchal SR, Heyland D. 'Not yet' and 'Just ask': barriers and facilitators to advance care planning – a qualitative descriptive study of the perspectives of seriously ill, older patients and their families. BMJ Support Palliat Care 2015;5(1):54-62.
19. Kermel-Schiffman I, Werner P. Knowledge regarding advance care planning: a systematic review. Arch Gerontol Geriatr 2017;73:133-42.
20. Howard M, Bernard C, Tan A, Slaven M, Klein D, Heyland DK. Advance care planning: Let's start sooner. Can Fam Physician 2015;61(8):663-5.
21. Demirkapu H, Van den Block L, De Maesschalck S, De Vleminck A, Colak FZ, Devroey D. Advance care planning among older adults of Turkish origin in Belgium: exploratory interview study. J Pain Symptom Manage 2021;62(2):252-9.
22. Spring J, McKinlay J, Puxty K, Metaxa V, Detsky M, Mehta S, et al. Perspectives on advance care planning for patients with hematologic malignancy: an international clinician questionnaire. Ann Am Thorac Soc 2021;18(9):1533-9.
23. Chan CWH, Ng NHY, Chan HYL, Wong MMH, Chow KM. A systematic review of the effects of advance care planning facilitators training programs. BMC Health Serv Res 2019;19(1):362.
24. Korfage IJ, Carreras G, Arnfeldt Christensen CM, Billekens P, Bramley L, Briggs L, et al. Advance care planning in patients with advanced cancer: A 6-country, cluster-randomised clinical trial. PLoS Med 2020;17(11):e1003422
25. Resick JM, Arnold RM, Sudore RL, Farrell D, Belin S, Althouse AD, et al. Patient-centered and efficacious advance care planning in cancer: Protocol and key design considerations for the PEACe-compare trial. Contemp Clin Trials 2020;96(12):106-71
26. American Academy of Pediatrics. Committee on Bioethics and Committee on Hospital Care. Palliative care for children. Pediatrics 2000;106(5):351-3.
27. van Driessche A, Gilissen J, De Vleminck A, Kars M, Fahner J, van der Werff Ten Bosch J, et al. The BOOST paediatric advance care planning intervention for adolescents with cancer and their parents: development, acceptability and feasibility. BMC Pediatr 2022;22(1):210.
28. Holland LR, Walker R, Henney R, Cashion CE, Bradford NK. Adolescents and Young Adults with Cancer: Barriers in Access to Psychosocial Support. J Adolesc Young Adult Oncol 2021;10(1):46-55.
29. Pinkerton R, Donovan L, Herbert A. Palliative care in adolescents and young adults with cancer-why do adolescents need special attention? Cancer J 2018;24(6):336-41.
30. Thompkins JD, Needle J, Baker JN, Briggs L, Cheng YI, Wang J, et al. Pediatric advance care planning and families' positive caregiving appraisals: An RCT. Pediatrics 2021;147(6):e2020029330. doi:10.1542/peds.2020-029330
31. Lotz JD, Jox RJ, Borasio GD, Führer M. Pediatric advance care planning from the perspective of health care professionals: a qualitative interview study. Palliat Med 2015;29(3):212-22.

32. Orkin J, Beaune L, Moore C, Weiser N, Arje D, Rapoport A, et al. Toward an understanding of advance care planning in children with medical complexity. Pediatrics 2020;145(3):e20192241.
33. Friedman SL. Parent resuscitation preferences for young people with severe developmental disabilities. J Am Med Dir Assoc 2006;7(2):67-72.
34. Bogetz JF, Bogetz AL, Gabhart JM, Bergman DA, Blankenburg RL, Rassbach CE. Continuing education needs of pediatricians across diverse specialties caring for children with medical complexity. Clinical Pediatrics 2015;54(3):222-27.
35. Almack, K, Cox, K, Moghaddam, N, Pollock K, Seymour J. After you: Conversations between patients and healthcare professionals in planning for end of life care. BMC Palliat Care 2012;11:15.
36. Abernethy, ER, Campbell, GP and Pentz, RD. Why many oncologists fail to share accurate prognoses: They care deeply for their patients. Cancer 2020;126(6):1163-5.
37. Lotz JD, Daxer M, Jox RJ, Borasio GD, Führer M. "Hope for the best, prepare for the worst": A qualitative interview study on parents' needs and fears in pediatric advance care planning. Palliat Med 2017;31(8):764-71.
38. Christenson K, Lybrand SA, Hubbard CR, Hubble RA, Ahsens L, Black P. Including the perspective of the adolescent in palliative care preferences. J Pediatr Heal Care 2010;24(5):286-91.
39. Belpame N, Kars MC, Beeckman D, Decoene E, Quaghebeur M, Van Hecke A, et al. "the AYA director": A synthesizing concept to understand psychosocial experiences of adolescents and young adults with cancer. Cancer Nurs 2016;39(4):292-302.
40. Baker JN, Hinds PS, Spunt SL, Barfield RC, Allen C, Powell BC, et al. Integration of palliative care practices into the ongoing care of children with cancer: individualized care planning and coordination. Pediatr Clin North Am 2008;55(1):223-50.
41. Wiener L, Bedoya S, Battles H, Sender L, Zabokrtsky K, Donovan KA, et al. Voicing their choices: Advance care planning with adolescents and young adults with cancer and other serious conditions. Palliat Support Care 2022;20(4):462-70.
42. Weaver MS, Heinze KE, Kelly KP, Wiener L, Casey RL, Bell CJ, et al. Palliative care as a standard of care in pediatric oncology. Pediatr Blood Cancer 2015;62(5):829-33.
43. Kassam A, Skiadaresis J, Habib S, Alexander S, Wolfe J. Moving toward quality palliative cancer care: Parent and clinician perspectives on gaps between what matters and what is accessible. J Clin Oncol 2013;31(7):910-15.
44. Kaye EC, Woods C, Kennedy K, Velrajan S, Gattas M, Bilbeisi T, et al. Communication around palliative care principles and advance care planning between oncologists, children with advancing cancer and families. Br J Cancer 2021;125(8):1089-99.
45. Mack JW, Fasciano KM, Block SD. Communication about prognosis with adolescent and young adult patients with cancer: information needs, prognostic awareness, and outcomes of disclosure. J Clin Oncol 2018;36(18):1861-7.
46. Wiener L, Zadeh S, Battles H, Baird K, Ballard E, Osherow J, et al. Allowing adolescents and young adults to plan their end-of-life care. Pediatrics 2012;130(5):897-905.
47. Weaver MS, Wiener L, Jacobs S, Bell CJ, Madrigal V, Mooney-Doyle K, et al. Weaver et al's Response to Morrison: Advance Directives/Care Planning: Clear, Simple, and Wrong. J Palliat Med 2021;24(1):8-10.
48. Fladeboe KM, O'Donnell MB, Barton KS, Bradford MC, Steineck A, Junkins CC, et al. A novel combined resilience and advance care planning intervention for adolescents and young adults with advanced cancer: A feasibility and acceptability cohort study. Cancer 2021;127(23):4504-11.
49. Heckford E, Beringer, A. Advance care planning: challenges and approaches for pediatricians. J Palliat Med 2015;17(9):1049-53.
50. Izumi S. Advance care planning: the nurse's role. Am J Nurs 2017;117(6):56-61.

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RECENT ADVANCES OF CHOLINESTERASE INHIBITORS PLAYING A CRITICAL ROLE IN THE TREATMENT OF ALZHEIMER'S DISEASE (2020-2022)

ALZHEİMER HASTALIĞININ TEDAVİSİNDE KRİTİK BİR ROL OYNAYAN KOLİNESTERAZ İNHİBİTÖRLERİNDEKİ SON GELİŞMELER (2020-2022)

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ABSTRACT

Alzheimer's disease (AD) is a common neurodegenerative disorder which has a catastrophic effect on the brain. It significantly affects people's daily life, especially the population over the age of 65, with problems such as neuron death, memory loss, cognitive disorders, and cholinergic dysfunction. However, despite the drug development studies which have been conducted recently quite intensively, an effective drug molecule that can completely cure people or stop the stage of the disease has not been found yet, unfortunately. One of the reasons for this is the complexity of understanding of how the pathology of the disease arises. The most accepted theory for AD is the cholinergic hypothesis. Acetylcholine (ACh), an important neurotransmitter, is metabolized by two cholinesterase (ChE) enzymes named acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). With AD, ChE activity increases and thus the degradation of acetylcholine is triggered. In addition, it is understood that the peripheral anionic region (PAS) of the AChE also caused the formation of Aβ-peptide fibrils. Therefore, based on this hypothesis, the most effective approach in treatment is the use of cholinesterase inhibitors (ChEI), which try to restore ACh levels by increasing them at cholinergic synapses. Furthermore, it focused on the design of multifunctional molecules. In conclusion, considering the complex nature of the disease and its effects, it is clear that more studies are needed in this area. In this study, important and remarkable studies in recent years have been included and it aimed to contribute to future drug research and development studies.
Keywords: Alzheimer's disease (AD), cholinesterase inhibitors (ChEIs), acetylcholinesterase inhibitors (AChEIs), butyrylcholinesterase inhibitors (BuChEIs), multifonctional drugs, multi-target directed ligands

ÖZ

Alzheimer hastalığı (AD) oldukça sık rastlanılan ve majör yıkıcı bir etkiye sahip olan nörodejeneratif bir hastalıktır. Özellikle 65 yaş üstü nüfusu büyük oranda tutan, hasta kişinin nöron ölümü, hafıza kaybı, bilişsel ve kolinerjik işlev bozuklukları gibi sıkıntılarla günlük yaşamını önemli derecede etkileyen bir durumdur. Fakat son dönemde yapılan ilaç geliştirme çalışmalarının oldukça yoğun yürütülmesine rağmen, hala kişileri tamamen iyileştirebilecek veya hastalığın evresini tamamen durdurabilecek etkili bir ilaç molekülü maalesef bulunamamıştır. Bunun sebeplerinden biri de hastalığın patolojisinin nasıl ortaya çıktığının anlaşılamaması ve karmaşıklığı ile ilgilidir. Alzheimer hastalığı için kabul gören en önemli teori kolinerjik hipotezdir. Önemli bir nörotransmitter olan asetilkolin (ACh), asetilkolinesteraz (AChE) ve bütirilkolinesteraz (BuChE) isimli iki kolinesteraz enzimi (ChE) tarafından metabolize edilir. AD ile birlikte ise ChE aktivitesi artmış ve dolayısıyla asetilkolinin yıkımı tetiklenmiştir. Ayrıca AChE'in periferal anyonik bölgesi (PAS)'nin Aβ-peptid fibrillerinin oluşmasına da neden olduğu anlaşılmıştır. Bu sebeple bu hipoteze dayanarak tedavideki en etkili yaklaşım, kolinerjik sinapslarda ACh seviyesini arttırarak eski haline döndürmeye çalışan kolinesteraz inhibitörlerinin (ChEI) kullanılmasıdır. Üstelik multifonksiyonel moleküllerin tasarımları üzerine de odaklanılmıştır. Sonuç olarak hastalığın karmaşık yapısı ve etkileri göz önüne alındığında, bu alanda daha fazla çalışma yapılması gerekmektedir. Bu çalışmada son yıllardaki önemli ve dikkat çeken çalışmalar yer almış olup, bundan sonraki ilaç araştırma ve geliştirme çalışmalarına katkı sağlaması amaçlanmıştır.
Anahtar kelimeler: Alzheimer hastalığı (AD), kolinesteraz inhibitörleri (ChEIs), asetilkolinesteraz inhibitörleri (AChEIs), butirilkolinesteraz inhibitörleri (BuChEIs), multifonksiyonel ilaçlar, çoklu hedefe yönlendirilmiş ligandlar

INTRODUCTION

Dementia, a major neurodegenerative disorder, is a disease that significantly affects a person's memory, behavior, mental abilities, and social life (1). In 1906, Alzheimer's disease (AD) was first defined as the condition of a serious disease of the cerebral cortex by the German psychiatrist Alois Alzheimer, after the examination of the brain autopsy of a patient who had some personality differences and lost his memory before he died (2, 3). It is the most common type of dementia worldwide,

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which is chronic-permanent and very common especially in people over the age of 65, although the exact cause is unknown, the pathology can first be detected by;

- the accumulation of intracellular tau proteins (by hyperphosphorylation) and extracellular beta-amyloid (Aβ) aggregates,
- the occurrence of neuronal degeneration with neurofibrillary tangles (NFTs) after upregulation of β-sesterase,
- oxidative stress,
- neuroinflammation,
- the low levels of acetylcholine (ACh) neurotransmitter in the hippocampal and cortical region of the brain (1, 4-8).

For this reason, neuronal death and damage to the hippocampal part of the brain occur with the changes as a result of AD. Besides, symptoms which are characterized by insidious onset and slow progress, such as agitation, disinhibition, apathy, difficulty in making decisions, difficulty in finding words, severe speech disorder and forgetfulness, psychosis, anxiety and sleep disturbance may occur and it is highly progressive (2, 4, 7). Physiological differences between the normal brain of a healthy person and the brain of a person with AD are shown in Figure 1. Advanced age, gender, genetic factors (70% probability), head injuries, vascular disorders, obesity, diabetes, infections, lifestyle and environmental factors (heavy metals, air pollution and others) can be counted as threatening situations that may cause the disease (3). Although it is considered to affect the elderly population, a familial AD caused by a gene mutation caused death in a 28-year-old (9). As stated in the World Alzheimer's report of 2021, there are more than 55 million Alzheimer's patients worldwide today, and this number is estimated to reach 78 million by 2030 (1). Dementia in the world population data in 2016; It was reported as the 5th largest cause of death among 2.4 million deaths and the 2nd largest one in people over 70 years of age (5). The World Health Organization has stated that AD may be more common than cancer, cardiovascular diseases and AIDS within the next century (10).

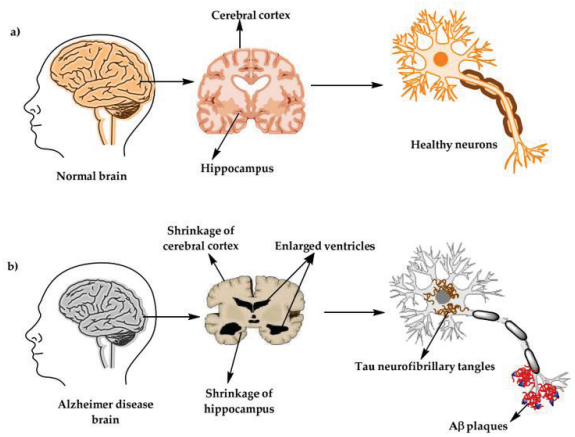


Figure 1: Physiological differences between the normal brain of a healthy person and the brain of a person with Alzheimer's disease (3).

Current Alzheimer's drug therapy

Acetylcholine is the key for both the central nervous system (CNS) and autonomous nervous system, the biomarker in AD, which is of such importance, and responsible for memory and learning tasks in the brain by providing communication between two nerve cells. Acetylcholinesterase enzyme plays an important role in the regulation of acetylcholine and the first theory is the cholinergic hypothesis based on it (4, 11). As it is known, cholinergic transmission is quite significant in cerebral cortical development and activity, cerebral blood flow, sleep-wake cycle, memory, learning and cognition. Furthermore, the biological response to acetylcholine-transmitted warning signals in the cholinergic system is given by a post-synaptic receptor (muscarinic or nicotinic receptor) (12). In order to increase the density of ACh, which decreases with the increase of acetylcholinesterase enzyme as a result of Alzheimer's disease in brain cholinergic synapses and also in neuromuscular junctions, to make up for the cholinergic loss, and to increase cholinergic transmission, it is attempted to block acetylcholinesterase and butyrylcholinesterase enzymes that break down this neurotransmitter into choline and acetate (2, 4, 13, 14). Butyrylcholinesterase enzyme, which is the isoenzyme of acetylcholinesterase, a member of the cholinesterase enzyme family (also known as serum cholinesterase and contains 65% homologous amino acid residues with AChE) is a minor element in the brain of a healthy person, while it is increased due to plaque and tangles in the brain of the patient with AD. And therefore impaired cholinergic transmission can also be treated by inhibition of BuChE (15, 16). But in advanced AD, on the contrary, the AChE level is severely reduced due to severely damaged neurons (15). Since the BuChE enzyme, which is also called as pseudocholinesterase, is synthesized in the liver and then distributed in the plasma, it is less specific to acetylcholine, and AChE activity plays a more dominant role in acetylcholine hydrolysis at neuromuscular junctions and cholinergic synapses (5, 15, 17). It was reported that BuChE-rich neurons in the human cerebral cortex were only half as large as neurons with dense AChE (15). Additionally, when the structure of AChE was examined, it was understood that the peripheral anionic site (PAS) of the enzyme also causes the formation of Aβ-peptide fibrils. Thus, it was also studied on the designs of molecules that would interact with the AChE enzyme binding site like the PAS and prevent the formation of Aβ-peptide fibrils. What makes AChEI the most effective and most successful treatment method is not only the fact that these drug molecules increase cholinergic transmission, but also the fact that it creates a dual solution by preventing Aβ synthesis, accumulation and formation of aggregates (18).

In summary, AChE enzyme is preferred as a target in drug development due to its structure and relationship with acetylcholine, but BuChE enzyme also gains importance as a target to be inhibited because it will increase to 105-165% of normal condition in advanced AD (15).

Researchers have discovered a catalytic anionic site (CAS) at the bottom of a narrow passage, in which the hydrophobic amino-

acid side chains are located. CAS consists of a few subsites: the anionic site, where the interaction with ACh takes place, the esteratic site (ES), where three residues of the catalytic triad are included, the oxyanion hole, and the acyl pocket, which provides substrate selectivity (12). The catalytic triad (serine, histidine and an acidic residue which is also a glutamate) is included in AChE enzym's catalytic site and has a crucial importance (12, 15). A choline-binding site have hydrophobic tryptophan residues while it is expected to have anionic groups. Another significant subunit known as the peripheral anionic site (PAS) has also been found approximately 15A˚from the CAS (12). Structural features of the AChE enzyme are shown in Figure 2.

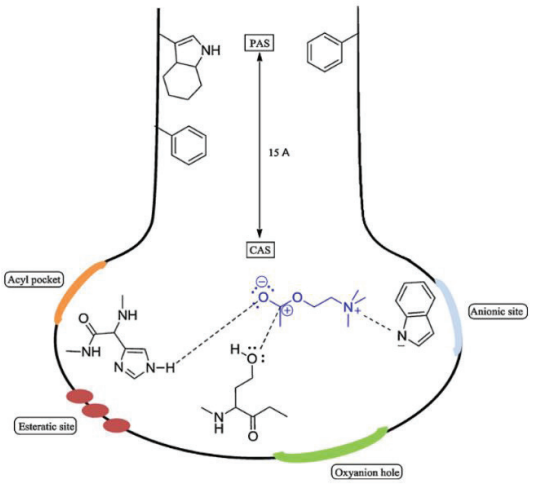


Figure 2: Structural features of the AChE enzyme (as known as serine hydrolase) (12).

Although the communication of the substrate with the active site seems to take a long time based upon the path it will take, it is considered that this reaction takes place very quickly thanks to the electric field of AChE. Despite electrical field reduction attempts, the rate of the enzyme has not changed. In the literature, it was noted that AChE hydrolyzes about 25000 AChs per second (19). The serine amino acid containing the hydroxyl group (acts as a nucleophile) and the histidine amino acid containing the imidazole ring (acts as a base) in the structure of the AChE hydrolyze ACh in its ester structure and allow the release of choline and the formation of the intermediate Acetly-AChE, and subsequently let the intermediate product hydrolyze to release acetate. The AChE reaction is shown in Figure 3.

The acetylcholine neurotransmitter is synthesized from choline and acetyl-coenzyme A in the cytoplasm of neurons by the enzyme choline acetyltransferase (ChAT) and is transported via the vesicular acetylcholine transporter (VAChT). According to Cholinergic Hypothesis; In the 1970s, it was thought that the source of the problem in cholinergic transmission was the enzyme choline acetyltransferase (ChAT), which enabled the synthesis of acetylcholine. However, later studies pointed out the AChE enzyme. Discovery of interactions between AChE and Aβ peptide supported this idea (3). The cholinergic synapse is shown in Figure 3.

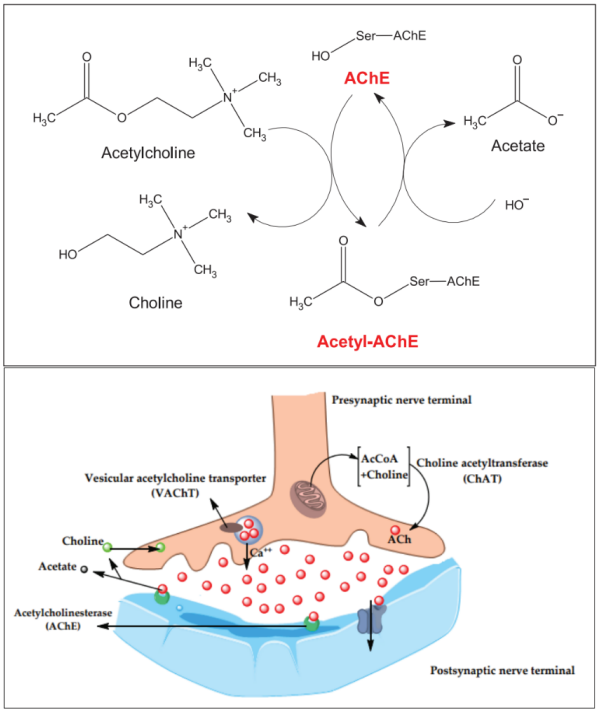


Figure 3: The acetylcholinesterase (AChE) reaction (19). And the cholinergic synapse (3).

Unfortunately, the progression of Alzheimer's disease cannot be stopped or completely cured with current medications, but they are helpful in reducing symptoms and improving the person's quality of life (8). In addition, cholinergic-acting cholinesterase enzyme inhibitor (ChEI) molecules, which are also approved by the Food and Drugs Administration (FDA), constitute an important group of drugs in the treatment in the early stages of Alzheimer's disease (2, 4, 16). These are:

- Tacrine: It has the tetrahydroacridine structure and it was the first FDA approved ChEI (in 1993). It inhibits both AChE and BuChE.
- Donepezil: It is an indanonebenzylpiperidine derivative (FDA approval in 1996) and it selectively inhibits AChE.
- Galantamine: It is an alkaloid derivative (FDA approval in 2003) and it selectively inhibits AChE.
- Rivastigmine: It is a carbamate derivative (FDA approval in 2006) and it makes non-specific inhibition (2, 9, 14).

Apart from ChEIs, memantine, a non-competitive antagonist of glutamate N-methyl-D-aspartate (NMDA) receptors, which was FDA-approved (in 2003) is also used in the treatment of AD (5, 8, 9). Nevertheless, as well as their benefits, these molecules may also have serious peripheral cholinergic side effects such as gastrointestinal discomfort, bradycardia, diarrhea, loss of appetite, nausea, vomiting, sweating, muscle cramps, fatigue, increased frequency of bowel movements, insomnia, headache, dizziness, loss of consciousness, excitement, extreme fear, hallucination, urinary incontinence and bronchoconstriction.

Thus, new drug molecules are being developed in order to improve the selectivity and side-effect profiles (2, 4, 6, 8, 13). The chemical structures of traditional ChEIs are shown in Figure 4.

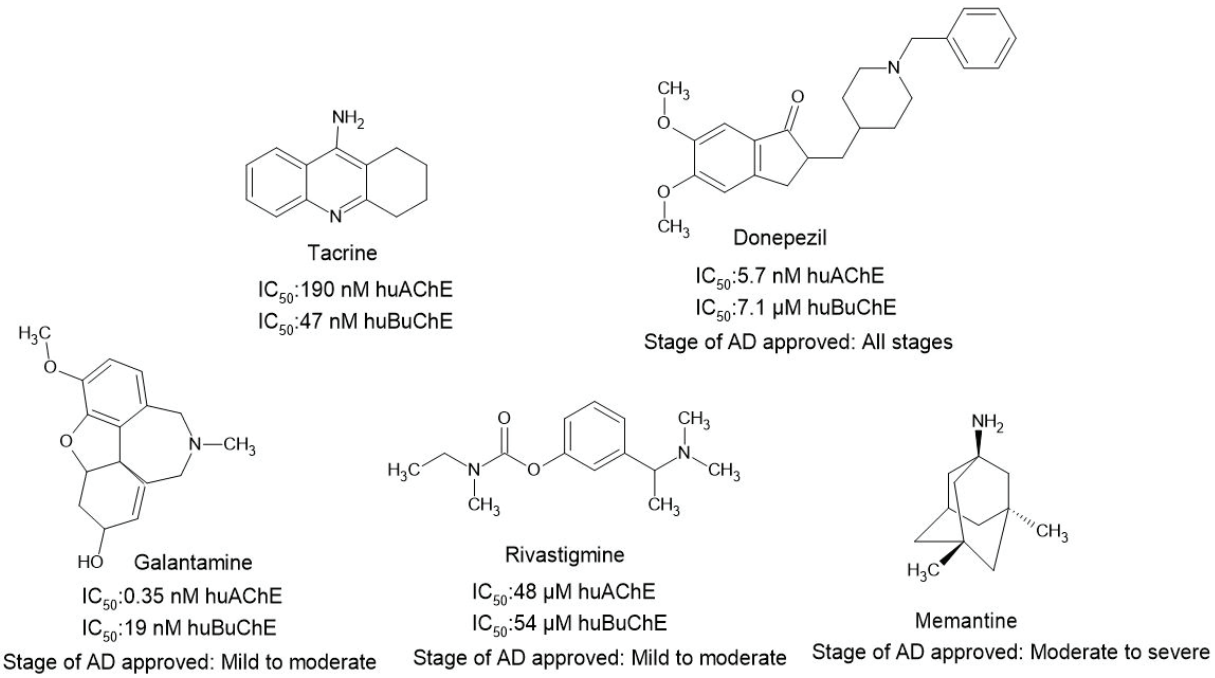


Figure 4: Chemical structures of cholinesterase enzyme inhibitor (ChEI) drug molecules and their inhibitory values against human acetylcholinesterase enzyme (huAChE) and human butyrylcholinesterase enzyme (huBuChE), and non-competitive antagonist of glutamate N-methyl-D-aspartate (NMDA) receptors, memantine (5, 14).

While donepezil, rivastigmine and galantamine are currently used clinically for Alzheimer's disease, tacrine was withdrawn from the market due to its severe hepatotoxic effect and drug-drug interactions (2, 14). In a study it was discovered that memantine and donepezil molecules can also be used as a dual combination in the treatment (8). In another study, it was noted that in a situation where donepezil or galantamine molecules cannot improve, cognition and behavior with the rivastigmine molecule was better, and if the progression of the disease continued, the combination therapy of adding memantine molecule to the treatment (rivastigmine + memantine) was also beneficial (20).

In drug research, AChE is an enzyme system targeted not only for AD, but also for the treatment of neurological diseases such as Lewy body dementia, Parkinson's disease dementia, schizophrenia, and diseases such as myasthenia gravis, glaucoma, and anticholinergic poisoning (21, 22).

The first of the methods focused on in drug development studies is the synthesis of analogs and derivatives of molecules synthesized from nature, the second is the synthesis of analogs and derivatives of molecules synthesized from existing drug molecules, the third is the design of molecules that will interact with the valuable parts of the AChE enzyme binding site like the PAS, and finally the use of methods such as structure-based pharmacophore modeling, molecular docking, etc. (22).

Therapeutic approaches that focus on solving and understanding AD and trying to stop or change the progress of the disease are; a) stopping oxidative damage to neurons; b) stopping or reducing the increase of amyloid protein; c) fixing the loss in the cholinergic system; d) increasing or restoring the amount of acetylcholine (3, 23).

It is understood from the studies conducted in recent years that both AChE and BuChE, which are important drug targets in the central nervous system, were of great importance. Although the focus is on AChE at the beginning of AD, while this enzyme decreases, the BuChE enzyme level increases and takes over the task of AChE. Therefore, ACh level is closely related to both enzyme systems. For this reason, the design of Dual-Target Inhibitors has gained importance and studied by researchers. The molecule **1** in the Figure 5 inhibited both AChE (eeAChE IC_{50} = 0.39 µM) and BChE (eqBChE IC_{50} = 0.28 µM) enzymes (by binding to the CAS and PAS regions of enzymes), and the molecule was reported to have lower cytotoxicity than tacrine. Therefore, it is thought to be promising in AD as a safe and multipotent molecule (24).

Considering the effective molecules in the treatment, some compounds formed as a result of hybridization (these are shown in Figure 5) have been discovered to be highly potent AChE and BuChE inhibitor molecules (14).

In a study where researchers designed and synthesized a series of acridine derivatives containing 1,3,4-thiadiazole moiety, it was observed that all new molecules inhibited both ChE enzymes but had high selectivity against AChE. It has been noted that the molecules **6** and **7** in Figure 6 have better inhibitory

power and much lower hepatotoxicity than tacrine. It has been reported that the molecules provide inhibition by binding to site 2, a new allosteric site of AChE, instead of site 1 (CAS/PAS) (25).

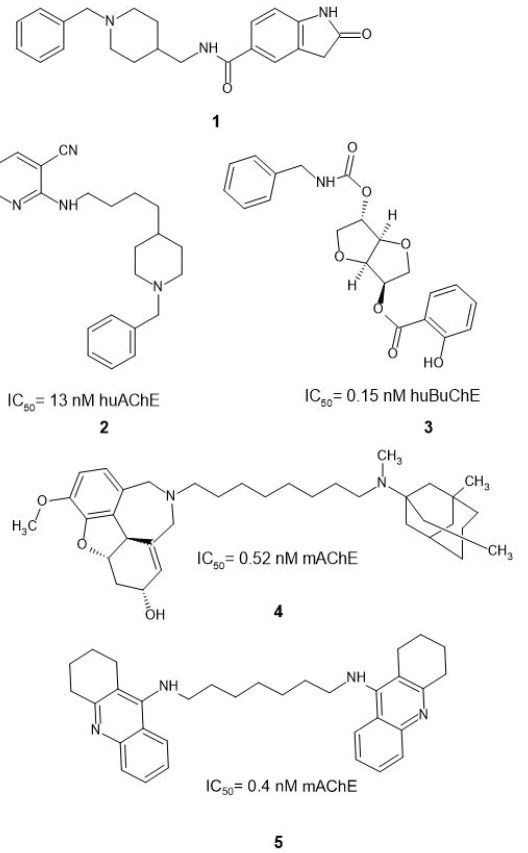


Figure 5: Examples of molecules and potencies discovered to inhibit potent AChE and BuChE (human AChE (huAChE), human BuChE (huBuChE), murine AChE (mAChE) (14, 24).

In order to prevent hepatotoxicity caused by the free amine group in tacrine, new tacrine analogues were looked for. It has been stated that these analogs are safer molecules with low toxicity compared to tacrine, and that molecules bind to the enzyme through hydrogen bond interactions and provide inhibition. The IC_{50} values of the compound **8** and **9** with piperazine containing acetamide and butyrylamide chains have shown in the Figure 6 were 0.52±0.03 and 0.73±0.04 µM, 0.71±0.04 and 1.01±0.03 µM, respectively, and showed an inhibition effect against AChE and BuChE respectively (10).

One of the AD treatment goals by researchers is to prevent Aβ aggregation. Normally, the Aβ peptide structure consists of 39-42 amino acids but it was discovered that oligomers consisting of the Aβ1–42 peptide are much more toxic, so the formation of Aβ1–42 must be stopped. For this purpose, in a study designed to evaluate novel multifunctional inhibitors, it was noted that the most potent molecule (molecule **12** is shown in Figure 7) had 100 times more selectivity against BuChE, while it showed IC_{50}: 180 nM value against AChE. In addition, it was understood that all four synthesized molecules showed preventive and neuroprotective activities against Aβ accumulation. It was an important step for drug candidate molecules that could be used in AD (26).

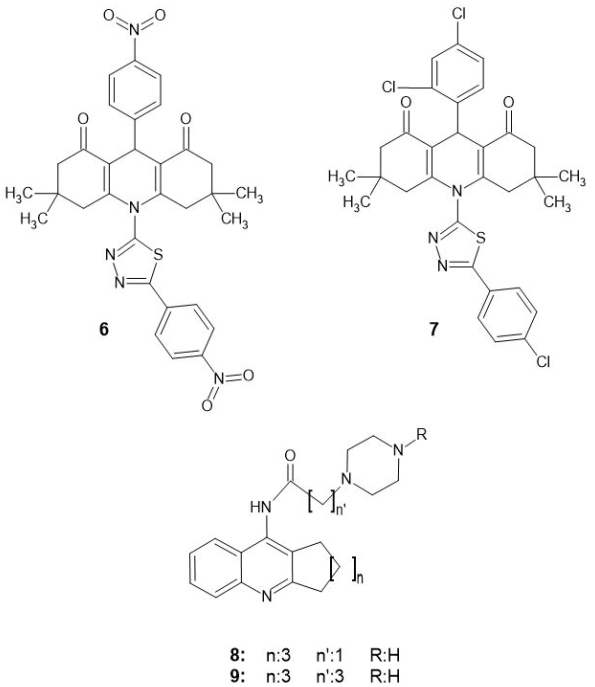


Figure 6: The chemical structures of acridine derivatives containing 1,3,4-thiadiazole moiety (molecule **6** and **7**), analogues of the tacrine (molecule **8** and **9**) (10, 25).

We are in an era when multi-target molecules are in demand. Therefore, based on tramiprosate, which has selective anti-Aβ oligomers aggregating activity, a series of novel compounds based on tramiprosate was designed and synthesized. In the designed compounds, the addition of a pyridinium/isoquinolinium ring to the tramiprosate moiety increased the efficiency of the molecules to the binding sites in ChE enzymes, but sulfonic acid moiety prevented this binding. It has been reported that new derivatives synthesized by removing this fragment inhibit over 10% Aβ aggregation at 1 µM concentration, while they can inhibit 70% Aβ on ChEs. The most effective molecule with dual inhibition (with >85% inhibition, at 100 µM), low cytotoxicity and anti-Aβ aggregation (18% inhibition) properties was shown in the Figure 7 as compound **15**. In addition, according to an experiment performed on AD mice, this molecule crossed the blood brain barrier, suggesting that it may be useful for drug molecules *in vivo* (27).

In another study, amine, oxime, ether, epoxy and acyl derivatives of the benzobicyclo[3.2.1]octene were synthesized and most of them were found to have a higher selectivity capacity against BuChE. Among the synthesized compounds, compound **16** in Figure 7 showed the highest AChEI effect with an IC_{50}=8.3 µM. Compound **17** in Figure 7, on the other hand,

has 5 times stronger inhibition properties against BuChE than Huperzine A (28).

Studies showed that zinc accumulates in high concentration in the brains of people with AD. While the Zn^{2+} ion is necessary for neural functions in a healthy individual under normal conditions, excessive accumulation of this ion causes the aggregation of neurotoxic Aβ, oxidative stress and the secretion of high levels of proinflammatory cytokines. For this reason, studies for metal chelation therapy are being tried. In a study synthesizing 8-substituted derivatives of the sampangine alkaloid, which had strong antibacterial and antifungal effects, multifunctional agents that could better cross the blood-brain barrier and selectively chelate Zn^{2+} ion, as well as high AChE enzyme inhibitory activity, were detected. The most successful derivative in the study is given in the Figure 7 as compound **19** with IC_{50}: 0.27±0.01μM against AChE (29).

AChEI and anti-Aβ aggregation properties of phosphoshazine and phosphazide derivatives were also discovered and synthesized. It was noted that a coumarin phosphazide derivative compound (it is shown in Figure 7 as compound **20**) could bind to MMP-2 (Matrix metalloproteinase-2) and Zn^{2+}-induced Aβ42 aggregation, PAS and CAS at the same time, which caused AChE inhibition and had low toxicity data. Matrix metalloproteinases, which should be at lower levels in the human brain under normal conditions, unfortunately increase in AD and cause neurotoxicity together with Aβ. According to this known fact, adding extra "MMP-2 inhibitory" activity to the discovered molecule is a great improvement in terms of multifunctional drug developments and should be studied (30).

In recent years, drug candidates obtained from natural sources related to AChE inhibition were investigated. Some examples are; the galantamine molecule that has been isolated from *Galanthus nivalis* and Huperzine A alkaloid obtained from a medicinal plant named *Huperzia serrata* (22). The chemical structures of Huperzine A and neuroprotective dimeric AChE-Is derived from the tacrine and/or huperzine A fragment are shown in Figure 8 (31). In addition, while the polysaccharides (are shown in Figure 8) contained in *Pleurotus ostreatus* (aka Oyster Mushroom) are known for their antitumor and antioxidant properties, they are proved to improve learning and memory impairment by reducing cognitive deficits, AChE activity and oxidative stress in AD (5).

Apart from plants, bioactive inhibitors were also found in marine sponges. Moreover, it was demonstrated that these compounds have a better side-effect profile than synthetically produced molecules and provide neuroprotective activity with a very good bioavailability. Examples of these are gracilin derived from *Spongionella gracilis*, a marine sponge that has therapeutic potential in AD. (The chemical structures of the Gracilin A derivative molecules are shown in Figure 8) For this reason, marine life has become very interesting for researchers due to its important effects on the pathogenesis of AD in various *in vivo* and *in vitro* studies (9).

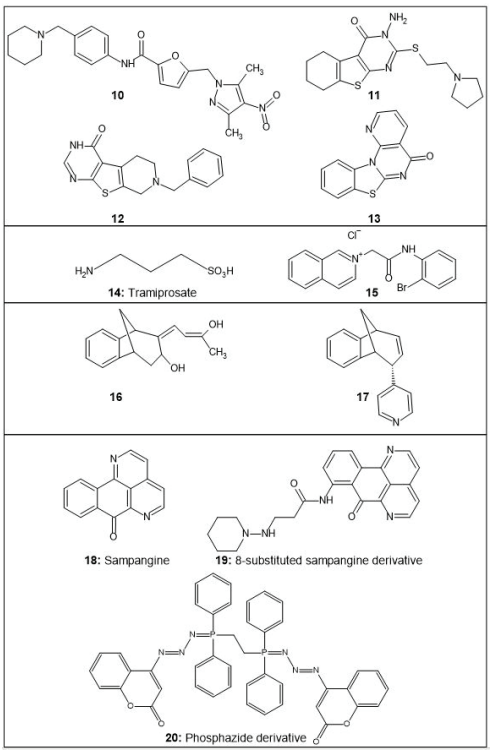


Figure 7: The chemical structures of novel multifunctional inhibitor molecules **10**-**13** (26). The chemical structures of tramiprosate and the novel compound based on tramiprosate (compound **15**) (27). The chemical structures of compound **16** and **17** (28). The chemical structures of sampangine, 8-substituted sampangine derivative and phosphazide derivative (29, 30).

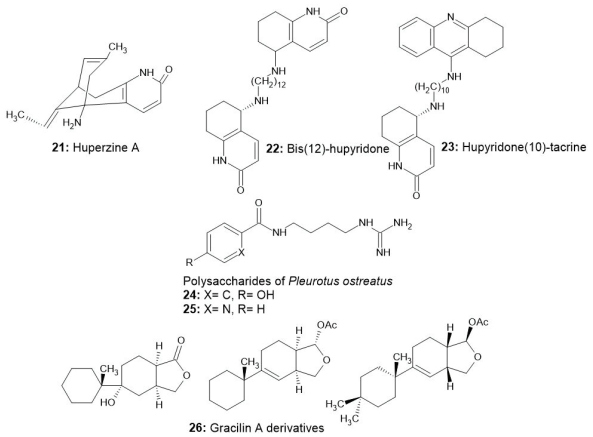


Figure 8: The chemical structures of Huperzine A, AChEIs derived from the tacrine and/or huperzine A fragment, polysaccharides of *Pleurotus ostreatus* and Gracilin A derivative molecules (5, 9, 31).

In the last decade, studies on the antimicrobial activity of silver salts have emerged. Since antimicrobial, antiproliferative, anticancer, anti-HIV, antioxidants, and anti-inflammatory properties of N-Heterocyclic Carbenes (NHC) are known, new NHC salts containing benzimidazole-moeities and their silver (I) complexes were prepared. Compounds **27** and **28** in Figure 9 were

measured with IC_{50} values of 8.56±1.17 μM and 5.05±0.30 μM against AChE and BChE, respectively, and were identified as potent inhibitors. They also showed moderate antibacterial and antifungal activities (32).

A number of 3-benzyloxyflavone derivatives have been synthesized and observed to have potent dual inhibitory effects against AChE and BChE. Overall, these compounds turned out to be more active against AChE than BChE. Due to the presence of a bulky and hydrophobic diphenylamino group substituent at the 4-position of the B ring, the molecule **29** in Figure 9 interacted with active pockets of both enzymes and became the most potent ChE enzyme inhibitor (33).

Polar nitro and amino groups substitutes were added to imidazo[1,2-b]pyridazine compounds, which is a pharmacophore group for analgesic and anti-inflammatory properties, and their biological activities were measured. The substituted 3-nitro-6-amino-imidazo [1,2-b] pyridazine compounds (**30** and **31**) in the Figure 9 showed strong AChE inhibition. In addition, it has been reported that these compounds are multifunctional compounds that cause antiproliferative, anti-migratory and anti-inflammatory activities at high doses (34).

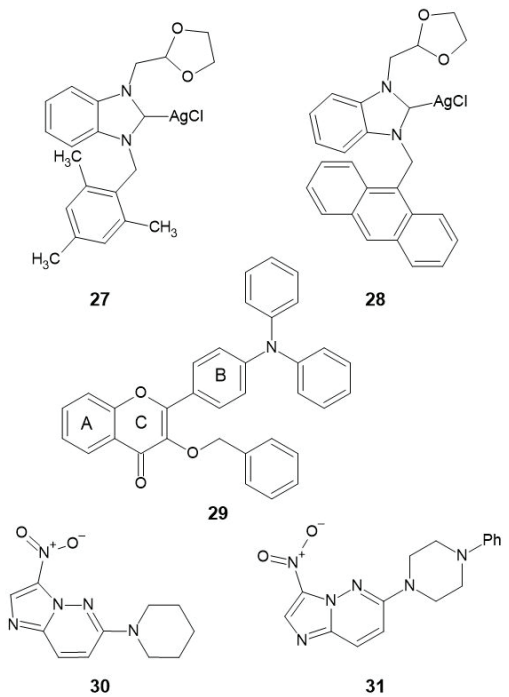


Figure 9: The chemical structures of N-Heterocyclic Carbenes salts containing benzimidazole-moeities and their silver (I) complexes (molecule **27** and **28**), the 3-benzyloxyflavone derivative (molecule **29**) and the substituted 3-nitro-6-amino-imidazo[1,2-b]pyridazine compounds (molecule **30** and **31**) are shown in Figure 9 (32-34).

Ladostigil, a synthetic molecule, is a pluripotential neuroprotective drug that inhibits cholinesterase enzymes and brain selective monoamine oxidase -A and -B. Therefore, it is thought to be beneficial especially for dementia with depression and it has come up to phase IIb studies (35). While some ChEIs such as rivastigmine have a short half-life and serious dose-related side effects are encountered in long-term use, the side-effect profile of Ladostigil has been found to be better. However, the potential of this compound to cross the blood-brain barrier is weak (21). The chemical structure of the Ladostigil is shown in Figure 10.

It has been reported that the N-substituted α-aminophosphonate-bearing chromone moiety molecule in Figure 10 (compound **33**) shows AChE enzyme inhibition with an IC_{50}=0.103±0.24 μM that is twice as potent as tacrine, 35 times more than galantamine, and 50 times more potent than rivastigmine. In addition, the compound has the ability to bind to the CAS and PAS regions of both ChE enzymes and has DNA damage protection efficacy. In the study, it has been also observed that the synthesized aliphatic amine analogs provide better inhibition against AChE, while aromatic amine analogs provide better inhibition against BuChE (36).

It has been discovered that enilconazole, one of the antifungal drugs, provides 43% inhibition of AChE at 0.3 μg/ml, tebuconazole provides dose-dependent inhibition of AChE and BChE and miconazole provides dual ChE inhibition, improving memory disorders in the brain of mice with AD. Based on the fact that the azole ring has ChE enzyme inhibitory activity as well as antifungal, antiviral, antibacterial, antitubercular, anticonvulsant, anti-inflammatory effects, compound **37** in Figure 10 that provides AChE inhibition with IC_{50}: 8.77μM has been reported. In particular, it has been reported that these molecules provide inhibition by allowing the imidazole ring to bind with important residues of ChE enzymes (17). The chemical structures of enilconazole, tebuconazole, miconazole and compound **37** are shown in Figure 10.

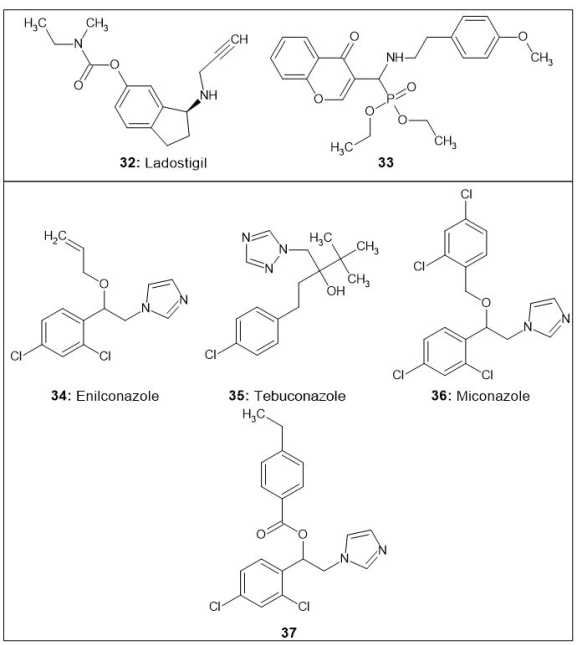


Figure 10: The chemical structure of Ladostigil and the N-substituted α-aminophosphonate-bearing chromone moiety molecule (compound **33**) (35- 36). The chemical structures of enilconazole, tebuconazole, miconazole and compound **37** (17).

The carbazole-coumarin hybrid compound shown in Figure 11 as compound **38** shows high inhibition and selectivity towards AChE and BuChE enzyme. According to the study, the length of the binding site affects the degree of AChE enzyme inhibition, and the moiety of coumarin affects the degree of BuChE enzyme inhibition. It has been discovered that the synthesized molecule provides this inhibition by interacting with amino acids in both the CAS and PAS regions of AChE. In conclusion, selective and dual binding site inhibitors of AChE are promising for future drug designs of AD, by increasing cholinergic stimulus and additionally preventing Aβ aggregation (37).

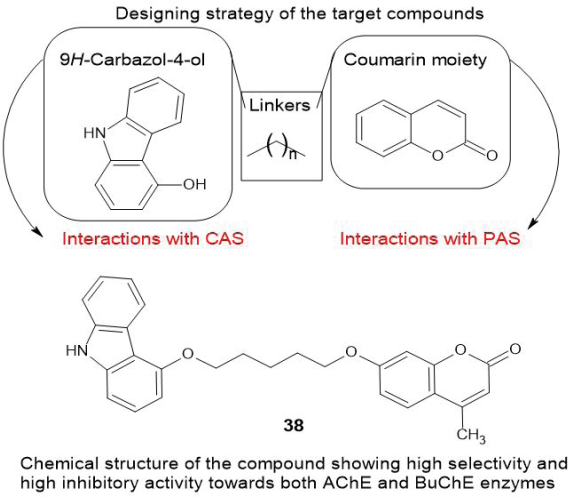


Figure 11: Designing strategy of the target compounds and the chemical structure of compound **38** showing high selectivity and high inhibitory activity towards both AChE and BuChE enzymes (37).

The compounds in Figure 12 are included in the literature as new original patented AChE inhibitor compounds (5).

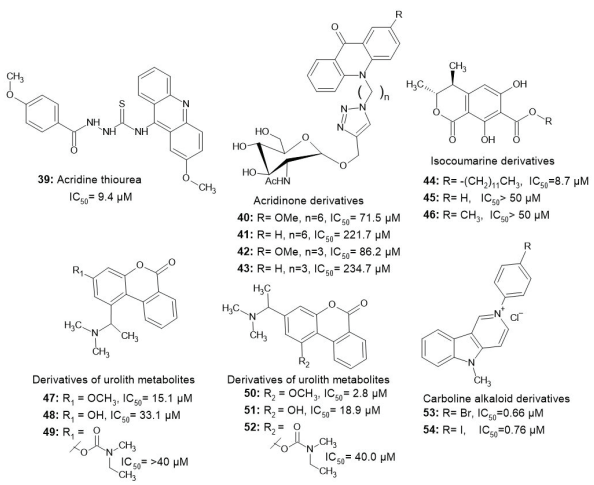


Figure 12: The chemical structures of new original patented AChE inhibitor compounds and their respective IC_{50} values for AChE) (5).

In a study based on the ChE enzyme inhibitor activities of rings containing nitrogen and sulfur atoms and the information that polyphenols inhibit oxidative stress, multi-targeted 1,3,4-thiadiazole-1,3-benzenediol conjugates **(55-59)** in Figure 13 were synthesized and it was discovered that they have many valuable biological activities. Molecules **55, 56, 57 and 58** were shown to have stronger and more selective inhibitory activity against the AChE enzyme (with an IC_{50} = 29–76 nM) than against the BuChE enzyme. This sensitivity to both enzymes was supported by docking studies and thus they were determined to be dual active inhibitors. In this case, the molecules containing amine groups (**55-58**), targeted the CAS region of AChE like the tacrine molecule. The thiadiazole ring and the phenyl groups in their structures showed activity by interacting with the Trp84 part and –Phe330 part of the enzyme, respectively. In addition, the interaction of OH functional groups by making hydrogen bonds was also important. Considering this data, the side-effect profiles of these molecules (**55-59**) are better, as they form reversible enzyme-inhibitor complexes with non-covalent interactions, and it was also observed that they did not show cytotoxicity at this concentration. Molecule **58** was also found to be better as BuChEI than the others. Besides ChE inhibition activity of synthesized molecules, it was also noted that their anti-amyloidogenic effects (except for molecule **58**) were better than curcumin, and molecules **55, 56 and 59** inhibited oxidation as well as quercetin. Another advantage of these molecules is that they have metal ion (such as Cu^{2+}, Fe^{3+} and Zn^{2+} ions) chelating activities and their blood-brain barrier permeability is reasonable (except for molecule **56**). Thus, it can be said that molecules **(55-59)** in this study are effective in many ways to target AD. And it has been a very important and valuable development for researchers (38).

In a study, a series of carbamate derivatives of N-salicyloyl tryptamine were synthesized. It was discovered that molecule **60** in Figure 13 showed properties as a mixed type reversible dual inhibitor of AChE and BChE, also increased anti-inflammatory cytokines IL-4 and inhibited $A\beta_{1-42}$ aggregation (39). In another study synthesizing a novel series of (4-(1,2,4-oxadiazol-5-yl) phenyl)-2-aminoacetamide derivatives, multifunctional agents that inhibit BuChE, have anti-Aβ aggregation properties and inhibit neuroinflammation was discovered. The most active molecule (BuChEI IC_{50}:1.28±0.18 μM) was noted as the compound **61** in Figure 13 (40).

In another study, acetylated triterpenoic acids and 1,3- or 1,4-diazabicyclo[3.2.2]nonanes were combined. It has been discovered that the synthesized compounds have low AChEI activity, but they are very strong active compounds to the BuChE enzyme (42). The chemical structures of triterpenoic acids are shown in Figure 14.

In another interesting study, tacrine-linked triazole glycoconjugates were synthesized and it was expected to reduce the effect of tacrine on known hepatotoxicity. It was reported that the desired effect (non-toxicity) is provided in the evaluated molecules and as a result of molecular modeling, molecule **62** in Figure 13 as the most active molecule as AChEI (41).

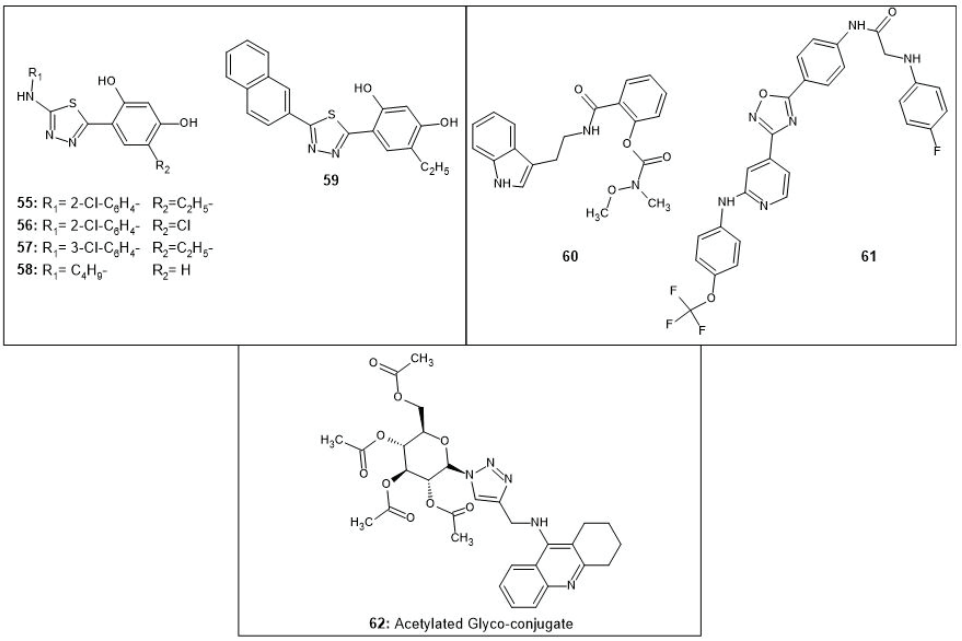


Figure 13: The chemical structure of 1,3,4-thiadiazole-1,3-benzenediol conjugates **55-59** (38). The chemical structures of carbamate derivative of N-salicyloyl tryptamine (compound **60**) and (4-(1,2,4-oxadiazol-5-yl)phenyl)-2-aminoacetamide derivative (compound **61**) (39- 40). The chemical structure of the tacrine linked triazole glycoconjugate (compound **62**) (41).

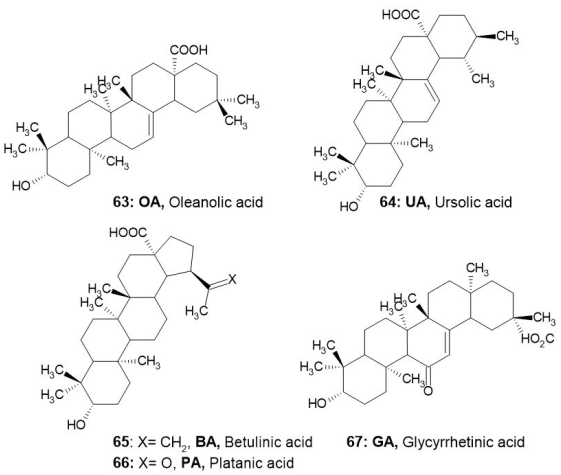


Figure 14: The chemical structures of triterpenoic acids (42).

Recent current studies have also aimed to treat multiple diseases. In a study considered in this context, both AChE and carbonic anhydrase (hCA) enzymes were studied to target people with both AD and Parkinson's disease. For this purpose, a group of new bis-ureido-substituted sulfaguanidine and sulfsoxazole derivatives were obtained. As a result of *in silico* and *in vitro* studies, it was found that compounds **68** and **69** in Figure 15 also have ABTS radical scavenging activity (at the rate of 70% and 78%, respectively) as well as enzyme inhibitor activity. Thanks to the elimination of free radicals that cause cellular damage and inhibitory effects of the molecules on hCAs and AChE enzymes, these molecules can be beneficial to both targeted diseases and shed light on the future (43).

In another study targeting dual enzyme inhibition, sulfonamide-pyrrole-3-one conjugates were synthesized. Inhibitory activities of the compounds against hCA I, hCA II and AChE enzymes were investigated. It has been of interest that many derivatives are more potent inhibitors than existing drugs. Compound **70** in Figure 15 is the best AChEI molecule in the study. With these discoveries, researchers are seeking solutions to many metabolic diseases (44).

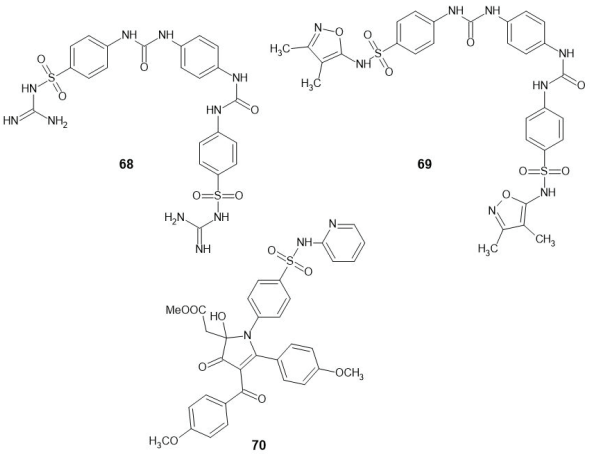


Figure 15: The chemical structures of bis-ureido-substituted sulfaguanidine and sulfsoxazole derivatives (compound **68** and **69**, respectively), sulfonamide-pyrrole-3-one conjugate (compound **70**) (43, 44).

Hybrids were designed in a different study after it was discovered that intervention in many aspects of AD, which has a comp-

lex multifactorial etiology, is important. The hybrid molecules consisted of TPPU, which is a soluble epoxide hydrolase (sEH) inhibitor, and 6-chlorotacrine (and huprine Y), which inhibits AChE enzyme. When the leader molecule given in Figure 16 as compound **73** was administered to a mouse with AD, it was observed that it had high brain permeability and water solubility and decreased toxicity in the nervous system (45).

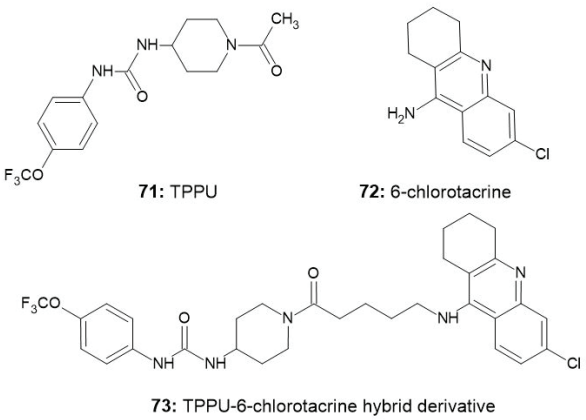


Figure 16: The chemical structures of TPPU, 6-chlorotacrine and TPPU-6-chlorotacrine hybrid derivative (45).

A group of researchers working on a series of pyrazole and pyrazolone derivatives proved that the compound **74** in Figure 17 provided a strong inhibition by connecting with the catalytic and important residues of the AChE enzyme (His 447 and Ser 203) through *in silico* studies. This data was also supported by *in vitro* studies, and it was discovered that compound **74** (IC_{50} value of 0.38 ± 0.019 mg/mL ($p < 0.05$) had close and good results with rivastigmine (IC_{50} value of 0.36 ± 0.018 mg/mL ($p < 0.05$) (46).

In a study in which another heterocyclic ring was involved and polysubstituted pyrroles were synthesized, it was found that compound **75** in Figure 17 was not very effective in BuChE inhibition, but it had affinity with H-bond and hydrophobic interactions especially against the AChE enzyme and provided significant inhibition (IC_{50} value of 2.95± 1.31 µM) appeared (47).

In another study, which was aimed to proceed based on Donepezil, an FDA-approved molecule, a change was made on the heterocyclic ring. A new compound was formed by adding the pyridine ring instead of the phenyl ring of the known donepezil molecule. It was revealed that this compound **76** in Figure 17 binds to PAS and CAS pockets of AChE and shows mixed inhibition as strong as the reference molecule (48).

In another important and valuable study, a group of 2-(2- oxoethyl)pyrimidine-5-carboxamide derivatives was synthesized based on the Donecopride molecule. According to studies in the literature, Donecopride is a serotonin subtype 4 receptor agonist, and a molecule that has been discovered to have low inhibition against BuChE and selective and strong enzyme inhibition against AChE. When the biological activities of the synthesized molecules were examined, it was understood that compound **78** in Figure 18 had lower BuChE enzyme inhibition, but had a stronger inhibition property against the AChE enzyme than Huperzine-A. As a result of this information, an *in silico* study was performed and it was revealed that compound **78** interacts with both the CAS and PAS regions of AChE and causes a complex inhibition. Researchers thought that the aryl ketone moiety of Donecopride was important in this activity. Moreover, the fact that the compound **78** has drug-like properties by providing Lipinski's rule of five is an important discovery for future research (49).

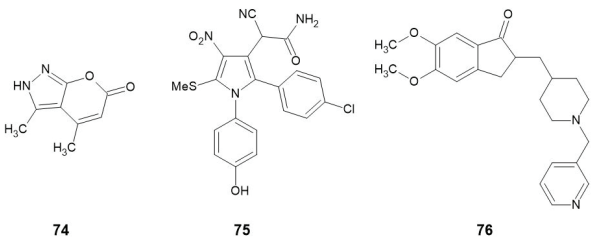


Figure 17: The chemical structure of 3,4-dimethylpyrano[2,3-c] pyrazol-6(2H)-one (compound **74**), polysubstituted pyrrole derivative (compound **75**) and compound **76** based on donepezil (46-48).

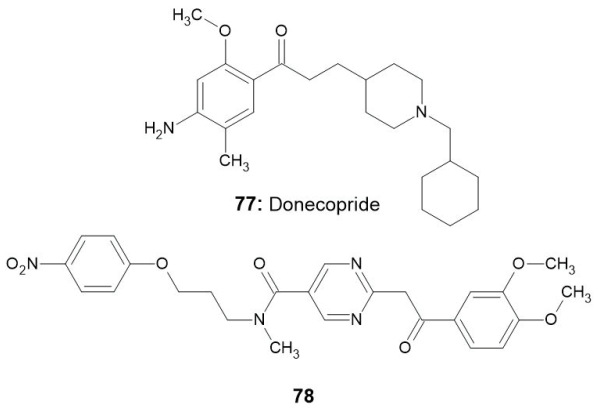
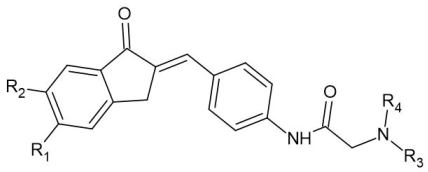


Figure 18: The chemical structures of donecopride and the novel compound based on donecopride (49).

In another recent study that adopted the "one drug for multiple targets" approach, a group of molecules containing an indan-1-one fragment was synthesized based on the donepezil molecule and these molecules were tested with *in vitro* and *in silico* studies. As a result of the study, it was found that some of the synthesized molecules inhibited both ChE enzymes and monoamine oxidase (MAO) B, had anti-amyloidogenic effects, and also prevented oxidative stress with antioxidation activity. The molecules **79-81** in Figure 19 inhibited the MAO-B enzyme as well as the AchE enzyme and for this reason, these molecules were found valuable in terms of AD. On the other hand, it was suggested that molecules **82-84** in Figure 19, which are the other molecules synthesized in the study, could be the leading drug molecules in the treatment of Parkinson's disease with their MAO B inhibition activities (50).



79: R_1= OCH_3 R_2= H R_3= CH_3 R_4= C_3H_7
80: R_1= H R_2= OCH_3 R_3= CH_3 R_4= C_3H_7
81: R_1= OCH_3 R_2= OCH_3 R_3= CH_3 R_4= C_3H_7
82: R_1= OCH_3 R_2= H R_3= CH_3 R_4= $CH_2CH_2CH_2N(CH_3)_2$
83: R_1= H R_2= OCH_3 R_3= CH_3 R_4= $CH_2CH_2CH_2N(CH_3)_2$
84: R_1= OCH_3 R_2= OCH_3 R_3= CH_3 R_4= $CH_2CH_2CH_2N(CH_3)_2$

Figure 19: The chemical structures of new donepezil-based indan-1-one derivatives (50).

CONCLUSION

In the light of the information obtained, while 24 million of the current world population has Alzheimer's disease and it is estimated that these Figures will increase 4 times in 2050, the drug groups approved to solve this problem that threatens public health are extremely limited (3). Researchers are trying to obtain molecules with potent and wide therapeutic potential through the development of novel multifunctional ChEIs. In addition, it is desired that these molecules have optimal pharmacodynamics and low side-effect profile. For this reason, in new drug development studies, researchers try to change the chemical structures of existing drugs and develop them by combination/hybridization. As a result of our study, it has been detected that as a multifactorial disease with complex pathology, AD has been demonstrated by important and remarkable literature studies in recent years that it can be targeted and resolved with multifunctional drugs and with the multi-target-directed ligand strategy. Using this strategy, the researchers have aimed to achieve multiple targets with one multifunctional molecule. The present review was written to draw attention to this issue.

Peer Review: Externally peer-reviewed.

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REFERENCES

1. Gauthier S, Rosa-Neto P, Morais JA, Webster C. World Alzheimer Report 2021: Journey through the diagnosis of dementia. London, England: Alzheimer's Disease International, McGill University 2021. Available from https://www.alzint.org/resource/world-alzheimer-report-2021/
2. Alptüzün V. Jenerik İlaçlar ve Akılcı Kullanım-Alzheimer Hastalığında Asetilkolinesteraz İnhibitörleri [Generic Drugs and Rational Use-Acetylcholinesterase Inhibitors in Alzheimer's Disease]. Turkish Pharmacy Association 8th Pharmacy Congress of Turkey, (28.10.2006; Session one); 2006.
3. Breijyeh Z, Karaman R. Comprehensive Review on Alzheimer's Disease: Causes and Treatment. Molecules 2020;25(24):5789.
4. Bhagat K, Singh JV, Sharma A, Kaur A, Kumar N, Gulati HK, et al. Novel series of triazole containing coumarin and isatin based hybrid molecules as acetylcholinesterase inhibitors. J Mol Struct 2021;1245:131085.
5. Bortolami M, Rocco D, Messore A, Di Santo R, Costi R, Madia VN, et al. Acetylcholinesterase inhibitors for the treatment of Alzheimer's disease – a patent review (2016–present). Expert Opin Ther Pat 2021;31(5):399-420.
6. Carcak-Yilmaz N, Altuntas M, Akyel YK, Uydes-Dogan BS. Pharmacist's role in pharmacotherapeutic management of Alzheimer's disease. Istanbul J Pharm 2017;47(1):1-4.
7. El-Khatabi K, El-Mernissi R, Aanouz I, Ajana MA, Lakhlifi T, Shahinozzaman M, et al. Benzimidazole derivatives in identifying novel acetylcholinesterase inhibitors: a combination of 3D-QSAR, docking and molecular dynamics simulation. Phys Chem Res 2021;10(2):237-49.
8. Nguyen TT, Nguyen TTD, Nguyen TKO, Vo TK, Vo VG. Advances in developing therapeutic strategies for Alzheimer's disease. Biomed Pharmacotherapy 2021;139:111623
9. Kabir MT, Uddin MS, Jeandet P, Emran TB, Mitra S, Albadrani GM, et al. Anti-Alzheimer's molecules derived from marine life: understanding molecular mechanisms and therapeutic potential. Mar drugs 2021;19(5):251.
10. Macha B, Kulkarni R, Garige AK, Pola S, Akkinepally R, Garlapati A. Design and Friedlander Reaction Based Synthesis of New Cycloalkyl Ring Fused Quinolines as Multifunctional Agents for Alzheimer's Treatment: In Silico Studies. Chemistry Select 2021;6(9):1947-57.
11. Arslan T. Design, synthesis of novel peripherally tetra-chalcone substituted phthalocyanines and their inhibitory effects on acetylcholinesterase and carbonic anhydrases (hCA I and II). J Organomet Chem 2021;951:122021.
12. Silva T, Reis J, Teixeira J, Borges F. Alzheimer's disease, enzyme targets and drug discovery struggles: From natural products to drug prototypes. Ageing Res Rev 2014;15:116-45.
13. João KG, Videira RA, Paiva-Martins F, Valentão P, Pereira DM, Andrade PM. Homarine Alkyl Ester Derivatives as Promising Acetylcholinesterase Inhibitors. Chem Med Chem 2021;16(21):3315-25.
14. Miles JA, Ross BP. Recent Advances in Virtual Screening for Cholinesterase Inhibitors. ACS Chem Neurosci 2021;12(1):30-41.
15. Li Q, He S, Chen Y, Feng F, Qu W, Sun H. Donepezil-based multifunctional cholinesterase inhibitors for treatment of Alzheimer's disease. Eur J Med Chem 2018;158:463-77.
16. Weinreb O, Amit T, Bar-Am O, Youdim MBH. Ladostigil: A novel multimodal neuroprotective drug with cholinesterase and brain-selective monoamine oxidase inhibitory activities for alzheimer's disease treatment. Curr Drug Targets 2012;13(4):483-94.
17. Barut B, Sari S, Sabuncuoğlu S, Özel A. Azole antifungal compounds could have dual cholinesterase inhibitory potential according to

virtual screening, enzyme kinetics, and toxicity studies of an inhouse library. J Mol Struct 2021;1235:130268.

18. Sayar-Gümüş G. Potansiyel Anti-Alzheimer Heteroaromatik Hidrazit-Hidrazon Türevi Bileşikler Üzerinde Sentez ve Biyoaktivite Çalışmaları [Synthesis and Bioactivity Studies on Potential Anti-Alzheimer Heteroaromatic Hydrazide-Hydrazone Derivatives]. (Doctoral dissertation): Ege Univ. 2017. URL: https://tez.yok.gov.tr/UlusalTezMerkezi/tezDetay.jsp?id=F9auTkEImHVKDNkQdlw08A&no=06ntbxASMObaT15BlhaGxA
19. Soreq H, Seidman S. Acetylcholinesterase-new roles for an old actor. Nat Rev Neurosci 2001;2(4):294-302.
20. Dantoine T, Auriacombe S, Sarazin M, Becker H, Pere J-J, Bourdeix I. Rivastigmine monotherapy and combination therapy with memantine in patients with moderately severe Alzheimer's disease who failed to benefit from previous cholinesterase inhibitor treatment. Int J Clin Pract 2006;60(1):110-8.
21. David B, Schneider P, Schäfer P, Pietruszka J, Gohlke H. Discovery of new acetylcholinesterase inhibitors for Alzheimer's disease: virtual screening and in vitro characterisation. J Enzyme Inhib and Med Chem 2021;36(1):491-6.
22. Vignaux PA, Minerali E, Lane TR, Foil DH, Madrid PB, Puhl AC, et al. The Antiviral Drug Tilorone Is a Potent and Selective Inhibitor of Acetylcholinesterase. Chem Res Toxicol 2021;34(5):1296-307.
23. Ujan R, Channar PA, Bahadur A, Abbas Q, Shahe M, Rashid SG, et al. Synthesis, kinetics and biological assay of some novel aryl bis-thioureas: A potential drug candidates for Alzheimer's disease. J Mol Struct 2021;1246:131136.
24. Guo Y, Yang H, Huang Z, Tian S, Li Q, Du, C, et al. Design, Synthesis, and Evaluation of Acetylcholinesterase and Butyrylcholinesterase Dual-Target Inhibitors against Alzheimer's Diseases. Molecules 2020;25(3):489.
25. Lotfi S, Rahmani T, Hatami M, Pouramiri B, Kermani ET, Rezvannejad E et al. Design, synthesis and biological assessment of acridine derivatives containing 1,3,4-thiadiazole moiety as novel selective acetylcholinesterase inhibitors. Bioorg Chem 2020;105:104457.
26. Liu G, Jiao Y, Lin Y, Hao H, Dou Y, Yang J, et al. Discovery and Biological Evaluation of New Selective Acetylcholinesterase Inhibitors with Anti-Aβ Aggregation Activity through Molecular Docking-Based Virtual Screening. Chem Pharm Bull 2020;68(2):161-6.
27. Chakravarty H, Ju Y, Chen W-H, Tam KY. Dual targeting of cholinesterase and amyloid beta with pyridinium/isoquinolium derivatives. Drug Dev Res 2020;81(2):242-55.
28. Cadež T, Grgi˘cevi´c A, Ahmetovi´c R, Bari´c D, Hrvat NM, Kovarik Z, et al. Benzobicyclo[3.2.1]octene Derivatives as a New Class of Cholinesterase Inhibitors. Molecules 2020;25(21):4872.
29. Xie RR, Su, CL, Li W, Zou XY, Chen YS, Tang H. Synthesis and biological evaluation of novel 8- substituted sampangine derivatives as potent inhibitor of Zn2+-Aβ complex mediated toxicity, oxidative stress and inflammation. Bioorg Chem 2021;109:104710.
30. El-Sayed NF, El-Hussieny M, Ewies EF, Fouad MA, Boulos LS. New phosphazine and phosphazide derivatives as multifunctional ligands targeting acetylcholinesterase and β-Amyloid aggregation for treatment of Alzheimer's disease. Bioorg Chem 2020;95:103499.
31. Mak S, Li W, Fu H, Luo J, Cui W, Hu S, et al. Promising tacrine/huperzine A-based dimeric acetylcholinesterase inhibitors for neurodegenerative disorders: From relieving symptoms to modifying diseases through multitarget. J Neurochem 2021;158(6):1381-93.
32. Lasmari S, Ikhlef S, Boulcinaa R, Mokrani, EH, Bensouici C, Gürbüz N, et al. New silver N–heterocyclic carbenes complexes: Synthesis, molecular docking study and biological activities evaluation as cholinesterase inhibitors and antimicrobials. J Mol Struct 2021;1238:130399.
33. Mughal EU, Sadiq A, Ayub M, Naeem N, Javid A, Sumrra SH, et al. Exploring 3-Benzyloxyflavones as new lead cholinesterase inhibitors: synthesis, structure–activity relationship and molecular modelling simulations. J Biomol Struct Dyn 2021;39(16):6154-67.
34. Sharma RK, Singh M, Ghimeray K, Juneja P, Dev G, Pulavarthi S, et al. Imidazopyridazine acetylcholinesterase inhibitors display potent anti-proliferative effects in the human neuroblastoma cell-line, IMR-32. Molecules 2021;26(17):5319.
35. Guieu B, Lecoutey C, Legay R, Davis A, Sopkova de Oliveira Santos J, Altomare CD, et al. First Synthesis of Racemic Trans Propargylamino-Donepezil, a Pleiotrope Agent Able to Both Inhibit AChE and MAO-B, with Potential Interest against Alzheimer's Disease. Molecules 2021;26(1):80.
36. Shaikh S, Dhavan P, Ramana MMV, Jadhav BL. Design, synthesis and evaluation of new chromone-derived aminophosphonates as potential acetylcholinesterase inhibitör. Mol Divers 2021;25(2):811-25.
37. Song M-Q, Min W, Wang J, Si X-X, Wang X-J, Liu Y-W, et al. Design, synthesis and biological evaluation of new carbazole-coumarin hybrids as dual binding site inhibitors of acetylcholinesterase. J Mol Struct 2021;1229:129784.
38. Skrzypek A, Matysiak J, Karpińska M, Czarnecka K, Kręcisz P, Stary D, et al. Biological evaluation and molecular docking of novel 1, 3, 4-thiadiazole-resorcinol conjugates as multifunctional cholinesterases inhibitors. Bioorg Chem 2021;107:104617.
39. Liu D, Zhang H, Wang Y, Liu W, Yin G, Wang D, et al. Design, synthesis, and biological evaluation of carbamate derivatives of N-salicyloyl tryptamine as multifunctional agents for the treatment of Alzheimer's disease. Eur J Med Chem 2022;229:114044.
40. Liu T, Chen S, Du J, Xing S, Li R, Li Z. Design, synthesis, and biological evaluation of novel (4-(1,2,4- oxadiazol-5-yl)phenyl)-2-aminoacetamide derivatives as multifunctional agents for the treatment of Alzheimer's disease. Eur J Med Chem 2022;227:113973.
41. Kaur Gulati H, Choudhary S, Kumar N, Ahmed A, Bhagat K, Singh JV, et al. Design, Synthesis, biological investigations and molecular interactions of triazole linked tacrine glycoconjugates as Acetylcholinesterase inhibitors with reduced hepatotoxicity. Bioorg Chem 2022;118:105479.
42. Heise N, Friedrich S, Temml V, Schuster D, Siewert B, Csuk R. N-methylated diazabicyclo[3.2.2]nonane substituted triterpenoic acids are excellent, hyperbolic and selective inhibitors for butyrylcholinesterase. Eur J Med Chem 2022;227:113947.
43. Lolak N, Akocak S, Durgun M, Duran HE, Necip A, Turkes C, et al. Novel bis-ureido-substituted sulfaguanidines and sulfisoxazoles as carbonic anhydrase and acetylcholinesterase inhibitors. Mol Divers 2022;4(1):1-15.
44. Gumus M, Babacan SN, Demir Y, Sert Y, Koca I, Gulcin I. Discovery of sulfadrug-pyrrole conjugates as carbonic anhydrase and acetylcholinesterase inhibitors. Arch Pharm 2022 (Weinheim);

355(1):e2100242. doi:10.1002/ardp.202100242

45. Codony S, Pont C, Grinan-Ferre C, Di Pede-Mattatelli A, Calvo-Tusell C, Feixas F, et al. Discovery and in vivo proof of concept of a highly potent dual inhibitor of soluble epoxide hydrolase and acetylcholinesterase for the treatment of alzheimer's disease. J Med Chem 2022;65(6):4909-25.
46. Messaad M, Dhouib I, Abdelhedi M, Khemakhem B. Synthesis, bioassay and molecular docking of novel pyrazole and pyrazolone derivatives as acetylcholinesterase inhibitors. J Mol Struct 2022;1263:133105.
47. Pourtaher H, Hasaninejad A, Iraji A. Design, synthesis, in silico and biological evaluations of novel polysubstituted pyrroles as selective acetylcholinesterase inhibitors against Alzheimer's disease. Sci Rep 2022;12(1):15236.
48. Makarian M, Gonzalez M, Salvador SM, Lorzadeh S, Hudson PK, Pecic S. Synthesis, kinetic evaluation and molecular docking studies of donepezil-based acetylcholinesterase inhibitors. J Mol Struct 2022;1247:131425.
49. Han C, Wei BB, Shang PP, Guo XY, Bai LG, Ma ZY. Design, synthesis and evaluation of 2-(2-oxoethyl)pyrimidine-5-carboxamide derivatives as acetylcholinesterase inhibitors. Bioorg Med Chem Lett 2022;72:128873.
50. Sağlık BN, Levent S, Osmaniye D, Evren AE, Karaduman AB, Özkay Y, et al. Design, Synthesis, and in Vitro and In Silico Approaches of Novel Indanone Derivatives as Multifunctional Anti-Alzheimer Agents. ACS Omega 2022;7(50):47378-404.

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Invited Review Article	5000	250	50	6	10 or total of 20 images
Case Report	1000	200	15	No tables	10 or total of 20 images
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Book Section: Suh KN, Keystone JS. Malaria and babesiosis. Gorbach SL, Barlett JG, Blacklow NR, editors. Infectious Diseases. Philadelphia: Lippincott Williams; 2004.p.2290-308.

Books with a Single Author: Sweetman SC. Martindale the Complete Drug Reference. 34th ed. London: Pharmaceutical Press; 2005.

Editor(s) as Author: Huizing EH, de Groot JAM, editors. Functional reconstructive nasal surgery. Stuttgart-New York: Thieme; 2003.

Conference Proceedings: Bengisson S. Sothemin BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Rienhoff O, editors. MEDINFO 92. Proceedings of the 7th World Congress on Medical

Informatics; 1992 Sept 6-10; Geneva, Switzerland. Amsterdam: North-Holland; 1992. pp.1561-5.

Scientific or Technical Report: Cusick M, Chew EY, Hoogwerf B, Agrón E, Wu L, Lindley A, et al. Early Treatment Diabetic Retinopathy Study Research Group. Risk factors for renal replacement therapy in the Early Treatment Diabetic Retinopathy Study (ETDRS), Early Treatment Diabetic Retinopathy Study KidneyInt: 2004. Report No: 26.

Thesis: Yılmaz B. Ankara Üniversitesindeki Öğrencilerin Beslenme Durumları, Fiziksel Aktivitelerive Beden Kitle İndeksleri Kan Lipidleri Arasındaki Ilişkiler. H.Ü. SağlıkBilimleriEnstitüsü, DoktoraTezi. 2007.

Manuscripts Accepted for Publication, Not Published Yet: Slots J. The microflora of black stain on human primary teeth. Scand J Dent Res. 1974.

Epub Ahead of Print Articles: Cai L, Yeh BM, Westphalen AC, Roberts JP, Wang ZJ. Adult living donor liver imaging. DiagnIntervRadiol. 2016 Feb 24. doi: 10.5152/dir.2016.15323. [Epub ahead of print].

Manuscripts Published in Electronic Format: Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis (serial online) 1995 Jan-Mar (cited 1996 June 5): 1(1): (24 screens). Available from: URL: http:/ www.cdc.gov/ncidodlElD/cid.htm.

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