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When a drug, product, hardware, or software program is mentioned within the main text, product information, including the name of the product, the producer of the product, and city and the country of the company (including the state if in USA), should be provided in parentheses in the following format: "Discovery St PET/CT scanner (General Electric, Milwaukee, WI, USA)"

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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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RESEARCH / ARAŞTIRMA
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COMBINED ANALYSIS OF LINKAGE AND WHOLE EXOME SEQUENCING REVEALS *CIC* AS A CANDIDATE GENE FOR ISOLATED DYSTONIA

BAĞLANTI VE TÜM EKZOM DİZİLEME ANALİZLERİNİN BİRLİKTE DEĞERLENDİRİLMESİYLE *CIC* GENİNİN İZOLE DİSTONİ ADAYI OLARAK BELİRLENMESİ

Barış SALMAN[1,2], Emrah YÜCESAN[1,5], Bedia SAMANCI[3], Başar BİLGİÇ[3], Haşmet HANAĞASI[3], Hakan GÜRVİT[3], Uğur ÖZBEK[1,4], Sibel UĞUR İŞERİ[1]

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ABSTRACT

Objective: To explore the underlying genetic variations and mechanisms in a family affected by isolated dystonia.

Material and Method: We employed whole genome Single Nucleotide Polymorphism (SNP) based linkage analysis along with whole exome sequencing (WES) in a consanguineous family presenting with isolated dystonia. An in-house pipeline compiled for WES analysis along with in-depth *in silico* prediction algorithms were used to assess the associated data produced in this study. Sanger sequencing was used for variant confirmation and segregation.

Results: Data analysis included locus oriented WES variant prioritization and cryptic splicing predictions. We detected a homozygous and synonymous variation rs748449895 (NM_015125.4: c.4143C>T; p.(Thr1381=)) in the capicua transcriptional repressor, CIC. This variation disrupts the YB-1 RNA recognition motif and creates an alternative SRp20 RNA recognition motif.

Conclusion: The resulting variant might cause the dystonia phenotype by affecting the alternative splicing of CIC transcript and altering the exon inclusion motif which may disrupt the ATXN1–CIC complex.

Keywords: Autosomal recessive dystonia, whole genome genotyping, linkage analysis, whole exome sequencing, alternative splicing

ÖZET

Amaç: İzole distoni hastalığından etkilenmiş bir ailede hastalığa neden olan genetik varyasyonları ve mekanizmaları keşfetmek.

Gereç ve Yöntem: İzole distoni hastalığı tanısı konmuş ve akraba evliliği bulunan bir ailede, tüm genom Single Nucleotide Polymorphism (SNP) temelli bağlantı analizi ile beraber tüm ekzom dizileme (TED) gerçekleştirildi. TED analizleri için laboratuvarımızda geliştirilen akış hattı ve in siliko tahmin algoritmaları bu çalışmada üretilen verinin ilişkilendirilmesinde kullanıldı. Sanger dizileme varyantların doğrulanması ve ayrımı için kullanıldı.

Bulgular: SNP dizimi ile genotipleme, bağlantı analizi ve ekzom dizileme analizleri sonucu rs748449895 (NM_015125.4: c.4143C>T;p.(Thr1381=)) homozigot sinonim varyantı tespit edildi. Devamındaki biyoinformatik analizler varyantın YB-1 RNA tanıma motifi olduğunu gösterdi. Bu varyant YB-1 RNA tanıma motifini bozarak, SRp20 RNA tanıma motifi oluşturmaktadır.

Sonuç: Bulunan varyant, ekzon katılma motifini değiştirerek CIC transkriptinin alternatif kırpılmasını etkileyip ATXN1-CIC kompleksini bozarak distoni fenotipine yol açabilir.

Anahtar Kelimeler: Otozomal resesif distoni, tüm genom genotipleme, bağlantı analizi, tüm ekzom dizileme, alternatif kırpılma

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INTRODUCTION

Dystonia is a group of movement disorders that is characterized by involuntary, chronic, twisting muscle contractions and which causes repetitive involuntary movements with temporary or permanent abnormal postures (1). It is a highly heterogeneous condition both in genetic and clinical dimensions (2). Nevertheless, a number of genes have been implicated in isolated dystonia, including *THAP1*, *GNAL*, *ANO3* and *TOR1A* (3, 4).

Linkage analysis is an old, but still useful and reliable approach to map the chromosomal coordinates of disease genes, particularly in extended families with monogenic conditions (5). This method has had a tremendous impact on autosomal recessive conditions in consanguineous families. In today's reality, where next generation sequencing (NGS) techniques seem to be the gold standard for disease gene identification, linkage analysis still has a role to serve: Linkage analysis pinpoints candidate chromosomal regions as localization filters for NGS data analysis. In this way, instead of evaluating a large number of samples and variants with unknown significance, a limited number of patients with targeted variants can be examined (6, 7). Whole exome sequencing (WES) is a popular tool among all NGS approaches because of its relatively low variant content and easy to handle analysis features compared to whole genome applications (8). Therefore, in the last decade there have been several reports that have combined linkage analysis with WES as a powerful tool to determine genes and variants associated with the specific diseases (9-11).

Herein, we present genetic studies including Single Nucleotide Polymorphism (SNP) array based linkage analysis and WES performed in a first degree consanguineous family from Turkey with three patients afflicted with isolated dystonia. This effort has led us to identify a novel variation that possibly disrupts an alternative splicing motif in *CIC*.

MATERIAL AND METHOD

In this study, a first degree consanguineous family from Turkey with three affected (Case 1, Case 2, Case 4) and one unaffected (Case 3) siblings along with their mother (Case 5) were evaluated. Clinical assessment was performed at Istanbul University, Faculty of Medicine, Behavioral Neurology and Movement Disorders Unit of the Neurology Department, Istanbul, Turkey. Physical and neurological examinations were performed for all available family members and detailed information on family history was collected. Informed consents were obtained from all five family members in accordance with Istanbul University, Istanbul Faculty of Medicine, Clinical Ethics Committee with consent certificate 2015/493 approved on 23/02/2015. The clinical features of the patients are compiled in Table 1.

DNA was extracted using the QIAamp DNA Blood Maxi Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's protocol. Afterwards, whole genome genotyping was performed for all five individuals using the Illumina HumanCytoSNP-12v2-1 300k BeadChip kit. Firstly, copy number variations (CNVs) were analyzed using Illumina GenomeStudio v2.0 using cnvPartition CNV Analysis Plugin v3.2.1. PLINK Input Report Plug-in v2.1.4 is used to convert the data to a text format for linkage analysis. Multipoint logarithm of the odds (LOD) score was calculated using GeneHunter v.2.1r5 (12) and run under EasyLinkage v5.08 (13) interphase assuming reces-

Table 1: Clinical characterization of affected siblings

	Case 1	**Case 2**	**Case 4**
Sex	M	F	F
Age at onset (year)	7	9	7
First detectable sign	Tremor	Tremor	Dysarthria, dysphagia, gait difficulty
Age at last examination	53	42	53
Progression	Yes	Yes	Yes
Pyramidal signs	Brisk reflexes	No	No
Oculomotor findings	Normal	Normal	Normal
ENMG study	Mild myopathic changes	Normal	N/A
MRI findings	Unremarkable	Unremarkable	N/A
Other findings	Dysarthria, dysphagia, dystonic tremor, axial dystonia, chorea, mild asymmetric bradykinesia	Dysarthria, dysphagia, dystonic tremor, axial dystonia, oromandibular dystonia, ataxia	Dystonia

sive inheritance with full penetrance. Computation was adjusted in sets of 100 markers and spacing 0.1 cM. In addition, Haplopainter v1.043 (14) was used to draw the pedigree diagram and visualize the resulting haplotypes.

Whole exome sequencing was performed for the two affected siblings (Case 2 and Case 4) from the family on an Illumina HiSeq2000 platform. Exonic DNA was captured using Agilent SureSelect Human All Exon V5 (Agilent Technologies, Santa Clara, CA, USA). Samples were sequenced for the targeted regions with a mean coverage of 53× and 88% of the reads were covered over 20×. Alignment to reference genome hg19 was done using Burrows-Wheeler Aligner v0.7.16a (15), sorting, marking duplicate reads and other bam manipulations were carried out using Picard tools v2.12.0 (16), variant calling was performed with the Genome Analysis Toolkit v3.6.0 HaplotypeCaller (17). The variants were annotated using the Ensembl Variant Effect Predictor (VEP) v101 (18). Variants with gnomAD and 1kG allele frequency less than 0.001 were filtered using VEP filter script. Filtering of the high LOD regions shared by two siblings was performed using python v3.9 script. Validation and segregation analyses for candidate variants were carried-out using Sanger sequencing. SpliceAid 2 and Splice AI were used to predict the splicing impacts of these variations (19, 20).

RESULTS

A consanguineous family with four siblings and their mother were studied. Three of the siblings (two of which were female and one was male), were affected and the other sibling was unaffected. All the affected siblings (Case 1, Case 2, Case 4), the unaffected sibling (Case 3), and the mother (Case 5), were examined at the Department of Neurology at Istanbul University Faculty of Medicine. Case 1 was admitted to our clinic at the age of 49 for the first time due to dystonia and involuntary movements in the whole body. He started having tremors in his right hand at the age of seven, and after two years, he also had tremor in his left hand, and his writing had gradually deteriorated. At the age of 12, gait difficulty started, and at the age of 15, he had dystonia in his whole body, mostly in his waist and neck, and rarely dysphagia. His complaints increased in cold and crowded environments. He had no other diagnosed diseases. His parents were cousins. He was the second of four siblings, and his two sisters had similar complaints (Case 2, Case 4). In his examination, he was disorientated, and his speech was dysarthric. Myerson was positive. There were diffuse dystonic and choreiform movements, (these were more prominent on the right side of the body), and dystonic tremors in the bilateral upper extremity. There was bilateral mild bradykinesia which was more prominent on the left side. Deep tendon reflexes were brisk. He had gait difficulty due to severe dystonia in the whole body. Cranial MRI and EEG examinations were normal. EMG and muscle biopsy revealed mild myopathic changes. Serum and urine copper and serum ceruloplasmin levels were normal. Ophthalmic examination was unremarkable. Bilateral p100 latencies were prolonged in the visual evoked potential test. Genetic investigation for Huntington's disease was negative. Despite the L-dopa, clonazepam, biperiden, baclofen, tetrabenazine and bornaprine treatments, his complaints were ongoing in his last examination at the 4th year of his follow-up. Case 2 was admitted to our clinic for the first time at the age of 38 with gait difficulty, tremors in the hands, and speech disorder. At the age of nine, she first started having tremors in her right hand and, after a while, in her left hand, and deterioration in writing was observed. Ten years later, speech and swallowing disturbances were added, and ten years later, ataxia and gait difficulties were observed. Her past medical history was unremarkable. Her speech was dysarthric in the neurological examination. There were oromandibular dystonia and bilateral upper extremity dystonic tremor, more prominent on the right. She had gait difficulty due to ataxia. Vitamin E and serum AFP levels were normal. Cranial MRI and EMG were unremarkable. The neurological examination findings of the patient, who did not attend follow-up examinations regularly and did not respond to L-dopa treatment, were the same in the 4th year follow-up. Case 4, who was admitted for the first time at the age of 53, had gradually progressing gait, speech, and swallowing difficulty, which started at the age of seven. The patient, who had dysarthria, generalized dystonia, and was unable to walk due to dystonia at the first examination, did not attend maintain their examinations. The unaffected sibling (Case 3) and the mother (Case 5) had no neurological complaints, and neurological examinations were normal.

Evaluation of CNVs using the SNP data did not suggest a shared CNV event in the affected siblings. Parametric linkage analysis in the family identified seven linkage peaks on chromosomes 1, 3, 4, 6, 10, 11 and 19, respectively with a maximum LOD score of higher than 2.5 (Figure 1). These coordinates (Table 2) were prioritized for WES

Table 2: Chromosomal positions (hg19) of regions with LOD score over 2.5

Chromosome	Start	End
chr1	94023997	110579200
chr3	150872381	153042330
chr4	140021705	140672561
chr6	133528828	134365722
chr10	117763432	120364746
chr11	59716220	84908270
chr19	33538792	43404463

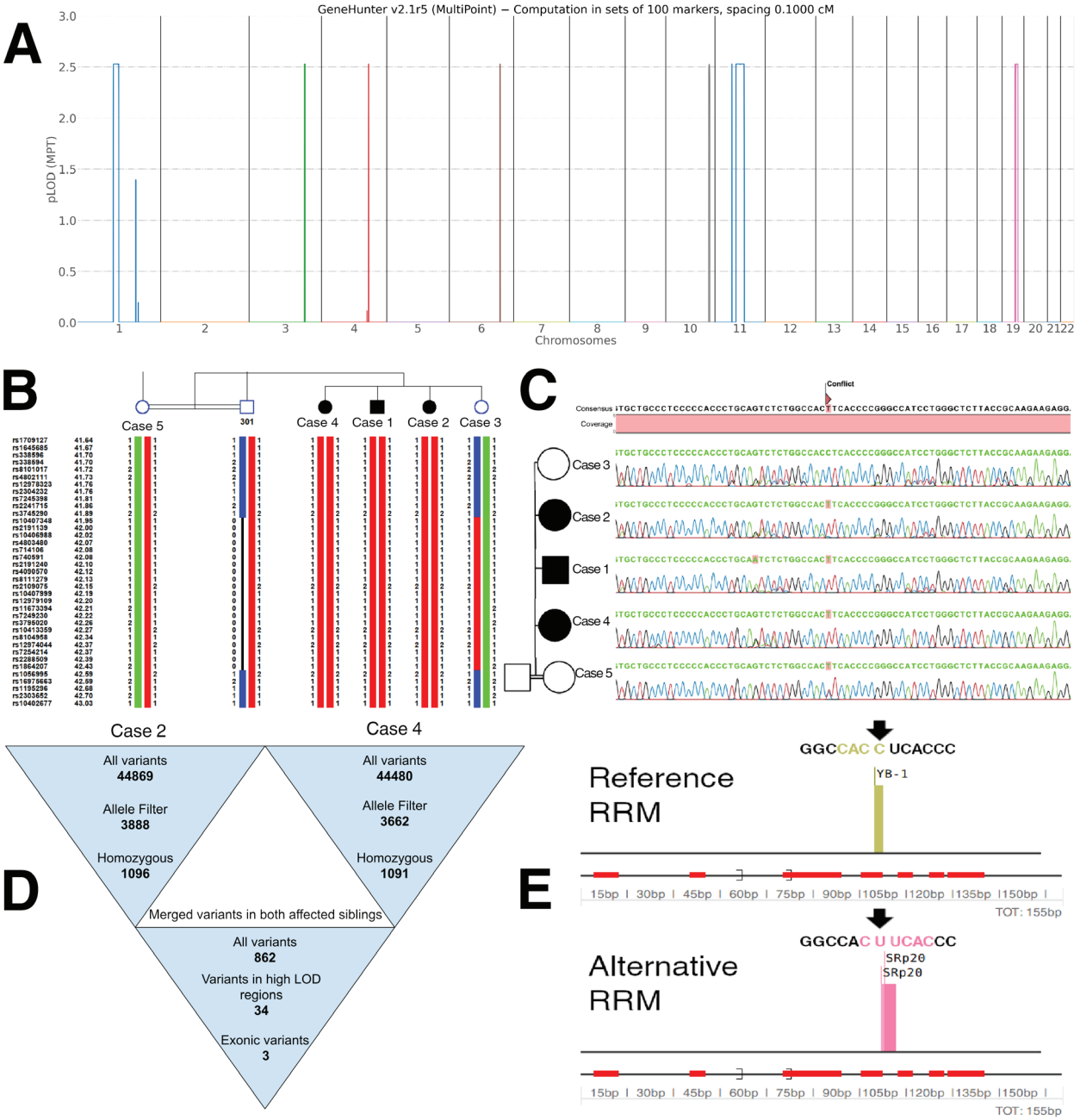

Figure 1: Genetic studies in the dystonia family. **A)** Multipoint LOD scores (GeneHunter) of the SNP data set along the autosomal chromosomes, **B)** SNP derived haplotype blocks (Haplopainter) on chromosome 19 around *CIC* gene, **C)** Segregation of the variant NM 015125.4:c.4143C>T within the family, **D)** Summary of the filtering steps to discover candidate variants, **E)** In-silico prediction (Splice Aid 2) analysis to show possible alternative splicing effect of the variant

analysis. Filtering process started with the evaluation of these regions and then continued with successive steps as presented in Figure 1. This filtering strategy led us to identify three synonymous candidate variants as annotated with VEP and presented in Table 3. None of these variants were present in the homozygous form in the gnomAD database. Among these genes, Capicua transcriptional repressor-*CIC* encodes a member of high mobility group-box (HMG-box) superfamily of transcriptional repressors and has shown to be a critical regulator of neuronal differentiation (21). Although the variant was annotated as synonymous with the VEP pipeline, the role of *CIC* gene product in neuronal differentiation has prompted us to perform familial variant segregation and in depth *in sili-*

Table 3: Remaining variants as result of the filtering strategy

	Position (hg19/hg38)	Gene Consequence	HGVSc HGVSp	Existing variation GnomAD_AF
1	chr11:g.73021267T>A chr11:g.73310222T>A	ARHGEF17 synonymous_variant	NM_014786.3:c.1584T>A NP_055601.2:p.Pro528=	rs553216026
2	chr19:g.36431638G>A chr19:g.35940736G>A	LRFN3 synonymous_variant	NM_024509.1:c.1311G>A NP_078785.1:p.Gly437=	rs144242723 0.0008631
3	chr19:g.42798189C>T chr19:g.42294037C>T	CIC synonymous_variant	NM_015125.5:c.4143C>T NP_055940.3:p.Thr1381=	rs748449895 0.00004778

co analyses. Sanger sequencing of this variant along with haplotype inspection of the linkage region confirmed segregation of ENST00000575354.6:c.4143C>T with the phenotype in the pedigree (Figure 1). According to *in silico* prediction tool SpliceAid 2 (19), the variant causes disruption of the existing RNA recognition motif (RRM) and creates a new different RRM (Figure 1). SpliceAI tool also predicts a minor acceptor loss effect (20).

DISCUSSION

Using two unbiased genetic approaches, SNP based linkage analysis and WES, we identified a variation that possibly affects an alternative splicing motif in the *CIC* gene that may be associated with dystonia. The *CIC* gene is a human ortholog of the Drosophila melanogaster *capicua* gene and belongs to the protein family of the high mobility group (HMG)-box superfamily of transcriptional repressors. Protein capicua homolog plays a role in development of the central nervous system (CNS) and is involved with brain development with ataxin-1 (22). *CIC* is known to form a transcriptional repressor complex with *ATXN1* which was previously demonstrated to cause several neurological diseases (23). Dystonias are a heterogeneous group of disorders as mentioned above. Dystonias may be classified according to the affected body part, e.g. focal, segmental, multifocal, hemidystonia and generalized. Furthermore, dystonias may also be classified by associated features, such as isolated, combined and complex (24). The most recent classifications of dystonias are recommended by the European Federation of Neurological Societies (25). Taken together, dystonias are clinically and genetically complex, which complicates gene identification studies. However, it may be possible to identify candidate genes in pedigrees with clear autosomal recessive inheritance caused by identical descent inheritance due to consanguinity. Recently, a number of dystonia related genes have been identified in parallel with the developments in NGS technologies, such as *CIZ1*, *ANO3*, *TUBB4A* and *GNAL* for primary dystonia, *PRRT2* for paroxysmal kinesigenic dystonia, and some other genes, *SLC30A10* and *ATP1A3* (26). Both dominant and recessive inheritance patterns are valid for genetic dystonias (27). Among the studies with autosomal recessive patterns, some patients have been reported in which the parents were consanguineous (28, 29).

Our study demonstrates a synonymous variation in *CIC*. This variation resides on the exon 17 of ENST00000575354.6 (NM_015125.5), which consists of 20 exons in total. Interestingly, it has been shown that dominantly affecting variations in *CIC* may cause intellectual disability (23). Likewise, in 2010, Vissers et al. demonstrated a heterozygous de novo missense variation (23, 30). In 2011 Bettegowda et al. reported *CIC* gene variations in six cases in their study on human oligodendroglioma (31). When considered overall, variations in the *CIC* gene exhibit autosomal recessive character. However, evaluating the nature and genetic heterogeneity of the disease, it should be considered that different inheritance patterns can be seen.

Although the homozygous variant detected herein is synonymous, our in depth *in silico* analyses have shown that this variation breaks the existing YB-1 RRM (GGCCACCUCACCC) (32) and creates a new motif (GGCCACUUCACCC), which is recognized by SRp20 splicing factor (33). YB-1 transcription factor is shown to stimulate exon inclusion while SRp20 stimulates the splicing of the exon (32, 34). Consequently, this apparently 'silent' variation may not be silent at the end of the day: It may result in cryptic splicing *via* recruitment of the SRp20 splicing factor. It is very well known that alternative splicing is an important mechanism that confers protein diversity and alterations in control of splicing may be associated with disease (35-37). Several genetic diseases which are molecularly undiagnosed have turned out to be associated with cryptic splicing due to 'silent' exonic or intronic variations that actually affect pre-mRNA splicing (38, 39). Some well characterized neurological diseases, such as frontotemporal dementia, parkinsonism, spinocerebellar ataxia 8 are thought to be associated with abnormal splicing, but the exact evidence is still unclear (40). Licatalosi et al. compiled neurological diseases, including ataxia-telangiectasia, myotonic dystrophy, FXTAS, SMA, SCA2-8-10-12 that are directly related to alternative splicing defects (41). After these findings, in 2013 Feng and Xie described different mechanisms of alternative splic-

ing that are related to neurological diseases (42). Alternative splicing variants are also shown to be a part of the disease mechanisms of neurological disorders with complex genetic etiologies including ataxia and dystonia (43, 44). In sum, although the mechanism of occurrence of many neurological diseases has been reported to be alternative splicing, there may be other many neurological diseases that are still not clarified.

CONCLUSION

In our findings we demonstrated that *CIC* may be associated with dystonia and other neurological phenotypes by mechanism of alternative splicing. Therefore, our report requires further functional studies both for *in vitro* and *in vivo* to define the exact features of the *CIC* gene.

Ethics Committee Approval: This study was approved by the Clinical Ethical Committee of the Istanbul University, Istanbul Faculty of Medicine (Date: 23/02/2015, No: 2015/493).

Informed Consent: Written consent was obtained from the participants.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- B.S., S.U.İ., E.Y., B.B., H.H.; Data Acquisition- E.Y., B.S., B.B., H.H., H.G.; Data Analysis/Interpretation- B.S., E.Y., B.S., B.B., H.H., S.U.İ.; Drafting Manuscript- B.S., E.Y., B.S., B.B., S.U.İ.; Critical Revision of Manuscript- B.S., E.Y., B.S., B.B., H.H., H.G., U.Ö., S.U.İ.; Final Approval and Accountability- B.S., E.Y., B.S., B.B., H.H., H.G., U.Ö., S.U.İ

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İSTANBUL ÜNİVERSİTESİ YAYINEVİ

RESEARCH / ARAŞTIRMA
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EFFECTS OF ERYTHROPOIETIN PRETREATMENT ON LIVER, KIDNEY, HEART TISSUE IN PENTYLENTETRAZOL-INDUCED SEIZURES; EVALUATION IN TERMS OF OXIDATIVE MARKERS, PROLIDASE AND SIALIC ACID

PENTİLENTETRAZOL-İNDÜKLÜ NÖBETLERDE ERİTROPOİETİN ÖN TEDAVİSİNİN KARACİĞER, BÖBREK, KALP DOKUSU ÜZERİNE ETKİLERİ; OKSİDATİF MARKIRLAR, PROLİDAZ VE SİALİK ASİT AÇISINDAN DEĞERLENDİRME

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ABSTRACT

Objective: The effects of erythropoietin (EPO) which has been frequently studied as an anti-epileptic agent, on peripheral tissues have not been investigated. This study investigated the effects on malondialdehyde (MDA), advanced protein oxidation products (AOPP), superoxide dismutase (SOD), prolidase and sialic acid (SA) levels in the heart, kidney and liver tissues of EPO pretreatment in pentylenetetrazole (PTZ)-induced seizures.

Material and Method: Thirty three male adult rats were divided into three groups. A saline-injected control group, a 60 mg/kg PTZ-injected group to induce seizures and a 3000 IU/kg EPO-injected group 24 hours before seizures. After seizure severity and seizure latency were scored, the rats sacrificed, the tissues were immediately removed for biochemical analyses.

Results: The PTZ-induced seizures increased MDA in kidney ($p<0.01$) and AOPP in liver ($p<0.05$) but didn't alter these markers in heart tissue. In all three tissues, SOD didn't change due to seizures. The SA levels increased in the heart ($p<0.001$), decreased in the kidney ($p<0.001$), and were unchanged in liver. Prolidase increased ($p<0.05$) only in kidney, and was unchanged in other tissues. EPO-pretreatment decreased seizure severity and increased seizure latency. It prevented the increase in MDA in the kidney ($p<0.01$) but increased AOPP ($p<0.05$) and

ÖZET

Amaç: Antiepileptik ajan olarak sıklıkla çalışılan eritropoietinin (EPO)'nun periferik dokular üzerindeki etkileri araştırılmamıştır. Bu çalışmada pentilentetrazol (PTZ) ile indüklenen nöbetlerde EPO ön tedavisinin kalp, böbrek ve karaciğer dokularında malondialdehit (MDA), ileri protein oksidasyon ürünleri (AOPP), superoksid dismutaz (SOD), prolidaz ve sialik asit (SA) seviyelerine etkisi araştırıldı.

Gereç ve Yöntem: Otuz üç erişkin erkek sıçan üç gruba ayrıldı. Salin enjekte edilmiş kontrol grubu, nöbetleri indüklemek için 60 mg/kg PTZ enjekte edilmiş grup, nöbetlerden 24 saat önce 3000 IU/kg EPO enjekte edilmiş grup. Nöbet şiddeti ve nöbet gecikmesi puanlandıktan sonra, sıçanlar sakrifiye edildi, dokular biyokimyasal analizler için hemen çıkarıldı.

Bulgular: Pentilentetrazol ile indüklenen nöbetler, böbrekte MDA ($p<0,01$) ve karaciğerde AOPP'yi arttırdı ($p<0,05$), ancak kalp dokusunda bu markırları değiştirmedi. Her üç dokuda da SOD nöbetler nedeniyle değişmedi. Kalpte SA arttı ($p<0,001$), böbrekte azaldı ($p<0,001$), karaciğerde değişmedi. Prolidaz sadece böbrekte arttı ($p<0,05$), diğer dokularda değişmedi. EPO ön tedavisi nöbet şiddetini azalttı ve nöbet latansını artırdı. EPO böbrekte MDA artışını engelledi ($p<0,01$), ancak AOPP'yi artırdı ($p<0,05$) ve SOD'u azalttı ($p<0,01$) ve prolidazı nöbetlerin arttırdı-

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decreased SOD (p<0.01) and further increased prolidase more than the seizures increased (p<0.01). EPO-pretreatment prevented the increase in AOPP in the liver (p<0.05) but was ineffective in PTZ-induced SA changes in the heart and kidney.

Conclusion: We think that the increase in the heart SA level in seizures is an original finding and deserves investigation in the context of seizure-related cardiac arrhytmias. Also, despite the EPO's anti-seizure effect, increased protein oxidaiton and prolidase, especially in the kidney, is an other important finding that needs further research.

Keywords: Erythropoietin, PTZ-induced seizures, oxidative stress markers, sialic acid, prolidase, peripheral tissues

ğından daha fazla artırdı (p<0,01). EPO ön tedavisi, karaciğerde AOPP artışını önledi (p<0,05), ancak kalp ve böbrekte PTZ'nin neden olduğu SA değişikliklerinde etkisizdi.

Sonuç: Nöbetlerde kalp dokusundaki SA artışının nöbet-ilişkili kardiak aritmiler bağlamında araştırmayı hak eden orijinal bulgu olduğunu düşünüyoruz. Ayrıca EPO ön tedavisinin nöbet engelleyici etkisine rağmen özellikle böbrek dokusunda artmış protein oksidasyonu ve prolidaz da ileri araştırmayı gerektiren diğer önemli bulgudur.

Anahtar Kelimeler: Eritropoietin, PTZ-indüklü nöbetler, oksidatif stres belirteçleri, sialik asit, prolidaz, periferik dokular

INTRODUCTION

It is demonstrated that epileptic seizures cause oxidative stress (OS) not only in the central nervous system but also in peripheral tissues (1). OS is an issue caused by an imbalance between the production of reactive oxygen species (ROS) in tissues and the capacity of endogenous antioxidant defense systems to remove these reactive products. Superoxide dismutase (SOD), one of the important antioxidant enzymes in the body, plays a key role in detoxifying superoxide anions and may prevent OS-induced cellular damage (2). It is shown that experimental induced epileptic seizures increase lipid peroxidation and decrease SOD enzyme activity in liver and kidney (2). On the other hand, it is reported that various anti-epileptic drugs (AEDs) are insufficient in preventing seizures and may trigger OS in brain and peripheral tissues, impair the endogenous antioxidative ability. Thus, epilepsy patients are suffering from hepatic and renal dysfunctions due to their current anti-epileptic drug treatment (1). For instance, it is shown that commonly used drugs such as sodium valproate is associated with serious hepatotoxicity (3). Thus, novel AED investigations that suppress seizures more efficiently with no or minimum adverse effects draw attention. Erythropoietin (EPO) is a hypoxia inducible hematopoietic factor, which is predominantly expressed in the kidneys. However, EPO and its receptors are widely expressed in many tissues including brain, liver, skeletal, heart, muscle and lungs (4). There are many studies showing the anti-epileptic effect of EPO (5-7). In our previous studies, we have shown its antioxidant and anti-inflammatory effects on brain tissue in addition to antiseizure effect of EPO treatment in a generalized acute tonic-clonic epilepsy model induced by pentylenetetrazole (PTZ) in rats (6, 7). However, although EPO's anti-epileptic property is shown, it is an important deficiency that its effects on peripheral tissues have not been investigated yet. EPO has been shown to have an antioxidant effect and a protective effect on heart tissue and kidney ischemia/reperfusion injury models (4, 8, 9). These reports led us to ask what effect EPO treatment might have on the OS that may occur in peripheral tissues in epileptic seizures.

Because of detoxification and excretion functions, the liver and kidney are two important organs that have been investigated in the context of side effects such as OS in experimental seizure models (2, 10). On the other hand, it is reported that drug refractory epilepsy patients have cardiovascular abnormalities and epilepsy is associated with the risk of cardiac ischemia (11). Also, it is known that sudden cardiac arrest and heart rhythm disorders are considered the most common cause of epilepsy-related deaths (12). However, the effects of seizures and anti-epileptic drugs on heart tissue have not been investigated. Voltage-gated sodium channels which include negatively charged sialic acid (SA) is vital for neuronal signal conduction and regular heart rhythms (13). Sialic acid can directly activate the voltage dependent sodium channel at lower depolarization via contributing to negative potential (14). In this context, if SA may affect heart muscle excitability, how epileptic seizures and anti-epileptic drugs affect SA levels in heart muscle is an issue that needs to be investigated, but as far as we know, no such study has been conducted before. Besides the effects of epileptic seizures and anti-epileptic drugs on heart tissue have not been investigated in terms of other biomarkers.

Prolidase is a metalloenzyme that is found in many tissues including the kidney, brain, heart, lungs, and pancreas (15). It plays a role in the recycling of proline from imidodipeptides for the resynthesis of collagen, the main component of the connective tissue and therefore it provides collagen turnover, and matrix remodeling (16). It is also reported that prolidase enzyme activity may be induced with OS and inflammation, also disturbances in prolidase enzyme activity can contribute to the damaging effects of free radicals through collagen breakdown and may play a role in the progress of various diseases (17). Besides, it is shown that serum prolidase enzyme activity and OS values increased in epileptic patients taking anti-epileptic drug and it is reported that this may be a risk factor for vascular damage because of the increase in the collagen cycle (18). To the best of our knowledge, there is no data on how the epileptic seizures affect prolidase

activity in kidney, liver, and heart tissues and how the EPO treatment changes it.

In light of this information, in this study, we essentially focused the effects of PTZ-induced acute generalized tonic clonic seizures as well as EPO pretreatment 24 hours before PTZ administration on liver, kidney and heart tissues. We evaluated these effects in terms of SA levels, prolidase activity, advanced oxidation protein products (AOPP; protein oxidation indicator), malondialdehyde (MDA; lipid peroxidaiton indicator) and SOD levels.

MATERIAL AND METHOD

Animals and experimental design

Male Wistar albino rats (200–250g) were housed in cages and 12 h light–dark cycle and an ad libitum feeding were maintained. Experiments were conducted in the morning to avoid circadian variations. All procedures were done in accordance with the guidelines of Bezmialem Vakif University Animal Experiments Local Ethics Committee (Date: 22.04.2016, No:128).

Thirty three rats were randomly divided into three groups (n=11/each group); 1: Control group (administered with 0.9% saline), 2: PTZ group (administered with 60 mg/kg PTZ to induce generalized tonic-clonic seizures), 3: EPO+PTZ group (administered with 3000 IU/kg EPO 24 h before PTZ injection). PTZ (Sigma, St. Louis, MO, USA), and Recombinant human EPO (r-HuEPO, Eprex; Epoetin alfa, Santa Farma, Turkey) were dissolved in 0.5 ml 0.9% saline and administered intraperitoneally. PTZ dose was selected as it achieves the most successful convulsive response with the least mortality (19). The EPO dose is the dose that does not have any side effects that do not affect the hematocrit values and has anticonvulsive activity (7).

PTZ-induced seizures

Pentilentetrazol is commonly used to create a model of generalized seizure in rats and to study the effectiveness of anti-epileptic drugs (7). A single dose 60 mg/kg PTZ intraperitoneally was injected to induce tonic-clonic generalized seizures. After the PTZ injection, the rats were placed in a plexiglass acrylic cage and seizure behavior was observed until convulsions stopped and was recorded with a camera. The severity of seizures were assessed, using scores based on modified Racine's scale (20), as follows: 1, ear and facial twitching; 2, head bobbing and repeated myoclonic jerks; 3, partial clonic forelimb convulsions in a sitting position; 4, major seizures (generalized tonic-clonic seizures whilst lying on the belly); 5, generalized tonic-clonic seizures; running, followed by the loss of righting ability, and then a tonic phase progressive to the clonus of all four limbs. Seizure latency was measured as the time between the injection of PTZ and the appearance of the first myoclonic jerks.

Biochemical analysis of AOPP, MDA, SOD, SA and prolidase enzyme activity in liver, heart, and kidneys

After the evaluation of seizure severity, rats were decapitated under anesthesia (50 mg/kg ketamine and 10 mg/kg xylazine) and the liver, heart and kidneys were removed immediately. The kidney, heart and liver tissues were washed in ice cold 0.01 M PBS (Phosphate Buffered Saline) to avoid blood contamination. All tissue samples were homogenized in 0.01 M PBS (pH 7.4) using Teflon/glass homogenizer and the homogenates were centrifuged at 5,000 g for 15 min at 4°C. The supernatants were aliquoted and stored at -80°C immediately until AOPP, MDA, SA levels and SOD, prolidase activity assay were performed. The total amount of protein was determined by the Lowry method (21).

AOPP assays were performed spectrophotometrically at 340 nm wavelength by Hanasand's modified method (22). AOPP concentrations were calculated from the standard curve graph and expressed as μmol/L chloramine-T equivalents.

Changes in tissue lipid peroxidation levels were evaluated by measuring MDA levels. Spectrophotometric analysis of MDA levels was performed by Beuge and Aust's method (23), determined by the quantity of thiobarbituric acid reactive products. The MDA concentration was calculated by its molar extinction coefficient (ε=155 $mM^{-1}cm^{-1}$) and expressed as nmol/mg protein.

SA levels were determined by the Tram method (24). B-formylpyruvic acid formed because of periodic acid oxidation was reacted with two molthiobarbituric acid. A colored compound was formed that gave maximum absorbance at 549 nm. Since this product is not stable, absorbances were recorded at 549 nm in the spectrophotometer by pulling into the cyclohex-zanon phase. The SA amount was expressed as μg/mg protein.

The total SOD enzyme activity was measured spectrophotometrically (25). The percent inhibition rate was calculated with the formula Ablank-Asample/Ablank x 100. 50% inhibition corresponds to 1 Unit of enzyme activity. Enzyme activity was given as U/mg protein.

Prolidase enzyme activity was determined according to the spectrophotometric method of Ozcan et al. 2007 (26), based on the measurement of proline levels, a prolidase product, produced by prolidase enzyme. Tissue prolidase activities were expressed in terms of nmol/min/mg protein.

Statistical analysis

The levels of AOPP, MDA, SOD, SA and prolidase enzyme activity in the liver, heart and kidneys were analyzed separately. The comparisons between groups were made by unpaired t tests in GraphPad Prism 8 software. A value of $p<0.05$ was considered statistically significant.

RESULTS

Evaluation of PTZ-induced seizures

A single dose of 60 mg/kg PTZ caused generalized tonic clonic epileptic seizures of 4-5 severity according to the Racine's scale. EPO pretreatment significantly reduced the severity of seizures and increased seizures latency (Table 1). As seizures were not observed in the control group, it was not included in the table.

Table 1: Seizure severity and Seizure latency in PTZ and EPO+PTZ groups

	PTZ	EPO+PTZ	p value
Seizure latency (Sec)	74.86±6.02	101.1±8.54	p<0.05
Seizure severity	4.8±0.4	3.4±0.5	p<0.01

PTZ: single dose of 60 mg/kg pentylenetetrazol was administered; EPO+PTZ: 3000 IU/kg erythropoietin was administered 24 hours before a PTZ injection. Seizures were not seen in control group, therefore they are not given in the table.

Evaluation of OS markers, prolidase and SA levels in peripheral tissues

When the changes in the OS markers, prolidase and SA levels in peripheral tissues were examined, different results were observed for all three organs. The variation of each parameter according to the groups was as follows.

AOPP; PTZ induced seizures caused an increase in AOPP in liver tissue compared to the control group (p<0.05) but did not change in heart and kidney AOPP levels compared to the control group (Figure 1). EPO treatment before seizures decreased AOPP level in the liver compared to the PTZ group (p<0.05). Conversely, it further increased AOPP level in kidney compared to the PTZ group (p<0.01) (Figure 1). EPO pretreatment did not significantly cause change in terms of AOPP in heart tissue.

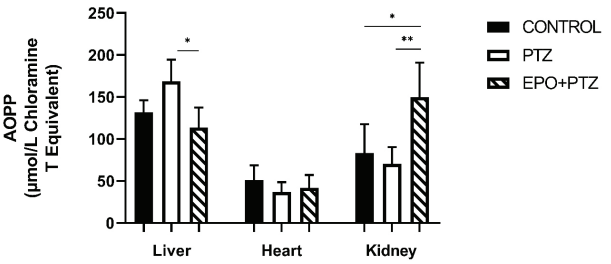

Figure 1: AOPP levels in liver, heart and kidney tissues in all groups

AOPP: Advanced oxidation protein products; Control: 0.9% saline was administered; PTZ: single dose of 60 mg/kg pentylenetetrazol was administered; EPO+PTZ: 3000 IU/kg erythropoietin was administered 24 hours before a pentylenetetrazol injection. Each column represents the mean±SD. *p<0.05; **p<0.01

MDA; PTZ-induced seizures increased kidney MDA level compared to control group (p<0.01). Conversely, it did not cause to any change in MDA levels in liver and heart tissues compared to the control group (Figure 2). EPO treatment before seizures significantly decreased MDA levels in heart (p<0.05) and kidney (p<0.01) compared to PTZ group (Figure 2). But EPO pretreatment did not cause any change in liver MDA level compared to other groups (Figure 2).

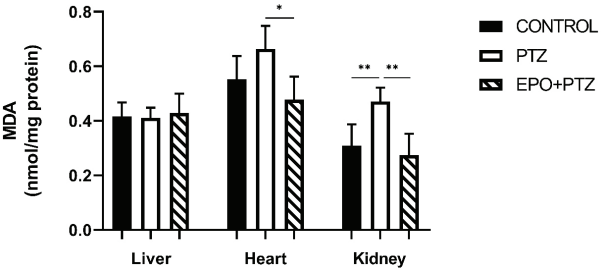

Figure 2: MDA levels in liver, heart and kidney tissues in all groups

MDA: Malondialdehyde. Control: 0.9% saline was administered; PTZ: single dose of 60 mg/kg pentylenetetrazol was administered; EPO+PTZ: 3000 IU/kg erythropoietin was administered 24 hours before a pentylenetetrazol injection. Each column represents the mean±SD. *p<0.05; **p<0.01

SOD; PTZ-induced seizures did not change SOD levels of liver, heart and kidney tissues according to the control group (Figure 3). EPO treatment before seizures did not cause significant difference in liver and heart tissues' SOD levels, but EPO pretreatment significantly caused a decreased SOD level in kidney compared to the PTZ and control groups (p<0.001 and p<0.01, respectively) (Figure 3).

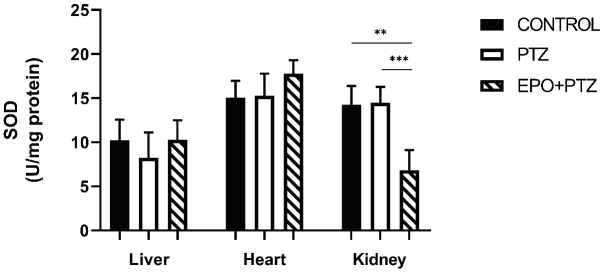

Figure 3: SOD levels in liver, heart and kidney tissues in all groups

SOD: Superoxide dismutase. Control: 0.9% saline was administered; PTZ: single dose of 60 mg /kg pentylenetetrazol was administered; EPO+PTZ: 3000 IU/kg erythropoietin was administered 24 hours before a pentylenetetrazol injection. Each column represents the mean±SD. **p<0.01; ***p<0.001

SA; PTZ induced seizures significantly caused an increase in the heart SA level compared to the control group ($p<0.001$). Conversely, it caused a significant decrease in the kidney tissue SA level compared to the control group ($p<0.001$). But it did not cause a difference in liver SA level compared to the control group (Figure 4). In the EPO pretreated group all tissue SA levels were the same as in the PTZ group. In other words, in the EPO treatment group, as in the PTZ group, the SA level in the heart tissue was higher than the control group ($p<0.05$), it was lower in the kidney tissue compared ($p<0.001$). EPO pretreatment also did not cause a significant change in the liver SA level as in the PTZ group (Figure 4).

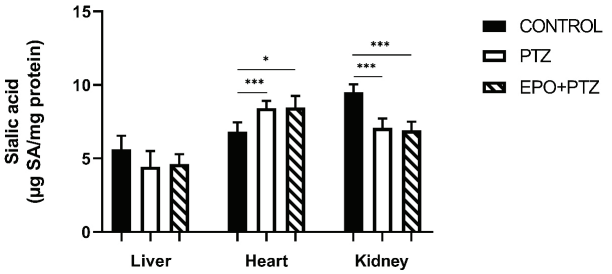

Figure 4: Sialic acid levels in liver, heart and kidney tissues in all groups

Control: 0.9% saline was administered; PTZ: single dose of 60 mg/kg pentylenetetrazol was administered; EPO+PTZ: 3000 IU/kg erythropoietin was administered 24 hours before a pentylenetetrazol injection. Each column represents the mean±SD. *p<0.05; ***p<0.001

Prolidase; PTZ-induced seizures did not cause a change in liver and heart tissues prolidase level compared to the control group but increased in the kidney prolidase level in comparison ($p<0.05$) (Figure 5). EPO treatment before seizures increased the kidney prolidase level ($p<0.05$), but EPO pretreatment did not cause a change in liver and heart tissues prolidase levels compared to other groups (Figure 5).

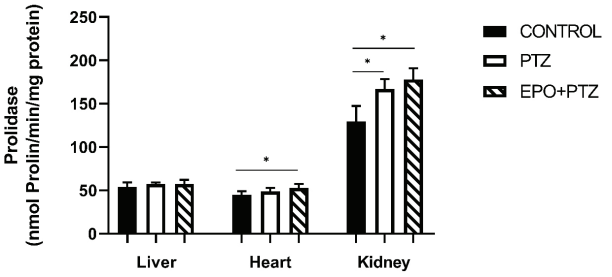

Figure 5: Prolidase levels in liver, heart and kidney tissues in all groups

Control: 0.9% saline was administered; PTZ: single dose of 60 mg/kg pentylenetetrazol was administered; EPO+PTZ: 3000 IU/kg erythropoietin was administered 24 hours before a pentylenetetrazol injection. Each column represents the mean±SD. *p<0.05

DISCUSSION

It is demonstrated that PTZ-induced seizures in rats increased the MDA level, which is a lipid peroxidation indicator in the liver and kidney and also the brain (10, 27). Although our findings are consistent with other studies in terms of the OS increase in the liver and kidney, it is original because it shows a difference because the oxidative biomarkers differ according to tissues. PTZ induced seizures did not cause significant changes in oxidative markers in heart tissue, but it increased MDA in the kidney and AOPP in the liver, it did not decrease SOD, which is an antioxidant enzyme in both tissues. Antioxidant markers are expected to decrease while oxidative markers increase (28). The fact that the SOD level was not decreased in our study suggested an increase in the activity of the endogenous defense system against the OS caused by seizures in tissues. Indeed, supporting our suggestion, studies done in patients with epilepsy have shown that there is an increase in oxidative markers in plasma that show protein, lipid and DNA oxidation, and an increase in antioxidant enzymes (29).

Another OS related parameter we investigated in our study was prolidase, which is one of the matrix metalloproteinases. It was shown that increased prolidase enzyme activity and total oxidant markers in the serum of epileptic patients taking the anti-epileptic treatment may have an increased risk for vascular damage associated with degenerated collagen turnover (18). In our study PTZ-induced seizures increased the level of prolidase only in kidney tissue, it did not change in other tissues. When we consider that PTZ-induced seizures cause an increase in MDA, which is lipid peroxidation indicator, in the kidney we suggested that an increase in prolidase may be associated with lipid peroxidation in particular.

Pentilentetrazol induce seizures by activating glutamate receptors and inhibiting GABA receptors (20). Glutamate receptors have been also identified in tissues such as those of the liver, kidney, lung, and heart and also the brain (30). Thus, seizures might affect peripheral tissues through glutamate receptors. Also, it is reported that N-methyl-D-aspartate (NMDA) receptors, which are a subtype of glutamate receptors could be one of the crucial mediators in the regulation of oxidative balance in many tissues. Although the functional significance of glutamate receptors for kidney, liver and other tissues is not exactly understood, it is suggested that sustained activation of these receptors induces changes in cellular calcium dynamics and in turn can activate free radical generation, that is lipid peroxidation (30). A study targeting glutamate receptors has reported that type-5 metabotropic glutamate receptor (mGlu5) antagonist (MPEP) administration might protect erythrocytes and liver tissue against anoxic damage and prevent increase in OS re-

vealed by PTZ-induced seizures in rats (10). In our study, the fact that epileptic seizures increased prolidase and MDA, especially in the kidney, may be due to containing more glutamate receptors of the kidney. Indeed, it is reported that the kidney contains more and all types of glutamate receptors (30). In another study it is reported that OS related tissue damage in renal cells may be caused by excessive activation of NMDA glutamate receptors (31).

In this study we also investigated the change of SA in peripheral tissues following seizures. We found that PTZ-induced seizures caused a significant increase in the level of SA only in the heart tissue. We think this is important because seizures related to cardiac rhythm disorders are an important problem and we suggest this may be related to SA. Indeed, it is proposed that the serum SA content is associated with cardiovascular diseases. It is shown that the SA level was elevated in serum after myocardial infarction (32). Moreover, a relationship between an increase in SA biosynthesis and cardiac hypertrophy was demonstrated (33). We think that studies investigating the significance of SA in heart rhythm disturbances or sudden deaths observed in epileptic seizures are needed.

As far as we know, this study is the first study investigating changes in SA levels in the heart and other tissues in epileptic seizures. Interestingly in our study PTZ-induced seizures lowered SA levels in the kidney. It is suggested that SA acts as a competitive antagonist at the glutamate binding site (34). Non-neural glutamate receptors may play a role in normal cellular functions such as cell to cell communication. Also, all the ionotropic glutamate receptors, especially NMDA receptors are nonselective cation channels, allowing the passage in small amounts of Ca^{+2} (30, 35). We think that the decrease in SA in the kidney tissue and thus the increase in Ca^{+2} entry into the cell may have triggered OS. On the other hand, PTZ-induced seizures increased the SA level in heart tissue. We thought that the increased SA in the heart tissue caused of seizures might have prevented Ca^{+2} entry into the heart cell, so the increase of OS products in the heart tissue may have been prevented. As a matter of fact, in our study, oxidative markers did not increase in the heart tissue because of the seizures. However, we thought that the excitability of the heart may change because of the increase in SA in the heart tissue that might prevents Ca^{+2} entry into the cell. From this point of view, the effects, and consequences of seizures on SA levels in the kidney and heart tissues are important subjects that require further investigation.

In our study, we also investigated the effects on peripheral tissues besides the anti-epileptic effect of EPO pretreatment in seizures. As previously shown by our studies (7, 19), also in the present study EPO pretreatment decreased the severity of PTZ-induced seizures and increased the seizure latency. EPO pretreatment prevented an MDA increase caused by seizures in the kidney, while increasing the level of AOPP, which was not changed with seizures. It has been reported that AOPP, which are protein oxidation products, are more reliable than lipid peroxidation products as OS indicators due to their stability and longevity (36). Therefore, although EPO pretreatment reduces MDA in the kidney, it has a significant oxidant effect in terms of AOPP. In addition, the decrease of SOD in the kidney with EPO pretreatment is another finding supporting that EPO therapy may cause OS in the kidney. Moreover, EPO pretreatment increased the prolidase level in the kidney more than the increase caused by seizures. It is reported that increased tissue prolidase levels in diseases such as diabetes, chronic liver disease are an indicator of OS and the reduction of antioxidant defense could cause increase in prolidase level (37). Indeed, in our study, in the EPO-treated group the kidney SOD levels reduced, while prolidase level increased. All these findings strengthen the theory that EPO pretreatment can cause OS in the kidney. The Kidneys are the primary site of EPO production and contain more EPOr, which have higher affinity. Therefore, we think that exogeneously given EPO may arise from the affinity differences of the EPOr in hematopoietic organ, the kidney and nonhematopoietic organs, and the differences in the dynamics of the signal pathways initiated through these receptors. Meanwhile, our findings revealed that EPO treatment before seizure is ineffective to changes in SA levels in the heart and kidney caused of seizures. Namely, the SA level was high in heart tissue and low in kidney tissue as in the PTZ group. On the other hand, interestingly EPO treatment prevented the increase in AOPP caused by PTZ in the liver. This finding at the same time shows the protective effect of EPO in the liver.

CONCLUSION

Our results clearly showed that seizures cause OS in the liver and kidneys and increase SA in heart tissue. We suggest that increased SA in the heart is an important and original result that may be critical for seizure related cardiac arrythmias and/or sudden deaths. Our study clearly showed that EPO suppresses PTZ-induced seizures, as we have shown earlier. Furthermore, our results revealed that EPO pretreatment affected the changes in OS markers caused by seizures in tissues, but this effect was different according to the tissue, increased protein oxidaiton and prolidase, especially in the kidney. While investigating the anti-epileptic effect of EPO in seizures, its effect on tissues has not been studied before, and thus limits our discussion. Although there is no parameter showing the effect of EPO on glutamate receptors in peripheral tissues in our study, we suggest that EPO may show different effects on tissues due to the different glutamate receptors expressed in peripheral tissues. Indeed, glutamate receptors, which play a critical role in the initiation

and spread of seizures, have been detected in peripheral tissues including the heart and kidney and it is reported that the glutamate receptors may mediate the functions of tissues (38). Our preliminary study clearly shows that while investigating the anti-epileptic effect of EPO, its effect on tissues must not be ignored. We think that further studies are needed to understand the mechanism.

Ethics Committee Approval: This study was approved from by the Bezmialem Vakif University, Animal Experiments Local Ethics Committee (Date: 22.04.2016, No:128).

Peer Review: Externally peer-reviewed.

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THE RELATIONSHIP BETWEEN THE EXPRESSION LEVELS OF TISSUE INHIBITOR OF METALLOPROTEINASES-3 (TIMP3) AND SEVERITY OF ATHEROSCLEROSIS

METALLOPROTEİNAZ-3 DOKU İNHİBİTÖRÜNÜN (TIMP3) İFADE DÜZEYLERİ İLE ATEROSKLEROZUN ŞİDDETİ ARASINDAKİ İLİŞKİ

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ABSTRACT

Objective: Tissue Inhibitor of Metalloproteinase-3 human (TIMP3) is one of tissue inhibitors of metalloproteinases (TIMPs), which binds to the components of the extracellular matrix, and has crucial roles in atherosclerogenesis and adipose tissue differentiation. In this study, it was aimed to determine the effects of *TIMP3* gene expression levels on severity of atherosclerosis in different tissues.

Material and Methods: The first group of the study (evaluated for coronary artery disease) were cases classified as high and low plaque scores according to the degree and location of atherosclerotic lesions. In the second group (post-mortem cases) were male cadavers who died due to coronary heart disease (CHD, n=26) and non-cardiac trauma (T-nonP, n=4). The *TIMP3* expression levels were examined in leukocyte and peri-coronary epicardial adipose tissues (EAT) samples (n=69 and n=34, respectively) of the first group and EAT and coronary artery samples (n=12 and n=30, respectively) of the second group using quantitative RT-PCR. In addition, the protein expressions of TIMP3 were analysed on artery sections by immunofluorestaining.

Results: In the post-mortem study group, the *TIMP3* expression levels were found to increase in no plaque segments of arteries of cases with CHD (CHD-nonP) compared to advanced atherosclerotic arteries (CHD-P) and normal arteries of T-nonP cases

ÖZET

Amaç: Hücre dışı matriksin bileşenlerine bağlanan TIMP3, metaloproteinazların doku inhibitörlerinden (TIMP'ler) biridir ve ateroskleroz gelişimi ile yağ dokusu farklılaşmasında rol oynamaktadır. Bu çalışmada, farklı dokulardaki *TIMP3* gen ifade düzeylerinin ateroskleroz şiddeti üzerine olan etkisinin belirlenmesi amaçlandı.

Gereç ve Yöntemler: Çalışmanın ilk grubu (koroner arter hastalığı için değerlendirilen), aterosklerotik lezyonların derecesine ve yerine göre yüksek ve düşük plak skoru olarak sınıflandırılan vakalardı. İkinci grup (post-mortem vakalar), koroner kalp hastalığı (KKH, n=26) ve kardiyak olmayan travma (T-nonP, n=4) nedeniyle ölen erkek kadavralardı. Birinci grubun lökosit ve peri-koroner epikardiyal yağ doku (EYD) örnekleri (sırasıyla, n=69 ve n=34) ile ikinci grubun EYD ve koroner arter örneklerinde (sırasıyla, n=12 ve n=30) *TIPM3* ifade düzeyleri kantitatif RT-PCR ile incelendi. Ek olarak, TIMP3 protein lokalizasyonları, immunfluoresans tekniği ile belirlendi.

Bulgular: Post-mortem çalışma grubunda, *TIMP3* ifade düzeylerinin KKH'lı vakaların plaksız arter segmentlerinde (CHD-nonP), ileri aterosklerotik arterlere (CHD-P) ve T-nonP vakaların normal arterlere kıyasla arttığı bulundu (sırasıyla, p=0,01 ve p=0,05). Lökosit ve EYD'lerdeki *TIMP3* gen ifadeleri, çalışma grupları arasın-

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(p=0.01 and p=0.05, respectively). The *TIMP3* expressions in EATs and leukocytes were not statistically significant between the study groups. In addition, TIMP3 protein was detected in normal arteries, peri-coronary EATs and mostly in macrophages-rich areas in advanced atherosclerotic arteries.

Conclusion: Tissue Inhibitor of Metalloproteinase-3 human (TIMP3) expression levels increase in normal coronary arterial segments of cases with CHD, which depict a protective role of TIMP3 in development of atherosclerosis.

Keywords: Atherosclerosis, TIMP3, gene expression level, coronary artery, adipocyte, leukocyte

da istatistiksel olarak anlamlı fark göstermedi. Ek olarak, TIMP3 proteini normal arter, peri-koroner EYD ve ileri aterosklerotik arterde makrofajların yoğun olduğu alanlarda tespit edildi.

Sonuç: KKH'lı vakaların normal koroner arteriyel segmentlerinde *TIMP3* ifadesindeki artışın ateroskleroz gelişimine karşı koruyucu bir etkisi olabileceğini göstermektedir.

Anahtar Kelimeler: Ateroskleroz, TIMP3, gen ifade seviyesi, koroner arter, adiposit, lökosit

INTRODUCTION

Atherosclerosis, which is the main causal mechanism underlying coronary heart diseases (CHD), is one of the leading causes of morbidity and mortality in the world. Atherosclerosis is a progressive arterial lesion that develops primarily from a series of reactions induced by endothelial injury. In this inflammatory-fibroproliferative response, the extracellular matrix (ECM) plays an important role in the intercellular network among smooth muscle cells, macrophages, T lymphocytes and endothelial cells (1-3). Tissue inhibitors of metalloproteinase (TIMPs) are responsible for inhibiting matrix metalloproteinases (MMPs), which are the main regulators of ECM (4). The interaction between MMPs and TIMPs regulates balance between ECM and its environment (5). It has been demonstrated in previous studies that this delicate balance is disturbed in cancer, myocardial infarction and inflammatory diseases, including atherosclerosis (6-8). To balance MMP levels in damaged tissues, exogenous therapeutic application of TIMP3 has been found to be beneficial in experimental studies (8). Secreted TIMP3 is the only ECM in the TIMP family in insoluble form and has been associated with decreased inflammation and the atherosclerotic plaque stability (9, 10).

Moreover, *TIMP3* expression remains low during the adipocyte differentiation; however, it increases when cell differentiation is disrupted (11). Adipose tissue has a crucial regulatory role in the development of atherosclerosis and diabetes due to inflammation (12-15). However, the role of TIMP3 in atherosclerosis, which has a modulatory role in adipose tissue formation around the atherosclerotic coronary artery, is unknown.

The influence of *TIMP3* expression, which has therapeutic significance in disease modulation, on different types of tissues and cells and advanced atherosclerosis has thus far not been addressed in the same study design. In this study, it was aimed to determine the effect of the *TIMP3* expression profiles in different cells and tissues on the atherosclerosis process. For this purpose, *TIMP3* expression levels in peripheral blood leukocytes and peri-coronary epicardial adipose tissue (EAT) were investigated in low and high score groups formed by Gensini and Syntax scoring according to the complexity and severity of coronary artery disease in the living cases of the study. On the other hand, we attempted to verify the pre-existing *TIMP3* relationships and determine the association between EAT and coronary arteries with advanced atherosclerotic plaques in post-mortem cases with CHD.

MATERIALS AND METHODS

All procedures performed in the living study groups involving human participants were in accordance with the ethics committee of the Faculty of Medicine Clinical Research Ethics Committee, Istanbul University (Date: 27.10.2011, No: 1822 and Date: 17.01.2014, No: 160) and followed the Declaration of Helsinki. The experimental protocol of post-mortem study groups was evaluated and approved by the Scientific Research Commission of Council of Forensic Medicine (B.03.1.ATK.0.01.00.08/863, 28.12.2010).

Samples of living cases

The first study group, named as living cases (total n=69) consist of participants who were evaluated for coronary artery disease (CAD) by performing invasive coronary angiography due to stable angina pectoris, ischemia, acute coronary syndrome and pre-surgical assessment. All cases in the first study group were divided into two groups according to complexity and severity of their coronary artery disease as low and high plaque score groups. Gensini and Syntax scores were calculated based on the degree and location of atherosclerotic lesions in coronary angiographic evaluation (16). Gensini and Syntax were considered to have a cut-off value of eight for both scores. The group with high plaque score (n=48) consisted of cases with CAD with at least one stenosis of least 50% in any coronary vessel. Twenty-five of 48 patients with CAD in this group were operated on for heart valve repair or replacement (n=5) and coronary artery by-pass surgery (n=20). Nine of the 21 cases in the low plaque score group without CAD (0% or <20% stenosis) were operated on for heart valve repair or replacement. Peripheral blood samples were collected for leukocyte separation from all cases. The peri-coronary epicardial adipose tis-

sues (EAT) from the proximal tract of the right coronary artery were obtained from 34 of 69 cases within the first 20 minutes during heart valve surgery (n=14) and by-pass surgery (n=20). The study design and the number of samples studied in subgroups is given in Figure 1.

Peripheral blood and tissue samples of cases were provided from the Istanbul University-Cerrahpasa, Department of Cardiovascular Surgery and Department of Cardiology. Written informed consent was obtained from every participant before blood and/or tissue samples were taken in surgical operation and coronary angiography.

Cases with atherosclerosis combined with various systemic autoimmune or chronic inflammatory diseases including rheumatoid arthritis, systemic lupus erythematosus, and antiphospholipid syndrome were excluded from the study.

Samples of post-mortem cases

The coronary artery samples and peri-coronary epicardial adipose tissues (EAT) were obtained from male autopsy cases (total n=30) within 24 hours post-mortem. Since female autopsy cases were extremely rare, they were not included in the study, and also cases with appropriate sampling time were not included in the study. All post-mortem samples were provided from the Republic of Turkey, Ministry of Justice Council of Forensic Medicine. The ethical approval was obtained for the post-mortem tissue collection from Scientific Research Commission of Council of Forensic Medicine (Project number and date: B.03.1.ATK.0.01.00.08/863, 28.12.2010).

Post-mortem cases were evaluated according to the autopsy report including the pathological, biochemical and toxicological analysis. The cases were classified according to causes of death as coronary heart disease (CHD, n=26) and non-cardiac trauma (T, n=4). The non-atherosclerotic coronary arteries (T-nonP) group included traumatic cases without coronary heart disease, whose coronary arteries were evaluated as normal in autopsy including histopathologic evaluations. The exclusion criteria for this study were: moderate to advanced putrefaction, drug poisoning or toxicity, and cases of homicide or suspected homicide. The characteristics of the study groups are shown in Table 1. The coronary artery samples were dissected according to their macroscopic features (plaque size, occlusion, presence of calcification for atheroma, and also anatomic location, artery diameter, artery wall thickness and normal appearance, etc.) and grouped as arteries with advanced atherosclerotic plaque (CHD-P) (n=26) and no plaque segments (CHD-nonP) (n=25) obtained from CHD cases and also non-atherosclerotic coronary arteries (T-nonP) (n=4). The peri-coronary EAT and arterial samples of the young T-nonP group who were reported to have no atherosclerosis in the autopsy report were used as controls for gene expression and histopathological examinations.

The post-mortem peri-coronary EAT samples were obtained from the surrounding tissue of the coronary artery of traumatic cases (n=2 from T-nonP group), the surrounding tissue of the coronary artery with plaque (n=10 from CHD-P group) and without plaque (n=10 from CHD-nonP) of CHD cases. Since tissue could not be obtained from some cases and there was insufficient tissue for

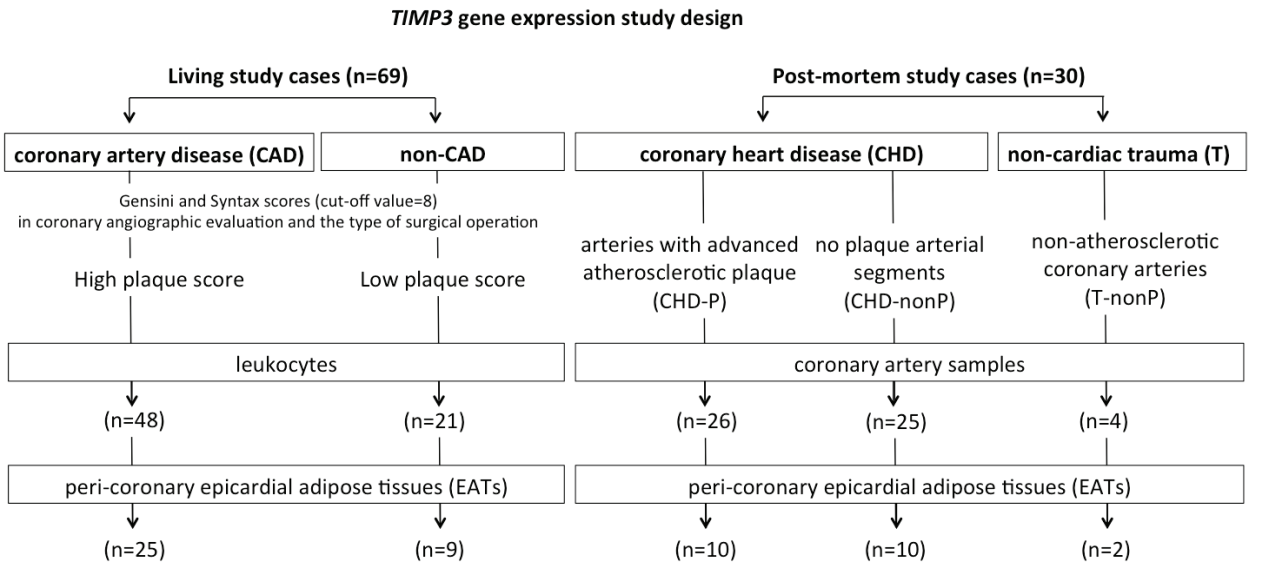

Figure 1: Flowchart of the *TIMP3* gene expression study design. This figure illustrates the study design of living study cases and post-mortem study cases and their subgroups. The numbers of peripheral blood leukocytes, peri-coronary epicardial adipose tissues (EATs) and coronary artery tissues from a total of 99 cases are shown.

RNA isolation in some cases, EAT sample numbers of the study groups were unequal.

Histopathology and immunofluorescence techniques

Small pieces of (approximately 3-4 mm) the non-atherosclerotic segments of coronary arteries (n=2 from T-nonP group and n=2 from CHD-nonP group) and the coronary arteries with advanced plaque including peri-coronary epicardial adipose tissues (n=4 from CHD-P group) of the post-mortem cases were separated to compare protein localizations between the groups for use in the immunofluorescence technique. These tissues were fixed in 5% paraformaldehyde, mounted within OCT Medium (Sakura/Tissue-Tek Company, Torrance), and then were dissected into 10 µm thick cross-sectional segments using a cryostat in -40°C.

In Hematoxylin and Eosin (HE) staining, at least three tissue sections of all fixed tissue samples were confirmed to have advanced atherosclerotic lesions and normal artery morphology with histopathologic evaluation (17).

The other tissue sections on poly-L-lysine coated glass slides were fixed in 3% paraformaldehyde in phosphate-buffered saline (PBS) pH 7.4 for 15 min at room temperature for immunofluorescence analysis of TIMP3 and tissue specific proteins. After rinsing twice with ice cold PBS, tissues on slides were subjected to heat-induced antigen retrieval by incubation in citrate buffer (10 mM citric acid in 1xPBS, pH 6.0). Next, immunofluorescence protocol was followed for immunostaining. The tissue sections were incubated first with primer antibody (diluted with antibody diluent solution, abcam, ab64211) overnight at 4°C, and then with specific secondary fluorescence antibody in the dark for one hour at room temperature. The sections were washed in PBS (3×2 minutes) and mounted Fluoroshield Mounting Medium with DAPI (4,6-diamino-2-phenyl indole) to identify the nuclei. Monoclonal mouse anti-CD68 antibody [KP1] (1/200 dilution rate, ab955), polyclonal rabbit anti-alpha smooth muscle actin (1/100 dilution rate, ab5694), polyclonal rabbit anti-human TIMP3 antibody (1/200 dilution rate, ab39184), goat anti-rabbit IgG H&L (Alexafluor 488 Green, 1/500 dilution rate, ab181448), goat anti-mouse IgG H&L (FITC, 1/1000 dilution rate, ab6785) and Fluoroshield Mounting Medium with DAPI (ab104139) was obtained from Abcam. Images were acquired using Confocal Microscope (Leica TCS-SPE).

Total RNA isolation

The coronary arteries and perivascular adipose tissues were carefully removed by dissection, immediately frozen in liquid nitrogen and stored at -80°C until further use. Total RNAs were extracted using the Trizol reagent (TRIZOL Reagent, Invitrogen, USA). Trizol reagent was added 1 mL (for each 100 mg) to the tissues and homogenized with tissue homogenizer.

The peripheral blood samples were collected into 10 ml Vacuette K_2EDTA tubes (BD, Franklin Lakes, NJ). Gey's Solution (155 mM NH_4Cl, 10mM $KHCO_3$ in DEPC treated distilled water) was added to a 2:1 ratio on whole blood. Then, samples were incubated at +4°C for 20 minutes. White blood cells (leukocytes) were isolated via centrifugation for 10 min at 1500 rpm at 10°C. The cell pellet was washed a second time in Gey's solution as equal volume of the blood. Next, 1 ml of 1x Phosphate Buffered Saline (PBS) was added to the pellet and centrifuged for 10 minutes at room temperature at 1500 rpm. The leukocytes pellet was homogenized with 1 ml Trizol Reagent.

Total RNA isolation from tissue and leukocyte homogenates was performed according to the Trizol RNA isolation protocol. The concentration and quantity of total RNA samples were measured at 260 nm and 280 nm (A260/280) using a Nanodrop 1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE).

Expression analysis with Quantitative Real Time PCR

The expression levels of *TIMP3* were determined in the leukocytes and peri-coronary EAT obtained from the living cases and in the coronary artery and peri-coronary EAT samples obtained from the post-mortem cases using quantitative Real Time-PCR (qRT-PCR). qRT-PCR were performed with the PCR Master primer-probe mixes of each gene transcripts with the Probe Master Mix in the LC480 instrument for the *TIMP3* and as a control for the *ACTB* (actin, beta, NM_001101.2) and *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase, NM_002046.3) as endogenous controls. cDNA was first synthesized from total RNA samples, and then qRT-PCR was performed, and the relative expression levels were calculated as $2^{-\Delta\Delta Ct}$ method. The difference in the threshold cycle between target (*TIMP3*) and reference genes (the mean of *B-actin* and *GADPH*) as ΔCt was calculated for all samples studied in duplicate. The sample with the lowest Ct mean of endogenous genes selected from the T-nonP group and low score group was used as the calibrator (reference sample) for post-mortem groups and living study groups, respectively. The relative expression of the *TIMP3* in all samples was compared to the calibrator and the results were expressed as relative quantification (RQ) values. Real Time ready Catalog Assay for *TIMP3* (Assay IDs: 101221), *ACTB* (Assay IDs: 143636), *GAPDH* (Assay IDs: 141139) and Light Cycler 480 Probes Master Kit was purchased from Roche Life Science for quantitative RT-PCR.

Statistical analysis

Comparison of expression levels of *TIMP3* was conducted by using RQ values, and results were expressed as mean and standard deviation (S.D.). Normality of distributions of the *TIMP3* expression levels and other continuous variables were assessed using the Shapiro-Wilk test. If vari-

ables were not normally distributed, Mann Whitney U-test was used for comparison between two groups, and Kruskal-Wallis test was used for multiple comparisons among three groups. Student t-test and ANOVA test was used to compare for the means of clinical characteristics that were normally distributed. The Chi Square Test or Fisher's Exact Test was used for categorical variables. Spearman's test was used for correlation analysis of Syntax and Gensini scores that were not normally distributed. All statistical analyses were performed using SPSS 14.0 (SPSS Inc., Chicago, IL, USA). Values of $p<0.05$ were considered statistically significant. There was at least 80% ($\alpha=0.05$) statistical power and an effect size of 0.5 and 0.8 in global effects when total sample size was at least 27 and 12, respectively. Power and sample size calculations were performed using the G*Power statistics software (18).

Table 1: The characteristics of living cases and post-mortem cases

	Living cases		
Characteristics	**Low plaque score (n=21)**	**High plaque score (n=48)**	**p-values**
Gender, % (men)	50%	64%	0.244
Age (means, years)	57.93±10.21	57.7±10.91	0.900
Gensini score	3.42±3.69	54.58±32.24	0.0001
Syntax score	2.33±2.95	19.90±9.12	0.0001
BMI (kg/m^2)	27.53±4.49	27.16±3.79	0.611
Diastolic blood pressure (mmHg)	75.54±9.43	75.83±9.46	0.870
Systolic blood pressure (mmHg)	124.08±16.34	123.13±15.21	0.756
Triglycerides (mg/dl)	163.59±94.22	170.73±95.10	0.645
Total cholesterol (mg/dl)	186.5±43.5	189.23±46.92	0.724
HDL cholesterol (mg/dl)	41.45±12.42	39.20±11.72	0.249
LDL cholesterol (mg/dl)	122.81±36.32	125.94±43.41	0.649
CRP (mg/dl)	7.84±11.38	12.02±20.33	0.214
Glucose (mg/dl)	107.55±29.37	122.96±43.75	0.008
Myocardial infarction, yes (%)	-	58.3%	0.0001
Hypertension, yes (%)	57.1%	47.9%	0.481
Diabetes mellitus, yes (%)	33.3%	31.2%	0.864
Type of surgery operation; Heart valve surgery, yes (%)	42.9%	10.4%	0.0001
Coronary by-pass surgery, yes (%)	-	41.7%	

Post-mortem cases

Characteristics	**CHD group (n=26)**		**T-nonP group with normal arteries (n=4)**	**p-values***	**p-values****
	with plaque segments (CHD-P) (n=26)	**arterial segments without plaque (CHD-nonP) (n=25)[♯]**			
Age (mean, years)	51.3±12.1	51.3±12.1	29.3±16.3	0.003	0.003
BMI (kg/m^2)	27.2±4.4	28.1±3.8	23.3±4.6	0.111	0.03
Heart weight (gr)	458.6±119.4	479.7±135.4	366.0±54.0	0.142	0.113
Positive family history, yes	53.8%	48.0%	0%	0.044	0.07
Plaque in aorta, yes	76.0%	70.8%	0%	0.003	0.007
Plaque in LCA, yes	88.5%	88.0%	0%	0.0001	0.0001
Plaque in RCA, yes	69.2%	60.0%	0%	0.009	0.026

[♯], A sufficient amount of arterial segment without a plaque could not be obtained in one CHD case. *CHD-P group vs. T-nonP group, **CHD-nonP group vs. T-nonP group; P; advanced atherosclerotic plaque, CHD; coronary heart disease

RESULTS

The basic clinical characteristics of living cases (n=69) and post-mortem cases (n=30) were shown in Table 1. There was a strong positive correlation between Gensini and Syntax scores (r=0.92; p<0.0001) of the living cases. The age, body mass index (BMI), blood pressures, and serum lipids except gensini/syntax scores and glucose levels were not statistically different between the low and high plaque score groups. In the post-mortem study groups, the traumatic cases in the T-nonP group (n=4) were younger than the coronary heart disease case groups (n=26) (p=0.003), and the heart and arteries were normal in the autopsy reports. This group (T-nonP) was used for calculation of RQ values in post-mortem samples.

TIMP3 expression levels in leukocytes and peri-coronary EAT of living cases

The leukocytes and peri-coronary EATs of living cases grouped as high and low plaque scores according to surgical operation, and invasive coronary angiography were investigated for the expression levels of *TIMP3*. The high score group consisted of 48 patients with high Gensini and Syntax scores (cut-off value ≥8 for both) who had coronary artery disease. The low plaque score group consisted of 21 cases that underwent heart valve surgery and with low Gensini and Syntax scores (cut-off value <8 for both). The peri-coronary epicardial adipose tissues (EAT) samples were obtained from 34 of the 69 cases, of which nine cases were in the low score group and 25 cases in the high score group.

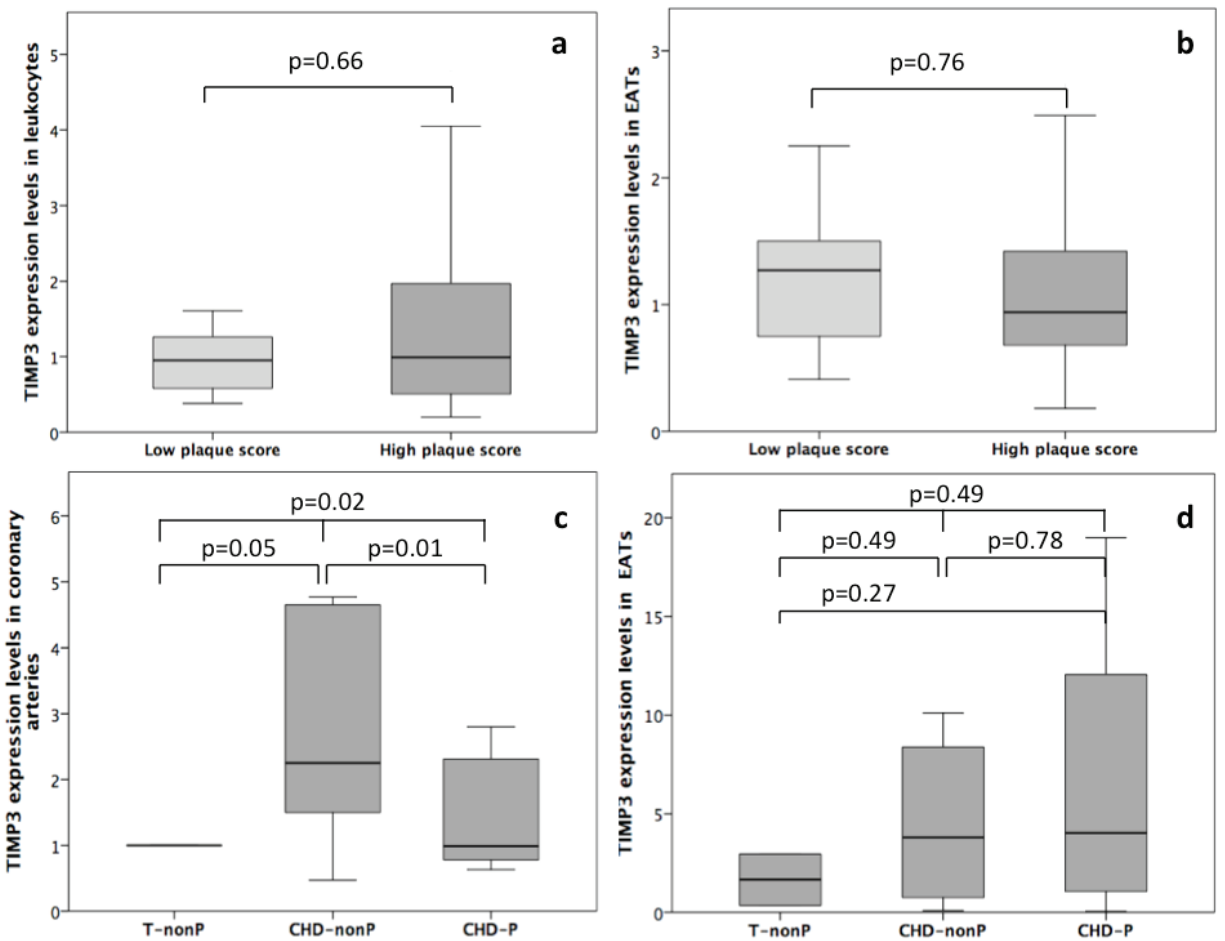

Figure 2: The comparison of *TIMP3* expression levels in study groups. *TIMP3* expression levels in leukocytes (a) and peri-coronary Epicardial Adipose Tissues (EAT) (b) according to plaque scores were shown. In post-mortem, *TIMP3* expression levels in coronary arteries (c) and peri-coronary EAT (d) were compared in tissues with advanced plaque (CHD-P) and without plaque (CHD-nonP) of cases with coronary heart disease (CHD) and non-cardiac trauma (T-nonP). Kruskal-Wallis test was used to make a comparison among three groups. Mann Whitney-U test was used to compare two groups. Mean±standard deviation of the expression levels was shown as the box-plots.

The expression levels of *TIMP3* were analysed in both leukocytes and peri-coronary EAT according to plaque scores. *TIMP3* expression levels of circulating leukocytes were not found statistically different in the high plaque score group (1.47±1.33, n=48) compared to the low plaque score group (1.08±0.70, n=21) (p=0.66) (Figure 2a). Moreover, *TIMP3* expression levels in peri-coronary EAT samples did not show any difference (p=0.76) between two groups. The expression levels of the low and high plaque score groups were 1.18±0.59 (n=9) and 1.29±0.99 (n=25), respectively (Figure 2b).

TIMP3 mRNA expression levels in coronary arteries and peri-coronary EAT of the post-mortem cases

The coronary artery and peri-coronary EAT samples of post-mortem cases were investigated for *TIMP3* mRNA expression levels. The expression levels of *TIMP3* in post-mortem coronary arteries showed statistically significant differences between three groups (2.99±2.62, n=25 for CHD-nonP group, 1.46±1.14, n=26 for CHD-P group and 1.13±0.32, n=4 for T-nonP, p=0.02) (Figure 2c). *TIMP3* expression was significantly higher in coronary artery segments without atherosclerotic plaques (CHD-nonP group) as compared to advanced plaques (CHD-P group) (p=0.01) and also non-atherosclerotic coronary arteries (T-nonP) (p=0.05) (Figure 2c). The expression levels of *TIMP3* in post-mortem EAT samples were shown in Figure 2d. The expression levels of *TIMP3* in peri-coronary EAT samples of CHD-P group (6.21±6.46, n=10) were found 1.4 fold higher compared to EAT samples of CHD-nonP group (4.53±3.85, n=10) and 3.8 fold higher compared to EAT samples of T-nonP group (1.65±1.86, n=2); however, both were statistically non-significant (p=0.27 and p=0.78, respectively). *TIMP3* expression levels in EAT samples were not comparable among three groups (p=0.49) (Figure 2d).

TIMP3 protein detection in coronary arteries of the post-mortem cases

Hematoxylin-Eosin (HE) staining was used in serial sections to determine the histological classification of the coronary arteries with and without plaque. Two randomly selected coronary arteries obtained from the non-cardiac traumatic cases (T-nonP group) and the cases with coronary heart disease (CHD-nonP group) were determined to have histologically normal morphology. Obvious differences between the arterial walls were shown as normal histological morphology (Figure 3a) and advanced lesion atherosclerotic morphology (Figure 3d). TIMP3 protein localization (Figure 3b) had a distribution that was similar to smooth muscle specific α-actin (Figure 3c) in medial and intimal smooth muscle cells of the non-atherosclerotic segments of coronary arteries. Although the TIMP3 protein was not stained as high as the α-actin signal, it was detected in all atherosclerotic plaque and normal arterial sections in areas of smooth muscle cells. On the other hand,

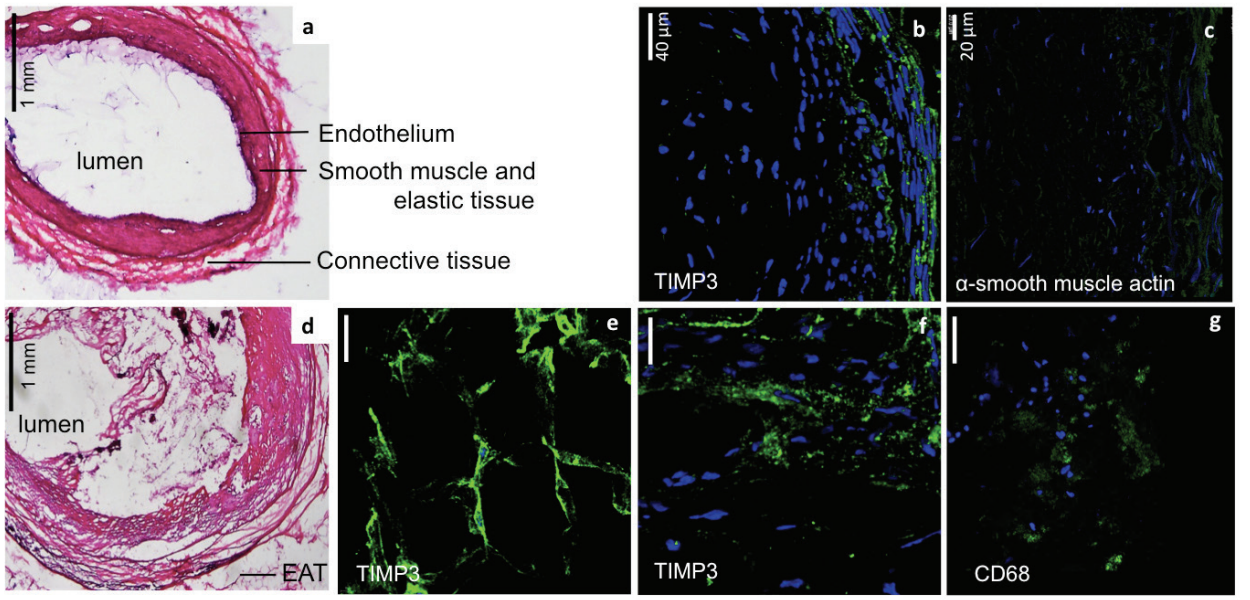

Figure 3: Immunostaining images of TIMP3 in EAT, normal and atherosclerotic artery of the post-mortem samples. Hematoxylin-Eosin (H-E) stained sections of normal artery (T-nonP group) (a) and artery with advanced atherosclerotic plaque (CHD-P) (d) were shown; pink shows the cytoplasmic area and blue represents the nuclei of cells in the artery sections. Post-mortem tissue samples were analyzed to determine specific proteins by staining Alexa Fluor 488 (green) and FITC (green) and also nuclei by staining DAPI (blue). TIMP3 was determined in normal arteries (b), in adipocytes of EAT (e) and in CHD-P (f). Smooth muscle specific α-actin was shown in normal arteryies (c). Macrophage specific CD68 was determined in CHD-P arteries (g)

in all sections obtained from CHD-P cases, the TIMP3 protein signals (Figure 3e and 3f, respectively) were observed at similar intensity than CD68 signal (Figure 3g) around the peri-coronary adipocytes and in macrophages-rich regions within fibrous plaques of advanced atherosclerotic arteries. Immunostaining results for localizations and distributions of all proteins were evaluated comparatively using serial sections obtained from the same tissue of each case.

DISCUSSION

In this study, evidence of reduced *TIMP3* expression in advanced atherosclerotic plaques when compared to histologically normal arterial segments obtained from post-mortem cases with coronary heart disease was demonstrated. In addition, TIMP3 protein was determined in macrophage-rich regions within fibrous plaques of atherosclerotic arteries. These results may indicate that higher macrophage density in advanced atheroma is associated with decreased *TIMP3* gene expression. Aso, although TIMP3 protein was shown around peri-coronary adipocytes for the first time in this study, it was determined that *TIMP3* expression levels in peri-coronary EAT samples and leukocytes had no effect on the severity of atherosclerosis.

Monocytes and macrophages have the crucial immunological roles in atherosclerotic plaque development (19). The circulating monocytes differentiate to macrophages, which migrate to the site of inflammation and disrupt extracellular matrix (ECM) barriers (20). In atheroma plaques, differentiated macrophages are most abundant cells that are formed by migration of circulating monocytes. Systemic insulin resistance and subclinical atherosclerosis was shown to be in association with decreased *TIMP3* expression in circulating monocytes (21). In this study, it was found that *TIMP3* expression levels did not show a statistically significant difference in circulating leukocytes between the plaque score groups. Since monocytes were not separated from peripheral blood leukocytes in this study, a similar association between expression levels of TIMP3 and severity of atherosclerosis could not be determined.

In studies on human advanced plaques, abundant macrophages were observed at the necrotic core and rupture-prone shoulders of the plaques (9). Decreased *TIMP3* expression was characterized by the proliferation of foam cell macrophages (FCMs), which are responsible for atherosclerotic plaque development (9, 10, 21, 22). Decreased *TIMP3* expression mainly was found in intimal macrophages that was previously reported to have higher MMP activities (9). In addition, decreased TIMP3 activity has been found to be associated with increased MMP9 level in the atheroma that is known to be a potential effect for plaque instability (23). In this study, as in previous results, *TIMP3* gene expression levels were decreased in atherosclerotic plaques and also TIMP3 protein was localized in macrophage-rich regions of atheroma. Elevated *TIMP3* expression level in rupture-prone sites of the atherosclerotic plaque has been claimed to play a protective role against plaque rupture (7, 24). In a previous study, TIMP3 protein levels in advanced human atheroma obtained from carotid endarterectomy have been shown to decrease compared to fibrous plaques with a small or absent lipid core (24). In this study, TIMP3 protein localization was confirmed by immunostaining in regions rich in SMC and macrophages. However, protein levels could not be compared between groups. Also, down regulated expression of *TIMP3* has been found in arterial tissues enriched with monocyte/macrophages of patients with metabolic and inflammatory diseases like Type 2 Diabetes Mellitus (10, 21). Other studies also support decreased *TIMP3* expression levels in macrophages differentiated to FCMs (9, 10, 22). Similar to these studies, in the post-mortem samples of this study, two fold decreased *TIMP3* expression was found in advanced atheroma plaque (CHD-P) compared to coronary artery segments without plaque (CHD-nonP). On the other hand, the cases in the T-nonP group were younger and had lower BMI than the cases with CHD. The reason for the lower *TIMP3* expression levels in the normal arteries of this young subject group (mean age 29.3 years) compared to the arteries of both CHD groups might be due to the early onset of the atherosclerotic process or to lifestyle and other unknown metabolic conditions.

It has been shown that EAT causes progression of atherosclerosis and finally cardiac complications (13, 14). EAT thickness and volumes are closely associated with coronary artery disease, metabolic syndrome and insulin resistance (14, 15, 22, 25-27). In addition, in post-mortem and clinical studies, increase in the size of adipose tissue around the atherosclerotic coronary artery was demonstrated (28-30). In the present study, the peri-coronary EAT volume in patients with CHD was higher than the non-atherosclerotic cases. In particular, adipose tissue has a crucial regulatory role for the development of atherosclerosis and provides micro-environmental homeostasis in vasculature (12). It has been reported that *TIMP3* expression decreases during adipocyte differentiation, but increases when cell differentiation is disrupted (11). In a study, it has been shown that overexpression of *TIMP3* in macrophages within white adipose tissue of transgenic mice protects from metabolic inflammation and is related to metabolic disorders such as diabetes and non-alcoholic steatohepatitis (31). However, the contribution of *TIMP3* expression level in peri-coronary EATs to the development of atherosclerosis is unknown. For the first time in this study, the relationship between *TIMP3* expression levels and atherosclerosis was investigated in peri-coronary EATs, but no statistically significant association in both post-mortem and living cases was demonstrated. In addition, TIMP3 protein using immunofluores-

cence technique in peri-coronary adipocytes surrounding advanced atherosclerotic arteries was demonstrated for the first time in this study. As a result, differences in TIMP3 expression profiles in adipocytes within peri-coronary EAT might not significantly contribute to the development of atherosclerosis.

Limitations

The coronary artery plaque samples from living cases using endarterectomy have not been collected for comparison with the post-mortem artery samples. *TIMP3* expression differences could not be investigated as monocytes were not isolated from circulating blood samples of living cases. In this study, data on medication use of the cases were not available. Other limitations of this study include heterogeneity of atherosclerotic plaque characteristics, difficulty of obtaining standard post-mortem tissues, and unknown lifestyles of post-mortem cases. Limitations of using post-mortem samples depend on many conditions such as storage time, temperature, time until it is frozen, pH, thawing, and pain level of death etc. Moreover, lifestyle of the cases also has effects on results such as physical exercise, legal/illegal substance use, and their diet (32). In this study, after the autopsy, a detailed death report was examined, extent of eligibility criteria was assessed, and standard time was applied for tissue supply. The number of young post-mortem non-cardiac trauma cases investigated is a major limitation of this study, and the *TIMP3* expression result of this group could not be discussed since there is no comparison publication on *TIMP3* expression levels in arterial tissues of different age groups. And also, interactions of TIMP3 with other MMPs could not be investigated in this study.

CONCLUSION

TIMP3 expression is significantly decreased in advanced atherosclerotic plaques. Increased *TIMP3* expression levels in normal coronary arterial segments of cases with CHD indicate that TIMP3 plays a protective role in development of atherosclerosis at the molecular level in these arterial areas. Although the association of *TIMP3* expression levels with the severity of atherosclerosis in circulating leukocytes and peri-coronary epicardial adipose tissue could not be demonstrated in this study, in the future, determination of differences in *TIMP3* expression and other interacting extracellular matrix proteins in circulating monocytes and other vascular cells involved in atheroma plaque development will help to understand atherosclerotic pathogenicity. Finally, in this study, the *TIMP3* expression level, which has therapeutic significance, on different types of tissues and cells was investigated with the same workflow, and these results were presented. The balance between MMPs and their inhibitors might be possible with exogenous administration of TIMP3 to ensure plaque stability in advanced atheroma plaque arteries in the future.

Ethics Committee Approval: TThis study was approved by the-Clinical Research Ethical Committee of the Istanbul University, Istanbul Faculty of Medicine (Date: 27.10.2011, No: 1822 and Date: 17.01.2014, No: 160) and Scientific Research Commission of Council of Forensic Medicine (Project number and date: B.03.1.ATK.0.01.00.08/863, 28.12.2010).

Informed Consent: Written consent was obtained from the participants.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- E.K.B., C.E.Y., M.Y.; Data Acquisition- F.G.G., D.Y., D.Ö., C.E.Y., M.Y., D.Ö., M.C., E.K.B.; Data Analysis/Interpretation- G.Ç., E.K.B., M.C., C.E.Y., M.Y.; Drafting Manuscript- G.Ç., E.K.B.; Critical Revision of Manuscript- E.K.B., M.C., C.E.Y., M.Y.; Final Approval and Accountability- G.Ç., F.G.G., D.Y., D.Ö., C.E.Y., M.Y., D.Ö., M.C., E.K.B.

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İSTANBUL ÜNİVERSİTESİ YAYINEVİ

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A NOVEL TRAINING ALTERNATIVE IN ORTHOGNATHIC MANDIBULAR OSTEOTOMY: AIR DRIED CLAY MODEL

ORTOGNATİK MANDİBULA OSTEOTOMİSİNDE YENİ BİR EĞİTİM SEÇENEĞİ: HAVA KURUTMALI KİL MODELİ

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ABSTRACT

Objective: Patient safety and low complication rates are indispensable in surgical training and models are among the main educational tools. The aim of this study is to assess the efficiency of a novel model for orthognathic mandibular osteotomy.

Material and Methods: A template and seventeen partial mandibular models (MM-17) were manufactured with air dried clay. The dimensions of the models were feasible for sagittal split ramus osteotomy (SSRO). Model surgery was performed by surgeons with a minimum of three years' experience in orthognathic surgery. Each surgeon operated four separate models and the following data were recorded: corticotomy and SSRO completion time, MM-17 fracture type, similarity value of MM-17 with native mandible, representation value of MM-17, and the training compatibility value of MM-17.

Results: The cost was 0.6 American Dollars. The mean corticotomy time was 126.75 seconds (110-150). Mean cortical resistance similarity value was 8.75 (8-10). The mean SSRO time was 288 seconds (205-401). Sixty percent of the fractures were seen in the outer cortex. The mean medullary resistance similarity value was 5 (4-6) and mean mandibular representation value was 5.25 (4-7). The training compatibility value was 8.25 (7-10).

Conclusion: Air dried clay demonstrated mechanical similarities with bone cortex and it was used for mandibular modelling

ÖZET

Amaç: Hasta güvenliği ve düşük komplikasyon oranları cerrahi eğitimde olmazsa olmazdır ve modeller temel eğitim yöntemleri arasındadır. Bu çalışmanın amacı, ortognatik mandibula osteotomisinde yeni bir modelin etkinliğini değerlendirmektir.

Gereç ve Yöntemler: Hava kurutmalı kilden bir şablon ve 17 kısmi mandibula modeli (MM-17) üretildi. Modellerin boyutları sagittal split ramus osteotomisine (SSRO) uygundu. Model cerrahisi ortognatik cerrahide en az üç yıllık deneyimi olan dört cerrah tarafından yapıldı. Her cerrah dört ayrı modelde çalıştı ve şu değerler kaydedildi: kortikotomi ve SSRO tamamlanma süresi, MM-17 kırığı ve türü, MM-17'nin mandibula ile benzerlik değeri, temsil değeri ve eğitim uygunluk değeri.

Bulgular: Maliyet 0,6 Amerikan Doları'ydı. Ortalama kortikotomi süresi 127,75 saniyeydi (110-150). Ortalama korteks direnç benzerliği değeri 8,75'ti (8-10). Ortalama SSRO süresi 288 saniyeydi (205-401). Kırıkların yüzde altmışı dış korteksteydi. Ortalama medulla direnç benzerlik değeri 5 (4-6) ve ortalama mandibula temsil değeri 5,25'ti (4-7). Eğitim uygunluk değeri 8,25'ti (7-10).

Sonuç: Hava kurutmalı kil, kemik korteksi ile mekanik benzerlikler göstermektedir ve mandibula modeli üretiminde ilk kez kullanılmıştır. Ayrıca, MM-17 diğer modellerden daha ucuzdur. Kortikotomi ve SSRO tamamlanma süreleri kısadır. Çünkü dissek-

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for the first time. MM-17 cost less than other devices. Corticotomy and SSRO completion times were short due to the lack of dissection and bleeding. Despite its drawbacks in SSRO, MM-17 is a versatile and low cost alternative in orthognathic mandibular corticotomy training. High power drill utilization skills may be gained with MM-17 before clinical practice.

Keywords: Air-dried clay, mandible, model surgery, orthognathic surgery, training

siyon ve kanama gibi hastaya bağlı etkenler yoktur. SSRO'daki yetersizliklerine rağmen MM-17 ortognatik mandibular kortikotomi eğitiminde çok kullanışlı ve düşük maliyetli bir seçenektir. Yüksek devirli motor kullanımı becerileri klinik uygulama öncesi MM-17 ile edinilebilir.

Anahtar Kelimeler: Eğitim, hava kurutmalı kil, mandibula, model cerrahisi, ortognatik cerrahi

INTRODUCTION

Orthognathic surgery is one of the main aspects of plastic reconstructive and aesthetic surgery. The management of dentofacial deformities that may be present in dental and facial contours is planned and performed by collaboration between orthodontists and plastic reconstructive and aesthetic surgeons. After appropriate orthodontic treatment, patients may be referred for orthognathic surgery (1). Double jaw surgery may be performed in order to address the deformities in both the maxilla and mandible whereas single jaw surgery is indicated for deformities involving only the mandible (2).

Sagittal split ramus osteotomy, first defined by Obwegeser and Trauner in 1955 is currently the most popular mandibular osteotomy (2, 3). Various authors including Hunsuck and Epker modified this osteotomy in order to adapt it to modern practice (4, 5).

Due to its anatomical structure, the mandible is suitable to be split through the sagittal plane from both rami. However, such splitting is technically demanding and it requires a level of expertise (1). Orthognathic surgery is not performed equally in quantity throughout the training facilities of Turkey and the number of orthognathic operations is insufficient in some centers (6). All surgical training begins in a clinical manner and novice and inexperienced surgeons, as they go through a learning curve, may perform procedures that result in complications . In order to overcome such shortcomings and to gain expertise, surgical residents must be trained on surgical models. Today, highly technological computer-based planning and simulation is utilized in orthognathic surgery and the training is supported by virtual reality and touch sensitive (haptic) devices (7, 8). However, such techniques require both expensive hardware and software and such an infrastructure is lacking in most training facilities.

With the help of a novel, low cost, air- dried clay model, the inexperienced surgeons may improve their skills using high-power surgical devices and progress to a level where they can perform actual surgical procedures.

The aim of this study is to assess the efficiency of this model in basic orthognathic mandibular osteotomy training.

MATERIAL AND METHODS

The study was presented to the local Ethic Committee in April 2019 and approval was not deemed necessary. In fact, the study was performed neither on humans nor other live subjects.

After the molding process, the air-dried clay (Hardpas, Argiles Bisbal, Spain) can harden without any extra treatment in 24 to 48 hours (9). In order to maintain standardization, an air- dried clay template was manufactured out of an artificial human mandibular model (1020159 [A20], 3B Scientific, USA) (Figure 1). The template was prepared to enable the production of partial mandible models that allowed sagittal split ramus osteotomy.

Figure 1: In order to maintain standardization of the model, an air dried clay template was manufactured out of an artificial human mandibular model (1020159 [A20], 3B Scientific, USA)

The template was filled with 75 grams of air dried-clay for each model and partial mandibles were produced. Each model was dried at room temperature for two days and they were weighed at the end of the second day. The mean mass of the models was 68 grams (65–70 grams). A total of 17 models were produced and they were named "Mandibular Model–17" (MM-17) (Figure 2).

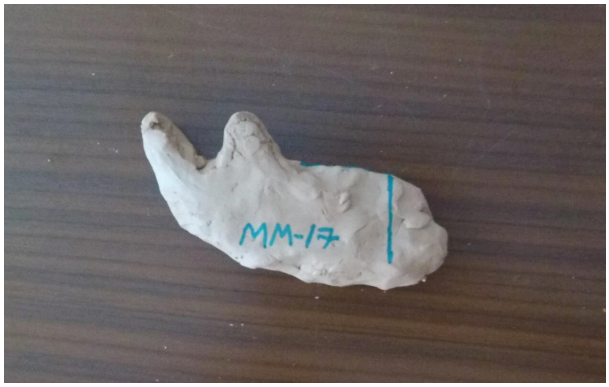

Figure 2: "Mandibular Model-17" (MM-17). The osteotomy lines were marked on the medial, lateral and upper marginal cortices on each model with a surgical marking pen.

Four plastic reconstructive and aesthetic surgeons with at least three years' experience in orthognathic surgery were invited to participate in the study by post . In order to demonstrate the surgical procedure, the first author performed the model surgery while the four surgeons observed. Demonstrative corticotomy and sagittal split ramus osteotomy were performed and the instructions were given to the surgeons. Each participant was given four MM-17's, a high-power drill and a cutting handpiece and they performed model surgeries individually. The participants were asked to operate on four models in order to increase the reliability of the study. The following data were recorded for each model: Corticotomy and SSRO completion time, MM-17 fracture type and time during corticotomy and SSRO, resistance similarity value of MM-17 cortex and medulla with native mandible, mandibular representation value of MM-17, and training compatibility value of MM-17. Resistance similarity and representation values were subjective measurements. A score of "0" represented no similarity and "10" total similarity and representation between MM-17 and native human mandible. Training compatibility value was another subjective measurement with a similar scale. Zero was total incompatibility whereas ten was total compatibility with orthognathic mandibular osteotomy training. The mean values were calculated and the results were compared. Corticotomies of the entire cortex without fractures were accepted as successful and complete bipartite sagittal split ramus osteotomies without fractures were accepted as successful.

RESULTS

The human mandibular anatomic model (1020159 [A20], 3B Scientific, USA), the high-power drill and the cutting handpiece belong to our institution and they were not included in the study costs. The only expense of the study was the air-dried clay (Hardpas, Argiles Bisbal, Spain) that was used for the production of both the template and the model. After the production of two templates and seventeen MM-17's, the cost of a single MM-17 was calculated as 0.6 American Dollars.

The results are listed in Table 1. All corticotomies were completed successfully (16/16) and no fractures were observed during the corticotomies (Figure 3). Mean corticotomy completion time was 127.75 seconds (110–150 seconds). Mean cortical resistance similarity value was 8.75 (8–10). Only three sagittal split ramus osteotomies were completed successfully (3/16) (19%) (Figure 4). The

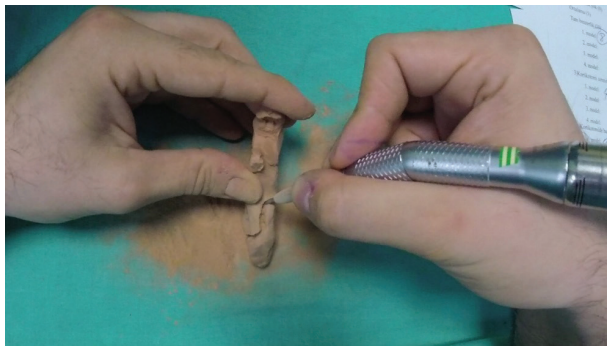

Figure 3: A successful MM-17 corticotomy.

Table 1: The mean values and results obtained from each surgeon after the model surgeries

	CCT (seconds)	CSV	SSROCT (seconds)	SSROSV	SSRO failure	RV	TCV
Surgeon 1	110	8	401	5	3	5	9
Surgeon 2	150	10	0	5	4	4	7
Surgeon 3	116	8	259	6	3	7	10
Surgeon 4	131	9	205	4	3	5	7
Mean	126,75	8,75	288	5	13 failures (81%) 3 success (19%)	5,25	8,25

CCT: Corticotomy completion time; CSV: Corticotomy similarity value; SSROCT: Sagittal split ramus osteotomy completion time; SSROSV: Sagittal split ramus osteotomy similarity value; SSRO failure: Sagittal split ramus osteotomy failure; RV: Representation value of MM-17; TCV: Training compatibility value of MM-17.

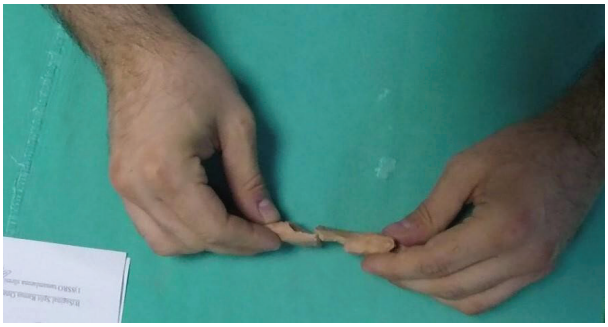

Figure 4: A successful MM-17 sagittal split ramus osteotomy.

outer cortex was affected in sixty percent of the fractures and the inner cortex was affected in forty percent of the fractures. Three successful SSRO's were completed in a mean time of 288 seconds (205-401 seconds). Mean medullary resistance similarity value was 5 (4–6). Mean mandibular representation value of MM-17 was 5.25 (4–7). Mean value of compatibility with orthognathic mandibular osteotomy training was 8.25 (7–10).

No technical problems were observed in either the high-power drills or the handpieces (Figure 5).

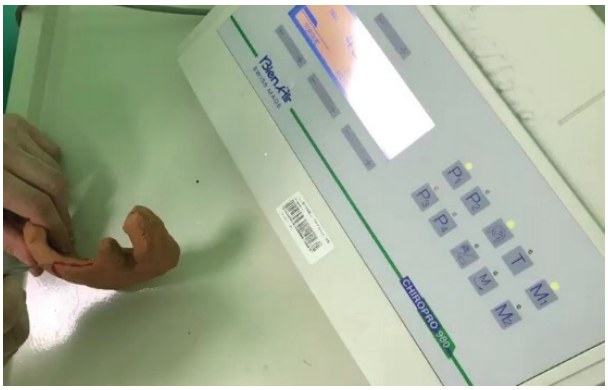

Figure 5: A high power drill that was utilized in the model surgery.

DISCUSSION

Structural deformities of the maxilla and the mandible may cause dental misalignment, malocclusion and alterations in facial appearance. Such deformities may be corrected with orthognathic surgery, ameliorating both form and function (2).

In sagittal split ramus osteotomy, the osteotomy of the medial cortex of the ramus is performed above the lingula and the lateral corticotomy is performed between the first and the second molar teeth. Finally, the upper margin of the ramus is corticotomized in order to connect the medial and the lateral corticotomy lines. After the corticotomies, a full-thickness osteotomy is performed in order to split the ramus into condylar and alveolar fragments. At the end of bilateral sagittal split ramus osteotomies, two condylar and one alveolar fragments are formed and the alveolar fragment may be positioned in three planes according to the orthodontic plan (1-5).

Sagittal split ramus osteotomy (SSRO) is prone to complications including unfavorable fractures, unfavorable splits, inferior alveolar and facial nerve injuries and internal maxillary artery injuries (10). The avoidance and management of these complications require technical experience in SSRO.

Although there are numerous plastic reconstructive and aesthetic surgery training institutions in Turkey, orthognathic surgery training is still limited and there are vast differences between centers (6). The main reasons for this limitation are requirements of coordination with orthodontics clinics and of specialized surgical instruments (2). In modern surgical training of the residents, maximal patient safety and minimal complication rates are basic principles (11). However, due to the aforementioned limitations, most residents can not have sufficient orthognathic surgical training in line with these principles (6). In order to maintain patient safety with minimal complications, orthognathic surgical models may be utilized.

Residents of all surgical fields may gain relevant skills with surgical models and they may support their theoretical knowledge before clinical practice. Palter et al. compared training results with low cost artificial models for fascial repair and the residents who had practiced with the models had a better surgical ability and understanding of the surgical procedure in the operating room (11). Easily reproducible and low cost models such as MM-17 may yield similar results in orthognathic surgery training. Plastic Reconstructive and Aesthetic surgery residents may gain relevant skills with MM-17 and continue clinical practice with more safety and fewer complications.

Cadavers and various artificial materials are regarded as the gold standard in all surgical training models (8). However, they may not be feasible for every training institution due to cost and availability. Three-dimensional virtual and solid models can be manufactured using thin sliced maxillofacial computed tomographs and model surgery can be practiced on such models (7). Currently, virtual reality is a popular alternative in orthodontic planning and orthognathic surgery training and it may be supported by touch-sensitive haptic simulators (12). With such simulators, the resident may visualize the relevant anatomic area with three-dimensional, real time detail through virtual reality and hand-held or wearable haptic devices may reflect the alterations in the target tissue (8, 13, 14). All such surgical models require specialized computer software, three-dimensional printers

and haptic receptors with very high costs. In contrast to these current and expensive models, the MM-17 cost less than an American dollar. Also, real time instrumental experience and skills may be gained during the model surgery with MM-17.

Various materials may be utilized in the production of sagittal split ramus osteotomy (SSRO) artificial models. Baccarin et al. compared three different materials in a model study that evaluated osseous fixation after SSRO: plastic, polyamide and polyurethane (15). None of the materials were biomechanically equivalent to the human mandible in aspects of elasticity, texture and load bearing capacity. All models were found to be relevant only for the simulatory training and for the preparation for clinical practice (15). The ideal material has not been found for the production of mandibular models. The air-dried clay of MM-17, which was utilized for the first time, needs to be evaluated objectively with biomechanical studies.

The literature has a limited number of studies on surgical models produced with air-dried clay. Kazum et al. designed a bone drilling model for orthopaedics surgery residents (16). In this design, the air-dried clay layer was attached to the posterior cortex of an artificial bone and the residents were asked to drill holes through the artificial bone. After the drilling process, the air dried-clay layer was checked for evidence of overdrilling through the posterior cortex and into this clay layer. Kazum et al. designed this study with respect to the similarity between the resistance and texture of the artificial bone cortex and the air-dried clay (16). In accordance with this study, successful corticotomies were performed on each MM-17 and the participants of the study evaluated the MM-17 cortex with a mean similarity value of 8.75 out of 10. According to the similar results of these two studies, air- dried clay represented the bone cortex effectively.

The corticotomy and sagittal split ramus osteotomy completion times were shorter than live surgery. These differences were due to the lack of soft tissue dissection, retraction, bleeding and assistance during the model surgery. Bleeding and the control of bleeding are major factors that prolong operative times. The SSRO success of MM-17 was 19% and numerous osteotomy failures, including outer cortical fractures, were seen during the model surgery. The main reason for these failures was the absence of osseous medulla in the uniformly constituted cortex MM-17 models . Therefore, the participants did not report the same procedural success for SSRO as the corticotomy and the SSRO similarity value was 5 out of 10. Also, the mandibular representation value of MM-17, measured at 5.25 out of 10, was lower because of the absence of osseous medulla. This important detail is a limitation of this surgical model. Also, the ramus of the model is slightly thicker than normal mandible. Due to this limitation, the model may be regarded as a simple tool for beginners. New designs may be required for completing the learning curve of inexperienced surgeons.

Mandibular corticotomies, which are performed with specialized instruments such as high-power drills, are the first steps of a successful orthognathic surgery. Experience and skills with such instruments should be gained before operating on real patients. Four experienced orthognathic surgeons evaluated the training compatibility value of MM-17, reporting a mean of 8.25 points out of 10. Thus, the learning curve of corticotomy with specialized instruments may be completed on MM-17 models.

Not every resident has the opportunity to train in an orthognathic surgery performing clinic, and clinical practice may not be possible for every surgeon. MM-17 may address the basic needs of a novice orthognathic surgery resident with its low cost and versatility in mandibular corticotomy.

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İSTANBUL ÜNİVERSİTESİ YAYINEVİ

RESEARCH / ARAŞTIRMA
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EVALUATION OF OUTCOMES OF PRIMARY PSEUDOPHAKIC RETINAL DETACHMENT SURGERY

PRİMER PSÖDOFAKİK RETİNA DEKOLMANI CERRAHİSİ SONUÇLARININ DEĞERLENDİRİLMESİ

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ABSTRACT

Objective: To evaluate the results of pars plana vitrectomy (PPV) alone and combined with circumferential scleral buckling (CSB) surgeries for aphakic or pseudophakic rhegmatogenous retinal detachment (RRD).

Materials and Methods: Thirty-seven eyes of 37 patients who underwent PPV (20 and 23 Gauge) alone or PPV combined with CSB due to pseudophakic or aphakic primary RRD were included in the study. Postoperative anatomical success (AS) and functional success (FS) were evaluated. The AS was defined as a completely flattened retina without any subretinal fluids after the removal of the silicone oil tamponade, if used. The FS was defined as two or more decimal improvements in the logMAR equivalent of Snellen visual acuity.

Results: The mean age of the patients was 62.43±11.40 (32-80) years, 21 (56.8%) patients were male, and 16 (43.2%) were female. The mean follow-up time was 21.35±16.86 (6-84) months. PPV combined with CSB were performed in 23 patients. AS was found to be 86.5% (32/37), FS was 49.9% (17/37). No statistically significant difference was observed in both AS and FS between the groups according to preoperative PVR presence (AS-p=0.61, FS-p=0.14), preoperative macular involvement (AS-p=0.98, FS-p=0.36), whether PPV combined with CSB (AS-p=0.97, FS-p=0.29), and the type of tamponade (p>0.05 in all).

Conclusion: PPV with or without CSB is safe and effective in cases with primary pseudophakic retinal detachment and achieves good AS without being affected by the presence of PVR or macular involvement. However, functional success may not always follow.

Keywords: Pseudophakic retinal detachment, pars plana vitrectomy, circumferential scleral buckling

ÖZET

Amaç: Afakik veya psödofakik regmatojen retina dekolmanı (RRD) için tek başına pars plana vitrektomi (PPV) ve çevresel skleral çökertme (ÇSÇ) ile kombine ameliyatların sonuçlarını değerlendirmek.

Gereç ve Yöntem: Psödofakik veya afakik primer RRD'ye nedeniyle tek başına veya ÇSÇ ile kombine PPV (20 ve 23 Gauge) uygulanan 37 hastanın 37 gözü çalışmaya dahil edildi. Postoperatif anatomik başarı (AB) ve fonksiyonel başarı (FB) değerlendirildi. AB, eğer kullanılmışsa silikon yağı tamponadının çıkarılmasından sonra hiç subretinal sıvının olmadığı tamamen yatışmış retina olarak tanımlandı. Ayrıca FB, Snellen görme keskinliğinin logMAR eşdeğerinde iki veya daha fazla ondalık artış olarak tanımlandı.

Bulgular: Hastaların ortalama yaşı 62,43±11,40 (32-80) yıl, 21'i (%56,8) erkek, 16'sı (%43,2) kadındı. Ortalama takip süresi 21,35±16,86 (6-84) aydı. 23 hastaya ÇSÇ ile kombine PPV uygulandı. AB %86,5 (32/37), FB %49,9 (17/37) olarak bulundu. Hem AB ile hem de FB ile; preoperatif PVR varlığı (AB-p=0,61, FB-p=0,14), preoperartif maküla tutulumu (AB-p=0,98, FB-p=0,36), PPV'nin ÇSÇ ile kombine olup olmaması (AB-p=0,97, FB-p=0,29), ve kullanılan tamponad tipi (tümü p>0,05) gibi durumlara göre oluşturulan gruplar arasında istatistiksel olarak anlamlı bir fark gözlenmedi.

Sonuç: Primer psödofakik retina dekolmanı olan olgularda PPV yalnız veya ÇSÇ ile kombine olarak uygulandığında, maküla tutulumundan veya PVR varlığından etkilenmeden iyi AB ulaşmak için etkili ve güvenlidir. Bununla birlikte, fonksiyonel başarı her zaman eşlik etmeyebilir.

Anahtar Kelimeler: Psödofakik retina dekolmanı, pars plana vitrektomi, çevresel skleral çökertme

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INTRODUCTION

Retinal detachment (RD) is a rare but severe complication of cataract surgery and, if left untreated, results in vision loss of the affected eye. Some researchers have pointed out that about 50% of RD develops within one year of cataract extraction surgery (1, 2). The incidence of RD after intracapsular cataract extraction surgery (ICCE) is 0.98-3.6%, 0.33-1.7% after extracapsular cataract extraction surgery (ECCE), and 1-1.17% after phacoemulsification. This incidence is eight times more than the normal population (3, 4). With the increase in the number of cataract surgeries performed in the last 20 years, the incidence of aphakic and pseudophakic rhegmatogenous RD (RRD) has also increased. Although it is known that with current phacoemulsification surgery, complications during and after surgery are reduced, retinal detachment is a complication that remains relevant, as it significantly reduces the patient's visual expectations. The number of people undergoing cataract surgery accounted for about 3% of the overall population, while 40% of patients with RRD had a history of previous cataract surgery. It is believed that removing the natural lens accelerates vitreous liquefaction, which causes premature posterior vitreous detachment and increases the risk of RD (2-4). The condition of the lens capsule also determines the vitreous liquefaction amounts. It is known that posterior lens capsule perforation during surgery and neodymium yttrium-aluminum-garnet (Nd-YAG) laser capsulotomy significantly increases RD incidence (5). Some potential risk factors are influential in RD development after cataract surgery. These factors were indicated as preoperative predisposing factors (myopia, lattice degeneration), intraoperative complications (posterior capsular rupture with or without vitreous loss), and postoperative factors (i.e., capsulotomy, vitreous hemorrhage, trauma) that are unrelated to the surgical procedure.

Evaluating fundus' appearance when examining pseudophakic RD patients may be tricky with opacification, reflections, and weak mydriasis. Circumferential scleral buckling (CSB), pneumatic retinopexy, primary pars plana vitrectomy (PPV) alone or with CSB are surgical options used to treat pseudophakic RD (6-8). The use of internal tamponades with PPV increases the success of this surgery (9). Although anatomical success rates are high after vitreoretinal surgeries, good visual outcomes are not always achieved as expected (10).

This study examined the clinical findings of primary RD in patients who developed RD after cataract extraction and underwent vitreoretinal surgeries in our clinic. Risk factors, surgery techniques and its results, and prognosis were evaluated.

MATERIALS AND METHODS

Approval was obtained from the Ethics Committee of Bezm-i Alem University Faculty of Medicine for this retrospective study. Informed consent forms were obtained from all patients in the study. The study was carried out in accordance with the tenets of the Helsinki Declaration.

Thirty-seven eyes of 37 patients who had been diagnosed with primary pseudophakic or aphakic RRD and had undergone PPV [20 or 23 Gauge (G)] with or without CSB between 2004 and 2011 were recruited to the study. This study evaluated demographic data, history of systemic and eye diseases, presence of myopia, time frame from cataract surgery to RD development, preoperative best-corrected visual acuity (BCVA), intraocular pressure (IOP), intraocular lens (IOL) condition, lens capsule integrity, and the presence of Nd-YAG laser capsulotomy, the condition of the retinal tears and detachment, the presence of macular involvement and proliferative vitreoretinopathy (PVR) were determined. Perioperative data [i.e., performing CSB and relaxing retinotomy-retinectomy, type of intraocular tamponade (IOT), complications], and postoperative data [BCVA, IOP, recurrence, anatomical success (AS), functional success (FS), and complications].

Exclusion criteria

Patients who underwent surgery due to recurrent detachment, underwent only scleral procedures without PPV, or were followed-up for less than six months were excluded.

Surgical technique

Standard PPV was performed, as under the wide-angle imaging systems, 20 G or 23 G (with trocar, transconjunctival) conventional three PPV ports were created. In the presence of advanced stage PVR, inferior retinal tears, or multiple tears in different quadrants, CSB was performed in the same session. ACCURUS ® Surgical System (Alcon, Fort Worth, Texas, USA) was used for vitrectomy. Standard surgical procedures were performed. Triamcinolone was used for visualizing posterior hyaloid and membranes. The vitreous base was shaved with indentation. Retinotomy/retinectomy was made when necessary. Sclerotomies were closed with 7.0 polyglactin sutures. As IOT, either 1000 cst silicone oil, 13% C3F8 gas, 18% SF6 or pure air were used to fill the entire vitreous cavity. If CSB was to be performed in the same session, limbal 360° peritomy was performed on the conjunctiva. A number 240 band was passed under the rectus muscles, and was sutured 13 mm away from the limbus with 5.0 ethibond in 4 quadrants. The tightening of the band was done after PPV. Conjunctiva was closed with 8.0 polyglactin sutures. After the surgery, rest in the appropriate position was recommended. In postoperative care, Tobramycin eye drops (four times a day for ten days), prednisolone acetate 1% drops (six times a day, tapered after one week and dis-

Table 1: Demographic and characteristic data of the patients

Variables	Results
Male/Female- n (%)	21 (56.8%)/16 (43.2%)
Mean age- (year)	62.43±11.40 (32-80)
Mean follow-up (months)	21.35±16.86 (6-84)
Duration after cataract surgery (months)	15.54±17.24 (3-72)
PPV alone/PPV with CSB- n (%)	23 (62.2%)/14 (37.8%)
PPV 20G/PPV 23G- n (%)	19 (51.4%)/18 (48.6%)
Type of cataract surgery (Phaco vs ECCE)- n (%)	36 (97.3%)/1 (2.7%)
Preoperative lens status (pseudophakic/aphakic)- n (%)	36 (97.3%)/1 (2.7%)
IOL localization (bag/sulcus/AC)- n (%)	28 (75.7%)/5 (13.5%)/3 (8.1%)

PPV: Pars plana vitrectomy, G: Gauge, Phaco: Phacoemulsification, ECCE: Extracapsular cataract extraction, IOL: Intraocular lens, AC: Anterior chamber

continued at the end of the one month) were prescribed. Antiglaucomatous drops were used if needed.

A detailed ophthalmological examination on the postoperative first day, first week, first, third, sixth months, and the following every six months were performed on all patients. BCVA values were measured with Snellen charts and converted into logMAR. The complications evaluated were corneal edema or opacity, hypotony (IOP ≤5 mmHg), elevated IOP (≥22 mmHg), fibrin formation, hyphemia, or vitreous hemorrhages. The surgeries performed between the study groups were compared in terms of AS, FS, BCVA, and complications. The AS was defined as a complete flattened retina without any SRF after removing the silicone oil (if used) in the final visit. Cases with two or more decimal improvements in the logMAR equivalent of Snellen BCVA were considered as FS.

The SPSS (Version 21, IBM Corp. Armonk, NY: USA) software statistic program was used for statistical analysis. The Wilcoxon Signed Ranks Test was used to compare descriptive statistics and the parameters that did not show normal distribution in the comparison of quantitative data. Fisher's Exact test and Chi-Square test was used to compare qualitative data. Pearson correlation analysis was used in correlation analyses. Statistically, significance was accepted as $p<0.05$.

RESULTS

Of the 37 patients included in the study, almost all had reduced vision at various levels at the time of admission. Photopsy-entopsy history was present in 6 (16.2%) cases, and 6 (16.2%) cases had a history of suspected head or ocular trauma.

High IOP was detected in 2 (5.4%) patients in preoperative examination. Five of the patients (13.5%) had a history of high myopia. Laser photocoagulation had been performed on two patients (5.4%) for retinal tears before the surgery. In 9 (24.3%) patients, there was a posterior lens capsule rupture and anterior vitrectomy history. A Nd-YAG laser capsulotomy history was also in 1 (2.7%) three months before RD. Three patients (8.1%) who had anterior chamber I OL had a peripheral iridectomy. Demographic data and characteristic distributions of patients a re summarized in Table 1.

Preoperative PVR was present in 10 (27.0%) patients, and PPV with CSB was performed on seven of these PVR cases. Posterior vitreous detachment (PVD) in 11 (29.7%), prominent inflammatory reaction in 7 (18.9%), and vitreous hemorrhage (VH) were present in 2 (5.4%) cases. Single retinal tears were detected in 23 patients and multiple retinal tears in 14 patients. The characteristics of retinal tears and RD are summarized in Table 2.

Table 2: Retinal tears and retinal detachment features

	n (%)
Numbers of retinal tear	
Multiple (3.3±1.1)*	14 (37.8%)
Single	23 (62.2%)
Localization of retinal tears	
Superior quadrants	9 (24.3%)
Inferior quadrants	8 (21.6%)
Both superior & inferior quadrants	20 (54.1%)
Involvement of retinal detachment	
Superior quadrants	9 (24.3%)
Inferior quadrants	8 (21.6%)
Both superior & inferior quadrants	20 (54.1%)

*: The average of only the multiple retinal tears patients

Table 3: Change of mean BCVAs over time

BCVA (logMAR)	Mean±SD	(min-max)	p*
Preop	2.41±1.01	(0.15-3.10)	
postop **1st week**	1.85±0.83	(0.40-3.10)	***0.035****
postop **1st months**	1.55±0.94	(0.30-3.10)	***0.001****
postop **3rd months**	1.42±0.76	(0.22-3.10)	***0.001****
postop **6th months**	1.38±0.77	(0.22-3.10)	***0.001****
postop **final examination**	1.21±0.85	(0.0-3.10)	**0.001****

*Wilcoxon Signed Ranks test, between preoperative mean values and postoperative mean values, preop: preoperative, postop: postoperative, **: p<0.001, BCVA: Best corrected visual acuity

In 75.6% of the patients, the final visit's BCVA values improved compared to the postoperative BCVA values. Significantly, the mean BCVA at all of the postoperative visits was better compared to the preoperative mean. The BCVA data before and after the surgery are summarized in Table 3.

The mean preoperative IOP was 13.04±4.11(6-30) mmHg. Although, in the postoperative first week, the mean IOP increased to 14.58±5.32 mm Hg, this increase was insignificant (p>0.05). However, in cases using an intraocular gas tamponade, a significant increase in IOP was observed. In the postoperative first month, the mean IOP was 17.04±7.94 mmHg, and in the sixth month, it was 15.78±5.12 mmHg; both were significantly high compared to the preoperative mean (both p<0.05). A high IOP was present in 7 (18.9%) patients during the postoperative period. Cyclophotocoagulation was performed in a patient whose IOP was not controlled despite all medical treatments. IOP control was achieved in all patients after the sixth month. The final visit mean IOP was 13.62±4.97 mmHg, with no significant difference between the preoperative mean (p>0.05).

In postoperative first day examinations, corneal epithelial defects were observed in four patients (10.8%), and corneal edema in 3 (8.1%) patients. All epithelial defects healed within the first week; even so, epithelial irregularity was prolonged until the third month in two patients. Prolonged corneal edema was observed in two patients but disappeared at the first-month examination.

Anterior chamber IOL was removed in 2 (5.4%) patients with anterior PVR whose vitreous base could not be shaved and visualized adequately. Relaxing retinotomy/retinectomy was performed in six of 10 PVR (+) cases. Drainage retinotomy was performed on three cases (8.1%). Two (5.4%) patients developed a limited amount of suprachoroidal hemorrhage as intraoperative complications.

Silicone oil extraction was performed in the postoperative period, through the anterior chamber from the aphakic eye and the pars plana in the pseudophakic eyes. Silicone oil was not removed due to the lack of postoperative light projection in one patient. During the follow-up, 4 (15.4%) of the 26 patients who underwent silicone oil removal developed recurrent RD (one patient on the first day, one in the 1st month, and two in the third month). Of the 11 patients treated by giving C3F8, SF6, or air, 1 (9.1%) patient given pure air developed recurrent detachment during the early follow-up. AS was achieved in 32 eyes (86.5%), and FS was achieved in 17 eyes (45.9%). The distribution of intraocular tamponade used is summarized in Table 4. Anatomical and functional success distribution is summarized in Table 5.

Table 4: Distribution of intraocular tamponade used intraoperatively

Tamponade	n	%
C_3F_8	4	10.8%
Air	1	2.7%
SF_6	6	16.2%
Silicone oil	26	70.3%

No statistically significant difference was observed in both AS and FS between the groups according to preoperative macular involvement (p=0.98, p=0.36 respectively), preoperative PVR presence (p=0.61, p=0.14 respectively), and whether PPV combined with CSB (p=0.97, p=0.24 respectively). There was no statistical significance in both AS and FS between the types of the IOT used (p>0.05 on both).

DISCUSSION

The risk of RD occurrence increases after cataract surgery. RD incidence is reported as 0.6%-1.7% in the first year after cataract surgery (11-13). Rowe et al. reported that the risk of retinal detachment was six times higher in those who underwent cataract surgery (14). Citirik et al. also stated that cataract surgery is the most common risk

Table 5: Distribution of the anatomical and functional success

Anatomical success		**Yes, n (%) total: 32/37 (86.5%)**	**No, n (%) total: 5/37 (13.5%)**	**++p**
Preop macular involvement	**Yes**	26 (86.6%)	4 (13.4%)	0.98
	No	6 (85.7%)	1 (14.3%)	
Preop PVR	**Yes**	8 (80%)	2 (20%)	0.61
	No	24 (88.8%)	3 (11.2%)	
PPV with or without CSB	**With**	20/23 (86.9%)	3/23 (13.1%)	0.97
	Without	12/14 (85.7%)	2/14 (14.3%)	
Functional success		**Yes, n (%) total:17/37 (45.9%)**	**No, n (%) total: 20/37 (54.1%)**	**++p**
Preop macular involvement	**Yes**	13 (43.3%)	17 (56.7%)	0.36
	No	4 (57.1%)	3 (42.9%)	
Preop PVR	**Yes**	7 (70%)	3 (30%)	0.14
	No	10 (37%)	17 (63%)	
PPV with or without CSB	**With**	11/23 (47.8%)	12/23 (52.2%)	0.24
	Without	6/14 (42.8%)	8/14 (47.2%)	

++Fisher's Exact and Chi-Square test between the groups in terms of the macular involvement, PVR presence, and whether PPV was combined with CSB; PVR: Proliferative vitreoretinopathy; CSB: Circumferential scleral buckling; preop: preoperative

factor for RRD, and 20-40% of RD patients had a history of cataract surgery (15). In our study, the time between cataract surgery and RD formation was an average of 15.5 months, and this period has been reported as 9.6-16 months in the literature (16, 17). In our study, 13.2% of patients had high myopia as the additional risk in pseudophakic RD etiology. Cankurtaran et al. also stated that if axial length is greater than 26 mm, the risk of pseudophakic RD is four times higher (18).

In our study, there was a lack of lens capsule integrity in preoperative 29% of patients. RD had developed with an average of 11.7 months after in the ruptured capsules. Like us, Citirik et al. also stated the importance of the posterior capsule integrity for pseudophakic RD etiology (15).

In pseudophakic RD, PPV is superior to scleral procedures; since PPV ensures that the vitreous opacities are cleaned, and subretinal fluid is drained in a controlled manner. Also, tears in the periphery can be found more easily. Moreover, there is no a clear crystalline lens and an accommodation capacity that should be protected in pseudophakic eyes (19). During the follow-up, 4 (15.4%) of the 26 patients who underwent silicone oil removal developed recurrent RD. It has been reported that the success rates of PPV alone and combined with CSB are between 88% and 100% (20-23). Bartz-Schmidt et al. reported that 94% AS with primary PPV achieved 100% with secondary PPV in pseudophakic RD (21). The AS ratio (86.5%) of primary PPV in our study is consistent with the literature. Dang Burgener et al. reported no difference in success rates and recurrences between primary PPV and PPV with CSB (19). Similarly, Kessner et al. stated no significant differences between both treatments in AS and FS rates for pseudophakic RD. We also observed no statistically significant difference (24). However, Joseph et al. reported better AS for PPV with CSB (92%) than PPV alone (84%), and reported similar visual outcomes (25).

Figueroa et al. reported that 6/12 and above BCVA was in 23% of eyes with macular involvement and 84.5% of eyes without macular involvement (26). Campo et al. reported a 65% preoperative macular involvement and that only 4% of these cases achieved 0.4 or more BCVA levels; however, these BCVA levels were 79% in cases without macular involvement (22). They also reported AS and FS rates as 86% and 62% in eyes with preoperative macular involvement and 91% and 82% in eyes without. However, we did not notice any statistical difference between those with or without preoperative macular involvement in AS and FS. This may be attributed to the unbalanced distribution of the groups with and without preoperative macular involvement and high preoperative BCVA value of the patients without involvement. Our AS rates are consistent with the literature, but FS is lower than reported in the literature, which can be explained by the preoperative macular involvement in most of our patients (81%).

However, in the present study, 75.6% of the patients, visual acuity improved compared to preoperative BCVA, but only 45.9% achieved FS criteria.

Dang Burgener et al. reported that the most critical factor affecting the surgical outcome is PVR (19). Çakır et al. reported a negative correlation between PVR grade and improvement of BCVA (27). Nevertheless, we observed no statistically significant difference between the PVR groups. This result may be explained as follows: PPV technique provides better shaving of the vitreous base in aphakic or pseudophakic eyes, better visualization of the peripheral retina, cleaning the membranes and bands, and flattening the retina by performing retinectomy/retinotomy more comfortably. Also, in advanced stage PVR, performing additional CSB and using a silicone oil tamponade provide better contact of retinal and retinal pigment epithelium and may improve the surgery results.

Temporary IOP elevation after PPV is a common complication, but it is rarely permanent. The increased IOP was reported 17.9%-48% after PPV with or without CSB in pseudophakic RD treatment (23, 28, 29). However, we observed an elevated IOP in 18.4% of patients, which is consistent with the literature. Angle-closure glaucoma after CSB may occur due to a forward rotation and congestion of the ciliary body, and elevated IOP could be controlled with medical treatment and/or laser iridotomy. Intraocular gas tamponades may cause secondary angle-closure glaucoma by expansion. Pupil blocks may occur due to silicone oil tamponade in aphakic eyes, or synechia in pseudophakic eyes. Also, the emulsified silicone passing into the anterior chamber (AC) or inflammatory cells in AC may give rise to obstruction of trabeculae, and formation of glaucoma (30).

This study's weaknesses are its retrospective design, low number of cases, imbalance in the distribution of preoperative subgroups (i.e., presence of PVR, involvement of macula), used IOT diversity, and a relatively short follow-up period. However, its strength is that it gives an idea about PPV surgery with or without CSB in treating pseudophakic RD. Prospective, randomized controlled trials with large case series and extended follow-ups will provide more accurate data.

CONCLUSION

According to the present study, PPV with or without CSB is effective and safe in primary pseudophakic RD cases. These treatments provide good (86%) anatomical success without being affected by preoperative PVR presence or macular involvement or the kind of endotamponade used. However, functional success may not always follow. The making-decision to surgical strategy (i.e., combining with CSB or the choice of IOT) should be made according to the eye's condition and RD's severity.

Ethics Committee Approval: This study was approved by the Ethics Committee of Bezm-i Alem University Faculty of Medicine (Date: 14.10.2009, No:10/12).

Informed Consent: Written consent was obtained from the participants.

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FIRST BRANCH OF OPHTHALMIC ARTERY AND ITS CLINICAL IMPORTANCE

ARTERIA OPHTHALMICA'NIN İLK DALI VE KLİNİK ÖNEMİ

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ABSTRACT

Objective: The ophthalmic artery and its branches have one of the most complex anatomy and embryology of all arteries. Although many studies have been done on its branches, different references have given different conclusions about the first branch of it. Therefore, it was aimed to investigate the first branch of the ophthalmic artery in this study.

Materials and Method: Forty-one orbits of the cadavers, which belonged to the Department of Anatomy of Istanbul Faculty of Medicine, were examined.

Results: It was observed that the first branch was the central retinal artery in 23 orbits (56%), the posterior ciliary arteries in 14 orbits (34%), the lacrimal artery in three orbits (7%) and the supraorbital artery in one orbit (3%).

Conclusion: It was observed that the first branch of the ophthalmic artery was the central retinal artery or posterior ciliary arteries substantially. Both arteries are essential for vision and are very important to be protected in surgeries involving this area.

Keywords: Ophthalmic artery, central retinal artery, posterior ciliary arteries, lacrimal artery

ÖZET

Amaç: Arteria ophthalmica ve dalları en karmaşık anatomiye ve embriyolojiye sahip arterlerden biridir. Bu arterin dallarıyla ilgili birçok çalışma yapılmış olsa da, ilk dalı hakkında farklı kaynaklarda farklı sonuçlar bulunmaktadır. Bu nedenle bu çalışmada arteria ophthalmica'nın ilk dalının araştırılması amaçlandı.

Gereç ve Yöntem: İstanbul Tıp Fakültesi Anatomi Anabilim Dalı'nda bulunan kadavralara ait olan 41 orbita incelendi.

Bulgular: Yirmi üç orbitada (%56) a. centralis retinae'nin, 14 orbitada (%34) aa. ciliares posteriores'in, üç orbitada (%7) a. lacrimalis'in ve bir orbitada (%3) a. supraorbitalis'in ilk dal olduğu gözlendi.

Sonuç: Arteria ophthalmica'nın ilk dalının yüksek oranda a. centralis retinae ya da aa. ciliares posteriores olduğu görüldü. Her iki arter de görme için temeldir ve bu bölgeyi ilgilendiren cerrahilerde bu arterlerin korunması oldukça önemlidir.

Anahtar Kelimeler: Arteria ophthalmica, arteria centralis retinae, arteriae ciliares posteriores, arteria lacrimalis

INTRODUCTION

The ophthalmic artery (OA) is the first intracranial branch of the internal carotid artery (ICA) (1). After originating from the ICA, it takes a short course in the cranium and then passes through the optic canal and enters the orbit. Here it gives branches that supply the eyeball, optic nerve (ON) and periophthalmic tissues such as eyelids, lacrimal gland and extraocular muscles.

Embryologically, the OA develops from three different arterial sources. Situations such as non-fusion of these arteries or persistence of the arteries that need to be regressed cause variations in the origin or branching of the OA, and these have been widely stated in the literature. These variations have been reported to pose a potential risk in craniotomy or endovascular treatment, such as obliteration of the middle meningeal artery, dural arte-

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riovenous shunt, tumor or epistaxis (2, 3). Therefore, the importance of OA anatomy has increased during the last decades for ophthalmologists, neurosurgeons and interventional neuroradiologists (4).

Branches of the OA are the central retinal artery (CRA), posterior ciliary arteries (PCA), lacrimal artery (LA), muscular branches, anterior and posterior ethmoidal arteries, palpebral arteries, supraorbital artery (SA) and its terminal branches, the dorsal nasal artery and frontal artery. It has been stated that CRA is the first branch of the OA with a frequency varying from 67% to 77.5% (1, 5). In cases where the CRA is the second or third branch, the probable first branch has been reported as the PCA (5). The CRA and PCA supply the retina and choroid respectively and therefore have been noted as critical in vision (1). For these reasons, it was aimed to investigate the morphology and morphometry of the OA and its first branch.

MATERIALS AND METHOD

The first branch of the OA was evaluated on 41 cadavers. All specimens were obtained from the Department of Anatomy of Istanbul Faculty of Medicine. All dissections were performed on cadavers used in undergraduate and post-graduate medical training. All cadavers were preserved with a formalin-ethanol-glycerin-phenol solution and kept in cold storage (5-8°C) after embalming.

The dissection process was carried out by preserving the integrity of the orbit and its internal structures, starting from the intracranial opening of the optic canal. When silicone injection was completed, the orbital roof and the bony structure of the optic canal were removed with precision and the orbit was reached (Figure 1). The OA

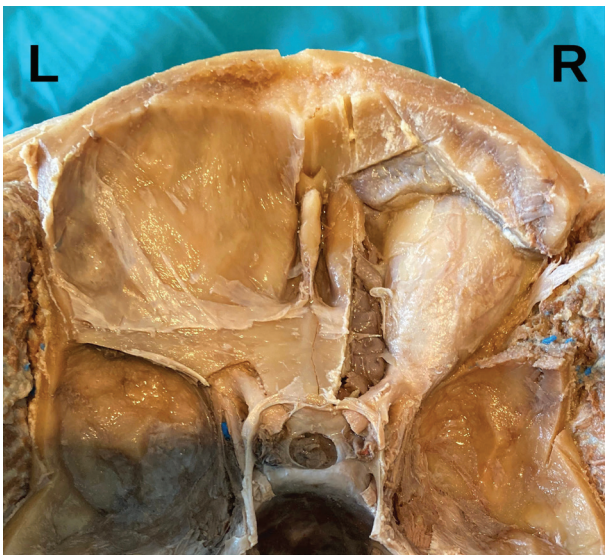

Figure 1: Exposition of orbita after removal of orbital wall
L: left, R: right

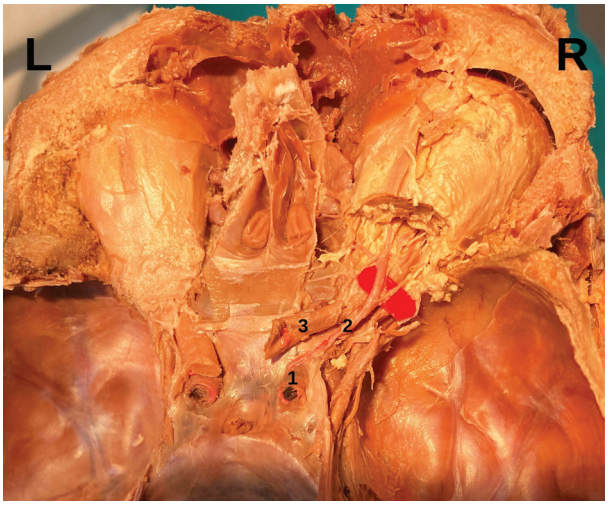

Figure 2: The dissection procedure of branches of ophthalmic artery
1: internal carotid artery, 2: ophthalmic artery, 3: optic nerve, L: left, R: right

and its branches and the ON were separated from other intraorbital structures by making delicate dissections, and were followed and recorded for each orbit until the OA gave its end branches (Figure 2). A total of 41 ophthalmic arteries were included in the present study of which 19 were bilateral and three were one-sided. The reason why those three arteries were studied unilaterally was that those three cadavers could not be evaluated bilaterally during dissection. The branches of the OA were evaluated after the colored latex silicone injection was made.

Ethical committee approval (Date: 16.04.2021, No: 172684) was obtained for our study.

RESULTS

In this study, the first branch was observed to be originating from the OA during its course in the orbital cavity. It was determined that the most frequent first branch of the OA was the CRA with a rate of 56% (n=23) (Figure 3). In total, 12 of the 23 CRA's were in the left orbit and 11 were in the right orbit. The CRA emerged as the first branch bilaterally in nine cadavers (totally 18), consequently the remaining five CRA were determined unilaterally in five cadavers. The second most frequent first branch was the PCA, observed in 14 orbits (34%) (Figure 4). Of these 14 arteries, eight were bilaterally (in four cadavers) and six were unilaterally traced as the first branch in dissected specimens. In those six cadavers, which had the PCA as the first branch of the OA unilaterally, on the contralateral side, the SA was the first branch in one case, the first branch could not be evaluated in one case and the CRA was the first branch in the remaining orbit (n=3). The third most common first branch was the LA with a rate of 7%

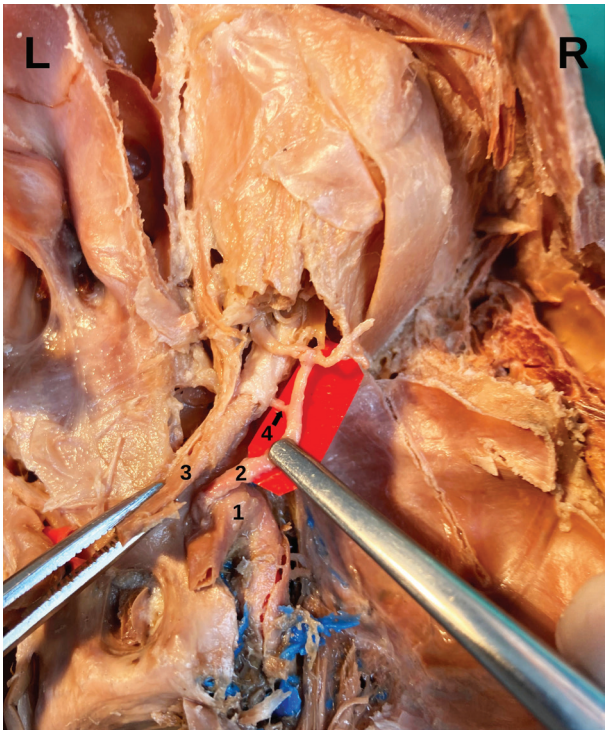

Figure 3: Exposition of central retinal artery as the first branch of ophthalmic artery
1: internal carotid artery, 2: ophthalmic artery, 3: optic nerve, 4: central retinal artery, L: left, R: right

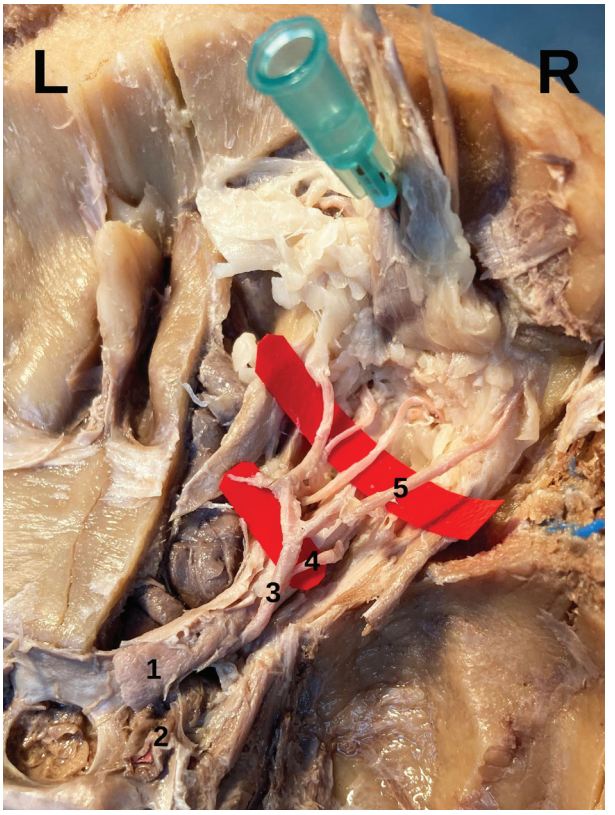

Figure 4: Exposition of posterior ciliary arteries as the first branch of ophthalmic artery
1: optic nerve, 2: internal carotid artery, 3: ophthalmic artery, 4: posterior ciliary arteries, 5: lacrimal artery, L: left, R: right

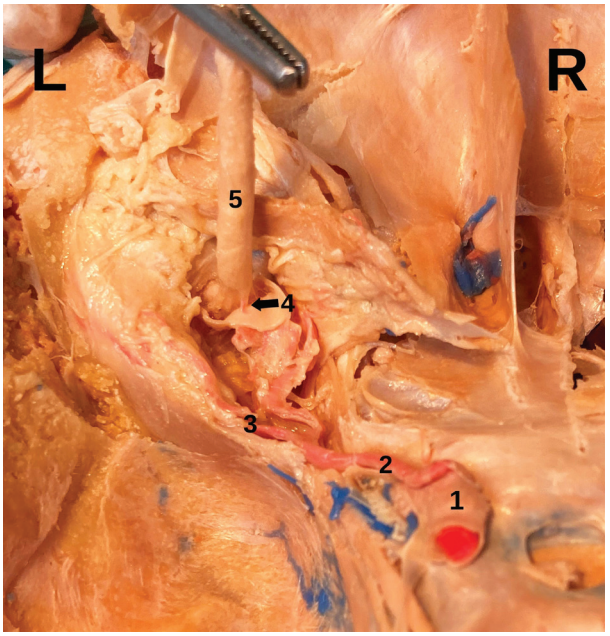

Figure 5: Exposition of lacrimal artery as the first branch of ophthalmic artery
1: internal carotid artery, 2: ophthalmic artery, 3: lacrimal artery, 4: central retinal artery, 5: optic nerve, L: left, R: right

(n=3) (Figure 5). Two of these three arteries were bilateral (in one cadaver) and the remaining artery was seen on the left side of one specimen. Finally, the least common (3%) first branch of the OA was the SA (n=1). Unfortunately we could not take a photograph of the cadaver which had the SA as a first branch unilaterally because when we passed on to the photograph taking stage of

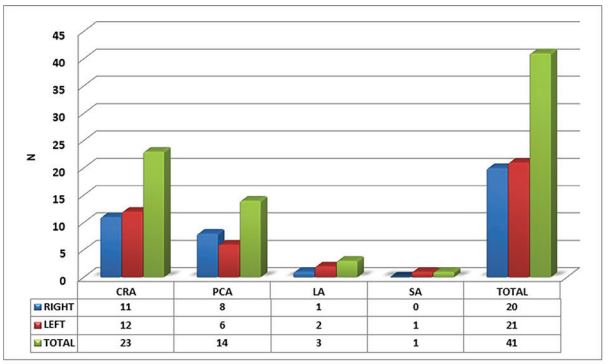

Figure 6: Numbers of different first branches of ophthalmic artery
CRA: central retinal artery, PCA: posterior ciliary arteries, LA: lacrimal artery, SA: supraorbital artery

Table 1: Numbers of each first branches originating from the ophthalmic artery, according to sides

No	The first branch of ophthalmic artery	
	Left	**Right**
1	CRA	CRA
2	CRA	CRA
3	LA	PCA
4	PCA	PCA
5	CRA	CRA
6	CRA	CRA
7	PCA	PCA
8	CRA	CRA
9	PCA	PCA
10	PCA	PCA
11	SA	PCA
12	LA	LA
13	PCA	CRA
14	CRA	CRA
15	CRA	PCA
16	PCA	CRA
17	CRA	CRA
18	CRA	CRA
19	CRA	CRA
20	Couldn't be evaluated	PCA
21	CRA	Couldn't be evaluated
22	CRA	Couldn't be evaluated

CRA: Central retinal artery, LA: Lacrimal artery, PCA: Posterior ciliary arteries, SA: Supraorbital artery

the study, we were faced with the fact that it had been harmed during routine practices. The numbers of each first branch originating from the OA, according to sides, are given in Figure 6. Additionally, our results concerning the first branch of the OA of each cadaver are given in Table 1.

DISCUSSION

Orbital dissection was first described by the German anatomist Meyer in 1887 (6). Meyer revealed the anatomical branching pattern and variations of the OA in a series of over 20 cases. Then, in a 3-series study conducted in 1962 by the ophthalmologist Sohan Sing Hayreh, the origin, course and branches of the OA were investigated (7-9). Around the same time period, Kuru, Moret, Vignaud, and Lasjaunias were also initiators in this field with their angiographic studies (10-13).

Knowledge of the embryological development of the OA is essential to understand its origin variations. It is stated that the OA is the craniofacial artery which has the most complex embryological development (4). The primitive OA is mainly formed by the primitive ventral ophthalmic artery (PVOA), the primitive dorsal ophthalmic artery (PDOA) and stapedial artery. In the 4-6 mm (crown–rump length) embryo, PDOA formation is observed from the bifurcation point of the primitive carotid artery in its caudal and cranial division (origin of the future PCA). The PDOA enters the orbit from the superior orbital fissure. At the 7-12 mm stage, the PVOA artery emerges from the cranial division of the primitive carotid artery and enters the orbit through the optic canal. At the 16-18 mm stage, the PVOA and PDOA combine to form the permanent stem of the primitive OA. In the 18-40 mm embryo, the stapedial artery is divided into maxillomandibular and supraorbital divisions. The maxillomandibular artery anastomoses with the pharyngeal arteries by extending beyond the head from the spinous foramen. The SA enters the orbit and divides into two branches called the ethmoidonasal and lacrimal arteries. The ethmoidonasal artery joins with the primitive OA at the level of the arterial ring around the ON. Thus, at the 40 mm stage, the OA reaches its adult configuration (1, 3, 4, 13).

This model explains most cases of the variant OA origin through migration, partial/complete regression, and persistence of primitive vessels, and/or remaining anastomotic loops (1).

The OA originates from the intradural part of the ICA (1, 4, 14, 15). In fact, there are many studies stating that they have not observed any source of origin other than the intracranial (intradural) segment of the ICA (5, 16-19). Also in this study, all of the examined ophthalmic arteries have originated from the intracranial segments of the ICA: distal to cavernous sinus.

For the purpose of description and clinical points of view, the ICA has been divided into 7 parts by neurosurgeon Bouthillerin in 1996 (20). According to this, the ICA is divided into its 1st or cervical segment, 2nd or petrous segment that gives origin to the caroticotympanic and vidian artery, 3rd or lacerum segment, 4th or cavernous segment that provides the meningohypophyseal and inferolateral trunk, 5th or clinoid segment, 6th or ophthalmic segment that gives the OA and superior hypophyseal artery, and 7th or communicating segment where the ICA, before its final division, gives origin to the anterior choroidal and posterior communicating artery (20). According to this classification, which is commonly used by surgeons and radiologists, the OA is defined as the first branch orig-

inating from the C6 segment of the ICA. Consequently the OA is also defined as the first intracranial or intradural branch of the ICA (1). Later, in 2009, Lehecka et al. divided the C4 segment into 3 portions as ophthalmic, communicating and choroid (21).

As the most common origin variation of the OA reported in the literature, it originates from the MMA (1, 3, 15). The MMA origin of the OA could be explained by: the absence of anastomosis between the supraorbital branch and the OA, or the persistence of the proximal stem of the supraorbital branch of the stapedial artery with regression of the primitive OA (4).

It has been reported that an OA originating from the cavernous part of the ICA is a rare variation and its incidence is estimated at 0.4% (22). In addition, Dilenge, Fiore, Parlato, Islak and Indo have also defined cases with the OA of cavernous (4th segment) origin (23-27). Toma reported that the OA arising from the cavernous segment of the ICA was defined as the second most common variation and added that this artery entered the orbit from the superior orbital fissure (3). Lasjaunias explained this variation by the embryological regression in the PVOA instead of the PDOA. In other words, this anatomical variation is explained by the embryological persistent PDOA (13).

In addition, origin of the OA from the clinoidal (5th segment) or communicating portions (7th segment) of the ICA has been recorded (3, 14, 28, 29).

The double origin of the OA is a very rare variant; its incidence is estimated to be around 0.2%, and only a few studies have been reported (4, 13, 22, 30, 31). Lasjaunias explained this variation by the lack of an anastomotic ring around the ON between the PDOA and the PVOA, or persistence of both of these arteries (1, 4, 13).

The rare origin variation of the OA from the MCA has been observed in the absence of the ICA (15, 32-34).

There have been reports of the ophthalmic artery originating from the ACA (27,35-37). This variation corresponds to the persistence of the PVOA (1).

It has been reported that it is encountered in the presence of ICA agenesis or hypoplasia (38-40).

OA originating from the basilar artery is an extreme variation. It has been stated that current embryological theories do not provide a satisfactory explanation for this formation (41-43).

OA originating from this artery has been reported in only 1 case (44).

After its origin, the OA follows a short intracranial course until it pierces the dura mater and reaches the optic canal. The course of the OA between its origin and optic canal is named as the intracranial course. This distance was calculated by Hayreh and Dass as 0.5–0.95 mm (8). Although the intracranial course of the OA is short, the knowledge of the anatomy of this course is of utmost surgical importance as surgical intervention for OA aneurysms takes place at that area (1).

After the intracranial course, the OA enters the optic canal together with the ON. This portion is defined as intracanalicular course.

Afterwards the OA exits the optic canal and enters the orbit. This is the intraorbital part of the OA**.** The intraorbital course of the OA divides into three segments. In the first segment, the OA exits the optic canal and proceeds parallel to the ON. The second segment courses medially passing above (83%) or below (17%) the ON (1). Finally, the third segment is separated to its branches medially to the ON, and terminates at the superomedial angle of the orbital opening (3, 4, 9).

Hayreh, who studied the OA branches in the orbits of 59 human cadavers, reported that localization and order of origin of these branches are not identical on either side of the same person (9). It is therefore stated that the branching patterns of the OA are very complex and unique.

Two different types of branching have been defined as the OA passes above or below the ophthalmic nerve (9, 15). According to this classification, if the OA crosses over the ON, the first branch is the medial posterior ciliary arteries (MPCA) in common with the CRA; if the OA crosses under the ON, the first branch is the lateral posterior ciliary arteries (LPCA). However, when the literature was examined, it was observed that there were branching patterns that did not fit this classification. Each most frequent first branch of the OA determined in our study is discussed separately.

The CRA is the most frequent first branch of the OA. It supplies blood to the retina, therefore it is critical for vision (1). It is one of the smallest branches of the OA and is a terminal artery. Due to its average diameter of 0.36 mm, the CRA is highly susceptible to occlusion (45). The CRA is commonly the first location where ischemic or embolic events make us notice the fact that there is serious vascular disease and high risk for an upcoming stroke. A central retinal artery embolus may produce a transient blindness in the affected eye, called amaurosis fugax, which lasts for several minutes but less than an hour (transient ischemic attack) (46). The CRA occlusion causes ischemia and results in infarction of the retina and as a result, sudden loss of vision with an incidence of 1–8/100,000 people occurs (1). Therefore, damage of the CRA usually results in blindness (18).

In the study by Tsutsumi et al. the CRA was found to be the first branch of the OA in 67%, the second branch in 28% and the third branch in 5% of the orbits they examined (5). In the present study, the CRA was observed as the first branch in 56% of cases.

The PCA are branches of the OA, ranging in number from 1 to 5, that supply the choroid and outer layer of the retina. It has been reported that in 80% of cases there are 2 or 3 PCA (1). The PCA are named as medial or lateral according to their location. Ciliary arteries supply the middle vascular tunic. Moreover, they take part in blood supply of the retina; if a detached retina occurs, this component of blood supply may be disrupted (46).

According to Hayreh, the first branch is either the CRA in common with the MPCA or only the LPCA (9, 15). In our study, the PCA were observed as the first branch with a frequency of 34%, as the second most frequent first branch of the OA.

The LA supplies the lacrimal gland, lateral rectus muscle, lateral part of the eyelids and gives off meningeal branches to dura mater (4). The LA is often referred to as the third or fifth branch of the OA (9). When the literature was reviewed, no case was observed in which the LA was reported to be the first branch of the OA. Therefore, this paper is the first one, in which the LA is reported to be the first branch of the OA (7% cases, n=3).

One of the limitations of our study is that we could not take a photograph of the cadaver which had the SA as a first branch. Moreover, we could not describe the practical locations for determining the first branch of the OA of the related region for surgeons.

In conclusion, the anatomy of the OA is quite complicated because it originates from three embryological sources, has a dual intracranial and extracranial course, and both itself and its branches are small in size. In addition, the location and proximity of structures such as the ON, and the critical importance of the CRA and PCA, which are usually the first branches of the OA, cause difficulties in surgical operations in this area. Knowledge of the anatomy and possible variations of the OA and its branches is essential for neurosurgeons and neuroradiologists while approaching pathologies such as retinoblastoma and CRA occlusion (5, 45).

Ethics Committee Approval: This study was approved by the Clinical Research Ethical Committee of the Istanbul University, Istanbul Faculty of Medicine (Date: 16.04.2021, No: 172684).

Peer Review: Externally peer-reviewed.

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COMPARISON OF SWALLOWING IN DIFFERENT TYPES OF PARTIAL LARYNGECTOMIES

FARKLI PARSİYEL LARENJEKTOMİ TEKNİKLERİNDE YUTMANIN KARŞILAŞTIRILMASI

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ABSTRACT

Objective: We designed this study to assess and compare the effects of different partial laryngectomy (PL) techniques on swallowing.

Material and Methods: Ten patients had laryngofissure with cordectomy, ten had frontal anterior laryngectomy with epiglottic reconstruction (FAL), ten had frontolateral laryngectomy (FLL), ten had cricohyoidopexy (CHP), ten had cricohyoidoepiglottopexy (CHEP), and ten had supraglotic laryngectomy. Swallowing was assessed with flexible endoscopy.

Results: Mild or moderate dysphagia for solid foods was discovered significantly more often in CHP patients compared to FLL and FAL ($p<0.05$) patients. Dysphagia discoveries for semi-solid and liquid food didn't significantly differ among PL's ($p>0.05$). Compared to other PLs, the penetration-aspiration test with 10 ml of water was distinctly lower in cordectomy and FLL patients ($p<0.05$).

Conclusion: Penetration and aspiration with 10 ml of water was marked lower in cordectomy and FLL patients matched to other PL patients. With studies involving more patients, it will be possible to increase the evidence value of our results.

Keywords: Partial laryngectomy, fiberoptic endoscopic evaluation of swallowing (FEES), deglutition disorders, dysphagia

ÖZET

Amaç: Bu çalışma, farklı parsiyel larenjektomi (PL) tekniklerinin yutma üzerindeki etkilerini değerlendirmek ve karşılaştırmak amacıyla tasarlandı.

Gereç ve Yöntemler: On hastada kordektomi ile laringofissür, onunda epiglotik rekonstrüksiyon (FAL) ile frontal anterior larenjektomi, on hastada frontolateral larenjektomi (FLL), on hastada krikohiyoidopeksi (CHP), on hastada krikohiyoidoepiglottopeksi (CHEP) ve diğer onunda supraglotik larenjektomi operasyonu yapılmıştı. Yutma fleksible endoskopi ile değerlendirildi.

Bulgular: Katı yiyecekler için hafif veya orta derecede disfaji, CHP hastalarında FLL ve FAL hastalarına kıyasla anlamlı olarak daha sık olduğu bulundu ($p<0,05$). Yarı katı ve sıvı gıdalar için disfaji araştırmasında, PL'ler arasında önemli ölçüde farklılık gösterilmedi ($p>0,05$). Diğer PL'lere kıyasla, 10 ml su ile penetrasyon-aspirasyon testi, kordektomi ve FLL hastalarında belirgin şekilde daha düşüktü ($p<0,05$).

Sonuç: Kordektomi ve FLL hastalarında diğer PL hastalarına göre 10 ml su ile penetrasyon ve aspirasyon testi, daha düşüktü. Daha fazla hastayı içeren çalışmalar sayesinde vermiş olduğumuz sonuçların kanıt değerinin artırılması mümkün olacaktır.

Anahtar Kelimeler: Parsiyel larenjektomi, fiberoptik endoskopik yutma değerlendirmesi (FEYD), yutma bozuklukları, disfaji

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INTRODUCTION

Laryngeal cancer is the most common malignancy of head and neck cancer in terms of frequency (1). Partial laryngectomy (PL) is indicated in the early stages and in some of the advanced stages of laryngeal cancer. PL has the advantage of preserving laryngeal functions, having lower morbidity, and increasing quality of life. However, swallowing dysfunction following PL is an important issue in some patients and may require a considerable amount of care and rehabilitation (2). The evaluation and treatment plan for swallowing difficulties has to be completed as soon as possible (3). Swallowing function, physiology, and aspiration can be effectively evaluated with fiberoptic endoscopic evaluation of swallowing (FEES). By assessing swallowing functions and implementing rehabilitation plans, patients could start a normal diet and potential complications such chronic aspiration, malnutrition, and dehydration could be prevented (3).

Objective evaluation and comparison of the effects of different PL techniques on swallowing functions is the purpose of this study.

MATERIAL AND METHODS

This study was done between July 2012 and February 2013. Sixty patients operated for laryngeal carcinoma were included in the study. Ten had laryngofissure with cordectomy, ten had frontal anterior laryngectomy with epiglottic reconstruction (FAL), ten had frontolateral laryngectomy (FLL), ten had cricohyoidopexy (CHP), ten had cricohyoidoepiglottopexy (CHEP) and ten had supraglottic laryngectomy. All of the patients were informed about the study and their informed consent was gained. This research was submitted to the Institutional Review Board and approved (Date: 14.09.2012, No: 12-7/80). Our study was completed within the framework of international ethical standards and the World Health Organization Helsinki Declaration.

Patient selection

Sixty patients, who underwent PL at least six months ago for histopathologically proven laryngeal cancer, were included in the study. Patients having symptoms of aspiration (coughing) and swallowing difficulties (dysphagia) were included. Patients with tumor recurrence, previous radiotherapy, and head and neck surgery other than PL were excluded from the study.

Instrumentation

Sociodemographic data was collected from patient records. Endoscopic assessment was carried out in an upright-seated position without using topical anesthesia to the nasal cavity. A video was recorded for each individual patient. We used a flexible nasopharyngoscope camera (KAY PENTAX Ltd, Montvale, NJ, USA) during the procedures.

Procedure

The test procedure comprised two administrations of 3 ml, 5 ml, and 10 ml of water stained with food dye (green) via an injector. Likewise, swallowing tests were completed with two administrations of one dessert spoonful of yoghurt (5 ml) colored with food dye and fish crackers. Premature spillage, retention-pooling, penetration, aspiration and reflex coughing were scored (from 1 to 5) with the scoring system established by Topaloglu et al. (Table 1) (4). An otolaryngologist and a physical therapy and rehabilitation specialist were present for the duration of each procedure and all procedures were video-recorded. As in our previously published study in different patient groups, the total test results were blindly evaluated by the same physician and unchanged otolaryngologist, who were focused and specialized in this field, regardless of the treatment procedures (5).

Statistical analysis

Computer software (SPSS version 22.0, SPSS Inc. Chicago, IL, USA) was conducted for statistical analysis. Comparison of categorical data was made with chi-square (X2) exact tests. According to the distribution pattern of the data, Wilcoxon and Mann-Whitney U tests were used in

Table 1: Swallowing grading scale developed by Topaloglu et al.

Points	Premature spillage of material	Retension/pooling of material and/or secretion	Penetration-aspiration reflex cough
1	Severe	Severe retention/pooling	Enterance of material into trachea; no reflex cough
2	Marked	Marked retention/pooling	Enterance of material into trachea; with reflex cough
3	Moderate	Mild retention/pooling	Enterance of material into larynx; remaining above the trachea no reflex cough
4	Mild	Coating residue/secretion	Enterance of material into larynx; remaining above the trachea with reflex cough forming
5	None	No retention/pooling	No enterance of material into larynx or trachea; no reflex cough

the analysis of nonparametric variables, and independent, and paired sample t-tests were used in the analysis of parametric variables. Determining the distribution pattern of the data was provided by the Shapiro-Wilk test. The distribution of the groups in our study was non-parametric. Considering the distribution of the data, Pearson or Spearman correlation analysis was used. Data was expressed as "median, interquartile range (IQR)". A p value of <0.05 was considered statistically significant.

RESULTS

Sixty patients treated with PL were involved in the study. Five (8.3%) of the patients were female and 55 (91.7%) were male, with a mean age of 59.87±7.93 years (range 33-79 years).

Forty-four (73.3%) patients reported coughing, two (3.3%) reported coughing and dysphagia, and 14 (23.3%) reported only dysphagia. The type of PL present in the patient and their symptoms were not statistically correlated (p>0.05). The presence and frequency of the subjective symptoms (difficulty in bolus control, need to clear throat, food gets stuck, choking) are presented in Table 2. Subjective symptoms and the type of PL were not statistically correlated (p>0.05).

Dysphagia for solid, semi-solid, and liquid food was evaluated and the data (according to the types of PL) is presented in Table 3. Mild or moderate dysphagia for solid foods was significantly more prevalent in CHP patients compared to FLL and FAL (p<0.05) patients. Dysphagia for semi-solid and liquid food did not significantly differ among different PL's (p>0.05).

Premature spillage, residue-secretion, and penetration-aspiration scores were evaluated with fiberoptic endoscopy. Scores for different types of PL are presented in Table 4. Penetration-aspiration with 10 ml water was meaningfully lower in cordectomy and FLL patients compared to supraglottic laryngectomy, CHP and CHEP patients (p<0.05). Other scores had no significant difference among the groups (Table 4) (p>0.05).

DISCUSSION

There are many reports assessing the swallowing function in a certain type of PL (6-12). While some researchers evaluated swallowing function with N/G tube removal time or gastrostomy tube removal rates in previous reports, others used quality of life measures (9, 12-15). Videofluoroscopy is also a widely used technique for the evaluation of swallowing (7, 8, 12, 16). However, a study,

Table 2: Operation types and subjective complaints

Complaint		Operation type						
		Supraglottic laryngectomy	Cordectomy	CHP	CHEP	FLL	FAL	p
Cough	N (%)	7 (70%)	8 (80%)	8 (80%)	8 (80%)	7 (70%)	6 (60%)	p>0.05
Cough, dysphagia	N (%)	0 (0%)	0 (0%)	2 (20%)	0 (0%)	0 (0%)	0 (0%)	
Dysphagia	N (%)	3 (30%)	2 (20%)	0 (0%)	2 (20%)	3 (30%)	4 (40%)	
Difficulty in bolus control	Not present (N;%)	9 (90%)	9 (90%)	9 (90%)	8 (80%)	10 (100%)	10 (100%)	p>0.05
	Present (N;%)	1 (10%)	1 (10%)	1 (10%)	2 (20%)	0 (0%)	0 (0%)	
Need to clear throat	Not present (N;%)	4 (40%)	7 (70%)	4 (40%)	8 (80%)	8 (80%)	7 (70%)	p>0.05
	Present (N;%)	6 (60%)	3 (30%)	6 (60%)	2 (20%)	2 (20%)	3 (30%)	
Sensation of a lump in the throat	Not present (N;%)	6 (60%)	7 (70%)	5 (50%)	7 (70%)	6 (60%)	5 (50%)	p>0.05
	Present (N;%)	4 (40%)	3 (30%)	5 (50%)	3 (30%)	4 (40%)	5 (50%)	
Sense of choking	Not present (N;%)	7 (70%)	8 (80%)	6 (60%)	9 (90%)	10 (100%)	10 (100%)	p>0.05
	Present (N;%)	3 (30%)	2 (20%)	4 (40%)	1 (10%)	0 (0%)	0 (%0)	

CHP: Cricohyoidopexy; CHEP: Cricohyoidoepiglottopexy; FLL: Frontolateral laryngectomy; FAL: Frontal anterior laryngectomy

Table 3: Dysphagia table

Operation type			Supraglottic laryngectomy	Cordectomy	CHP	CHEP	FLL	FAL	Total
Dysphagia to solid food	Not present	n	8	7	5	7	9	9	45
		%	80	70	50	70	90	90	75
	Mild or moderate	n	2	3	2	3	1	1	12
		%	20	30	20	30	10	10	20
	Severe	n	0	0	3	0	0	0	3
		%	0	0	30	0	0	0	5
	Total	n	10	10	10	10	10	10	60
		%	100	100	100	100	100	100	100
Dysphagia to semisolid food	Not present	n	6	8	6	8	10	9	47
		%	60	80	60	80	100	90	78.3
	Mild or moderate	n	4	2	4	2	0	1	13
		%	40	20	40	20	0	10	21.7
	Severe	n	0	0	0	0	0	0	0
		%	0	0	0	0	0	0	0
	Total	n	10	10	10	10	10	10	60
		%	100	100	100	100	100	100	100
Dysphagia to liquid food	Not present	n	5	10	8	4	8	8	43
		%	50	100	80	40	80	80	71.7
	Mild or moderate	n	4	0	2	6	2	2	16
		%	40	0	20	60	20	20	26.7
	Severe	n	1	0	0	0	0	0	1
		%	10	0	0	0	0	0	1.7
	Total	n	10	10	10	10	10	10	60
		%	100	100	100	100	100	100	100

CHP: Cricohyoidopexy; CHEP: Cricohyoidoepiglottopexy; FLL: Frontolateral laryngectomy; FAL: Frontal anterior laryngectomy

Table 4: P values regarding different laryngectomy types and swallowing scores

	Premature spillage	Residue, secretion	Penetration, aspiration, reflex cough
	p value	p value	p value
3 ml water	.539	.294	.097
5 ml water	.490	.471	.163
10 ml water	.305	.614	.024
5 ml yoghurt	.385	.303	.083
Fish cracker	.540	.314	.066

that compared swallowing functions in different types of PL with FEES, is missing. A study conducted by Alicandri–Ciufelli et al. compared swallowing functions of supraglottic laryngectomy and supracricoid partial laryngectomy (SCPL) patients with FEES (12). Other studies usually evaluated swallowing in only one type of PL or compared supracricoid laryngectomy with total laryngectomy (12,16). Alicandri-Ciufelli et al. found no statistically significant difference between supraglottic laryngectomy and supracricoid laryngectomy patients regarding swallowing functions evaluated with FEES (12). They also evaluated cases with both preserved arytenoids, radiotherapy, different ages, and a different time interval after surgery, and it was concluded that only radiotherapy had a significant negative effect on supracricoid laryngectomy patients with FEES (4). Premature spillage, residue-se-

cretion, and penetration-aspiration scores were higher in patients with both arytenoids preserved and in patients that did not receive radiotherapy, but this difference was not statistically significant (4). Because patients with a history of radiotherapy were excluded and both arytenoids were preserved in all patients, these were not evaluated in this study.

Another important step in preserving the swallowing function is to preserve the superior laryngeal nerve. If this nerve is damaged, the cricopharyngeal sphincter and the cough reflex will be negatively affected and the patient won't be able to recognize aspiration (3, 17, 18). This may hamper subsequent swallowing rehabilitation and may extend adaptation time. All effort should be taken to preserve both superior laryngeal nerves in laryngeal conservation surgery.

Zacharek et al. evaluated the swallowing function of 10 supracricoid laryngectomy patients with FEES and modified barium swallow studies (19). They reported swallowing difficulties in all patients. Supraglottic sensory loss secondary to unilateral or bilateral damaged superior laryngeal nerve, changed base of tongue/vallecular anatomy after extraction of epiglottis, physiologic insufficiency of the neoglottal valve, or a combination of these three mechanisms were the proposed mechanisms for swallowing difficulties (15, 18). All of the patients in this study tolerated oral food intake and were decanulated. None of the patients developed aspiration pneumonia. These findings show that all patients should have a sufficient cough reflex to protect their lungs from aspiration pneumonia and an active tracheopulmonary mucociliary clearance system. In addition, these studies emphasize the importance of adequate respiratory function in patients undergoing SCPL. In a study evaluating the swallowing function of 116 SCPL patients, 45 patients that had aspiration in videofluoroscopic study were assessed with high-resolution computed tomography and no statistically noteworthy difference was established among these patients and control groups regarding the radiographic images (19).

This is the first research to compare swallowing functions of different types of PL with FEES. A limited number of patients and inadequate randomization are the limitations of this study. Objective evaluation of swallowing in PL patients provides valuable data and feedback for both the doctor and the patient.

CONCLUSION

In conclusion, penetration and aspiration with 10 ml of water was meaningfully lower in cordectomy and FLL patients compared to supraglottic laryngectomy, CHP, and CHEP patients. This study is important because it is the first study, which evaluated swallowing objectively in patients who underwent six different PL. The most important result of this study, regardless of which PL technique is applied, is that aspiration problems are not caused, other than high-volume water ingestion, after the 6th postoperative month in patients, who have preserved both arytenoids and have not applied radiotherapy. We think that we can reduce the concern of surgeons with this objective study about swallowing that will occur as a result of the PL technique preference. Further studies with larger patient groups are warranted to obtain more reliable results.

Informed Consent: Written consent was obtained from the participants.

Ethics Committee Approval: This study was approved by the Clinical Research Ethical Committee of the Ege University School of Medicine (Date: 14.09.2012, No: 12-7/80).

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THE PRONATING RADIUS OSTEOTOMY FOR CORRECTING THE SUPINATION DEFORMITY IN BRACHIAL PLEXUS BIRTH PALSY

DOĞUMSAL BRAKİYAL PLEKSUS PARALİZİSİNDE SUPİNASYON DEFORMİTESİNİ DÜZELTMEK İÇİN RADİUS ROTASYON OSTEOTOMİSİ UYGULAMASI

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ABSTRACT

Objective: Severe supination deformity may be seen in brachial plexus birth palsy (BPBP). The aim of this study was to determine the efficacy of pronating radius osteotomy in the management of this deformity.

Material and Methods: BPBP patients with severe supination deformity were included in this study and they were operated between November 2003 and December 2015, by the same operative team. Pronating radius osteotomy was performed and internal fixation was maintained either by Kirschner wires or semitubular plates. In some patients, tendon transfers were performed during the same operation for the restoration of shoulder and thumb abduction and wrist extension.

Results: Forty one patients had supination deformities caused by BPBP. The mean age was 9.2 years (4-22). The mean follow-up was 5 years (1-7). The mean active pronation was -60° before the operation, and the passive one was -10°. The mean active pronation of the patients was 9° after the operation, and the passive one was 45°. The mean active supination of the patients was 75° before the operation, and the passive one was 85°. The mean active supination of the patients was 45° after the operation, and the passive one was 65°. One malunion was detected at the second year after the operation (1/41). Three patients had low pronation degrees during the follow-up (3/41).

Conclusion: Satisfactory postural and functional improvement can be achieved with the use of pronating radius osteotomy for patients with severe supination contractures.

Keywords: Brachial plexus birth palsy, contracture, pronating radius osteotomy, supination deformity, tendon transfer

ÖZET

Amaç: Doğumsal brakiyal pleksus paralizisi (DBPP) şiddetli supinasyon deformitesi ile seyredebilir. Bu çalışmanın amacı, bu deformitenin tedavisinde radius pronasyon osteotomisinin etkinliğini saptamaktır.

Gereç ve Yöntemler: Şiddetli supinasyon deformiteleri olan DBPP hastaları bu çalışmaya eklenmiştir ve bu hastalar Kasım 2003 ila Aralık 2015 tarihleri arasında, aynı ekip tarafından ameliyat edilmiştir. Radius pronasyon osteotomisi yapılmıştır ve kemiksel sabitleme Kirschner telleri veya semitübüler plaklar ile sağlanmıştır. Bazı hastalarda, omuz ve başparmak abdüksiyonu ve el bileği ekstansiyonunun sağlanması için aynı ameliyatta tendon transferleri de yapılmıştır.

Bulgular: Kırk bir hastada DBPP'ye bağlı supinasyon deformiteleri saptanmıştır. Hastaların ortalama yaşı 9,2 yıldır (4-22 yıl). Hastaların ortalama takip süresi 5 yıldır (1-7 yıl). Ortalama ameliyat öncesi etkin pronasyon -60° iken pasif değer -10°'dir. Ortalama ameliyat sonrası etkin pronasyon 9° iken pasif değer 45°'dir. Ortalama ameliyat öncesi etkin supinasyon 75° iken pasif değer 85°'dir. Ortalama ameliyat sonrası etkin supinasyon 45° iken pasif değer 65°'dir. Bir hastada ameliyat sonrası ikinci yılda malünyon saptanmıştır (1/41). Üç hastada ameliyat sonrası dönemde yetersiz pronasyon elde edilmiştir (3/41).

Sonuç: Şiddetli supinasyon kontraktürü olan hastalarda, radius pronasyon osteotomisi ile tatmin edici bir görünüm ve işlevsel düzelme elde edilebilir.

Anahtar Kelimeler: Doğumsal brakiyal pleksus paralizisi, kontraktür, radius pronasyon osteotomisi, supinasyon deformitesi, tendon transferi

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INTRODUCTION

Supination deformity is a challenge in the management of brachial plexus birth palsy. The pronator quadratus and the pronator teres muscles may be affected in the plexus palsies with the involvement of the C5-T1 nerve roots (1). The unopposed supination forces keep the forearm in supination, and with time, rigid deformity develops with the involvement of the interosseous membrane (1). At its earlier stages, the deformity may be corrected passively, but it generally progresses to become fixed over time (2).

The supination deformity affects the patient both functionally and aesthetically. For this reason, many surgical interventions have been described. Although some deformities may be treated with tendon transfers and interosseous membrane releases, more rigid deformities require bone interventions such as osteotomy (2, 3). In fact, the management strategies of the supination deformity vary according to the congruence of the radioulnar joint. Zancolli classified distal radioulnar joint congruence in three types such as type Ia, Ib and II. In type II (fixed deformity), the patients do not have passive pronation and supination in their distal radioulnar joints, and osseous procedures are required in such patients (4). In 1940, Blount suggested osteoclasis of both radius and ulna (5). In 1956, Burman described a method for rotation osteotomy in which the distal fragment of radius is brought to dorsal and ulnar angulation with non-axial torsion (6).

In this study, an overview of radius pronation osteotomy technique for supination deformities in brachial plexus birth palsy was made, and postoperative results were reported.

MATERIAL AND METHOD

Brachial plexus birth palsy (BPBP) patients who had pronating radius osteotomy for severe supination deformity of the forearm were included in the study (Figure 1). The patients were operated on between November 2003 and December 2015 by the same operative team. The following were the indications for radius pronating osteotomy: a lack of active and passive pronation of the forearm (fixed supination deformity), intact sensibility and good grasp/release function of the hand. In some patients, tendon transfers were performed during the same operation for shoulder, thumb and wrist function. All goniometric evaluations were made by the same hand therapist. Preoperative and postoperative active/passive pronation and supination degrees of the forearm were assessed by goniometric measurements (Figure 2). A low pronation degree was defined as passive pronation less than 30 degrees because such a range of motion is required in healthy individuals.

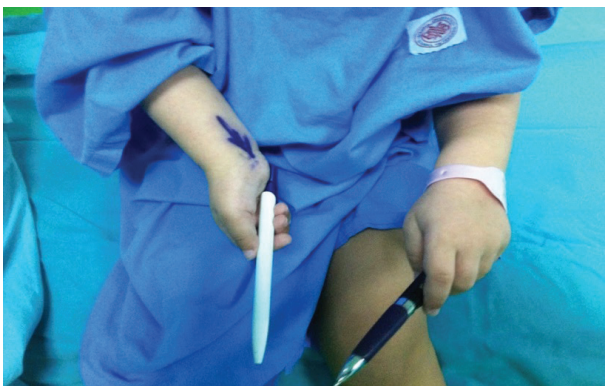

Figure 1: The preoperative photograph of a patient with right sided obstetric brachial plexus paralysis with severe supination deformity is shown. The patient can not pronate his forearm actively

Figure 2: The postoperative photograph of the same patient is shown. The patient can actively pronate his right forearm

The Institutional Ethics Committee approved the study (Date: 26.02.2018, No: 279). Informed consent was obtained from the guardian of each patient.

Surgery was performed under general anesthesia and a pneumatic upper arm tourniquet was used. The linear incision was performed on the volar and radial aspect of the forearm. The intersection point between the proximal and the middle one thirds of the radius was reached through this incision (Figure 3). Due to the robust blood supply of this point, rapid bone healing may be ensured. Radial osteotomy was performed distal to the pronator teres enthesis and the forearm was pronated to the maximal limit. Bone fragments were fixated either by Kirschner wires or by semitubular plates (Figure 4).

Following skin closure, a long-arm cast was applied. The elbow was flexed in 90 degrees and the forearm was positioned in maximal pronation. The wrist was extended in 30 degrees. The cast immobilization was continued for six weeks and the cast was changed with a thermoplastic splint after the sixth postoperative week. Simultaneously a rehabilitation program was started. The thermoplastic

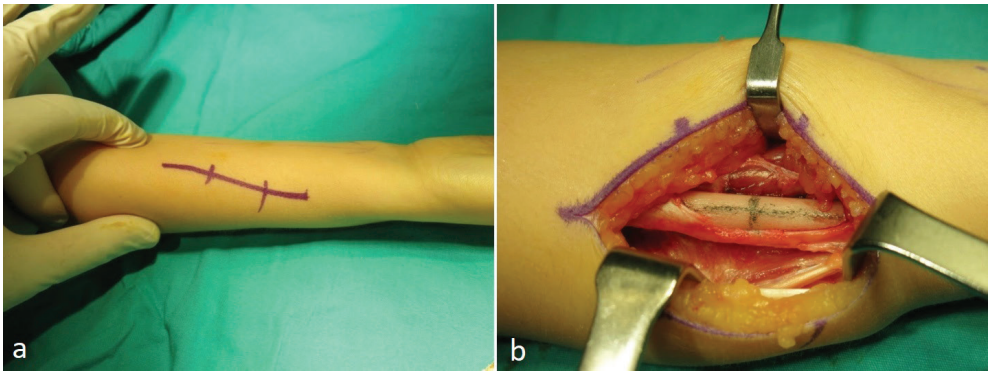

Figure 3: The operative photograph demonstrates our incision of preference (a) and the area of osteotomy on the radius (b)

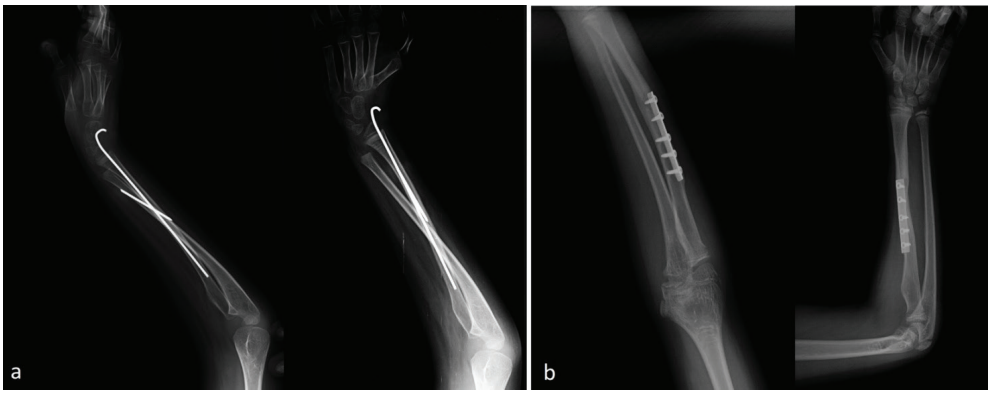

Figure 4: The postoperative radiograph of two patients are shown. (a) Kirschner wire (b) Semitubular plate

splint was used until the direct graphs revealed evidence of bone healing. Kirschner wires were usually removed at the postoperative second month according to X-ray follow-ups. Plate and screws were removed after 1-2 years if the patients had complaints such as prominence of the plate under the skin.

RESULTS

Forty one patients had supination deformities caused by BPBP. The mean age was 9.2 years (4-22). Twenty three patients were female whereas 18 patients were male. The mean follow-up was 5 years (1-7). Shoulder, thumb and wrist functions were augmented with tendon transfers in nineteen patients (19/41). There were no patients who underwent tendon transfers for forearm rotation before rotation osteotomy.

The angle 0 was defined as neutral. The negative values stood for supination whereas the positive values stood for pronation (Figure 5). The mean active pronation was - 60° (range - 80° to - 45°) before the operation, and the passive one was - 10° (range - 30° to 5°) (Figure 6). The

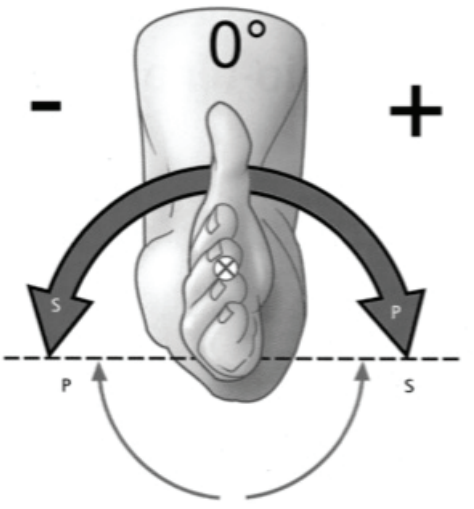

Figure 5: The angle 0 was defined as neutral. The negative values stood for supination whereas the positive values stood for pronation

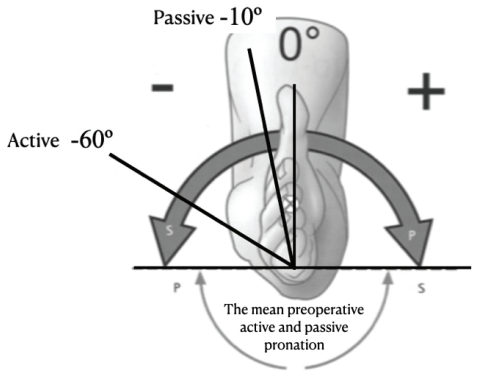

Figure 6: The mean pronation values (active and passive) are presented for the preoperative period

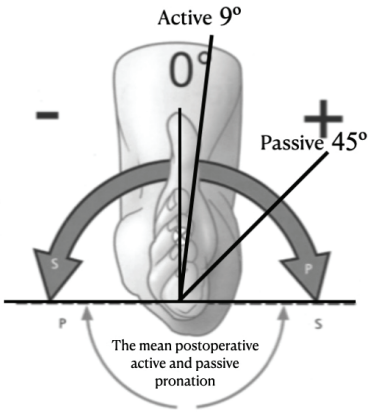

Figure 7: The mean pronation values (active and passive) are presented for the postoperative period

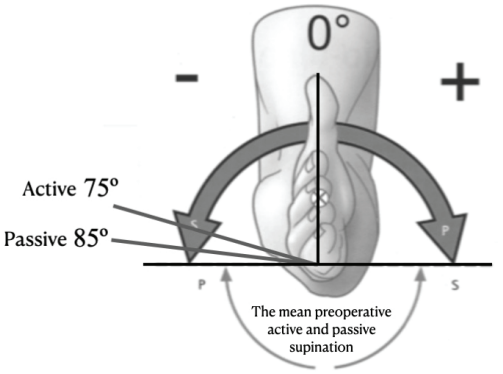

Figure 8: The mean supination values (active and passive) are presented for the preoperative period

mean postoperative active pronation was 9° (range - 10° to 20°), and the passive one was 45° (range 25° to 60°) (Figure 7). The mean active supination was 75° (range 35° to 85°) before the operation, and the passive one was 85° (range 45° to 90°) (Figure 8). The mean active supination was 45° (range 20° to 60°) after the operation, and the

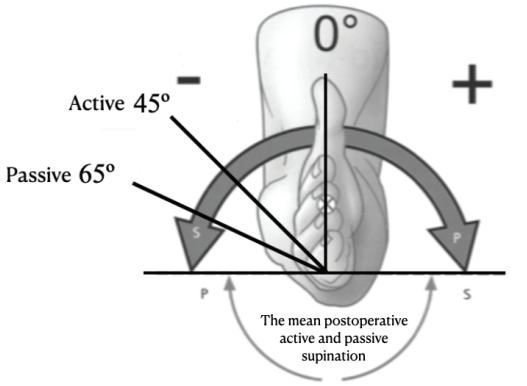

Figure 9: The mean supination values (active and passive) are presented for the postoperative period

passive supination was 65° (range 40° to 80°) (Figure 9). On the other hand, three patients had low pronation degrees (3/41).

One malunion was detected at the second post-operative year (1/41). This patient was treated with open reduction and internal fixation. One patient had a non-union following radius osteotomy due to insufficient plate fixation and the patient was treated by changing the semitubular plate with a strong locking compression plate. The osteosynthesis was performed with plates and screws in 30 patients and removal surgery was performed on 12 patients.

DISCUSSION

Tendon transfers, soft tissue releases and corrective osteotomies may be performed for supination deformities (1-5). The Zancolli procedure is one of the tendon transfers that enhance pronation. After incising the cubital area, the soft tissue contractures are released and the biceps insertion is detached from the ulnar aspect of the radius. The tendon is passed over the radial aspect of the radius to be inserted to the radial side of the radial head (2). This transfer turns the supinatory effect of the biceps muscle to a pronatory one. Although this technique is widely accepted, it was not useful for this study because all the patients had fixed supination deformities refractory to soft tissue reconstruction.

In 2004, Ozkan et al. suggested a new technique of active pronation maintenance (3). The rigid supination was overcome with interosseous membrane section and the brachioradialis tendon was transferred from the dorsal forearm to the volar side of the distal radius (3). However, the deformity was too rigid in this study's population and such soft tissue procedures were not enough to restore the pronation. Thus, tendon transfers, interosseous membrane releases and tendon lengthening procedures

were performed in conjunction with the rotational osteotomy in order to optimize the results of this study.

The rotational osteotomy is an important part of the reconstructive armamentarium and the benefits of the rotational osteotomies were accepted by the reconstructive community. Zaoussis performed radius rotation osteotomies on six patients with forearm supination deformities caused by BPBP (7). Zaoussis designed the radius rotation osteotomies according to malunion correction principles and his technique was different than Blount osteoclasis technique that preferred a closed approach (5, 7). Zaoussis performed open radius osteotomy distal to the radial tuberosity; however, in this study, osteotomies were performed just distal to the enthesis of pronator teres. Also, some authors combined the distal radial osteotomies with proximal humeral osteotomies in severe cases. Goddard et al. performed humerus rotational osteotomies in 10 BPBP patients and their findings supported the need for osteotomy procedures in severely disabled patients who were not good candidates for only soft tissue procedures (8).

Metsaars et al. compared the results of osteotomy with biceps rerouting procedure and they evaluated their results according to the severity of the contractures. Both techniques increased the pronation and the gain was proportionate to the severity of the deformity. The patients with more severe supination deformities benefited more from the rotational osteotomy. Although the recurrence rate was 20-40% in the osteotomy group and hardware complications were observed by Metsaars et al., such a high recurrence and complication rate were not seen in this study (9).

Bahm et al. operated on 40 patients with severe supination deformities and 23 of their patients were operated on either with Blount osteoclasis or open radial osteotomy (10). At a mean follow up of four years, their final postoperative pronation was 17 degrees. However, they had five recurrences in the osteotomy group. According to Bahm et al., age at the time of operation was significant because it affected the compliance of the patients with physiotherapy (10). In 2004, Allende et al. published their results on 66 patients with supination deformities caused by BPBP. Forty four of their patients were operated on with rotation osteotomies whereas 22 of them only had soft tissue procedures. In a mean follow up of 64.3 months, they obtained a postoperative passive pronation of 92 degrees. They had nine recurrences, two delayed unions and one malunion in the osteotomy group (11). In this study, the mean postoperative active pronation was 9 degrees and the mean postoperative passive pronation was 45 degrees. Also, low pronation degrees and recurrence were seen in three patients and malunion was seen in only one patient. The results of this study were comparable with recent literature.

CONCLUSION

Interosseous membrane releases and tendon transfers are preferred treatments in flexible supination deformities. Rotational osteotomies may be preferred in fixed deformities, and the complication rate is relatively low with this technique.

Ethics Committee Approval: This study was approved by the Clinical Research Ethical Committee of the Istanbul University, Istanbul Faculty of Medicine (Date: 26.02.2018, No: 279).

Informed Consent: Written consent was obtained from the participants.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- H.Ö.B., B.E.A., E.K., S.Ö., A.A.; Data Acquisition- H.Ö.B., B.E.A., E.K., S.Ö., A.A.; Data Analysis/Interpretation- H.Ö.B., B.E.A., E.K., S.Ö., A.A.; Drafting Manuscript- H.Ö.B., B.E.A., E.K., S.Ö., A.A.; Critical Revision of Manuscript- H.Ö.B., B.E.A., E.K., S.Ö., A.A.; Final Approval and Accountability- H.Ö.B., B.E.A., E.K., S.Ö., A.A.

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İSTANBUL ÜNİVERSİTESİ YAYINEVİ

RESEARCH - ARAŞTIRMA
DOI: 10.26650/IUITFD.2021.964078
İst Tıp Fak Derg 2021 / J Ist Faculty Med 2021

PROXIMAL FEMORAL NAILING VERSUS DYNAMIC HIP SCREW IN MANAGEMENT OF STABLE INTERTROCHANTERIC FEMUR FRACTURES: A COMPARISON OF CLINICAL AND RADIOLOGICAL OUTCOMES

STABİL İNTERTROKANTERİK FEMUR KIRIKLARININ TEDAVİSİNDE PROKSİMAL FEMORAL ÇİVİ İLE DİNAMİK KALÇA VİDASI: KLİNİK VE RADYOLOJİK SONUÇLARIN KARŞILAŞTIRILMASI

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ABSTRACT

Objective: The aim of this study was to compare the cost-effectivity, clinical and radiological outcomes of the proximal femoral nail (PFN) and Dynamic hip screw (DHS) in the management of stable intertrochanteric femur fractures (SIFFs).

Material and Methods: Patients who underwent surgical treatment for a SIFF in our department were retrospectively identified and then divided into two groups according to the treatment modality: Group 1, 57 patients (36 female, 21 male; mean age = 74.5±9.9 years) treated with PFN and Group 2, 65 patients (34 female, 31 male; 72±10.2 years) treated with DHS. Primary outcome measures were: estimated blood loss (EBL), total operating time (TOT), duration of hospital stay (DHS), rate of postoperative complication, rate of mortality, and treatment cost. Radiographic assessment included anteroposterior/lateral tip-apex distance (TAD) and amount of limb length discrepancy (LLD).

Results: No significant differences were observed in demographic characteristics between the two treatment groups (p>0.05). The mean follow-up was 44.2±31 months in group 1 and 53.7±38 months in group 2 (p=0.077). The mean TOT and

ÖZET

Amaç: Bu çalışmanın amacı, stabil intertrokanterik femur kırıklarının (SIFF) tedavisinde proksimal femur çivisi (PFN) ve dinamik kalça vidasının (DHS) maliyet-etkililik, klinik ve radyolojik sonuçlarını karşılaştırmaktı.

Gereç ve Yöntemler: SIFF nedeniyle cerrahi tedavi uygulanan hastalar geriye dönük olarak belirlendi ve tedavi şekline göre iki gruba ayrıldı: Grup 1, 57 hasta (36 kadın, 21 erkek; ortalama yaş = 74,5±9,9 yıl) PFN ile tedavi edilen ve Grup 2, DHS ile tedavi edilen 65 hasta (34 kadın, 31 erkek; 72±10,2 yıl). Birincil sonuç ölçütleri; tahmini kan kaybı (EBL), toplam ameliyat süresi (TOT), hastanede kalış süresi (DHS), ameliyat sonrası komplikasyon oranı, ölüm oranı ve tedavi maliyeti olarak yapıldı. Radyografik değerlendirme, ön-arka/yan uç-apeks mesafesini (TAD) ve uzuv uzunluk uyumsuzluğu miktarını (LLD) karşılaştırıldı.

Bulgular: İki tedavi grubu arasında demografik özelliklerde anlamlı bir farklılık gözlenmedi (p>0,05). Ortalama takip süresi grup 1'de 44,2±31 ay ve grup 2'de 53,7±38 ay idi (p=0,077). Ortalama TOT ve EBL, grup PFN'de grup DHS'ye göre anlamlı olarak daha kısaydı (p<0,001 ve p=0,03). Ortalama hastanede kalış süresi, postoperatif komplikasyon oranı, mortalite oranı ve tedavi mali-

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EBL were significantly shorter in group PFN than in group DHS (p<0.001 and p=0.03). No significant difference was observed in the mean duration of hospital stay, rate of postoperative complication, rate of mortality, and treatment cost between the two treatment groups (p>0.05). The post-operative complication rate was 9.5% in group PFN and 8.3% in group DHS, with no significant difference (p=0.83). There were significant differences in neither TAD nor LLD between the two treatment groups (p=0.69 and p=0.87, respectively).

Conclusion: The two treatment modalities seem to have similar effect to maintain stability for patients with stable IFFs. However, less EBL and shorter operation time can be expected from PFN compared to DHS in such patients.

Keywords: Intertrochanteric fracture, dynamic hip screw, proximal femoral nailing, cost-effectivity

yeti açısından iki grup arasında anlamlı fark gözlenmedi (p>0.05). Ameliyat sonrası komplikasyon oranı grup PFN'de %9,5 ve grup DHS'de %8,3 idi ve anlamlı bir fark yoktu (p=0,83). İki tedavi grubu arasında ne TAD ne de LLD'de anlamlı farklılıklar yoktu (sırasıyla p=0,69 ve p=0,87).

Sonuç: İki tedavi modalitesi, stabil IFF'leri olan hastalarda stabiliteyi sürdürmek için benzer etkiye sahip görünmektedir. Ancak bu tür hastalarda DHS'ye kıyasla PFN'den daha az EBL ve daha kısa operasyon süresi beklenebilir.

Anahtar Kelimeler: İntertrokanterik kırık, dinamik kalça vidası, proksimal femoral çivileme, maliyet etkinliği

INTRODUCTION

Over the past decades, hip fractures have emerged as a major health problem worldwide confronted by orthopedic surgeons as a consequence of the enhanced longevity of the population and increased incidence of osteoporosis (1). Intertrochanteric hip fractures account for nearly half the hip fractures in elderly patients and lead to obviously diminished life expectancy as well as dramatic impairment in social, economic, and health circumstances (2).

Over the past 30 years, the dynamic hip screw (DHS) has become the implant of choice for the effective treatment of stable intertrochanteric femoral fractures (IFFs) (Arbeitsgemeinschaft für Osteosynthesefragen/Orthopaedic Trauma Association (AO/OTA) 31-A1.1–A1.2), with favorable clinical and radiographic outcomes (3, 4). Nonetheless, proximal femoral intramedullary nails (PFNs) have been more widely used for such fractures in recent years, despite the lack of strong evidence to support their superiority over the DHS (3, 4).

Current literature illustrates the pros and cons of each treatment modality (1-4). However, to the best of our knowledge, evidences from the existing literature to determine the more advantageous implant to be used in the treatment for stable IFFs are controversial.

Therefore, the primary aim of this study was to compare the efficacy of DHS versus PFN for the management of stable IFFs based on the several clinical and radiographic outcome measures.

MATERIALS AND METHODS

Data collection and setting of the study

This retrospective comparative study was conducted on patients who underwent surgical treatment with either PFN or DHS for the treatment of IFFs between 2005 and 2013 at the department of Orthopedics and Traumatology in a single tertiary referral center. Inclusion criteria for the study were (4):

1. a diagnosis of unilateral stable IFF (AO/OTA 31–A1),
2. treated with either DHS or PFN
3. an age above 50 years old
4. a good cognitive function
5. adequate medical and radiographic records.

Exclusion criteria were:

1. a diagnosis of a pathological fracture
2. history of malignancy-metabolic disease or che mo-/radiotherapy
3. history of polytrauma
4. a fracture secondary to high energy trauma (mo tor vehicle accident, fall from a distant height gun-shot injury)
5. bilateral fractures
6. presented with an ASA score IV or higher.
7. Based on the above eligibility criteria, 66 patients were excluded from the study.

Participants

After obtaining institutional review board approval (Date: 04.04.2021, No: 117), a total of 188 patients were assessed retrospectively according to the above eligibility criteria. After excluding 66 patients, the remaining 122 patients meeting the eligibility criteria were enrolled in the study (Figure 1). All the patients include in the study were then divided into two groups according to the implemented treatment modality: Group 1 (PFN) including 57 patients treated with PFN and group 2 (DHS) including 65 patients treated with DHS.

Surgical technique

All patients were hospitalized from the emergency room and were managed with low molecular weight heparin and thromboembolic-deterrent stockings for prophylaxis of deep venous thrombosis. Then, all stable IFFs were treated operatively at the earliest opportunity, by three

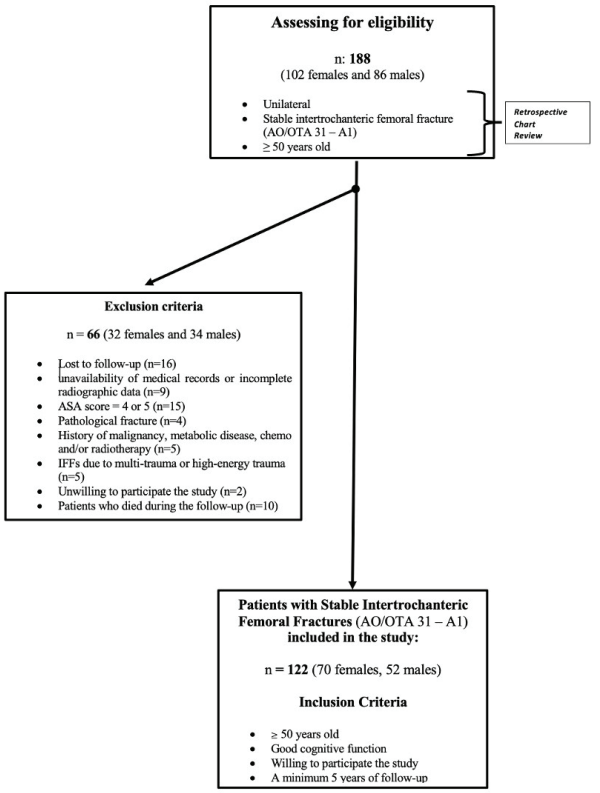

Figure 1: Flowchart of the study population, together with the inclusion and exclusion criteria

experienced orthopedic trauma surgeons either executing the operation or in attendance.

The type of implant used was decided based on the surgeon's preference and experience. After patients were placed supine on an orthopedic traction table, fractures were reduced by traction and internal rotation with the injured limb in a slightly adducted or neutral position to allow an introduction to the greater trochanter. The reduction was then checked, and implants were applied under image intensifier.

In group DHS, a DHS with a four-hole and 135 degrees plate (Dynamic Hip System screw/blade; Synthes GmbH, Basel, Switzerland) was inserted without any additional anti-rotational screw. In group PFN, proximal femoral nail anti-rotation (PFNA) (Synthes GmbH, Oberdorf, Switzerland) was performed (Figure 2, 3). In all patients, efforts had been paid to ideally place the tip of the screw within the subchondral bone of the femoral head with a combined tip-apex distance measuring less than 25 mm on anteroposterior and lateral radiographs.

Postoperative management and follow-up period

Patients were mobilized with partial weight bearing as tolerated at the second day after surgery, which was increased gradually according to the stability of the fracture and preference of the operating surgeon in PFN group. Patients were mobilized at the second day after surgery with no weight bearing until the bony union was confirmed radiographically in DHS group. All patients were recalled for regular follow-up examinations at the second, 6th, 12th, 24th week and once a year. During each follow-up visit, patients' functional status was assessed. To prevent any bias while comparing the total costs of two groups, implant costs were excluded.

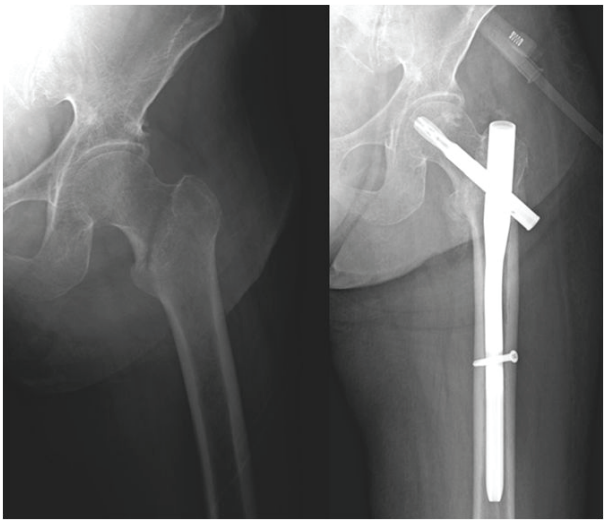

Figure 2: Pre- and post-operative AP X-ray of a 78-year-old patient with stable intertrochanteric femur fracture that was treated with PFN

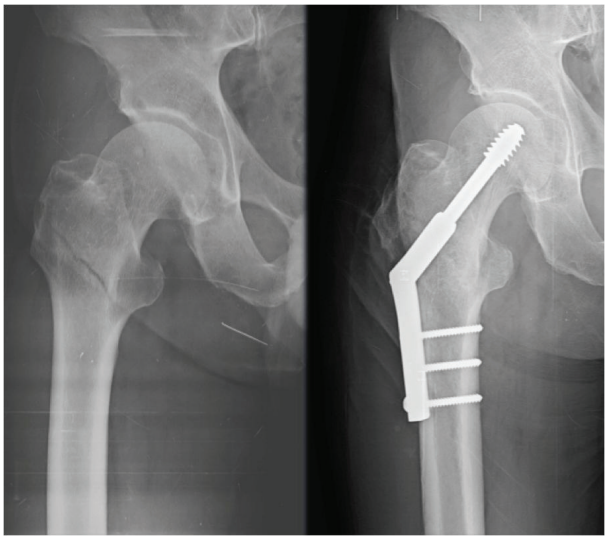

Figure 3: Pre- and post-operative AP X-ray of an 81-year-old patient with stable intertrochanteric femur fracture that was treated with DHS

Outcome measures

- Clinical assessment

The invasiveness of each operation was assessed based on the data including total operating time (TOT), estimat-

ed blood loss (EBL), duration of hospital stays (DHS), rate of postoperative complications, rate of mortality, and total hospital cost. All the relevant data were documented from our institution medical records including plain radiography, operative notes, information on demographic characteristics, discharge reports, and progress notes. EBL was calculated using the Mercuriali's formula (5). To prevent any bias while comparing the total costs of two groups, implant costs were excluded.

- Radiographic assessment

The position of the implants was examined measuring the tip-apex distance (TAD) on latest postoperative follow-up radiographs of the patients including anteroposterior (AP) pelvis and lateral hip views as per the method of Baumgaertner et al. (6). Also, femoral neck-shaft angle was measured on the AP hip radiographs.

Statistical analysis

For the statistical analysis, SPSS software (Version 22.0; SPSS Inc, Chicago, IL, USA) was used. Normality of distribution was tested using the Shapiro-Wilk test. Student's t-test was used to compare quantitative data, normally distributed variables of descriptive statistics (mean, standard deviation, median, frequency, rate, minimum and maximum). Pearson chi-square test, Fisher-Freeman-Halton test, and Fisher's exact test were used to compare qualitative variables. A p-value less than 0.05 was considered as statistically significant.

RESULTS

Baseline characteristics

No significant differences were observed in demographic characteristics between the two treatment groups (p>0.05). Group PFN consisted of 36 females and 21 males, and there were 34 females and 31 males in group DHS (p=0.227). The mean age was 74.7±10 years in group PFN and 72±9.9 years in group DHS (p=0.166). The mean follow-up was 44.2±31 months in group PFN and 53.7±38 months in group DHS (p=0.077) (Table 1).

Clinical outcomes

The mean TOT was significantly shorter in group PFN (90.3±19 minutes) than in group DHS (107.1±26 minutes) (p<0.001). The mean EBL was significantly less in group PFN (361±79 ml) than in group DHS (535±90 ml) (p=0.03). No significant difference was observed in the mean duration of hospital stay between the two treatment groups (6.62±1.8 days for group PFN vs 7.5±3 days for group DHS [p=0.165] (Table 2).

The post-operative complication rate was 9.5% in group PFN and 8.3% in group DHS, with no significant difference (p=0.83). Two patients developed pulmonary embolus, and three patients superficial wound side problems in group PFN. Two patients developed pulmonary embolism and three patient superficial wound side problem in group DHS. The mortality rate was 73.6% (42 patients) in group PFN and 67.6% (44 patients) (p=0.469).

The mean total hospital cost 1546.86±444.2 USD in group PFN and 1508.59±444.21 USD in group DHS. No significant difference was observed in terms of hospital costs between both groups (p=0.828).

Radiographic outcomes

The mean femoral-neck-shaft angle was 132.5° (range= 121°-139°) in group PFN and 133.6° (range=127°-138°) in group DHS (p=0.83). The mean AP/lateral tip apex distance was, respectively, 10.3 mm (range=4.2-13.1) and 9.9 mm (range=6.1-12.7) in group PFN as well as 11.8 mm (range=8.3-15.2) and 10.4 mm (range=6.5-14.2) in group DHS. There were significant differences in neither AP nor lateral tip apex distances between the two treatment groups (p=0.69 and p=0.87, respectively). The mean postoperative LLD was 0.78 mm (range=0-1.4) in group PFN and 0.8 mm (range=0-1.6) in group DHS, with no significant difference (p=0.74) (Table 2).

DISCUSSION

Over the last two decades, there has been a great controversy regarding the optimum treatment of stable IFFs PFN and DHS have been compared each other with respect to the clinical outcomes, biomechanical strength, rates of failure, rates of implant-related (blade cut- out, peri-prosthetic fracture) and patient-related (morbidity-mortality) complications, as well as the technical difficulties (7, 8). Current literature illustrates the pros and cons of each treatment modality. However, to the best of our knowledge, evidences from the existing literature to determine the more advantageous implant to be used in the treatment for stable IFFs are controversial. Data obtained from the current study have found no significant differences in clinical and radiological outcomes except EBL and TOT which were higher in group DHS.

According to a large Cochrane review, DHS was regarded as the gold standard for the treatment of stable IFFs because of the lower rates of complications and reoperations compared to PFN (8). These results were also supported by many other studies (8-10, 11). In the present study, no statistically significant difference was found with regard to complication rates between the two groups (p=0.83).

Many studies underlined that there was no significant difference between PFN and DHS with regard to bleeding which was another important intra- and post-operative concern (7, 10). EBL was found higher in group DHS compared to PFN detected with a low statistical significance (p=0.048). We believe that this small difference may have possibly occurred because of the technical principles of conventional DHS application. As shown in the literature, performing the

Table 1: Demographic data of both groups

	PFN group		DHS group		P value
	Mean±SD	Min–Max	Mean±SD	Min-Max	
Age (years)	74.7±10	50–94	72±9.9	53-97	0.166
Gender (F/M)	36/21		34/31		0.227
Follow-up (months)	44.2±31	12–140	53.7±38	12–144	0.077
Side (R/L)	29/28		36/29		0.619

SD: Standard Deviation; Min: Minimum; Max: Maximum; F: Female; M: Male; R: Right; L: Left

Table 2: Summary of mean clinical parameters

	Group 1 (PFN) Mean±SD	Group 2 (DHS) Mean±SD	P value
Duration of surgery (min)	90.3±19	107.1±26	<0.001
Estimated blood loss (ml)	361±79	535±90	0.03
Duration of hospital stay, (day)	6.62±1.8	7.5±3	0.165
Post-operative complications, (%)	9.5	8.3	0.83
Mortality, (%)	73.6	67.6	0.469
Time to unassisted mobilization	32.17±6.39	32.78±5.39	0.93
Mean total hospital costs when the implant costs are excluded	1546.86±444.2	1508.59±444.21	0.828
Femoralneck-shaft angle, (°)	132.5±4.2	133.6±3.1	0.83
AP tip apex distance, (mm)	10.3±2.7	11.8±3.2	0.69
Lateral tip apex distance, (mm)	9.9±1.9	10.4±3.7	0.87

SD: Standard deviation; min: minute; ml: mililiter; mm: milimeter; USD: United Stade Dollars

DHS minimally invasively may reduce the amount of total average blood loss in such patients (11, 12).

In the recent literature, duration of operations which directly influenced patients' prognosis and surgeon's performance was found to be similar between the two treatment modalities in concordance with the results of the present study (13, 14). This was probably due to experienced surgical team which could perform both surgeries with similar precision in terms of the surgical technique. The recent literature failed to show any significant difference between the two groups with regard to the duration of hospital stay, while some studies detected that the PFN group had a significantly longer hospital stay (7, 8, 15). The results of our study have shown that patients receiving DHS were hospitalized for a longer duration as compared to those receiving PFN, while no statistical significance was detected.

Without the exclusion of the implant costs, total hospital costs of PFN were previously reported more expensive than DHS (8, 16-20). We considered that the inclusion of implant cost in the comparison may have causes a high susceptibility to bias because of higher implant costs of PFN as compared to DHS. Accordingly, to prevent such a bias, we excluded the costs of the implants and found similar total hospital costs regardless of the implant type. Also, we believe that this was an expected result because of the demographical comparability of the two groups.

Memon et al. reported a study which investigated the fracture union and collapse, femur neck shortening, implant position and failure or collapse (cut out risk) (21). In that study, they found that PFN group demonstrated no implant cut out and less mean limb length shortening. Ricci et al. reported a study which investigate the secondary collapse is related to fixation method in 2-part intertrochanteric femur fractures in patients treated with PFN versus DHS (22). In that study, these fractures are not necessarily stable when treated with DHS and dual screw PFN seems to be most effective to maintain stability for patients with this fracture pattern.

Finally, some limitations and strengths of the current study should be taken into account. The main limitation was the retrospective nature of the study with a relatively low number of cases. Another limitation is that this study did not include the Oxford, Harris, Modified Harris,

HOOS, WOMAC scores to perform a functional evaluation. Bone quality of patients were not investigated which effect the bone union and radiological parameters. Lastly, other factors affecting mortality, such as chronic steroid use, sarcopenia, and cancer were not investigated. The main strength of this study was to provide a solution for the controversy in the literature in terms of the ideal management strategy of sIFFs by underlining that DHS was a more cost-effective method of treatment. However, it should also be highlighted that unstable IFFs were diagnosed more frequently in older patients as compared to stable IFFs, while intramedullary nailing was considered as the gold standard for the management of unstable IFFs for its biomechanical properties (17-20). To confer a better understanding about the optimal treatment of stable IFFs, large prospective randomized studies are needed.

The present study concluded that, for stable IFFs, fixation with dynamic hip screw versus proximal femoral nail had no significant difference with regard to duration of hospital stays, rates of post-operative complications and mortality and time until unassisted mobilization, while both groups were noted to have similar radiographic results. Within 40 years following surgery, the two treatment modalities have similar effective to maintain stability for patients with this fracture pattern and complication rate (19-22). However, PFN have lower duration of operation and blood loss than DHS.

Informed Consent: Written consent was obtained from the participants.

Ethics Committee Approval: This study was approved by the Ethical Committee of the Istanbul University Istanbul Faculty of Medicine (Date: 04.04.2021, No: 117).

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- T.P., S.B., M.C., M.D.; Data Acquisition- S.B., M.C., E.K.; Data Analysis/Interpretation- T.A., A.S., S.B., M.D.; Drafting Manuscript- T.P., S.B., M.D., T.A.; Critical Revision of Manuscript- Ö.Y., T.A., A.S.; Final Approval and Accountability- T.P., S.B., M.D., M.C., E.K., A.S., T.A., Ö.Y.

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İSTANBUL ÜNİVERSİTESİ YAYINEVİ

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NEUROENDOCRINE CARCINOMA OF THE BREAST: 18 CASES WITH LONG-TERM FOLLOW-UP

MEMENİN NÖROENDOKRİN KARSİNOMU: 18 HASTA VE UZUN DÖNEM SONUÇLARI

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ABSTRACT

Objective: Primary neuroendocrine carcinoma of the breast (NECB) is a rare distinct type of breast carcinoma. There is limited data about the optimal management, treatment, and prognosis. Therefore, we analyzed the clinicopathological features, management and the clinical outcome of this rare breast carcinoma.

Material and Methods: Patients diagnosed as NECB between July 2008 and January 2018 were included in the study. Medical records were retrospectively reviewed.

Results: A total of 4,896 breast cancer patients were reviewed and 18 NECB (0.4% of all cases) were extracted. The median age was 61.5 (30-82). Thirteen cases (72.2%) underwent breast conserving surgery. Eight patients had axillary lymph dissection. All of the cases were pathological T1 and T2. Only one patient was pathological stage 3. Median tumor size was 20.5mm (10-45). Only two cases presented with small cell subtype, the rest were well-differentiated. Hormone receptor was positive and HER2/neu was negative for all cases. Of the 15 patients with known Ki-67, three had high expressions (≥20%). No local or distant disease recurrences and death related with NECB were detected at a median follow-up period of 101 months (33-148).

Conclusion: NECB is more likely to be hormone receptor positive and HER2/neu negative as luminal A or B subtype. An excellent clinical outcome is remarkable despite a substantial number of patients with axillary lymph node positivity specifically for well-differentiated subtype. Less invasive treatment options should be kept in mind.

Keywords: Neuroendocrine carcinoma, breast neoplasm, prognosis

ÖZET

Amaç: Nöroendokrin meme karsinomu (NMK) nadir görülen ve özellikli bir meme tümörüdür. Bu alt tipin tedavisi ve prognozu ile ilgili bilgiler sınırlıdır. Çalışmamızda, bu nadir görülen tümörün klinikopatolojik özelliklerini, tedavisini ve klinik sonuçlarını inceledik.

Gereç ve Yöntemler: Temmuz 2008 ve Ocak 2018 tarihleri arasında NMK tanısı alan hastaların verileri retrospektif olarak incelendi.

Bulgular: Toplam kayıtlı 4896 meme kanseri hastasının 18'i NMK idi (toplam vakaların %0,4'ü). Ortanca yaş 61,5 (30-82) olarak bulundu. On üç hastaya (%72,2) meme koruyucu cerrahi uygulandı. Sekiz hastaya ise aksiller lenf nodu diseksiyonu yapıldı. Sadece bir hasta patolojik evre 3 iken tüm hastalar patolojik olarak T1 ve T2 idi. Ortanca tümör boyutu 20,5 mm (10-45) olarak bulundu. Sadece iki hasta küçük hücreli alt tipi iken 16 hasta iyi-diferansiye alt tipindeydi. Tüm hastalar hormon reseptör pozitif ve HER2/neu negatifti. Ki-67 değeri bilinen 15 hastadan 3 tanesinde yüksek Ki-67 değeri (>%20) mevcuttu. Ortanca 101 (33-148) aylık takip süresinde lokal-bölgesel veya uzak rekürrens görülmedi. On sekiz hastada hastalığa bağlı ölüm görülmedi.

Sonuç: NMK genellikle hormon reseptör pozitif ve HER2/neu negatif olacak şekilde luminal A veya B olarak tespit edilmektedir. Aksilla pozitif hastalar olsa da özellikle iyi-diferansiye alt grupta sağ kalımlar çok iyidir. Bu hastalarda daha az girişimsel tedavi seçenekleri göz önünde bulundurulmalıdır.

Anahtar Kelimeler: Nöroendokrinkarsinom, meme neoplazmı, prognoz

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INTRODUCTION

Primary neuroendocrine carcinomas of the breast (NECB) are rare neoplasms of the breast with similar morphological features to neuroendocrine tumors of the gastrointestinal tract and lung (1). The World Health Organization (WHO) described neuroendocrine marker expression in at least 50% of the total cell population of NECB. Expression of neuroendocrine markers, specifically synaptophysin and chromogranin A are generally present (2). According to the WHO classification, NECB has three subtypes based on the morphology as well-differentiated neuroendocrine tumors, small cell neuroendocrine carcinomas, and invasive carcinomas of the breast with neuroendocrine differentiation (3).

NECB is the disease of the sixth and seventh decade of females who are generally postmenopausal (4). Although the prevalence was reported between 2 and 5% in the WHO 2003 classification, recent studies demonstrated that it is between 0.1 and 0.5% (4-6).

NECB is associated with a more aggressive behavior, higher propensity for local recurrence, and poorer prognosis than ductal carcinoma (7). The optimal treatment for primary NECB is poorly known because of limited reports. These tumors can be misdiagnosed due to the lack of distinguishing features on presentation and imaging. It is important to recognize these tumors and distinguish them from other poorly differentiated tumors of the breast and metastatic small cell carcinomas from the lung in order to avoid diagnostic errors that could have therapeutic and prognostic implications for these patients. In this study, we demonstrated the clinicopathological characteristics, management and clinical outcomes of this rare breast carcinoma.

MATERIALS AND METHODS

Patients with a diagnosis of NECB between July 2008 and January 2018 were retrospectively evaluated. Demographic features, clinicopathological factors, treatment modalities, and outcomes of patients were recorded. Each patient was carefully managed in order to rule out breast metastasis of neuroendocrine carcinomas from other organs such as the gastrointestinal tract or lung. All patients were managed as breast carcinoma not otherwise specified (NOS) in concordance with international guidelines.

Up-to-date WHO criteria for NECB definitions were used. Tumors were stained by immunohistochemistry for neuroendocrine markers, synaptophysin and/or chromogranin-A along with estrogen receptor (ER) and progesterone receptor (PR), HER2/neu expression, and Ki-67 levels. Adjuvant treatment (chemotherapy, radiotherapy and endocrine therapy) was decided as in invasive ductal carcinoma of the breast.

Categorical and continuous variables were summarized using descriptive statistics (e.g. median, range, frequency, and percentage). Kaplan-Meier method was used for survival analysis. All statistical analyses were performed using the SPSS program (Version 22.0, SPSS Inc., Chicago, IL, USA).

RESULTS

Between July 2008 and January 2018, 4,896 breast cancer patients were treated in our clinic and 18 cases were identified that fulfilled the 2003 WHO criteria for NECB. The incidence of NECB was 0.4% and all cases were female. The median age was 61.5 (30-82). All cases underwent radiologic evaluation by mammogram, ultrasound, and magnetic resonance imaging (MRI) if needed. If axillary lymph nodes were clinically positive, positron emission tomography and computed tomography (PET/CT) were also performed.

Most of the cases (75%) underwent breast conserving surgery. Axillary lymph node dissection (ALND) was performed for eight patients. All patients had sentinel lymph node biopsy, except one patient that had clinically stage II nodal disease. She underwent direct ALND. The median tumor size was 20.5 mm (10-45). Pathological stage II nodal disease was also present in one patient who was mentioned before. Of the 18 cases, 16 were classified as well-differentiated and two were small cell neuroendocrine carcinoma according to the WHO criteria. All cases were hormone receptor positive and HER2/neu negative. Only two patients that demonstrated with small cell neuroendocrine carcinoma had higher levels of Ki-67 (60% and 80%). Synaptophysin and chromogranin-A expression were present in all cases. Details of clinical and pathologic features are presented in Table 1.

The median follow-up time was 101 months (33-148). Adjuvant treatment was administered as for the same principles for invasive ductal carcinoma of the breast. Seventeen patients had endocrine therapy with tamoxifen in premenopausal and anastrazole in postmenopausal patients. The only patient who did not received endocrine therapy was an 82-year old patient with co-morbidities. There was no mortality associated with NECB. One patient died of cardiac events. No local recurrences or distant metastases were detected (Table 2).

DISCUSSION

Primary NECB is a rare tumor. On specimens, if more than 50% of the cells express any of neuroendocrine markers (chromogranin-A, chromogranin-B, neuron specific enolase, and synaptophysin), it can be described as primary NECB (1). This criteria distinguishes NECB from other breast carcinomas that show only neuroendocrine morphological features or focal (i.e.,<50%) neuroendocrine

Table 1: Demographic and pathologic features of cases (n=18)

Factors	Category	n	%
Median age		61.5 (30-82)	
	<60	8	44.4
	≥60	10	55.6
AJCC cT stage	I	7	38.9
	II	10	55.6
	III	1	5.6
AJCC cN stage	0	13	72.2
	I	4	22.2
	II	1	5.6
Operation type	BCS	13	72.2
	Mastectomy	5	27.8
Axillary surgery	SLNB	10	55.6
	ALND	1	5.6
	SLNB + ALND	7	38.9
Median tumor diameter (mm)		20.5 (10-45)	
AJCC pT stage	I	9	50
	II	9	50
AJCC pN stage	0	11	61.1
	I	6	33.3
	II	1	5.6
AJCC p stage	I-A	6	33.3
	II-A	8	44.4
	II-B	3	16.7
	III-A	1	5.6
ER status	Positive	18	100
	Negative	0	0
PR status	Positive	17	94.4
	Negative	1	5.6
HER2/neu status	Positive	0	0
	Negative	18	100
Ki-67 (n=15)	Positive (≥20%)	3	20
	Negative (<20%)	12	80
Factors	**Category**	**n**	**%**
MBR grade	I	0	0
	II	15	83.3
	III	3	16.7
WHO classification	Well-differentiated	16	88.9
	Small cell	2	11.1
Adjuvant treatment	**Chemotherapy**		
	Yes	9	50
	No	9	50
	Radiotherapy		
	Yes	11	61.1
	No	7	38.9
	Endocrine therapy		
	Yes	17	94.4
	No	1	5.6

AJCC: American Joint Committee on Cancer, BCS: Breast conserving surgery, SLNB: Sentinel lymph node biopsy, ALND: Axillary lymph node dissection, ER: Estrogen receptor, PR: Progesterone receptor, HER2/neu: Human epidermal growth factor receptor-2, MBR: Modified Bloom-Richardson, WHO: World Health Organization

Table 2: 5-year and 10-year survival features of the patients

Median follow-up (month)	101 (33-148)
5-year disease free survival	100%
5-year overall survival	94.1%
10-year disease free survival	100%
10-year overall survival	94.1%

differentiation. The rate of NECB was 0.4% in our series, that is similar to previously published series (0.1-0.5%) (4-6).

NECB is detected as especially round spiculated masses on mammogram (5). They generally present homogeneous echogenicity with some posterior acoustic enhancement on sonography. It morphologically seems like triple negative breast tumors. However, these findings are not specific for NECB. As diagnosis and differential diagnosis is made by pathologic evaluation with neuroendocrine markers, metastasis from a primary neuroendocrine tumor other than breast should be excluded, and a core biopsy is recommended (8). Almost two thirds of NECB are associated with ductal carcinoma *in-situ* that distinguishes these masses from metastases (9). We performed core needle biopsy for all cases. On advanced stages, PET/CT can be used for systemic evaluation. We performed PET/CT for five clinical axilla positive patients and there were no distant metastasis. The surgical treatment strategy is generally similar with the management of ductal carcinoma. As many

patients are early stage, specifically T1 and T2 cases, we performed breast conserving surgery for 13 (72%) patients. Although only seven patients had pathological axillary lymph node positivity, we performed ALND on eight patients. One patient whose final pathologic nodal evaluation was negative reported as positive in intraoperative pathologic evaluation of the sentinel lymph node. So that ALND was decided upon.

The WHO has classified NECB into three groups as well-differentiated neuroendocrine tumors, small cell neuroendocrine carcinomas, and invasive carcinomas of the breast with neuroendocrine differentiation (3). Sixteen patients in our cohort were well-differentiated and two were small cell. Most of the primary NECB express ER up to 90-100% and PR up to 80-90% (7). This level of hormone receptor positivity is significant for survival. HER2/neu overexpression in NECB is very rare so that most studies in literature classified NECB as luminal A or sometimes luminal B type (10-12). In large series by Bogine et al. and Lavigne et al. they reported that almost 50% of cases are luminal A and 50% are luminal B (11, 12). In our series, only three cases of the 15 with a known Ki-67 status were luminal B (high Ki-67 ≥20%). All the cases were hormone receptor positive and there were no HER2/neu positivity.

As there is a lack of information due to the low incidence of NECB cases, chemotherapy regimens are not standardized. Nevertheless, general recommendation is treating it similarly to the treatment standards for ductal neoplasms (13). If chemotherapy is administered, the first line treatment choice is anthracycline and taxane based regimens (8). Almost all of the cases reported in the literature are luminal type so the adjuvant endocrine therapy is also a standard of care for most of the cases (13). All patients, except an 82-year old female with co-morbidities, received endocrine therapy in our series. Radiotherapy also must be taken into consideration for patients with breast conserving surgery and for advanced stage patients in the light of international guidelines. We administered adjuvant radiotherapy to 11 of our patients.

There are different survival reports for the outcome and prognosis of NECB patients. Some of them demonstrate worse survival and few of them demonstrate better survival. Most of the studies provide worse survival rates when compared to invasive ductal carcinomas of the same stage. Wang J et al. reviewed the surveillance, epidemiology, and end results (SEER) database for NECB. They indicated that the overall survival and disease specific survival were significantly worse in NECB (n=142) compared with invasive mammary carcinoma, not otherwise specified at the same stage (4). Another study by Zhang et al. also presented that NECB have a higher rate of local recurrence and lower rate of overall survival (14). On the other hand, recent studies express that poorer local control and survival outcomes are associated with small cell subtype of the NECB, not for the well-differentiated subtype (12, 15). These results are more reasonable as we know that morphology is the key for survival of the patients. In our series, we reported excellent survival outcomes with a median follow-up time of 101 months. There was only one death and it was associated with a cardiovascular event. The 5-year and 10-year overall survival rates were both 94.1%. There was no locoregional or distant recurrence during the follow-up time (Disease free survival and disease specific survival were 100%). This is probably because 16 of the 18 patients in our cohort were well-differentiated subtype. Even so, two patients with small cell subtype had 99 (AJCC stage 2A) and 62 (AJCC stage 3A) months of follow-up time with no locoregional or distant recurrences. In the light of the results of these recent studies and our study, the need for chemotherapy for well-differentiated NECB should be questioned. Additionally, we can even consider omitting surgical axillary lymph node staging for patients with early stage and favorable tumor biology as demonstrated by Özkurt et al. for tubular breast cancers (16).

However, we should always keep in mind that small cell subtype is aggressive and can metastasize even after several years from initial diagnosis so that long-term close follow-up is recommended.

In conclusion, NECB is a different subtype and rare variant of breast carcinoma. Our results suggest that NECB is more likely to be ER/PR positive and HER2/neu negative as luminal A or B subtype. An excellent clinical outcome is remarkable despite a substantial number of patients with axillary lymph node positivity specifically for well-differentiated subtype. This favorable prognosis might be due to good tumor biology profile associated with hormone receptor positivity with a substantial benefit from hormone therapy and other adjuvant therapies. Finally, we must be aware of the small cell subtype of NECB and follow-up closely for a long time as it can present with advanced stage disease and distant metastasis.

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İSTANBUL ÜNİVERSİTESİ YAYINEVİ

RESEARCH - ARAŞTIRMA
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EVALUATION OF $^{68}Ge/^{68}Ga$ GENERATOR PRE-ELUTION EFFICIENCY ON METALLIC IMPURITIES IN THE COMPOSITION OF $^{68}GaPSMA$-11 RADIOPHARMACEUTICAL IN NUCLEAR MEDICINE PET CHEMISTRY

NÜKLEER TIP PET KİMYASINDAKİ $^{68}GAPSMA$-11 RADYOFARMASÖTİK BİLEŞİMİNDEKİ METALİK KİRLİLİKLER ÜZERİNDE $^{68}Ge/^{68}Ga$ JENERATÖRÜNÜN ÖN ELÜSYON ETKİNLİĞİNİN DEĞERLENDİRİLMESİ

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ABSTRACT

Objective: The presence of metallic impurities can pose a serious concern due to their interaction with receptor-specific biomolecules that require the highest possible specific activity, especially in radioactive labeling. There are studies in which Zn and other toxic metal pollution increases if the generator is not used for a long time. In this study the effect of pre-elution on chemical and radiochemical impurities in the synthesis product was investigated.

Material and Methods: In the study, $^{68}GaPSMA$-11 labeling was performed with the generator eluate that was not used for one month (sample 1). A blank elution was taken 24 hours after using the generator (sample 2). In the next 24 hours, the second $^{68}GaPSMA$-11 labeling was performed (sample 3). Six hours after this labeling, another $^{68}GaPSMA$-11 synthesis was performed (sample 4). Eluates were analyzed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

Results: Results was reported as a table in µg/ml. ^{68}Ge levels determined at 0.018 ppm, 0.012 ppm and 0.009 ppm levels in the labeled products were determined below the pharmacopoeia limits. Zn(II) impurities were found as 0.625 ppm in the labeling performed without generator pre-elution. In generator pre-eluted labelings; Zn(II) impurities were determined as 0.133 ppm and 0.108 ppm.

Conclusion: Pre-elution of the generator prior to synthesis does not seem to be a chemical requirement other than zinc

ÖZET

Amaç: Metalik safsızlıkların varlığı, özellikle radyoaktif etiketlemede mümkün olan en yüksek spesifik aktiviteyi gerektiren reseptöre özgü biyomoleküllerle etkileşimleri nedeniyle ciddi bir endişe oluşturabilir. Jeneratörün uzun süre kullanılmaması durumunda Zn ve diğer toksik metal kirliliğinin arttığı çalışmalar da bulunmaktadır. Bu çalışmada ön elüsyonun sentez ürünündeki kimyasal ve radyokimyasal safsızlıklar üzerindeki etkisi araştırılmıştır.

Gereç ve Yöntemler: Çalışmada, $^{68}GaPSMA$-11 işaretlemesi, bir ay kullanılmayan jeneratör eluatı (örnek 1) ile yapıldı. Jeneratör kullanıldıktan 24 saat sonra boş bir elüsyon alındı (örnek 2). 24 saat içinde, ikinci $^{68}GaPSMA$-11 işaretlemesi gerçekleştirildi (örnek 3). Bu etiketlemeden 6 saat sonra başka bir $^{68}GaPSMA$-11 sentezi gerçekleştirildi (numune 4). Elüatlar, Endüktif Olarak Birleştirilmiş Plazma Kütle Spektrometresi (ICP-MS) ile analiz edildi.

Bulgular: Sonuçlar µg/ml cinsinden tablo olarak rapor edildi. İşaretli ürünlerde 0,018 ppm, 0,012 ppm ve 0,009 ppm seviyelerinde belirlenen ^{68}Ge seviyeleri farmakope limitlerinin altında belirlendi. Jeneratör ön elüsyonu yapılmadan yapılan işaretlemede Zn(II) kirliliği 0,625 ppm bulundu. Önceden yıkanmış jeneratör eluatı ile işaretlemelerde; Zn(II) kirliliği 0,133 ppm ve 0,108 ppm olarak belirlenmiştir.

Sonuç: Jeneratörün sentezden önce ön elüsyonu, çinko kirliliği dışında kimyasal bir gereklilik gibi görünmemektedir.

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pollution. ^{68}GaPSMA-11 can be pre-eluted for high labeling efficiency, but it is thought that pre-elution is not significant in terms of chemical and radiochemical contamination.

Keywords: Radiopharmaceutical product, ^{68}GaPSMA-11, metallic impurities, ^{68}Ge/^{68}Ga generator

^{68}GaPSMA-11, yüksek işaretleme etkinliği için önceden ayrıştırılabilir, ancak ön elüsyonun kimyasal ve radyokimyasal kontaminasyon açısından önemli olmadığı düşünülmektedir.

Anahtar Kelimeler: Radyofarmasötik ürün, ^{68}GaPSMA-11, metalik safsızlık, ^{68}Ge/^{68}Ga jeneratör

INTRODUCTION

Germanium is a rare element; it represents 10-11% of the earth's crust. There are four different stable isotopes of germanium; ^{70}Ge (20,55%), ^{72}Ge (27.37%), ^{73}Ge (7,67%), ^{74}Ge (36,74%). ^{68}Ge can be produced with the reactions ^{75}As(d,α2n) ^{68}Ge(79) or ^{69}Ga(p,2n) ^{68}Ge (80). However, recently a way to produce carrier-free germanium by reaction ^{66}Zn (a, 2n) ^{68}Ge has been obtained by liquid-liquid extraction with CCl_4 and Xylene (1).

The use of ^{68}Ge/^{68}Ga generator systems that do not require cyclotron has been a source of motivation for the evolution of ^{68}Ga-radiopharmacy (2-4). Gallium-68 ($^{68}Ga^{3+}$, β^+=89%, Eβ^+ max=1.90 MeV) is usually obtained through the electron capture decay of germanium-68), absorbed on an appropriate solid phase (i.e. generator produced). The half-life and other physical properties of both mother and daughter nuclei make ^{68}Ge/^{68}Ga generators suitable for clinical application in cyclotron-free imaging facilities. The daughter exists in secular equilibrium with the ^{68}Ga (t1/2=68.1 min), the mother ^{68}Ge (t1/2=271 days) (5).

In the ^{68}Ge/^{68}Ga generator system, ^{68}Ge is loaded into column material based on mineral oxides or organic extracts, and ^{68}Ga can be eluted using acidic media that vary depending on the generator material (6). Injection of $^{68}Ga^{3+}$ in the ionic form results in its very rapid association to native transferrin. The well stable binding requires that $^{68}Ga^{3+}$ radiopharmaceutical preparations be free from metallic impurities to ensure that the background blood radioactivity is as low as possible. With the increase in the age of the generator and the increase in the number of elations performed, the ^{68}Ge value may increase in addition to the regular activity. Metal impurities from the generator may not just be radionuclides. Toxic metals coming from the column material are also among the pollutants which can compete with gallium in the complexation reaction. Zn formation occurs with the decay of ^{68}Ga. It is known that the accumulation of Zn(II) in the generator column is constantly increasing. During equilibrium, when the maximum amount of ^{68}Ga accumulates, the amount of Zn(II) exceeds 10 times of ^{68}Ga. This excess is discarded when the generator is eluted and the ^{68}Ga/Zn(II) ratio is still above after ~2 half-life. Therefore, there are studies showing that regular elution before synthesis reduces the Zn(II) concentration 4-5 times (1, 3). The presence of non-radioactive metals such as Pb, As, Ni, which are considered metallic impurities in the ^{68}Ge/^{68}Ga generator eluate, is known. The presence of these metallic impurities could cause serious concern, particularly due to its interaction in radioactive labeling with receptor-specific biomolecules that require the highest possible specific activity (7). Therefore, secondary purification prior to labeling is an important step in radiopharmaceuticals labeled the used ^{68}Ga for clinical applications (8-10). Different methods used for these processes are based on anion exchange chromatography, cation exchange chromatography, or a combination thereof (11). The intended use of the cation exchange resin is to chemically distinguish between ^{68}Ga(III) and ^{68}Ge(IV). The aim is to quantitatively separate ^{68}Ga on the cartridge from ^{68}Ge completely passed through the cation exchange resin.

Radiolabeled PSMAs are the most important targets for imaging diagnosis and targeted radionuclide therapy of PC and its metastases because of their rapid and effective localization in tumors (12, 13). In particular, gallium, as a radionuclide, is used to label PSMAs to obtain suitable imaging ligands for PET/CT imaging. It shows higher tumor uptake and provides more acceptable background clarity. In this context, between ^{68}Ga-PSMA11, ^{68}Ga-PSMA617, and ^{68}Ga-PSMA I&T, ^{68}Ga-PSMA I&T as therapeutic agents are widely used for PET/CT imaging (14-16).

In addition to these disadvantages, studies on the long shelf life of the generator, the radiological stability of the column material, metal cationic impurities, the sterility of the eluate, and the long-life ^{68}Ge waste management are continuing. The development of stable materials addressing the aforementioned issues is an inevitable process that requires continuous improvement efforts by researchers.

In this study; ^{68}GaPSMA-11 synthesis was performed without pre-elution of the generator, which was not used for one month. With the same generator, ^{68}GaPSMA-11 synthesis was carried out by pre-elution at different day and time intervals. A comparison of the metal impurities in the composition of the ^{68}GaPSMA-11 radiopharmaceuticals was made at the present work.

MATERIALS AND METHOD

Chemicals

All reagents to be used for synthesis and quality control were purchased from Merck in high purity pharmaceutical grade. Kit equipment for the synthesis of ^{68}Ga peptides using cationic purification were obtained from ABX D-01454 Radeberg (Germany). The kit contains chemicals, hardware, and the cassette required for radiosynthesis of ^{68}Ga peptides the Scintomics GRP synthesizer using cationic purification. The kit components are cassette, PSH$^+$ cartridge, 5 M sodium chloride solution, ethanol, ethanol/water(1/1), phosphate buffered saline, 1.5 M HEPES buffer solution, and water for injections. The cassette exhibits are disposable and therefore made for single use. Reference PSMA-11 peptide was purchased from ABX D-01454 Radeberg (Germany) and stored at -20°C. Dilutions of PSMA11 were prepared with Farmako brand sterile water (1:1). Hydrochloric acid (0.1 N ultra-pure HCl) and 1.5 M HEPES (2-[4-(2-hydroxyethyl) piperazin-1-yl] ethane sulfonic acid) buffer solutions were obtained from ABX D-01454 Radeberg (Germany).

Instruments

$GaCl_3$ eluates were obtained from PARS Isotope-GalluGEN (Iran) ^{68}Ge/^{68}Ga generator with HCl solution in Scintomics GmbH GRP module 4V synthesis module.

^{68}Ge/68Ga Generator Certificate Properties; Column material SnO_2, TiO_2; HCl eluate concentration 0,1 N; Elution volume 7 ml; Generator age 6 month old, 120th elution; Chemical Impurity Zn<10 µg/GBq, Fe<10 µg/GBq.

Labelling of PSMA 11 with $^{68}GaCl_3$ elution in automated synthesis module

Cation exchange cartridge (PSH$^+$, no-preconditioned) was used to remove trace metals in $GaCl_3$ solution eluted from ^{68}Ge/^{68}Ga generator. $GaCl_3$ eluted from the PSH$^+$ cartridge with 5.0 M NaCl was added to the reaction vial containing 25 µg peptide dissolved in HEPES buffer. The mixture was then heated for 15 minutes at 90°C for labeling of the peptide with ^{68}Ga(III). Solution of ^{68}GaPSMA-11 in the reaction vial was passed through the C18 ion exchange cartridge to remove the unbound free ^{68}Ga ions. The retained ^{68}GaPSMA-11 was eluted from the C18 ion exchange cartridge with 2.0 ml ethanol/water (1/1). The product was passed through the 0.22 µm filter syringe and collected in the final vial.

Sample analysis

In the study, ^{68}GaPSMA-11 labeling was performed with the generator eluate that was not used for two months (sample 1). A blank elution was taken 24 hours after using the generator (sample 2). In the next 24 hours, the second ^{68}GaPSMA-11 labeling was done (sample 3). Six hours after this labeling, another ^{68}GaPSMA-11 synthesis was performed (sample 4).

Qualitative and quantitative analyzes of metal contents in ^{68}GaPSMA-11 eluate was made at the µg/ml(ppm) level and reported with the ICP-MS device located in the Advanced Technology Application and Research Center of the university. Some parameters of the device used are shown in Tables 1. With the ICP-MS analysis method; Germanium (Ge), Manganese (Mn), Zinc (Zn), Tin (Sn), Cobalt (Co), Nickel (Ni), Lead (Pb), Aluminum (Al) metal presence was examined. ICP-MS standard solutions were obtained from Perkin Elmer. Certified levels of standard solutions Ge: 999 µg.ml^{-1}±5 µg/mL. Mn: 1002 µg.ml^{-1}±5 µg/mL, Zn: 998 µg.ml^{-1}±5 µg/mL, Sn: 1002 µg.ml^{-1}±5 µg/mL, Co: 999 µg.ml^{-1}±5 µg/mL, Pb: 999 µg.ml^{-1}±5 µg/mL, Al: 1002 µg.ml^{-1}±5 µg/mL and Ge, Mn, Zn, Sn, Co, Pb and Al were used as internal standards for ICP-MS analysis. Analyzed metals; during extraction of nat-Ga to ^{68}Ge in cyclotron; column matrices in the ^{68}Ge/^{68}Ga generator and environmental factors have been selected for consideration. The results were recorded in the table format of the device and reported in excel format in the order of µg/ml.

Table 1: The operating conditions of the ICP-MS device

The operating conditions	
Rf Powers	1300 W
Gas flow rate	1.5 ml/min
Plasma gas flow	15 ml/min
Auxiliary gas flow	0.2 ml/min
Nebulizer gas flow	0.65 ml/min
Sample flow rate	1.5 ml/min
Flush time	20 sec
Read time	3 s

RESULTS

The purification method of ^{68}Ga eluate from the generator has been standardized using an automated system. The Ga^{3+} form of Ga is easily converted into other forms for use as a radiopharmaceutical. ^{68}Ga-gallate in HEPES medium was complexed by heating to convert to ^{68}GaPSMA-11. Therefore, no additional quality control was required for method validation. The automated synthesis was performed within 32 min. The pH of the final products was determined to be in between 6 and 7. The radiochemical yield of ^{68}GaPSMA-11 was > 99% by RP-HPLC. All samples were analyzed for metallic contamination by ICP-MS. The results are shown in Table 2.

Non-radioactive metal ions such as Zn(II) (produced by the decay of ^{68}Ga), Sn(IV) (produced from the SnO_2 column-based ^{68}Ge/^{68}Ga generator) and Mn, Fe(III) general

Table 2: ICP-MS analysis of ^{68}GaPSMA-11 radiopharmaceuticals and generator eluate

Metal pollution	^{68}GaPSMA-11 labeling (without pre-eluted, µg/ml)	Pre-elute of the generator (µg/ml)	^{68}GaPSMA-11 labeling (pre-eluted, µg/ml)	^{68}GaPSMA-11 labeling (After 6 hours, µg/ml)
Ge	0.018	0.325	0.012	0.009
Co	ND	0.001	ND	ND
Zn	0.625	1.382	0.133	0.108
Al	0.012	0.708	0.015	0.010
Pb	<0.001	0.008	<0.001	<0.001
Sn	0.004	0.003	0.001	0.001
Mn	<0.001	<0.001	<0.001	<0.001

Ge: Germanium; Co: Cobalt; Zn: Zinc; Al: Aluminum; Pb: Lead; Sn: Tin; Mn: Manganese

chemical impurity for labeling of radiopharmaceutical precursors it represents the metal ions that can compete with Ga^{3+}. Other chemical non-radioactive impurities arise in the production of ^{68}Ga; Nb, Ni or Cu.

^{68}Ge radioactive impurity was detected 0.325 ppm in the generator elution. It separated ^{68}Ge of the PSH^{+} cartridge and the liquid were sent to waste. ^{68}Ge levels determined at 0.018 ppm, 0.012 ppm, and 0.009 ppm levels in the labeled products were determined below the pharmacopoeia limits. And there was no significant difference between labeled products with elutions of pre-eluted generator and non-pre-eluted generator. Cobalt impurity occurs during the extraction of 68Ge from the cyclotron. Contamination detected during generator elution is within limits and there is no detection in marked products. Since Zn (II) is a metal that competes with ^{68}Ga, it is known that it can cause the reaction efficiency to decrease. Zn (II) contamination was found as 0.625 ppm in the labeling performed without generator pre-elution. In generator pre-eluted labeling; it was determined as 0.133 ppm and 0.108 ppm. No significant difference was found between the Zn impurities detected in the labeling product. Aluminum impurity occurs at column material in ^{68}Ge/^{68}Ga generator and during ^{68}Ge extraction from cyclotron. Aluminum impurity occurs at column material in ^{68}Ge/^{68}Ga generator and during ^{68}Ge extraction from cyclotron. It appears that the PSH^{+} cartridge does not purify this contamination. When the study data were examined, lead impurity was determined that although it was in generator elution, it did not switch to radiopharmaceutical. Tin metal pollution caused by the column material of the generator was detected at very low values. Manganese pollution was determined at values less than <0.001 ppm.

DISSUCUSION

Fifty percent of the 68Ga activity in the generator column is produced within a half-life. The generator can be decomposed every 3.5 hours to provide almost full (90%) radioactivity. Thus, the generator perfectly allows three separate elutions per day.

In this study, three different labeling processes were successfully performed in the automatic synthesis module with 99% efficiency. The chemical and radiochemical impurities examined that may pass into product compositions were compared with the limit values of European Pharmacopoeia monograph and IAEA Safety Standards. Chemical separations with PSH+ cartridge have been reported to reduce the amount of impurities to ppm levels.

The limit value of ^{68}Ge fraction in a ^{68}Ga solution used in radiopharmaceuticals labeling is stated as 0.001% in the European Pharmacopoeia monograph (4). Zn(II) (2 ppm), Nb (<7 ppm) and Cu, Pb, Co, Cr, Cd, Ni, Fe, Mn and Al (all <1 ppm) (4). According to the European Pharmacopoeia, the total radionuclide impurity limit in the [^{68}Ga] GaCl3 solution for radiolabeling cannot exceed 0.1% [17]. Lead is in the heavy metals class and is a highly toxic metal [18]. In terms of human health, this metal should not be in the radiopharmaceutical composition. The van der Waals radius of the Ga^{3+} (62 pm) ion is very similar to Mn^{+3} (64 pm), so they compete with ^{68}Ga in labeling, reducing the labeling efficiency of the generator.

It is thought that pre-elution of the generator before labeling increases the labeling efficiency when labeling PSMA-11 with ^{68}Ga. However, when the analysis results of the labeled products were examined, no significant difference was observed in chemical and radiochemical impurities between the product labeled with pre-elution generator elution and the labeled product with non-elution generator elution.

CONCLUSION

The stability and robustness of generator performance is important for product quality, patient safety, and to process traceability. There are known methods for obtaining the final product (labeled radiopharmaceutical) with the

least possible metal contamination. All of these methods cannot completely prevent the presence of metal cation impurities and ^{68}Ge radioactive impurity. In radiopharmaceutical production, these sources of contamination are considered a GMP issue controlled by process validation.

In the reported study, it was examined whether these metal impurities were contaminated in the product profile. In addition, whether generator pre-elution is important for contamination was evaluated. Pre-elution of the generator prior to synthesis does not seem to be a chemical requirement other than zinc pollution. Pre-elution can be made for ^{68}GaPSMA-11 in high yield, but no difference was observed in pre-elution in chemical and radiochemical terms.

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MDR1 C3435T POLYMORPHISM: A PRELIMINARY STUDY ON ITS RELATIONSHIP WITH THE RISK OF COLORECTAL CANCER

MDR1 C3435T POLİMORFİZMİ: KOLOREKTAL KANSER RİSKİ İLE İLİŞKİSİ ÜZERİNE BİR ÖN ÇALIŞMA

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ABSTRACT

Objective: Colorectal carcinoma (CRC) is the third most frequent cancer in the world and a heterogenious disease which aroze from one or a combination of different genetic mechanisms. The multiple drug resistance-1 (*MDR1*) gene which encodes P-glycoprotein (P-gp) plays a part in the bioavailability of drugs and cell toxicity. In the current study, we aimed to investigate the possible relation between the *MDR1* gene C3435T polymorphism and CRC risk in the Turkish population.

Material and Methods: Forty three patients (26 men, 17 women) with CRC and 48 healthy controls (34 men, 14 women) were included in the study. The *MDR1* C3435T genotypes were determined by the Restriction Fragment Length Polymorphism (RFLP) method.

Results: Statistical significance was obtained in terms of genotype distributions of *MDR1* C3435T genotypes between study groups. The frequencies of *MDR1* C3435T CC, TT and CT genotypes in the CRC patient group were found as 16.3%, 32.6% and 51.2%, respectively. The frequency of the homozygous TT genotype was found as 32.6% in CRC patients while it was identified as 14.6% in controls (p=0.04; OR=2.82, 95% CI: 1.01-7.87). When the patients were compared with the healthy population, TT genotype carriers of *MDR1* C3435T polymorphism were found at 2.8-fold (p<0.05) increased risk for the development of CRC.

Conclusion: Our results show that the *MDR1* C3435T polymorphism might be one of the genetic risk factors for CRC development in the Turkish population.

Keywords: *MDR1*, C3435T, polymorphism, colorectal cancer, Turkish population

ÖZET

Amaç: Bir veya çoklu genetik mekanizmaların kombinasyonuyla oluşan heterojen bir hastalık olan kolorektal kanser (KRK) dünyada en sık görülen üçüncü kanserdir. P-glikoproteini (P-gp) kodlayan çoklu ilaç direnci geni-1 (Multiple drug resistance-1 (*MDR1*)) ilaçların biyoyararlanımı ve hücre toksisitesinde rol oynar. Bu çalışmada, Türk popülasyonunda *MDR1* geni C3435T polimorfizmi ve KRK riski arasındaki olası ilişkiyi araştırmayı amaçladık.

Gereç ve Yöntemler: Çalışmamıza KRK hastası (26 erkek, 17 kadın) 43 kişi ve 48 sağlıklı kontrol (34 erkek, 14 kadın) dahil edilmiştir. *MDR1* C3435T genotipleri Restriksiyon Fragman Uzunluk Polimorfizmi RFUP) yöntemiyle belirlenmiştir.

Bulgular: *MDR1* C3435T genotiplerinin dağılımı açısından çalışma grupları arasında istatistiksel anlamlılık elde edilmiştir. KRK hasta grubunda *MDR1* C3435T CC, TT ve CT genotiplerinin sıklığı sırasıyla %16,3, %32,6 ve %51,2 olarak bulunmuştur. Homozigot TT genotipi frekansı KRK hastalarında %32,6 bulunurken kontrollerde %14,6 (p=0,04; OR=2,82, 95% CI: 1,01-7,87) olarak tespit edilmiştir. Hastalar ve sağlıklı kontroller karşılaştırıldığında *MDR1* C3435T polimorfizmi TT genotipi taşıyıcıları KRK gelişimi için 2,8 kat (p<0,05) artmış risk altında bulunmuştur.

Sonuç: Sonuçlarımız *MDR1* C3435T polimorfizminin Türk popülasyonunda KRK gelişimi için genetik risk faktörlerinden biri olabileceğini göstermektedir.

Anahtar Kelimeler: *MDR1*, C3435T, polimorfizm, kolorektal kanser, Türk popülasyonu

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INTRODUCTION

Colorectal carcinoma (CRC) is the third most frequent cancer in the world which is responsible for 9.2% of death from cancer in both sexes according to Globocan 2018 data (1). Approximately 90% of the CRC cases are sporadic, while less than 10% of them have genetic predisposition (2). CRC is a heterogenious disease which arose from one or a combination of different genetic mechanisms, such as chromosomal instability (CIN), CpG island methylator phenotype (CIMP), or microsatellite instability (MSI) leading to malign transformation. Several genes are found in association with the risk of CRC development in numerous studies (2, 3).

Multidrug resistance (MDR) proteins are part of the ATP-binding cassette (ABC) transporter superfamily and characterized as three different forms: atypical MDR, classical MDR and non-Pgp MDR (4). Many investigations have been performed regarding their function in transporting cytotoxic drugs out of the cell and their potential role as a target in cancer therapy (5).

The *MDR1/ABCB1* gene which is known as the "Classical" MDR phenotype, is located on chromosome 7p21.12 and is highly polymorphic. It is first described in cancer cells and encodes P-glycoprotein (P-gp) which is a transmembrane protein with 170 kd molecular weight (6, 7). P-gp is principally expressed in the excretory organs like the kidney, liver, and intestines and promotes the excretion of drugs and xenobiotics into the bile and urine, thereby limiting intestinal drug absorption (8-10). Moreover, P-gp, as a gate-keeper of the blood-brain barrier, prevents the delivery of several drugs into the central nervous system (9).

Several single nucleotide polymorphisms (SNP) have been investigated within the *MDR1* gene up to now. Most of them are found as intronic or silent, while some of them have been shown to be related to the changes in expression of the P-gp which affect tissue concentration of P-gp substrates (11-13).

A silent polymorphism in the middle of exon 26 of the *MDR1* gene (C3435T) rs1045642 was found to be in relation with different types of human cancers such as breast, kidney, and liver (14-17). Reduced levels of the intestinal P-gp in TT genotype carriers was compared to subjects with CC genotype in the study of Hoffmeyer et al. (11). Contrary to several studies which demonstrated the relation between the 3435TT genotype and decreased levels of P-gp expression, Nakamura et al. found that the CC genotype was associated with decreased P-gp expression levels (11-13). Ethnic differences in allelic frequencies of the C3435T polymorphism were also observed (18-20).

The aim of the current study was to investigate the possible association between *MDR1* C3435T SNP and CRC development in the Turkish population.

MATERIALS AND METHODS

Patient selection

Forty-three CRC patients (26 men, 17 women) diagnosed by radiologic and endoscopic methods, and surgical findings together with a pathologic examination were included in the study. Collection of blood samples were performed before cancer therapy. Patients who applied for non-neoplastic diseases such as trauma or inguinal hernia to general surgery and orthopedia clinics in the same hospital were included in the control group (34 men, 24 women). Ethical approval of the study was obtained from the Ethical Committee of the Istanbul Education and Research Hospital (Date: 23.06.2017, No: 1015). All procedures involving human participants were in accordance with the ethical standards of the institutional committee and the Declaration of Helsinki. Written informed consent was received from all participants prior to collecting their biological samples.

DNA extraction

Blood samples were collected into EDTA containing tubes, and the DNA was isolated from peripheral blood according to a salting out procedure (21).

Genotyping

Genotypes of the *MDR1* C3435T polymorphism were identified with a restriction fragment length polymorphism (RFLP) (22). The following primer sets were used for polymerase chain reaction (PCR); P1 (5'-ACTCTTGTTTTCAGCTGCTTG-3') and P2 (5'-AGAGACTTACATTAGGCAGTGACTC-3'). PCR was carried out using 200 ng of DNA, 200 µmol/l of dATP, dCTP, dGDP and TTP (MBI Fermantas, Vilnius, Lithuania), 250 ng of primers; 1.5 mmol/l of magnesium chloride, and 2U of *Taq* DNA polymerase (MBI Fermantas, Vilnius, Lithuania) in a total volume of 100 µl at GeneAmp PCR Systems 9700 thermal cycler (Perkin Elmer). Cycling conditions of PCR were as follows: 94°C (2 min) (initial denaturation) followed by 35 cycles at 94°C (30 s) (denaturation), at 56°C (30 s) (annealing), and at 72°C (30 s) (extension). The terminal elongation step was applied at 72°C (7 min). The C3435T genotypes were determined by digestion of a PCR product with *DpnII (MboI)* restriction enzyme at 37°C (4 h). Agarose gel with a density of 3% was used for seperation of DNA fragments.

Statistical analysis

SPSS (version 20.0) was used for statistical analyses. A chi-square test (X^2) was performed for the comparison of genotype and allele distributions in study groups. 'Gene counting methods' were performed for the calculation of allele frequencies. Biochemical features of the study

groups were compared by the Mann Whitney U test. A p-value of less than 0.05 was considered for statistical significance.

A sample size calculation was performed using the "PS Power and Sample Size Calculation" package program, with inputs of p0 (probability of exposure in controls) and p1 (probability of exposure in cases) from the Ensemble genome browser. The type I error probability (α) was used as 0.05. The statistical power of the relationship between SRBI rs5888, rs4238 MDR1 C3435T variation and colorectal cancer risk was obtained as 52.7% in the study.

RESULTS

Demographic and biochemical features of the study groups are given in Table 1. Mean age of the control subjects and CRC patients were 57.31±9.99 years and 54.55±13.44 years, respectively. Sex and age frequencies were similar in both groups. Alanine transaminase (ALT) ($p<0.001$), alkaline phosphatase (ALP), and blood urea nitrogen (BUN) ($p<0.001$) values were found higher in CRC group as compared to the control. The *MDR1* C3435T genotype and allele frequencies of the study groups are presented in Table 2. The genotype distributions were found to be significantly different between CRC patients and the control group. The frequencies of the CC, TT and CT genotypes of *MDR1* C3435T among CRC patients were 16.3%, 32.6% and 51.2%, respectively. The homozygous TT genotype was identified in 32.6% of CRC patients while it was found in only 14.6% in the control group ($p=0.04$; OR=2.82, 95% CI:1.01-7.87). The heterozygous genotype (CT) was observed in 51.2% of CRC patients while it was detected in 68.8% among the control group.

DISCUSSION

The product of the *MDR1* gene, P-gp, which is an integral membrane protein, actively transports relevant drugs from the inside to the outside of the cell, thereby playing a role in preventing the accumulation of toxic and carcinogenic substances inside the cell (9, 10, 23, 24). It also limits success of cancer therapy by removing chemotherapeutic drugs from tumor cells (25). In several cancer studies, P-gp over-expression has been related to the poor outcome of chemotheraphy in breast cancer, acute myeloid leukaemia, and childhood tumors (10, 16, 26). Altered P-gp expression and function due to genetic polymorphisms as well as physiological and environmental factors have been identified in several studies (8, 26, 27).

The C3435T is a "silent" polymorphism which has no effect on the amino acid sequence of the *MDR1* gene and is not expected to have a direct effect on P-gp expression (18, 28). The C3435T polymorphism has been found to have different effects on gene expression and protein formation in previous studies. This synonymous polymorphism could show its effect by involving in different mechanisms such as mRNA splicing, protein folding, and modification of translation efficiency (11-13, 28, 29).

Table 2: Genotype and allele frequencies of MDR1 C3435T in the study groups

Group	Control (n=48)	CRC patients (n=43)
Genotypes		
CC	16.7 % (8)	16.3% (7)
TT	14.6% (7)	32.6% (14)*
CT	68.8% (33)	51.2% (22)
Alleles		
C	51.04% (49)	41.86% (36)
T	48.95% (47)	58.13% (50)

n: number of individuals, CRC: Colorectal Cancer; *, p=0.04; OR=2.82 95% CI:1.01-7.87

Table 1: Demographic and clinical features of the study groups

	Control (n=48)	CRC patients (n=43)	p value
Age (year)	57.31±1.44	54.55±2.87	0.621
Sex (female/male, n)	14/34	17/26	0.297
Smoking (%)	34.5	33.3	0.933
ALT (U/L)	32.50±9.31	44.70 ±2.97	**0.001**
AST (U/L)	23.67±1.57	26.10±4.93	0.409
ALP (U/L)	79.50±9.12	102.11±4.55	**0.039**
Creatinine (mg/dL)	0.78±0.02	0.92±0.07	0.616
BUN (mg/dL)	0.88±0.02	1.13±0.28	**0.001**

The results are shown as mean ±SEM (standard error). Mann Whitney U test was performed for the comparison between groups. n: number of individuals, CRC: Colorectal Cancer, ALT: Alanine transaminase, AST: Aspartate transaminase, ALP: alkaline phosphatase, BUN: Blood urea nitrogen

In the study of Hoffmeyer et al, it was firstly showed that the *MDR1* C3435T polymorphism was associated with *MDR1* expression in the human duodenum. The study indicated that 3435TT carriers have decreased expression and P-gp function (11). Similarly, in the study of Eichelbaum et al. lower P-gp expression and function was found in *MDR1* 3435 TT genotype carriers (28). Some studies demonstrated the relation between the 3435TT genotype and decreased levels of P-gp expression, while Nakamura et al. found a positive correlation between CC genotype and decreased P-gp expression (11-13, 28).

There are several studies investigating the relationship between the *MDR1* C3435T polymorphism and CRC risk (8, 12, 31, 32). Kurzawski et al. first reported that carriers of the *MDR1* 3435TT genotype in the Polish population have a 2.7-fold increased risk for colon cancer development (12). Similarly, Ambudkar et al. showed that the *MDR1* 3435TT genotype is associated with an increased risk of developing CRC (8). The increased risk has been attributed to the formation of functional defects in the barrier of epithelial cells as a result of downregulation of P-gp and thus exposure of patients to higher damaging toxin levels (8). Humeny et al. studied the C3435T polymorphism in tumor and normal tissues of CRC patients, and reported no change in the genotypic frequency of polymorphism between tumor and normal samples during colorectal tumorigenesis (30).

Zhao et al. performed a meta-analysis study including 5,485 cases and 5,854 controls and found no significant associations between the *ABC1/ MDR1* C3435T polymorphism and CRC susceptibility by obtaining similar results for both Caucasian and Asian populations (31). However, in the the meta-analysis study of Jin et al. including 4818 individuals, T allele carriers of the *MDR1* C3435T polymorphism had significant lower risk for CRC development in the Asian population according to both homozygous comparison (TT vs CC) and recessive model (TT vs TC+CC). (32). In the study of Ozhan et al., although no significant association was observed between the C3435T polymorphism and CRC risk in the Turkish population, the *ABCB1* haplotype C_{1236}-G_{2677}-T_{3435} was found significantly more frequently in CRC patients as compared to the control group (p=0.0004, OR=11.96, 95% CI=2.59–55.32) in the haplotype-based analysis (33). The effect of SNPs on CRC development differs according to ethnicity in various studies in the literature. As an example, the MDM2 SNP309 and x-ray repair cross-complementing group 1 (XRCC1) Arg399Gln SNPs were found associated with the risk of CRC in the Asian population, while the same results were not found in European populations (34, 35). Additionally, in the study of Wang et al., the frequency of the MDR1 3435TT allele among healthy individuals was found to be higher in Asians and Caucasians ((27.8%, 49.4% respectively) (36).

In the present study, the distrubution of the C3435T alleles in CRC patients was significantly different from healthy subjects. According to the results of this study, a 2.8-fold increased risk was found for CRC development in patients who have the TT genotype of *MDR1* C3435T polymorphism. This finding may support the hypothesis that the 3435TT genotype plays a negative role in the expression of P-gp which is involved in the defense mechanism against detrimental compounds and carcinogens. However, the frequency of genotypes in the present study were found to be different from previous studies (19-21, 31, 36). Half of the individuals were heterozygous carriers of the variant, while more than 32% were homozygous carriers of the variant in the patient group. Also, this control group showed a different distribution of genotypes as compared with other studies. The difference might be due to the different number of subjects and/or different ethnic populations as well as the heterogenous nature of the disease and the effect of environmental factors.

The major limitation of the study includes small sized study groups. In order to shed more light on the association of the *MDR1* C3435T polymorphism with CRC risk, this work would be better extended with a larger study group that includes the results of this polymorphism on the expression levels of the MDR1 gene and protein levels.

To conclude, this is a preliminary study to demonstrate whether the *MDR1* C3435T polymorphism has an effect in CRC pathogenesis in the the Turkish population. Based on these findings, we propose that the *MDR1* gene C3435T polymorphism had a possible effect in the development of CRC in the Turkish population.

Ethics Committee Approval: This study was approved by the Clinical Research Ethical Committee of the Istanbul Education and Research Hospital (Date: 23.06.2017, No: 1015).

Informed Consent: Written consent was obtained from the participants.

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Author Contributions: Conception/Design of Study- H.Y.A., İ.Y.; Data Acquisition- S.A., G.Ö., İ.Y.; Data Analysis/Interpretation- H.Y.A., T.İ.; Drafting Manuscript- G.Ö., İ.Y., S.A., T.İ., H.Y.A.; Critical Revision of Manuscript- H.Y.A., T.İ.; Final Approval and Accountability- G.Ö., İ.Y., S.A., T.İ., H.Y.A.

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HEARING STATUS OF CHILDREN WITH BEHÇET'S DISEASE: A PROSPECTIVE PRELIMINARY STUDY

BEHÇET HASTALIĞI OLAN ÇOCUKLARIN İŞİTME DURUMU: PROSPEKTİF BİR ÖN ÇALIŞMA

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ABSTRACT

Objective: We aimed to evaluate the hearing status of children with Behçet's Disease (BD).

Materials and Method: It is a prospective, cross-sectional, controlled study. Pure-tone audiometry including high-frequencies and distortion product otoacoustic emission (DPOAE) were performed in 15 children with BD and 13 healthy controls.

Results: Although both groups had normal hearing levels, the pure tone average of the study group was higher than the healthy controls. Hearing thresholds at 500 Hz and 4000 Hz were statistically significantly higher in children with BD. There was a significant difference in DPOAE levels at 1000 Hz and 4000 Hz. Children with BD had lower levels at these frequencies.

Conclusion: In spite of the statistical differences in pure tone audiometry between the two groups, the delta was about 5 dB, which might not have clinical importance. However, this is the first study in children, and further studies are required.

Keywords: Hearing evaluation, Behçet's disease, otoacoustic emission, children

ÖZET

Amaç: Behçet Hastalığı (BH) olan çocukların işitme durumunu araştırmayı amaçladık.

Gereç ve Yöntem: Bu çalışma prospektif, kesitsel, kontrollü bir çalışmadır. On beş BH olan ve 13 sağlıklı çocuğa yüksek frekansları içeren saf ses odyometrisi ve distorsiyon ürünü otoakustik emisyon (DPOAE) uygulanmıştır.

Bulgular: Her iki grup da normal işitme seviyelerine sahip olmasına rağmen, çalışma grubunun saf ses ortalaması sağlıklı kontrollerden daha yüksekti. 500 Hz ve 4000 Hz'de işitme eşikleri BH'li çocuklarda istatistiksel olarak anlamlı derecede daha yüksekti. 1000 Hz ve 4000 Hz'de DPOAE seviyelerinde önemli fark vardı. BH olan çocuklar bu frekanslarda daha düşük seviyelere sahipti.

Sonuç: İki grup arasındaki saf ses odyometrisindeki istatistiksel farklılıklara rağmen, delta yaklaşık 5 dB idi ve bunun klinik önemi olmayabilir. Ancak bu çocuklarda yapılan ilk çalışmadır ve daha fazla çalışmaya ihtiyaç vardır.

Anahtar Kelimeler: işitme değerlendirmesi, Behçet hastalığı, otoakustik emisyon, çocuklar

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INTRODUCTION

Behçet's disease (BD) is a chronic multisystemic vasculitis and defined by Hulusi Behcet in 1937 (1). The onset of BD is usually in the 2nd decade of life, but although rare, manifestations may start during childhood (2). Although the clinical features may trace each other, the diagnosis of pediatric BD is a challenge. There is limited data about the course of BD in children. The main clinical features are mucocutaneous and ophthalmological lesions. However, vascular, pulmonary, gastrointestinal, musculoskeletal, and neurological involvement might also be noted during the course of the disease (3). Since it is a heterogenous disease with diverse organ involvement, audiological impact may participate the disease course. The incidence of hearing impairment was reported at between 12 to 80% in several adult studies (4-7). However, there is only one case report presenting a 15-year-old boy with BD and audiovestibular symptoms in the literature (8). According to our knowledge, there are no studies showing the status of hearing thresholds in children with BD who do not have audiovestibular symptoms. In this preliminary study, the objective was to evaluate the hearing functions of children with BD by using high frequency pure tone audiometry (PTA) and distortion product otoacoustic emmisions (DPOAE) and to compare these results with healthy controls.

METHODS

Ethical considerations

The current study protocol proceeded in accordance with the ethical standards in the Declaration of Helsinki 1964 and were approved by the local ethical committee (Date/No: 2017/1066). The informed consent of the parents and/or children was obtained.

Participants

In this prospective, cross-sectional, controlled study, 15 consecutive children diagnosed with Behçet's disease at a tertiary pediatric rheumatology center and 13 healthy controls were enrolled into the study. The diagnosis of BD was done according to the classification criteria of the International Study Group for BD (9).

Main outcome measures

Both patient and control groups were evaluated for hearing functions at the otorhinolaryngology unit of the Health Science University Istanbul Training and Research Hospital. The medical records of the patients were reviewed, and socio-demographic characteristics and clinical and laboratory findings were noted. The diagnosis age of all patients was under 18 years, and all were under colchicine treatment. HLA-B51 positivity was checked in all patients.

A clear past history regarding the ear infection, usage of ototoxic medication, trauma, and any other accompanying systemic disease that may affect hearing thresholds was taken from all participants. Additionally, a detailed clinical and audiological evaluation was performed by an otorhinolaryngologist. A high-frequency PTA and DPOAE were performed at the otorhinolaryngology department in the Health Science University Istanbul Training and Research Hospital. These tests were performed in a soundproof room. We performed a high frequency PTA using an AC40 Diagnostic Audiometer (Interacoustic Company, Denmark). The measured frequencies of routine PTA include 250, 500, 1000, 2000, and 4000 Hz, and high frequency PTA include 8000, 10000, 12500, and 16000 Hz. All these frequencies were evaluated in each patient. We calculated the PTA average by using hearing thresholds between 500 and 2000 Hz. The hearing level lower than 25 dB in each of these frequencies was defined as normal hearing. Otoacoustic emission detects the outer hair cell function via detection of the reaction generated by the cochlea across to sound signals. It can be used to differentiate the hearing loss type and predict cochlear sensitivity by early detection of the cochlear damage. DPOAEs were measured at frequencies of 1000, 1400, 2000, 2800, and 4000 Hz using Otodynamics ILO-288 Echoport equipment (Otodynamics Ltd., Hateld, UK). The ratio of a signal to the noise floor was defined as signal-to-noise ratio (SNR). The high SNR ratio indicates a high number of transmitted signals. This condition supports the DPOAE reliability. Also, the high SNR was shown for high DPOAE reliability.

Statistical analysis

The statistical analysis was performed using the SPSS22.0 program. In the evaluation of the data, a descriptive statistical method was used for mean, standard deviation, median, lowest, highest, frequency, and ratio values. The distribution of the variables was measured by the Kolmogorov Simirnov test. The Mann-Whitney U test was used in the analysis of quantitative independent data, and a Chi-square test was used in the analysis of qualitative independent data.

RESULTS

Fifteen children (30 ears) with BD (4 female and 11 male) and 13 healthy controls (26 ears) (5 female and 8 male) were enrolled in this study. The mean age of the children with BD and control group were 15.60±1.92 years and 13.92±2.56 years, respectively. The age and sex distributions were similar in both groups. The mean age of the onset of the disease was 10.9±3.01 years, and the mean age at diagnosis was 12.3±2.7 years. The mean duration of the disease was 4.6±2.6 years. All patients were under colchicine therapy. Nine (%60) of the patients had HLA B51 positivity. The clinical characteristics and demographic data of the patients are shown in Table 1. None of the children with BD had neurologic involvement. One of

Table 1: Demographic and clinical features of children with Behçet's disease

	Number of patients	%
Consanguinity	6	40
Family history of Behçet's disease	4	26.6
Oral aphthosis and ulcers	15	100
Genital ulcers	10	66.6
Ocular lesions	5	33.3
Vascular lesions	2	13.3
Skin lesions	12	80
Pathergy	7	46.6
Arthritis	2	13.3

the patients was using azathioprine, one was using azathioprine and prednisolone, and another was using azathioprine and warfarin sodium (because of deep venous thrombosis in the lower extremity) at the time of the study.

Audiologic data

The mean PTA values were in the normal hearing range in both groups whereas the patient group had higher values which were statistically significant (p=0.028). Hearing thresholds at 250 Hz, 1000 Hz, 2000 Hz, 8000 Hz, 10000 Hz, 12500 Hz, and 16000 Hz were in the normal range in both groups. Although within normal levels, hearing thresholds at 500 Hz and 4000 Hz were statistically significantly higher in children with BD, (p=0.037 and 0.031 respectively) (Table 2). DPOAE values at frequencies of 1400 Hz, 2000 Hz, and 2800 Hz were similar between the patient and control groups. However, patients had statistically significantly lower levels at 1000 and 4000 Hz in DPOAE than the healthy controls (p=0.040 and 0.028 respectively) (Table 3). SNR evaluations in both groups were similar.

DISCUSSION

In this preliminary study, we evaluated the hearing status of children with BD by using both objective and subjective audiological tests to detect early cochlear involvement. We found higher thresholds at 500 and 4000 Hz in PTA, despite being normal levels in both patient and control group, and also lower levels at the frequencies of 1000 and 4000 Hz in the DPOAE test in children with BD. Although these differences were statistically significant, the delta of 5 dB reported in this study as "statistically significant" and the lower levels at 2 frequencies of DPOAE testing might be meaningless in clinical practice. This is because the standards of most experts are not outside the range of test-retest variability; for instance, the test-retest reliability of an audiogram is 5 dB.

BD is a chronic disease with multiple organ-specific symptoms. It is most commonly seen in the Mediterranean region, the Middle East, and the Far East (the Silk Road). The prevalence was reported at 42/10000 in a study conducted in Istanbul, the largest cosmopolitan city in

Tablo 2: Pure Tone Average values including high frequencies of control group and Behçet's disease (BD)

		BD		Control group		
		Mean (±SD)/n	Median	Mean (±SD)	Median	p
Age		15.6±1.92	16.00	13.9±2.56	14.00	0.094[m]
Sex	Girl	n=4 (26.7%)		n= 8 (61.5%)		0.063[x2]
	Boy	n=11 (73.3%)		n= 5 (38.5%)		
PTA		9.17 ±2.86	9.00	6.88±3.18	6.00	***0.028[m]***
250 Hz		12.17±3.26	12.50	11.77±5.79	14.00	0.098[m]
500 Hz		11.50±3.25	10.00	8.92±3.12	10.00	***0.037[m]***
1000 Hz		8.67±3.39	7.50	7.69±2.75	9.00	0.425[m]
2000 Hz		7.33±3.59	5.00	6.00±5.87	5.00	0.148[m]
4000 Hz		9.83±5.04	10.00	5.73±6.44	6.00	***0.031[m]***
8000 Hz		5.33±4.62	5.00	7.88±6.99	7.00	0.429[m]
10000 Hz		3.83±6.19	2.50	4.31±5.37	5.00	0.674[m]
12500 Hz		2.83±8.01	2.50	5.62±6.42	2.50	0.226[m]
16000 Hz		4.70±7.33	5.00	4.96±5.23	2.50	0.907[m]

m: Mann whitney u test x2: chi square test

Table 3: DPOAE and SNR levels of control group and Behçet's disease

	Behçet's disease		Control group		
	Mean	**Median**	**Mean**	**Median**	**p**
DP1000 Hz	2.58±6. 97	3.45	7.58±5.93	8.90	***0.040***
DP1400 Hz	6.14±8.61	6.70	7.7±7.84	7.15	0.764
DP2000 Hz	2.60±9.44	4.10	5.60±6.70	5.30	0.661
DP2800 Hz	-1.14±11.21	0.90	1.18±12.44	3.50	0.300
DP4000 Hz	-0.55±12.16	1.40	8.62±5.25	7.30	***0.028***
SNR1000 Hz	4.59±9.16	5.60	8.90±7.54	8.00	0.112
SNR1400 Hz	10.75±10.49	15.00	13.16±8.69	9.80	0.908
SNR2000Hz	9.32±11.72	11.40	10.50±10.05	11.05	0.908
SNR2800 Hz	2.32±10.90	10.35	7.03±13.27	9.70	0.836
SNR4000 Hz	8.38±12.58	10.35	14.88±7.54	11.25	0.345

Turkey (10). The disease often begins early in childhood but may not be diagnosed for many years (11, 12). There are numerous studies comparing adult and pediatric patients with BD regarding demographics, clinical features, and course of the disease (13, 14). Several studies have evaluated the hearing impairment in adults with BD, but there is no reported data concerning the audio-vestibular involvement in pediatric patients with BD. Because of this deficiency in past literature, we evaluated the hearing function in children with BD in the present study which is the first study about this issue.

The reports evaluating the inner ear involvement in adults with BD are contradictory. Soylu et al. observed hearing loss in 20 of 72 adults with BD at frequencies of 0.25, 0.5, 2, and 4 kHz while Sonbay et al. reported 23% of sensorineural hearing loss (4, 5). They pointed out that the most significant loss was at the 4 and 8 kHz frequencies. The authors usually used a standard PTA between the frequencies of 250 and 8000 Hz in order to evaluate audio-vestibular involvement, and some of them determined a down slope at high frequencies (15). According to these data, recent studies investigated higher frequencies up to 16000 Hz and suggested that the first affected hearing thresholds are usually higher frequencies. Bakhshaee et al. performed a high frequency audiometry (up to 12000 Hz) in 27 patients with BD and showed that 60% of their cases had hearing loss at high frequencies (16). Süslü et al. investigated frequencies up to 16000 Hz, finding higher hearing thresholds at frequencies of 250, 1000, 2000, 4000, and 8000 Hz in patients with BD in comparison to normal subjects although all subjects had normal hearing levels at these frequencies (<20 dB) (7). Based on these reports, we also measured high frequencies (measured 9 consecutive frequencies between 250 and 16000 Hz) by a detailed PTA. Similar with Süslü et al., all of our patients had normal hearing levels at all frequencies, but inconsistent with this study, we observed statistically significant differences at frequencies of 500 and 4000 Hz between the patient group and the healthy controls. Moreover, although the hearing levels were in normal limits, we found higher mean PTA thresholds in children with BD. We did not determine hearing impairment in contrast to the previous adult studies. Furthermore, our findings do not support the theory that hearing loss begins firstly at high frequencies in BD. The reason for this may be due to the younger age, shorter duration of disease in our patients in comparison to adult studies, and also the small number of patients in the present study. The inconsistency our results with previous studies showing higher ratios of hearing loss can be explained by the low ages of the patients in the present study. Several reports detecting a relationship between hearing loss and age support this hypothesis (5, 15).

There are many studies measuring the auditory status of patients with BD by using PTA whereas some authors have used DPOAEs in auditory evaluation. Sonbay et al. reported significantly lower responses of DPOAEs at all frequencies in BD patients in comparison to healthy subjects (4). Dağlı et al. also found similar results and noted that the DPOAE findings were not correlated with the disease course (17). In the present study, we found that DPOAE responses in only frequencies of 1000 Hz and 4000 Hz were lower in children with BD in comparison to the controls. However, this difference between two groups did not reach statistical significance in the other measured frequencies, and the reason might be the small number of patients of the study group. We know that a normal DPOAE indicates the normal functioning of outer hair cells of cochlea. Based on this fact, our findings may be a sign of weaker outer hair cell motility in pediatric patients with BD. However, the number of patients in the present study was small, and therefore, further studies with larger groups should be performed to verify

this theory. According to the study of Süslü et al., it was demonstrated that the outer hair cells of patients with BD had less physiological motility (7). This condition was attributed to SNR values that were much lower in comparison to the control group in patients with BD. They underlined that decreased DPOAE responses with normal hearing thresholds in BD could be suggested as one of the early signs of subclinical cochlear involvement. Kemal et al. compared patients with BD and healthy subjects in terms of transient evoked otoacoustic emission (TEOAE) values and revealed that the difference in SNR between the two groups was significant at 4 kHz (6). They suggested that it is an indicator of cochlear involvement with reproducibility parameters together. In our study, there was no statistically significant difference between two groups in terms of SNR 1000 Hz, SNR 1400 Hz, SNR 2000 Hz, SNR 2800 Hz, and SNR 4000 Hz values ($p>0.05$).

Sensorineural hearing loss in autoimmune diseases is thought to be originated from vasculitic mechanisms. In different autoimmune diseases, different regions of cochlea were observed to be affected at varying degrees. Hearing loss in BD is also associated with vasculature due to perivascular immunocytic infiltration (7). Three of our patients were using azathioprine and/or warfarin sodium and/or prednisolone. In the literature, there is no data showing the effect of these drugs on hearing functions. All patients with BD in this study were under colchicine treatment. Keskindemirci et al. evaluated the hearing function of children with Familial Mediterranean Fever (FMF) that were using colchicine at cumulative doses of 1.5±14 mg and found no hearing loss (18). Therefore, weaker outer hair cell motility may not be related to these medications.

We measured the hearing levels by both a detailed PTA including high frequencies and DPOAE. Although we found higher mean PTA values and higher thresholds at specifically two frequencies, this difference might not be clinically significant. As vasculitic mechanisms are the major etiopathogenesis of BD, the involvement of cochlear blood vessels may induce cochlear pathology and progress to hearing loss.

The small number and normal hearing levels of children with BD are the most significant limitations of our study. Another limitation is that we did not make a speech discrimination test, and therefore, we could not use a standardized reporting format while evaluating our data. This study may be accepted as a pilot study showing the necessity of evaluation of the cochlear function in children with BD at a larger cohort.

CONCLUSION

We found a statistically significant difference in the audiologic tests of the diseased group in comparison to the control group. However, all subjects had normal limits of hearing levels and the ~5 dB difference may not be clinically important. The data of our study may be available as a baseline hearing status of children with BD. Therefore, this is the preliminary study on this issue, and future studies with larger groups should be performed in order to verify our results.

MAIN POINTS

- In this prospective, cross-sectional, controlled study the hearing status of 15 children with Behçet's Disease and 13 healthy controls were evaluated.
- Although both groups had normal hearing levels, pure tone average of the study group was statistically significantly higher than the control group. However the ~5 dB difference may not be clinically important.
- This is the preliminary study about this issue, and future multi-center studies with larger subjects should be performed.

Ethics Committee Approval: This study was approved from local ethics committee (Date/No: 2017/1066).

Informed Consent: Written consent was obtained from the participants.

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THE EFFECT OF SMARTPHONE APPS AND TECHNOLOGY COMPATIBILITY ON DIABETES CONTROL IN DIABETIC PATIENTS USING INSULIN

İNSÜLİN KULLANAN DİYABET HASTALARINDA AKILLI TELEFON UYGULAMALARI VE TEKNOLOJİYE UYUMUN DİYABET KONTROLÜ ÜZERİNE ETKİSİ

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ABSTRACT

Objective: This study was done to determine the need for technological systems in diabetes patients using insulin in Turkey, and to investigate the relationship between diabetes control and their smartphone apps.

Material and Methods: This descriptive cross-sectional type of study was carried out with 506 diabetic patients using insulin who were followed up at the Diabetes Outpatient Clinics within the Department of Internal Medicine, Istanbul University Istanbul Medical Faculty, between March and September 2017. The data were obtained with a face-to-face interview of the physician using the data collection form.

Results: The mean age of the participants was 54.23±15.23 (18-89) years. The investigation of distribution in accordance with age showed that 71 patients (14%) were in the 18-34 age group, 302 (59.7%) in the 35-64 age group, and 133 (26.3%) were aged 65 years and above. The rate of those with Type 1 diabetes was 22.9% (n=116) and the rate of those with Type 2 diabetes was 77.1% (n=390). Four-hundred and sixty-four (92.5%)

ÖZET

Amaç: Türkiye'de insulin kullanan diyabet hastalarında teknolojik sistemlere duyulan ihtiyacın belirlenmesi ve akıllı telefon uygulamalarının diyabet kontrolü ile ilişkisinin araştırılması amaçlanmıştır.

Gereç ve Yöntemler: Tanımlayıcı kesitsel tipte tasarlanan çalışma Mart ve Eylül 2017 tarihleri arasında Istanbul Üniversitesi, İstanbul Tıp Fakültesi, İç Hastalıkları Bölümü bünyesindeki Diyabet Polikliniklerinde takip edilen ve insulin kullanmakta olan 506 diyabet hastası ile gerçekleştirildi. Veriler, hazırlanan veri toplama formu yardımı ile görevli doktor tarafından yüz yüze görüşme yöntemi kullanılarak elde edildi.

Bulgular: Katılımcıların yaş ortalaması 54,23±15,23 (18 ile 89) yıl idi. Yaş kategorilerine göre dağılım incelendiğinde; 71'i (%14) 18-34, 302'si (%59,7) 35-64 yaş aralığında, 133'ü (%26,3) ise 65 yaş ve üstü grupta bulunmaktaydı. Tip 1 diyabet olanların oranı %22,9 (n=116), tip 2 diyabet olanlar ise %77,1 (n=390) idi. Hastaların 468'i (%92,5) diyabet hastalarının yaşam kalitesini artıracak ülkemize özgü bir hasta takip sisteminin faydalı olacağını

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of the patients stated that a patient follow-up system specific to our country would be useful in increasing the quality of life for diabetic patients. Three-hundred and twenty-one (63.4%) of the patients stated they have been using applications on - technological and telecommunication devices, and 185 patients (36.6%) stated they did not use the applications. In the model that examined the factors affecting the use of smartphone applications (Nagelkerke R2=44.6%, sensitivity=82.9% specificity=69.2%), it showed that higher education students' rate of using smartphone applications was 14.7 times higher than those with lower education; and those with an income level between 2 and 4 times above minimum wage were found to be 2.5 times higher than those with an income level of or below minimum wage.

Conclusion: It is anticipated that a system specific to our country will be needed for diabetic patients using insulin and that educated young patients with middle income will be highly interested in this system.

Keywords: Insulin-dependent diabetes mellitus, technological innovations, applications

belirtti. Hastaların 321'i (%63,4) teknolojik iletişim ve haberleşme cihazlarındaki uygulamaları kullandığını, 185'i (%36,6) ise kullanmadığını belirtti. Akıllı telefon uygulaması kullanımını etkileyen faktörlerin incelendiği modelde (Nagelkerke R2=%44,6, duyarlılık=%82,9 özgüllük=%69,2) yüksek eğitim görenlerin akıllı telefon uygulaması kullanma oranı, eğitimi olmayanlara göre 14,7 kat; gelir seviyesi asgari ücretin 2 ile 4 katı arasında olanlar da ise gelir seviyesi asgari ücret ve altında olanlara göre 2,5 kat daha yüksek bulundu.

Sonuç: İnsülin kullanan diyabet hastaları için ülkemize özgü bir sisteme ihtiyaç olduğu ve bu sisteme genç eğitimli ve gelir seviyesi orta yüksek olan hastaların ilgilerinin yüksek olacağı öngörülmektedir.

Anahtar Kelimeler: İnsülin bağımlı diyabet, teknolojik yenilikler, uygulamalar

INTRODUCTION

Diabetes is a metabolic disease that emerges with absolute or relative insulin deficiency or inadequate efficiency of insulin characterized by an increase in the blood glucose level which requires continous follow-up because of its chronic nature and the complications that can ensue (1).

Tha data of the World Health Organisation (WHO) published in 2014 showed that the number of diabetic patients was more than 422 million worldwide. The estimates of WHO showed that approximately 3.4 million of diabetes patients died of hyperglycemia each year (2). The recently published 9th Diabetes Atlas by the International Diabetes Federation reported that the prevalance of type 2 Diabetes has been increasing, and 79% of diabetes patients have been living in low-middle income countries. The same publication reported that approximately 700 million individuals diagnosed with diabetes are expected to reach the ages of 20-79 years in 2045 (3).

The TURDEP-II study performed in 2010 in Turkey reported that the prevalance of diabetes increased to 13.7%, and the number of patients diagnosed with diabetes was estimated to be 6.5 million (4). Performing the same ratio in the population at present showed that the number of diabetic patients can exceed 8.5 million (5).

The cost of chronic diseases was investigated in the report named "Chronic disease: an economic perspective" prepared with the cooperation of the World Health Organisation and The Oxford Health Alliance. The health spending per individual was calculated as $9,206 and higher in the mid-high income group of developed countries, between $2,976 - $9,205 in the low-mid income group countries, and $2,975 and below in low income group countries in the report in accordance with the data of the World Bank (6).

Diabetes is one of the biggest problems increasing healthcare costs, causing a significant burden for the global world and all countries. The cost of diabetes to the world economy in 2015 has a mean of approximately 1.32 trillion American Dollars (7). The total health expenditures for diabetes was 23.8% for the age group of 20-79 years in Turkey. In 2018, the data in Turkey showed that the annual cost of diabetes and its complications was 40 billion Turkish liras (3).

The use of mobile technological devices in the follow up and treatment of chronic diseases, especially in the follow up and treatment of diabetes has become more popular. The use of telemedicine practices and mobile applications can overcome geographical burdens, provide close follow-up, and create an opportunity for a patient to give feedback about their diabetes management (8). In addition, health applications offered for the use of diabetic patients provide management of insulin need and automatic feedback on blood glucose levels. Mobile applications with more features were reported to be used by an increasing number of individuals, enabling better self-management of their diabetes management (8). This study was designed to investigate the use of smart phone applications developed for use in Turkey of insulin dependent diabetes patients for diabetes control.

MATERIALS AND METHODS

The research design

The study designed as a descriptive cross-sectional type was conducted with 506 diabetic patients who used insulin, and were followed up in Istanbul University Istanbul

Faculty of Medicine Department of Internal Medicine from March 2017-September 2017.

The participants were informed about the study, and their informed consents were obtained. The data were obtained with a face-to-face interview method by the associated physician with the prepared data form. The use of applications by patients in smart phone or similar devices (tablet, smart watch, and smart wrist band, etc.) was taken as the basis for technology use criteria.

The ethics board approval was granted from Istanbul University Clinical Research Ethics Board with (Date: 10.03.2017, No: 268). The study was reported in appropriate with the STROBE guide.

The environment of the research

The Department of Internal Medicine in Istanbul Faculty of Medicine is a unit which serves more than a half million people annualy with 25 outpatient clinics with a 226 bed capacity.

Participants

The study was conducted with diabetic patients who used insulin, and were followed up in the diabetes outpatient clinic of Istanbul University, Istanbul Faculty of Medicine Department of Internal Medicine from March 2017-September 2017. The systematic sampling method was used in selection of the participants. Accordingly, patients with five or over registrations were included in the study.

Inclusion criteria

1. Having received insulin treatment in the last six months
2. Older than 17 years

Exclusion criteria

1. Pregnant
2. Paralysis
3. Unable to speak Turkish at a level to understand questions, and to respond

Variables

The primary result criterion of the study was identified as glycolysated hemoglobin (HbA1c). In addition, age, sex, height, weight, diseases, education and income data, received treatments, compliance to treatment, the ability to count carbonhydrates, severe hypoglycemia experiencing conditions, the reasons for experiencing hypoglycemia and hyperglycemia symptoms, physical activity conditions, and technological device use conditions of the participants were noted on the prepared data forms. The body mass index (BMI: Body weight/Height^2) was calculated. The glycolysated hemoglobin (HbA1c), fasting blood glucose (FBG), lipid profile (HDL, and LDL chloresterol, triglyceride), creatinine, and microalbuminuria levels, glomerulary filtration rate (GFR), and systolic/diastolic blood pressures (BP) calculated in the last three months were evaluated from the patient files. The cutting points for evaluating the urinary albumin excretion were taken as normo 30 mg/g, micro between 30-299 mg/g, and macroalbuminuria ≥300 mg/g.

Diabetes associated complications (retinopathy, neuropathy, and nephropathy) were investigated from the patient files as appropriate with current standard descriptions. The patients were asked whether they omitted insulin doses, whether they forgot and reinjected their injections for evaluating the compliance of diabetic patients to treatment protocols, and for investigating new technological methods for enabling patients to adhere to blood glucose regulations. Smart phone application use was checked to see whether individuals used the e-pulse, blood glucose follow up or other health practices on their phones during any period of the disease.

Sample size

The sample measuring of the research was performed using the G*Power 3.1.9.7 program. Because the main variable of the study was numerical, the model which can compare the means between two independent groups based on the t test assumptions was selected. A difference of 0.6 unit for HbA1c between the groups, and taking the standard deviations as 2.0 showed the effect width as 0.3. Accordingly, 484 individuals were required to obtain a strength of 95% in the analysis conducted with the assumption of the effect width as 0.30 (small-medium), and the α error as 0.05.

Statistical analysis

The data were analysed using the Statistical Package for the Social Sciences 21.0 software (SPSS Inc., Chicago, IL, USA). The continous data in the descriptive statistics were given with mean±standard deviation (SD), and categorical data were given with number, and percentage values. The Kolmogorov-Smirnov analysis was used to evaluate the appropriateness of the normal distribution for continous data in the statistical comparison of the data. The t test was used for parametric data in the presence of two independent groups in the comparison, the Mann-Whitney U test for nonparametric data, and the Chi square test was used in the comparison of categorical data.

The multivariate logistic regression analysis was performed using the independent variables detected to be effective on technology use in univariate analyses for evaluating the effects of these independent variables on one another, and for detecting which of these technology use behaviors were mostly effective. The model with the highests descriptive feature among these created models was used. Hosmer-Lemeshow test was used for model compliance. P value smaller than 0.05 was accepted adequate for the statistical significance.

RESULTS

Descriptive results

The mean age of the participants was 54.23±15.23 (18 to 89 years) years. The distribution in accordance with the age categories showed that 71(14%) were between 18-34 years old, 302 (59.7%) were between 35-64 years old, and 133 (26.3%) were aged 65 years and over. The rates of patients diagnosed with type 1 diabetes (T1DM) was 22.9% (n:116), and the rates of patients with type 2 (T2DM) diabetes was 77.1% (n:390). Three-hundred and twenty-one of the patients (63.4 %) were reported to have used applications on technological communication and telecommunication devices, however 185 patients (36.6%) reported not to have used these devices. Ninety-nine percent of participants (n:467) were reported to have communicated with their physician following up their diabetes by presenting to the outpatient clinic. Four-hundred and sixty-eight of the patients (92.5%) reported that a patient follow up system to increase the quality of life for diabetes patients specific to Turkey would be beneficial.

Paired comparisons

The age, BMIs, microalbumin, creatinine, triglyceride, and systolic blood pressures of patients who used smart phone applications were found lower when compared with nonusers, however their GFRs were found higher as compared with the nonusers. There was no statistically significant difference detected in the evaluation of the HbA1c levels in the last three months (Table 1).

Although the rate of type 1 DM was higher in patients who used smart phone applications as compared with patient nonusers, the prevalence in detecting complications was lower. However, no statistically significant difference was detected in the comparison of sex between the groups (Figure 1) (Table 2).

The conducted analyses revealed that the patients who used smart phone apps were more educated, with higher incomes, who performed more exercises, and were detected to have less complications (Table 2).

Multivariant analyses

The logistic regression model developed to estimate the smart phone application users was detected to have the Nagelkerke R^2 value of 44.6%, sensitivity of 82.9%, and specificity of 69.2% (Table 3).

The model investigating the factors affecting the use of smart phone applications showed that the smart phone application use ratios of patients with higher education were 14.7% higher compared with the ratios in patients with less education; and was 2.5% higher in patients with an income level between 2-4% above minimum wage as compared with the ratios of patients with minimum wage income or less (Table 3).

DISCUSSION

Key findings

Four-hundred and sixty-eight of the patients (92.5%) indicated that a patient follow up system specific to our

Table 1: The comparison of the laboratory results in accordance with the smart phone application use of patients

	The use of smart phone application					
	No		Yes			
	Mean	SD	Mean	SD	Z/t	p
Age (year)	63	10	49	15	10.148	**<0.001**
BMI (kg/m^2)	30.65	6.22	28.73	6.66	3.275	**0.001**
DM period (year)	18	10	14	9	4.509	**<0.001**
FBG (mg/dL)	181	82	192	90	1.414	0.157
HbA1c (%)	9.4	2.4	8.9	2.0	1.467	0.142
Microalbuminuria (mg/day)	181.4	486.6	119.0	375.9	3.290	**0.001**
Creatinine (mg/dL)	1.1	0.9	0.9	0.8	3.381	**0.001**
GFR (%)	80.64	37.86	110.98	39.39	4.305	**<0.001**
SBP (mmHg)	131	18	126	18	2.860	**0.004**
DBP (mmHg)	75	12	77	11	1.798	0.072
Triglyceride (mg/dL)	188	119	176	156	2.435	**0.015**
HDL Chloresterol (mg/dL)	46	15	49	18	0.919	0.358
LDL Chloresterol (mg/dL)	120	38	113	41	1.815*	0.070

SD: Standard deviation, Z: Mann-Whitney U test value, t: t test value in independent groups, BMI: body mass index, DM: Diabetes mellitus, GFR: Glomerulary filtration rate, SBP: Systolic blood pressure, DBP: Diastolic blood pressure

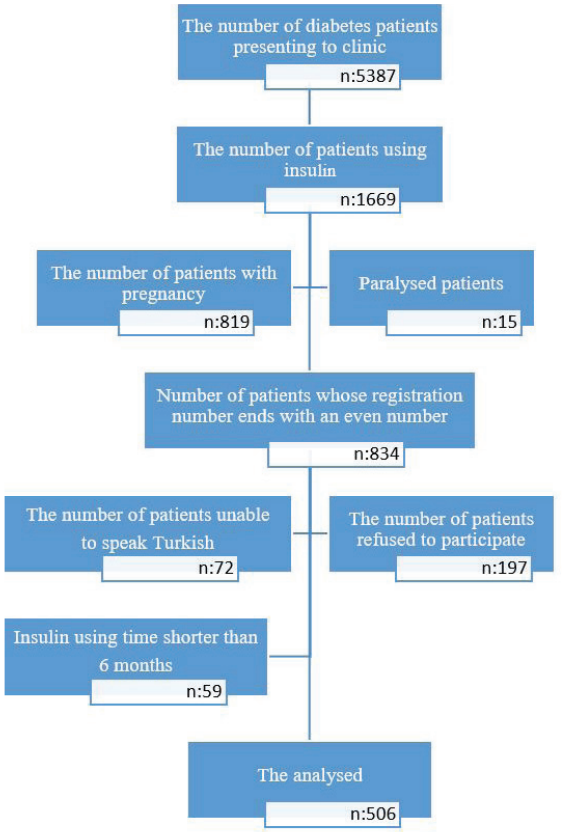

Figure 1: Study flow chart

country would be beneficial to increase the quality of life of diabetic patients. Of the participants 63.4% used smart phone applications. The patient group using smart phone applications were younger, and their BMIs and systolic BP levels were lower. T1DM patients more frequently used smart phone applications as compared with the levels in T2DM patients. The frequency of diabetic complications were lower in these patients, however this difference disappeared in the model which used the age as the confounding factor. The rate of smart phone application use of patients with higher education was found to be 14.7 fold higher compared with the rate in patients with less than primary education; and the rate in patients with income level between 2 to 4 fold of minimum wage was found to be 2.5 fold higher when compared with the rate in patients with an income level of minimum wage and below minimum wage.

Limitations

The patient selection method which minimises the effect of the confounding factors such as age, education status, and diabetes process was not used, therefore this was a factor limiting the study. One another limitation was that the smart applications used by patients could not be detailed.

Interpretations

The diabetes prevalance for all age groups was estimated as 2.8% worldwide in 2000, and the estimates for the year 2030 are 4.4% (9). Type 2 diabetic patients consist the majority of the patients. The USA Center for Disease Control and Prevention report showed that the mean ratios for T1DM, and T2DM in all diagnosed cases were 5.8%, and 90.9%, and the remaining 3.3% group consisted of other diabetes types (10). The reason for the detection of the prevalance of T1DM (22.9%) as higher than the literature in our study group, and less detection of T2DM (77.1%) and other diabetes types was suggested to be due to performing the research with patients who had used insulin.

Various technological devices used by diabetic patients in their daily life were included in the study. The self efficacy of patients who received special messages through a mobile phone significantly improved (11). The motivational effect on the users of a smart phone app developed for diabetic patients was investigated in a study. The software based system which was designed for recording the data of the glucose measurements, pedometer, food habits, and enabling feedback was found successful (12). A similar system was also tested on younger adults. Participants accepted that the use of glucometer was easy, and the system was useful in treatment of diabetes (13). In addition, a meta-analysis investigating 22 studies reported strong evidence that mobile phone directing caused statistically significant improvement in glysemic control, and self efficacy in diabetes care (14). In another meta-analysis, the technological applications were reported to be useful in improving symptom management (15).

The ratio of the smart phone application use of patients was found lower (63.4%) in our study. In addition, 92.5% reported that the patient follow-up system compatible with the smart phone applications specific to our country would be useful. However, none of our participants were detected to have used medical devices associated with smart phones. These results indicated that there was a need for enhancements in motivating the participation of patients to the diabetes treatment, and for development of technological systems.

The comparison of the type 1, and type 2 Diabetes patient groups showed that the patients in T1DM group were younger compatible with the literature (16). This age change contributed to the significant difference in the use of smart phone applications possibly in favor of T1DM patients. In addition, the exercising frequency of the app users might have affected their being more advantageous for microalbuminuria, systolic blood pressure, creatinine, and triglyceride. However, no significant difference was detected for the frequency

Table 2: The comparison of the characteristics of the participants in accordance with the smart phone app use

		The use of smart phone app					
		No		Yes			
		n	%	n	%	χ^2	p
Sex	Male	53	28.6	104	32.4	0.771	0.380
	Female	132	71.4	217	67.6		
DM type	Type 1	9	4.9	107	33.3	53.831	**<0.001**
	Type 2	176	95.1	214	66.7		
DM complication	yes	171	92.4	235	73.2	27.351	**<0.001**
	No	14	7.6	86	26.8		
The use of glucometer at home	Yes	180	97.8	319	99.4	2.396	0.122
	No	4	2.2	2	0.6		
Carrying glucometer with them	Yes-always	36	19.6	112	34.9	20.260	**<0.001**
	Yes-frequently	12	6.5	30	9.3		
	Yes-sometimes	34	18.5	62	19.3		
	No-never	102	55.4	117	36.4		
Aware of e-pulse	Yes	5	2.7	80	24.9	41.456	**<0.001**
	No	180	97.3	241	75.1		
Exercise	Yes	72	38.9	163	50.8	6.637	**<0.010**
	No	113	61.1	158	49.2		
Aware of CH count	Yes	16	8.6	92	28.7	27.996	**<0.001**
	No	169	91.4	229	71.3		
Performing CH counting	Yes	14	56.0	76	73.8	3.049	0.081
	No	11	44.0	27	26.2		
Albuminuria	Normal	33	45.2	88	63.7	6.739	**0.034**
	Micro	30	41.1	38	27.5		
	Macro	10	13.7	12	8.6		
Age group	18-34 years	3	1.6	68	21.2	72.170	**<0.001**
	35-64 years	99	53.5	203	63.2		
	65 years and above	83	44.9	50	15.6		
Educational status	Less than primary education	49	26.5	14	4.4	77.822	**<0.001**
	Primary and secondary education	127	68.6	218	67.9		
	Higher education	9	4.9	89	27.7		
Income level	Minimum wage and less than min. wage	49	26.5	47	14.6	33.556	**<0.001**
	Between minimum wage and 2 fold	105	56.8	144	44.9		
	2- 4 fold of the min. wage	25	13.5	93	29.0		
	Higher than 4 fold of minimum wage	6	3.2	37	11.5		

χ^2: Chi-square test value, DM: Diabetes mellitus, CH: Carbonhydrate

Table 3: Logistic regression computerised outcome

	B	Wald	p.	Exp (B) Lower	95% CI Upper	
Age	-0.068	34.226	**<0.001**	0.935	0.914	0.956
Education status (Reference category: Less than primary education)		28.459	**<0.001**			
Education status (secondary education)	1.241	12.721	**<0.001**	3.458	1.749	6.838
Education status (Higher education)	2.688	28.184	**<0.001**	14.709	5.452	39.687
Income (Reference category: Minimum wage and less)		8.812	**0.032**			
Income (Between Minimum wage and its 2 fold)	0.193	0.418	0.518	1.213	0.676	2.174
Income (2-4 fold of minimum wage)	0.948	6.643	**0.010**	2.580	1.255	5.306
Income (Higher than 4 fold of minimum wage)	0.850	2.015	0.156	2.341	0.723	7.573
Aware of e-pulse (1)	-1.654	10.569	**0.001**	0.191	0.071	0.519
DM type (Type 1 DM)	-0.692	2.403	0.121	0.501	0.209	1.201
Consonant	5.540	59.609	**<0.001**	254.565		

CI: Confidence interval

of the variables of HbA1c, fasting blood glucose, and diastolic blood pressure between patients who used or who did not use the applications. The applications were reported to have an effective component for helping to control HbA1c in type 2 diabetes patients (17). The contradicting of the result in our study with this outcome might be due to having patients who used insulin.

The key to success is providing the use of accurate technological devices, preparing education appropriate for the use of technological devices, and having regular communication with the patient for treatment protocol regulations (18). The results of our study revealed that the application types used by patients were inadequate for directly and significantly contributing in the control of diabetes. The development of technological devices specific to disease (smart insulin pen, noninvasive glucose sensor, insulin pump, various applications, etc) was evaluated to be beneficial for the regulation of diabetes treatment in insulin user patients. The reminder messages, and communication were suggested to be effective for motivating the patient.

Researchers in a study reported that the mean diabetes time was 13.5 years in T2DM patients, and the macrovascular complications in older patients were more common. However, microvascular complications were detected more frequently in patients who were diagnosed with diabetes at a younger age (19). Similarly, the microvascular complication risk in T1DM patients was reported to have increased in a mean of 14 years (20). The results in our study were evaluated as compatible with the literature. The complication detection frequency in younger individuals who used smart phone applications was found lower.

Our study showed that smart phone applications were more frequently used by younger, more educated patients with better economic welfare. In addition, the participants who used more technology were the patients who were more aware of the electronic health register system (e pulse) used in the country. This was compatible with the data in the literature (21, 22). However, the interactions between the investigated variables must be searched with other studies where the confounding effects were minimised.

As Gallagher et al. reported, the possibility of the use of a mobile technology of individuals aged below 56 years was 4 fold higher than the individuals aged over 69 years, and the use of technology owing to the health reasons was 3 fold higher. In addition, researchers reported in the same study that the possibility of using mobile technology high school graduate participants was 2 fold higher than the possibility of individuals who did not graduate from high school, and the possibility of mobile technology use due to health reasons was 5 fold higher (23). Compatible with the previous studies, our study results show an increased 14.7 fold in higher education patients as compared with individuals who did not receive higher education.

CONCLUSION

The frequency of diabetes has been increasing, the treatment costs and the burden in the health system has increased owing to complications emerging after poor follow-up of the disease. In addition, the quality of life, and also the life expectancy of patients have been shortened owing to the complications. Therefore, the development of innovative approaches for patients are important.

In addition, access to the health system, and contact with a diabetes team may be difficult in some pandemia and disaster conditions such as the example of the new coronavirus disease (COVID-19) pandemic caused by the new coronavirus SARS-COV-2. The distant follow-up of patients by physicians with mobile technological applications, the integration of patient measurements, and treatments with the laboratory and outpatient clinic recordings provide an innovative approach for improving diabetes treatment in such conditions.

The use of high level mobile technology must be enabled for diabetes treatment, and preventing complications, must be combined with the appropriate health education, and the patients must be motivated. Considering that our study center is a tertiary care referral institution, the lack of the use of technology of patients is striking. There is an important path to pursue for the integration of the ready for use blood glucose measurement devices and other technological devices in the management of diabetes treatment.

We suggest that there is a need for a system specific to our country for diabetic patients who used insulin, and the interest to this system will be higher in younger educated patients with mid to higher income level.

Ethics Committee Approval: This study was approved by the Clinical Research Ethical Committee of the Istanbul University Istanbul Faculty of Medicine (Date: 10.03.2017, No: 268).

Informed Consent: Written consent was obtained from the participants.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- M.K., R.Ç., K.K., İ.S., Ş.Ö., M.A.K.; Data Acquisition- M.K.; Data Analysis/Interpretation- M.K., R.Ç., K.K., İ.S., Ş.Ö., M.A.K.; Drafting Manuscript- M.K., R.Ç., İ.S.; Critical Revision of Manuscript- K.K., İ.S., Ş.Ö.; Final Approval and Accountability- M.K., R.Ç., İ.S., K.K., Ş.Ö., M.M.Ö., M.A.K.

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İSTANBUL ÜNİVERSİTESİ YAYINEVİ

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A CROSS-SECTIONAL STUDY ON FACTORS AFFECTING DIETARY QUALITY OF ADOLESCENTS WITH TYPE 1 DIABETES

TİP 1 DİYABETLİ ADÖLESANLARIN DİYET KALİTESİNİ ETKİLEYEN FAKTÖRLER ÜZERİNE KESİTSEL BİR ARAŞTIRMA

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ABSTRACT

Objective: The diet quality of adolescents with type 1 diabetes is shaped by some individual factors. These include age, gender, sociodemographic characteristics, lifestyle habits, and adaptation to diabetes treatment. This study aims to investigate the factors affecting the diet quality of adolescents with type 1 diabetes.

Material and Methods: The sample in this study consisted of adolescents with type 1 diabetes who were followed in the Department of Pediatric Endocrinology of the Faculty of Medicine at the University of Ankara between July 2017-January 2018. The research data was collected using the face-to-face interview technique with a questionnaire. The physical activity levels of the individuals were determined using a '24-hour physical activity level detection form (short)'. Three-day food consumption records were taken and evaluated via BeBiS. The Healthy Eating Index-2010 was used to determine diet quality.

Results: The study was conducted with a total of 110 adolescents (M:51.8%; F:48.2%) with type 1 diabetes in the 10-19 age range (mean age:14.0±2.40 years). Only 15.5% of all individuals have good diet quality. In a linear regression model formed by the variables of exercise status, physical activity type, and PAL value of individuals, a positive significant relationship was found between exercise status and diet quality (χ^2 (1,n=110)=1.392, p<0.05).

Conclusion: As a result, it was found that the majority of individuals needed to improve their diet quality and that exercise affected the diet quality of type 1 diabetic adolescents. In addition, exercise levels, which have an important role in both diabetes management and improvement of diet quality, should be increased.

Keywords: Type 1 diabetes, adolescents, diet quality, exercise

ÖZET

Amaç: Tip 1 diyabetli adölesanların diyet kalitesi bazı bireysel faktörlere göre değişebilmektedir. Bunlar yaş, cinsiyet, sosyodemografik özellikler, yaşam tarzı alışkanlıkları ve diyabet tedavisine uyumu içerir. Bu çalışmanın amacı, tip 1 diyabetli adölesanların diyet kalitesini etkileyen faktörleri incelemektir.

Gereç ve Yöntemler: Bu çalışmanın örneklemini, Temmuz 2017-Ocak 2018 tarihleri arasında Ankara Üniversitesi Tıp Fakültesi Çocuk Endokrinolojisi Anabilim Dalı'nda takip edilen tip 1 diyabetli adolesanlar oluşturmuştur. Araştırma verileri yüz yüze görüşme tekniği kullanılarak toplanmıştır. Bireylerin fiziksel aktivite düzeyleri '24 saatlik fiziksel aktivite düzeyi tespit formu (kısa) 'ile belirlenmiştir. Üç günlük besin tüketim kayıtları alınmış ve BeBis ile değerlendirilmiştir. Diyet kalitesini belirlemek için Sağlıklı Yeme İndeksi-2010 kullanılmıştır.

Bulgular: Çalışma, 10-19 yaş aralığında (ortalama: 14,0±2,40 yıl) 57'si erkek (%51,8), 53'ü kadın (%48,2) olmak üzere toplam 110 tip 1 diyabetli adolesan ile gerçekleştirilmiştir. Tüm bireylerin sadece %15,5'i iyi diyet kalitesine sahipti. Diyet kalitesinin cinsiyete göre dağılımı istatistiksel olarak anlamlı değildi. (p>0,05). Bireylerin egzersiz durumu, fiziksel aktivite türü ve PAL değeri değişkenlerinden oluşan doğrusal regresyon modelinde egzersiz durumu ile diyet kalitesi arasında pozitif yönde anlamlı bir ilişki bulunmuştur (χ^2 (1,n=110)=1,392, p<0,05).

Sonuç: Sonuç olarak, bu çalışmada, katılımcıların çoğunun diyet kalitelerini iyileştirmesi gerektiği ve egzersizin tip 1 diyabetli adölesanların diyet kalitesini etkilediği saptanmıştır. Buna ek olarak, hem diyabet yönetiminde hem de diyet kalitesinin iyileştirilmesinde önemli rolü olan egzersiz düzeylerinin artırılması gerektiği sonucuna varılmıştır.

Anahtar Kelimeler: Tip 1 diyabet, adolesanlar, diyet kalitesi, egzersiz

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INTRODUCTION

One of the basic elements necessary for metabolic control in adolescents with type 1 diabetes is medical nutrition therapy (1). The main goals of medical nutrition therapy for children and adolescents with diabetes are to achieve glycemic control through accessible and applicable meal plans, to minimize acute and chronic complications, and to maintain normal growth and development (2).

Diet quality, one of the components of medical nutritional treatment of adolescents with type 1 diabetes, has an important role in the management of diabetes (3). Good diet quality is defined as hygienic, nutritious, balanced, diverse, satisfying the needs of the individual, and supporting growth and development (4). Although healthy eating plays a crucial role in the management of type 1 diabetes, research shows that adolescents with type 1 diabetes have poor diet quality (4-6). The diet quality of adolescents with type 1 diabetes is shaped by some individual factors. These include age, gender, sociodemographic characteristics, lifestyle habits, and adaptation to diabetes treatment (7). According to a study conducted with individuals with type 1 diabetes, young girls (19-21 years) have higher diet quality compared to boys and younger age groups (13-15 years) (8). According to another study, parents' educational levels and diet quality of adolescents with type 1 diabetes have a positive correlation (9). According to a study by Granado-Casas et al., diet quality and physical activity levels of individuals with type 1 diabetes are associated (10). Because of the limited number of studies in which the factors affecting the diet quality of adolescents with type 1 diabetes are evaluated, this study aims to investigate the factors affecting the diet quality of adolescents with type 1 diabetes.

MATERIALS AND METHODS

Subjects

The sample of this study consisted of adolescents with type 1 diabetes aged 10-19 years who were followed up in the Department of Pediatric Endocrinology of the Faculty of Medicine at the University of Ankara between July 2017 and January 2018. The population of the study consisted of adolescents who attended the hospital and provided inclusion criteria during the given time. The purposive sampling method was used for sampling. Individuals who were diagnosed with type 1 diabetes at least one year ago, have a daily insulin dose of >0.5 units/kg, and receive intensive insulin therapy or have used an insulin pump for at least three months were included in the study. Those who were diagnosed with celiac and hyperlipidemia, and have been on an insulin pump for less than three months and use premixed insulin, and who were <10 years old and >19 years old were excluded from the study.

Data collection

The research data was collected using the face-to-face interview technique with a questionnaire. Sociodemographic characteristics of individuals and some data related to diabetes were obtained through the questionnaire. The physical activity levels of the individuals involved in the study were determined using the '24-hour physical activity level detection form (short)'. Using this form, the physical activity level (PAL) values of the individuals were calculated and classified as light/mild, moderate, and vigorous (11). All participants were asked whether hypoglycemia, defined as fasting blood glucose <70 mg/dL, occurred in the last one month. Food consumption status of the participants was determined by 3-day food consumption records. Dietary intakes were evaluated via the Nutrition Information System for Turkey. The Healthy Eating Index (HEI)-2010 was used to determine how much adolescents comply with dietary guidelines and dietary recommendations in the food pyramid and to measure diet quality (12). The amounts consumed were evaluated on the basis of nutrients in grams per 1000 kcal. Diet quality of individuals was defined according to the total HEI score: ≤50 is "poor diet quality," 51-80 is ''needs improvement in diet quality," and 81-100 is "good diet quality" (13).

Statistic analysis

The analysis of the data obtained from the survey was conducted with the SPSS statistical package program. Descriptive statistics were shown as mean±standard deviation for the variables with a normal distribution, as median and the interquartile range values for the variables with non-normal variables, and the number and percentages (%) of the cases for the nominal variables. The statistically significant difference between qualitative variables, if the assumptions of normal distribution are provided, was determined with the help of the Mann Whitney U-Test. The relationship between two categorical variables was analyzed by the Chi-Square Test. To reveal whether there was a statistically significant relationship between two quantitative variables, the Pearson Correlation Coefficient was used when at least one of the variables provided a normal distribution assumption, but Spearman's Correlation Coefficient was used when the above-mentioned assumption was not met. Logistic regression analysis was performed to determine the factors affecting diet quality. The confidence interval was accepted as 95.0% in all statistical tests and evaluated at $p<0.05$ significance level.

RESULTS

The study was conducted with a total of 110 adolescents with Type 1 diabetes in the 10-19 age range, 57 of whom were male (51.8%) and 53 of whom were female (48.2%). Of the participants 46.4% were in high school and 5.5%

were undergraduates. Parents were generally primary/secondary education graduates, with 14.5% of mothers and 21.8% of fathers having a bachelor's degree (Table 1).

When the data related to diabetes were examined, the mean age of the diabetes was statistically significant between groups (4.6±3.32, p<0.05). 98.2% of the participants received diabetes education. 41.3% of them met with a dietitian and 39.0% with a nurse. 60.2% of the trainings received were individual training. 47.2% of the participants counted carbohydrates. In the last month, the rate of individuals who had hypoglycemia was 65.5%. 60.0% of them experienced it 1-3 times and 10.0% experienced it 10 times or more (Table 2).

It was found that 84.5% of all participants needed to improve their diet quality. When this was evaluated by gender, it was determined that 86.0% and 83.0% of boys and girls, respectively, needed to improve diet quality. The ratio of those with good diet quality in boys and girls was 14.0% and 17.0%, respectively. Only 15.5% of all individuals had good diet quality. Distribution of diet quality by gender is not statistically significant (p>0.05) (Table 3).

There was only a weak and negative correlation between diet quality score and age in the general sample (p<0.05) (Table 4).

In the model formed by the variables of exercise status, physical activity type, and PAL value of individuals, a positive significant relationship was found between exercise status and diet quality ((1,n=110)=1.392, p<0.05) (Table 5).

DISCUSSION

The adolescent period is a term in which daily energy and nutrient requirements increase with growth and development, and the attitudes and behaviors gained, including nutrition, affect the individual in the short and long term (14). Since type 1 diabetes is a lifelong disease, acquiring healthy eating habits is extremely important in terms of achieving metabolic control. As a result of our study, it was found that there was a weak and negative correlation between the age and diet quality score of adolescents with type 1 diabetes. This shows that it is more important to provide motivation for treatment rather than age for people with diabetes. In the literature some studies suggest a positive relationship between age and diet quality, and some studies suggest the exact

Table 1: Distribution of individuals' education, parents' education by gender and mean ages

	Male (n=57)		Female (n=53)		Total (n=110)		χ^2
	n	%	n	%	n	%	p[a]
Education status							
Primary education	9	15.8	2	3.7	11	10.0	4.07 0.13
Secondary education	22	38.6	20	38.0	42	38.1	
High school	25	43.8	26	49.0	51	46.4	
Undergraduate	1	1.8	5	9.3	6	5.5	
Mother's education status							
Primary/secondary education	28	49.1	26	49.1	54	49.1	1.82 0.61
High school	22	38.6	17	32.0	39	35.5	
Undergraduate	7	12.3	9	17.0	16	14.5	
Graduate	-	-	1	1.9	1	0.9	
Father's education status							
Primary/secondary education	21	36.8	20	37.7	41	37.3	1.84 0.60
High school	21	36.8	14	26.4	35	31.8	
Undergraduate	11	19.4	13	24.6	24	21.8	
Graduate	4	7.0	6	11.3	10	9.1	
Age (years)							
$\bar{x}$±SD	14.6±2.14		13.5±2.53		14.0±2.40		**1141.00**
Median	13.0		15.0		14.0		**0.02[u]***
Min-Max	10.0-18.0		10.0-19.0		10.0-19.0		

[a]Chi-Square Test [u] Mann Whitney U Test *p<0.05 $\bar{x}$: Arithmetic mean; SD: Standard deviation

Table 2: Distribution of individuals' diabetes-related data by gender

	Male (n=57)		Female (n=53)		Total (n=110)		u p
Diabetes diagnosis age (years)							
$\bar{x}$±SD	9.5±3.63		9.1±3.55		9.3±3.58		1431.00
Median	10.0		10.0		10.0		0.63[b]
Min-Max	3.0-15.0		1.0-15.0		1.0-15.0		
Diabetes age (years)							
$\bar{x}$±SD	4.0±3.18		5.2±3.38		4.6±3.32		**1165.00**
Median	3.0		5.0		4.0		**0.03[b]***
Min-Max	1.0-13.0		1.0-14.5		1.0-14.5		
	n	**%**	**n**	**%**	**n**	**%**	**χ^2 p[a]**
Nutrition education							
Did not take	-	-	2	3.8	2	1.8	2.19
Took**	57	100.0	51	96.2	108	98.2	0.13
Dietician	35	41.2	36	41.4	71	41.3	
Nurse	34	40.0	33	37.9	67	39.0	2.70 0.60
Doctor	16	18.8	18	20.7	34	19.7	
**Type of training ** **							
Group training	27	39.7	26	40.0	53	39.8	0.31
Individual training	41	60.3	39	60.0	80	60.2	0.85
Carbohydrate counting							
Counting	23	40.4	29	54.7	52	47.2	
Not counting	24	42.1	15	28.3	39	35.5	2.68 0.26
Sometimes counting	10	17.5	9	17.0	19	17.3	
Hypoglycemia in the last 1 month							
Did not happen	19	33.4	19	33.0	38	34.5	0.07
Happened**	38	66.6	34	67.0	72	65.5	0.78
1-3 times	21	55.0	22	64.9	43	60.0	
4-6 times	8	21.0	4	11.7	12	17.0	1.68 0.64
7-9 times	6	16.0	4	11.7	10	13.0	
10 times and higher	3	8.0	4	11.7	7	10.0	

[a]Chi-Square Test [b]Mann Whitney U Test *p<0.05 $\bar{x}$: Arithmetic mean; SD: Standard deviation; **whose answered yes.

Table 3: Diet quality score and evaluation of individuals by gender

Diet quality	Male (n=57)		Female (n=53)		Total (n=110)		
	n	**%**	**n**	**%**	**n**	**%**	
Needs improvement	49	86.0	44	83.0	93	84.5	χ^2=0.18
Good	8	14.0	9	17.0	17	15.5	p=0.66[a]
$\bar{x}$±SD	68.95±10.23		70.61±9.03		69.75±9.66		u=1358.00
Median	20.00		69.95		69.68		p=0.36[b]
Min-Max	42.74-90.03		47.28-92.11		42.74-92.11		

[a]Chi-Square Test [b] Mann Whitney U Test, $\bar{x}$: Arithmetic mean; SD: Standard deviation

Table 4: Correlation of diet quality score of individuals with some parameters by gender

Some parameters	Male (n=57)	Female (n=53)	Total (n=110)
	r p[a]	r p[a]	r p[a]
Age (years)	-0.237 0.075	-0.170 0.224	-0.191 0.046*
Diabetes age (years)	-0.187 0.164	0.204 0.142	0.020 0.838
Diabetes diagnosis age (years)	-0.021 0.876	-0.254 0.067	-0.125 0.195

[a] Spearman Correlation Test *p<0.05

Table 5: Determination of variables which can be effective in classification of individuals in terms of diet quality by logistic regression analysis

Variables	β	Wald	S.S	p	Odds ratio	Coefficient interval for 95% odds ratio	
						Min	Max
Sociodemographic variables							
Age	0.137	0.393	1	0.531	1.147	0.747	1.760
Gender	0.341	0.374	1	0.541	1.407	0.471	4.203
Education status	-1.242	1.916	1	0.166	0.289	0.050	1.676
Mother's education status	-0.269	0.323	1	0.570	0.764	0.303	1.930
Father's education status	-0.390	1.161	1	0.281	0.677	0.333	1.376
Constant	0.821	0.149	1	0.699	2.273		
Variables about diabetes							
Diabetes diagnosis age	-0.038	0.100	1	0.752	0.963	0.763	1.216
Diabetes age	-0.105	0.632	1	0.427	0.900	0.694	1.167
Frequency of hypoglycemia	-0.337	3.516	1	0.061	0.714	0.502	1.015
Carbohydrate counting status	0.605	2.664	1	0.103	1.831	0.886	3.787
Presence of diabetes in the family	0.758	1.605	1	0.205	2.134	0.661	6.896
Constant	-40.378	0.000	1	0.999	0.000		
Variables about exercise							
PAL value	0.568	0.078	1	0.780	1.765	0.033	94.287
Exercise status	1.392	5.421	1	0.020*	4.024	1.246	12.992
Type of physical activity	0.264	0.060	1	0.806	1.302	0.158	10.739
Constant	-3.511	0.908	1	0.341	0.030		

* p<0.05, PAL: Physical activity level

opposite (8, 15-17). In a study, it was determined that the education given to adolescents with type 1 diabetes at an early age increases the motivation for treatment and can be an effective factor in achieving glycemic control and preventing complications (15). According to another study, the age of children and adolescents between the ages of 4-16 increase their adaptation to diet at earlier ages. According to another study, children and adolescents between the ages of 4-16 years increase their ability to adapt to diets at earlier ages (16). According to Kleiser et al., it was found that healthy eating scores decreased in adolescence compared to childhood (17). In conclusion, it is thought that it will be easier to adapt to nutritional therapy by giving responsibility not only to

parents but also to children, and enabling earlier self-care of diabetes in adolescents with type 1 diabetes.

The delivery of diabetes and nutritional education by an experienced pediatric diabetes team to adolescents with type 1 diabetes is crucial in terms of achieving glycemic control and improving the prognosis of diabetes (18). In this study, it was found that the most of the participants received nutrition education and almost half of them received it from a dietician. The reason why the majority of adolescents need improvement in their diet quality may be related to their not having met with a dietician. This is because the dietician, while ensuring the optimal body weight of the diabetic, also improves the compliance of the diabetic with the individual meal plan. Carbohydrate counting applications can determine the appropriate insulin dose for the carbohydrate taken and prevent acute and chronic complications. In addition, the adherence of adolescents to treatment is monitored during frequent follow-up and the meal plan is updated when necessary (2). In a study conducted by Karagüzel et al., the diabetes team, consisting of two dieticians, provided diabetes education to adolescents with type 1 diabetes who were taken to diabetes camp for seven days. HbA1c levels of the participants were significantly lower in the 6th and 12th months compared to the pre-camp levels (19). These results show that it is important to provide nutritional education at certain intervals as of the time of diagnosis in order to improve the diet quality of adolescents with diabetes.

In our study, the educational status of the adolescent and his family did not affect the diet quality score. The reason for this is the homogeneous distribution of diabetic subjects in terms of diet quality. Similarly, according to the study by Lipsky et al., educational level does not affect diet quality (20). However, in another study, adolescents whose parents were educated at university and above had higher diet quality (21). According to a similar study, higher education level is associated with better diabetes self-care, and this plays an important role in improving diet quality and thus in achieving glycemic control (22). In addition, parents with a high level of education are thought to be efficent in understanding the treatment of type 1 diabetes and helping the adolescent to make it a lifestyle. Parents mostly play an active role in the treatment of type 1 diabetes, especially in childhood and early adolescence. In this context, high educational level of the parents is very important for the prognosis of treatment.

Exercise, the third major treatment component for type 1 diabetes after nutrition and insulin, is a specific form of physical activity planned to improve physical health. Exercise was established to play an important role in providing glycemic control, to decrease the risk of cardiovascular disease and to be protective against obesity (18). Exercise therapy and medical nutrition therapy should not be considered separately. For example, exercise may cause hypoglycemia or even coma if enough carbohydrates are not taken (23). In our study, logistic regression analysis found that the exercise status of individuals had a statistically significant effect on diet quality. Accordingly, exercise status increases good diet quality by 4.024 times. In a study by Storey et al., a significant severe relationship was found between poor diet quality of individuals with insufficient physical activity level (24). In another study, similar results were obtained and good diet quality was associated with moderate to vigorous physical activity (20). In An's study, inadequate physical activity and poor diet quality were associated with obesity (25). This suggests that physically active individuals pay more attention to their diet and regular exercise facilitates adherence to diabetes treatment.

As a result, in this study, it was found that the majority of individuals needed to improve their diet quality, and exercise affected the diet quality of type 1 diabetic adolescents. Therefore, healthy food choices and diet quality of adolescents with type 1 diabetes should be improved. In this context, it is important to include dieticians, who are involved in the regulation of medical nutrition therapy, in the diabetes treatment team. Treatment should continue with regular, effective, and frequent trainings. In addition, exercise levels, which have an important role in both diabetes management and the improvement of diet quality, should be increased.

Ethics Committee Approval: Thist study was approved by the Ankara University Clinical Research Ethics Committee (Date: 22.05.2017, No: 10-526). A 'Research Permit' dated 12.04.2018 and numbered 15255985-302.01.08[774.99]-E.10299 was obtained from Ankara University Faculty of Medicine Cebeci Hospital Pediatric Endocrine Polyclinic.

Informed Consent: Written consent was obtained from the participants.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- G.Ş.B., A.K.; Data Acquisition- G.Ş.B., Z.Ş., M.B.; Data Analysis/Interpretation- G.Ş.B.; Drafting Manuscript- G.Ş.B., A.K.; Critical Revision of Manuscript- G.Ş.B., A.K.; Final Approval and Accountability- G.Ş.B., A.K., Z.Ş., M.B.

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İSTANBUL ÜNİVERSİTESİ YAYINEVİ

RESEARCH / ARAŞTIRMA
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EPIDEMIOLOGICAL AND CLINICAL CHARACTERISTICS, CAUSATIVE DRUGS, AND DIAGNOSTIC CHALLENGES OF FIXED DRUG ERUPTION IN ELDERLY PATIENTS: IS BULLOUS TYPE A MORE COMMON CLINICAL PHENOTYPE?

YAŞLI HASTALARDA FİKS İLAÇ ERÜPSİYONUNUN EPİDEMİYOLOJİK VE KLİNİK ÖZELLİKLERİ, ETKEN İLAÇLAR VE TANISAL ZORLUKLAR: BÜLLÜ TİP DAHA SIK GÖRÜLEN BİR KLİNİK FENOTİP MİDİR?

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ABSTRACT

Objective: The present study aimed to evaluate the epidemiological and clinical features, causative drugs, and diagnostic challenges of Fixed drug eruption (FDE) in elderly patients.

Material and Methods: In this single-center, retrospective, cross-sectional study, we evaluated data of all consecutive adult patients (aged >18 years) with an established diagnosis of FDE between 1996-2018 in our tertiary referral center in Turkey. Elderly patients who were aged ≥60 years at the time of diagnosis were allocated to the study.

Results: Among 182 adult FDE patients, 14 (7.7%) patients (male/female=1/1.8) were in the elderly group (mean age=66.1±6.9 years). Fix drug eruption was induced by intermittently taken drugs in all patients, 50% of whom had polypharmacy (concurrent use of ≥5 medications). Trimethoprim-sulfamethoxazole (42.9%) and non-steroidal anti-inflammatory drugs (35.7%) were the main causative drugs, while ornidazole was a remarkable novel FDE inducer since 2011. Fix drug eruption was mostly located on the trunk and extremities. Mucosal involvement was less frequent. Ten (71.4%) patients had bullous FDE (BFDE). There were no statistically significant differences between the gender and mean ages of the patients with and without BFDE.

Conclusion: Our long-term experience demonstrated that FDE may also affect elderly patients. Most of the patients had BFDE raising the question of whether the bullous type is a more common clinical phenotype in these patients. Among the important

ÖZET

Amaç: Bu çalışmanın amacı, yaşlı hastalarda fiks ilaç erüpsiyonunun (FİE) epidemiyolojik ve klinik özelliklerinin, etken ilaçlarının ve tanısal zorluklarının değerlendirilmesidir.

Gereç ve Yöntemler: Tek merkezli, retrospektif kesitsel çalışmamızda, Türkiye'de üçüncü basamak bir referans merkezi Alerji kliniğinde, 1996-2018 yılları arasında FİE tanısı konulmuş olan erişkin hastaların (>18 yaş) dosyaları incelenmiştir. FİE tanısı konulduğu sırada 60 yaş ve üzerinde olan yaşlı hastalar çalışmaya dahil edilmişlerdir.

Bulgular: Toplam 182 erişkin FİE hastası arasından, 14 (%7,7) hasta (erkek/kadın=1/1,8) yaşlı grubundaydı (ortalama yaş=66,1±6,9). Fiks ilaç Erüpsiyonu, hastaların tümünde aralıklı olarak kullanılan ilaçlara bağlı gelişmişti. Bu hastaların %50' sinde polifarmasi (eş zamanlı ≥5 ilaç kullanımı) mevcuttu. Fiks ilaç Erüpsiyonunun en sık etkenleri, trimetoprim-sülfametoksazol (%42,9) ve non-steroidal antienflamatuvar ilaçlar (%35,7) olmakla birlikte 2011 yılından itibaren ornidazol de yeni ve dikkat çekici bir Fiks ilaç Erüpsiyonu etkeni olarak karşımıza çıkmıştı. Fiks ilaç Erüpsiyonu lezyonları sıklıkla gövde ve ekstremitelerde yerleşmekteydi. Mukozal tutulum daha nadirdi. On (%71,4) hastada büllü FİE (BFİE) saptandı. Büllü FİE olan ve olmayan hastalar arasında cinsiyet ve ortalama yaş açısından istatistiksel olarak anlamlı farklılıklar saptanmadı.

Sonuç: Uzun-dönem klinik tecrübemiz ışığında, FİE yaşlı kişilerde de görülebilen ve hastaların büyük bir çoğunluğunda büllü

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diagnostic challenges of FDE in this age group, e.g., polypharmacy, multimorbidity, recall problems, cognitive disorders, frailty, and immunosenescence, BFDE should also be kept in mind as it may clinically mimic Stevens-Johnson syndrome/toxic epidermal necrolysis, and autoimmune bullous diseases.

Keywords: Drug eruptions, aged, vesiculobullous skin diseases, trimethoprim-sulfamethoxazole drug combination, non-steroidal anti-inflammatory agents, ornidazole

morfolojinin (BFİE) eşlik ettiği bir ilaç döküntüsü olarak karşımıza çıkmıştır. Bu durum, BFİE'nin bu yaş grubunda sık görülen bir klinik fenotip mi olduğu sorusunu akla getirmiştir. BFİE'nin klinik olarak Stevens-Johnson sendromu/toksik epidermal nekroliz veya otoimmün büllü hastalıklar ile ayırıcı tanıya girmesi tanıda zorluklara yol açabilmektedir. Fiks ilaç Erüpsiyonu şüphesi olan yaşlı hastalarda polifarmasi, multimorbidite, hafıza problemleri, kognitif bozukluklar, kırılganlık ve immün sistemin yaşlanması (immunosenescence) gibi tanıyı güçleştirebilecek durumlar arasında BFİE de akla gelmelidir.

Anahtar Kelimeler: İlaç erüpsiyonları, yaşlı, büllü deri hastalıkları, trimetoprim-sülfametoksazol, non-steroidal antienflamatuvar ilaçlar, ornidazol

INTRODUCTION

Population ageing is a global phenomenon driven by decreasing fertility rates and remarkable improvements in life expectancy (1, 2). According to The United Nations, the share of the world's population over 60 years is growing rapidly and is expected to reach up to 21.2% by 2050 (3). This ongoing demographic transformation poses special challenges for clinicians worldwide.

With advancing age, medication use is steadily rising in the elderly, highlighting the need for further research on adverse cutaneous drug reactions (ACDRs) which are often underreported in this age group (4). Compared with young adults, older people are at an increased risk for developing ACDRs, due to the high prevalence of multimorbidity, polypharmacy, and age-related changes in pharmacokinetics and pharmacodynamics (4-6). Moreover, evaluation of elderly patients with ACDRs may be challenging, especially of those with cognitive impairment, frailty, and immunosenescence. Most of the ACDRs are not life-threatening and spontaneously resolve after the discontinuation of the responsible agent. However, delayed diagnosis may result in recurrences which constitute an important source of morbidity, further decreasing the quality of life in elderly individuals (6).

Fixed drug eruption (FDE) is one of the most common drug eruptions in Turkey (7). It is a T-cell mediated (type IVc) hypersensitivity reaction characterized by the recurrence of skin and/or mucosal lesions at the same sites, following each exposure to the offending drug (7, 8). Its clinical spectrum is quite broad, ranging from typical solitary or multiple, sharply defined, erythematous or violaceous plaques to various atypical variants and even generalized bullous lesions (7, 8). The inducers of FDE show significant geographical and temporal variations depending on the most frequently used drugs in a given time period (9). Fix drug eruption (FDE) may occur throughout life; however, aside from a few anecdotal case reports, there is lack of data regarding its characteristics in the older population (10-12).

The present study aimed to investigate the epidemiological and clinical features, and culprit drugs of FDE in the elderly, with a special focus on bullous FDE (BFDE), which is a rare, but challenging FDE variant clinically mimicking severe and potentially life-threatening drug reactions, such as Stevens-Johnson syndrome and toxic epidermal necrolysis, and autoimmune bullous diseases, such as pemphigus vulgaris and bullous pemphigoid.

MATERIAL AND METHODS

In this single-center, retrospective, cross-sectional study, we evaluated the data of all the consecutive adult patients (aged >18 years) with FDE between 1996 and 2018 in our Allergy unit, a tertiary referral center in Turkey. Exclusion criteria were pediatric patients (aged ≤18 years) and patients in whom oral/topical provocation tests could not be performed, or failed to identify the causative drugs.

Among a total of 182 adult patients with an established diagnosis of FDE, only those who were ≥60 years of age at the time of diagnosis were allocated to the study. In all patients, FDE was diagnosed upon the results of oral/topical provocation tests which were performed during the remission phase of FDE, after a verbal or written informed consent had been obtained.

For the oral provocation test, a 1/8 dose of a certain drug was initiated, gradually increasing to 1/4, 1/2, and one full dose at 12 to 24 hour intervals. A positive test result was characterized by flaring up of the old FDE sites showing erythema and/or edema and accompanying itching and/or burning that started within 10-30 minutes up to a few hours after taking the test doses of the suspected drug (7). On the other hand, topical provocation tests were performed by applying the suspected drugs at concentrations of 1-10% in white petrolatum to the old FDE sites as occlusive or open patch testing, as described before (7). The development of erythema with/without induration in an old FDE lesion within 24 hours and persistence for at least 6 hours was considered a positive test result (7).

The files of the patients were analyzed regarding demographic, clinical, histopathological features, and causative drugs, and for patients with trimethoprim-sulfamethoxazole-induced FDE, additionally for the presence of genetic markers, i.e., HLA-A30 B13 Cw6 haplotype, and HLA-B55 antigen (split of B22). A lymphocytotoxicity assay was performed for Class I HLA typing (13).

Generalized FDE was defined as the presence of widespread FDE lesions involving ≥10% body surface area on ≥3 of 6 different anatomic sites, including the head and neck, anterior trunk, posterior trunk, upper extremities, lower extremities, and genitalia (14).

This study was approved by the local ethics committee (Date: 18.03.2021, No: 139758), and conducted in accordance with the ethical standards of the Declaration of Helsinki.

Statistical analysis

Statistical analysis was performed using SPSS (Version 22, IBM). Quantitative data were expressed as means±standard deviation (SD) and qualitative data as number (n) and percentage (%). Means were compared using Student's t-test (for normal distributions) or Mann-Whitney U test (for non-normal distributions), while frequencies were compared using the chi-square or Fisher's exact test. A 2-tailed P-value <0.05 was accepted for statistical significance.

RESULTS

Of 182 adult patients with FDE, 14 (7.7%) patients (male/female ratio 1/1.8) were in the elderly group. The mean age of the patients was 66.1±6.9 years. Two (14.3%) patients had a history of allergic rhinoconjunctivitis, while none of the patients had a family history of FDE. The characteristic features of 14 elderly patients with FDE are presented in Table 1.

The duration of the disease ranged from 1 month to 240 months, with a median of 18 months. In 12 (85.7%) patients, the disease duration was ≥1 year at the time of diagnosis. The number of attacks ranged from 1 (n=1) to 10 (n=2), with a median of 3. Seven (50%) patients reported an increase in the number and/or size of FDE lesions with subsequent attacks.

Table 1: Characteristic features of 14 elderly patients with an established diagnosis of fixed drug eruption between 1996 and 2018

Case no (year of diagnosis)	Age (years)	Gender	Atopy	Causative drug	Number of lesions	Typical plaques	Bullae	Mucosal involvement
1 (1996)	66	Male	No	Trimethoprim-sulfamethoxazole	2-10	Yes	Yes	No
2 (1996)	60	Male	No	Trimethoprim-sulfamethoxazole	Solitary	Yes	No	No
3 (1997)	62	Female	No	Naproxen	2-10	Yes	Yes	Oral
4 (1997)	60	Female	No	Trimethoprim-sulfamethoxazole	Solitary	Yes	No	No
5 (1999)	67	Female	AR	Trimethoprim-sulfamethoxazole	>10	Yes	Yes	No
6 (1999)	60	Male	No	Trimethoprim-sulfamethoxazole	Solitary	Yes	Yes	Genital
7 (2004)	65	Female	No	Dipyrone	>10	Yes	Yes	No
8 (2005)	60	Female	No	Piroxicam	2-10	Yes	Yes	Oral
9 (2008)	68	Male	No	Trimethoprim-sulfamethoxazole	2-10	Yes	Yes	Genital
10 (2008)	60	Male	AR	Indomethacin	Generalized	Yes	No	No
11 (2011)	77	Female	No	Ornidazole	Generalized	Purpuric	Yes	No
12 (2016)	72	Female	No	Naproxen	>10	Yes	Yes	No
13 (2017)	67	Female	No	Dimenhydrinate	>10	Yes	Yes	Genital
14 (2018)	82	Female	No	Ornidazole	Generalized	Yes	No	No

AR: Allergic rhinoconjunctivitis

Before referral to our clinic, FDE lesions of seven patients had been clinically diagnosed as drug eruption of an unknown nature (n=2), eczema (n=1), eosinophilic cellulitis (n=1), Sweet syndrome (n=1), pemphigus and erythema multiforme (n=1), and mycosis fungoides (n=1).

In all patients, the diagnosis of FDE was based upon the results of oral provocation tests which revealed a reactivation of old lesions between 30 minutes and 12 hours, following the intake of the responsible drug. Six patients also underwent topical provocation testing which was positive in only one of them. Histopathological examination was performed in eight patients and showed findings consistent with FDE, including an interface dermatitis characterized by dyskeratosis, basal cell vacuolar degeneration, melanophages in the upper dermis, and perivascular mononuclear inflammatory infiltration.

The main cause of FDE in elderly patients was trimethoprim sulfamethoxazole (n=6) which was used for the treatment of infections (urinary tract infection, bronchitis, and tooth infection) or postoperative infection prophylaxis. The second most common cause was nonsteroidal anti-inflammatory drugs (NSAIDs) comprising naproxen (n=2), dipyrone (n=1), piroxicam (n=1), and indomethacin (n=1), mainly administered for headache and arthralgia. Other causes included ornidazole (n=2) and dimenhydrinate (n=1), which were used to treat urinary tract infection and diverticulitis, and motion sickness, respectively. Between 1996 and 2003, the main culprit drug was trimethoprim-sulfamethoxazole (5/6=83.3%) which was superseded by NSAIDs (4/8=50%) between 2004 and 2018.

More than half (n=4/6; 66.7%) of the patients with trimethoprim-sulfamethoxazole-induced FDE were males, while more than 3/4 (n=4/5; 80.0%) of the patients with NSAIDs-induced FDE were females. However, these differences showed no statistical significance.

Three patients had solitary lesions located on the cheek, foot, and penis, respectively, induced by trimethoprim-sulfamethoxazole. On the other hand, three patients had generalized FDE caused by ornidazole (n=2) and indomethacin (n=1).

FDE presented with typical sharply defined, erythematous to violaceous plaque lesions in all patients (Figure 1 and Figure 2), except for one patient who had purpu-

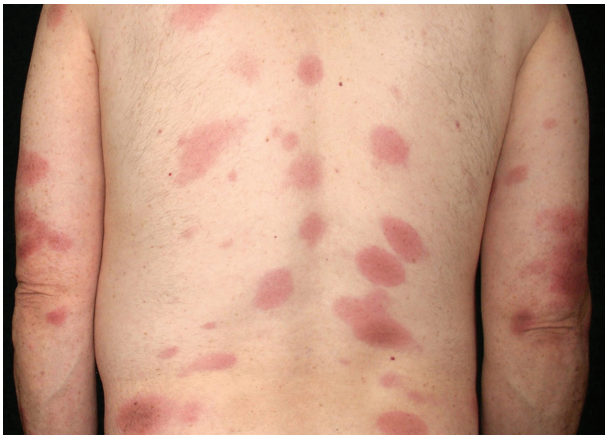

Figure 1: Typical plaque lesions of fixed drug eruption on the trunk

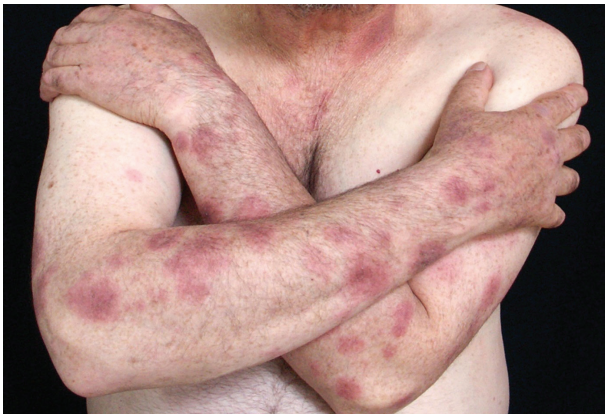

Figure 2: Typical plaque lesions of fixed drug eruption on the upper extremities

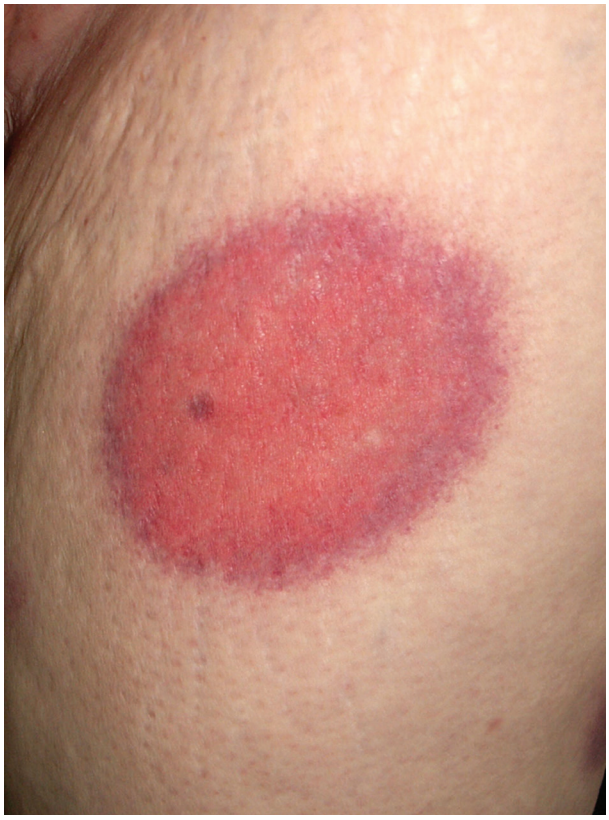

Figure 3: Purpuric fixed drug eruption on the left upper thigh

ric plaques (Figure 3). All patients suffered from pruritus which was accompanied by a burning sensation in 12 patients. In nearly all patients (n=13), FDE lesions healed with postlesional hyperpigmentation.

Bullous skin lesions overlying plaques (Figure 4) were observed in 10 (71.4%) patients. Bullae were present since the first attack (n=8), or developed during the subsequent attacks (n=2). The main causative drugs were trimethoprim-sulfamethoxazole (n=4) and NSAIDs (n=4), followed by dimenhydrinate (n=1) and ornidazole (n=1). Generalized bullae were seen in only one patient with ornidazole-induced FDE. Two patients with BFDE had concurrent bullous/erosive oral mucosal lesions. There were no statistically significant differences between the patients with and without BFDE with respect to the mean age, gender, and presence of atopy (Table 2).

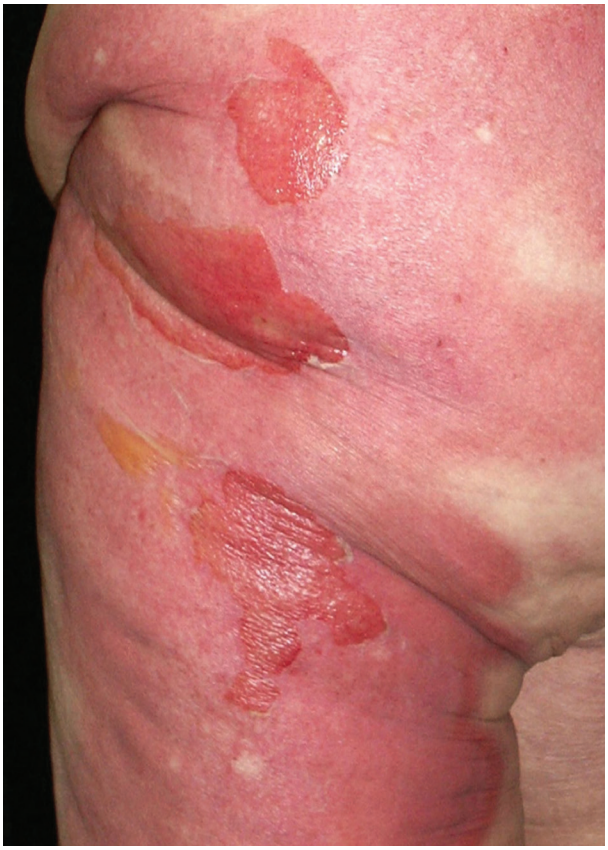

Figure 4: Bullous fixed drug eruption on the left upper thigh

Nine patients (64.3%) had skin lesions only, while 4 (28.6%) patients had coexistent skin and mucosal lesions, and one patient (7.1%) had mucosal lesions only. Localization and causative drugs of FDE are presented in Table 3.

With regard to skin involvement (n=13), the most commonly involved sites were the trunk and extremities (n=7) with a significant involvement of hands (n=7) and feet (n=4). Some patients showed specific patterns of skin involvement, such as finger/toe webs due to trimethoprim-sulfamethoxazole (n=3), naproxen (n=1), and dipyrone (n=1), vermillion border of lips due to trimethoprim-sulfamethoxazole (n=1) and naproxen (n=1), nasal philtrum due to dipyrone (n=1), eyelid due to trimethoprim-sulfamethoxazole (n=1), and inner eye canthus due to dipyrone (n=1).

Genital FDE was observed in three patients. Two patients had penile involvement due to trimethoprim-sulfamethoxazole, whereas one patient had vulvar involvement due to dimenhydrinate. Penile involvement was the sole presentation of FDE in one patient. On the other hand, oral mucosal FDE was observed in two female patients with multiple bullous/erosive lesions. One patient had buccal mucosa involvement due to naproxen, while the other patient had buccal mucosa, hard palate, and tongue involvement due to piroxicam.

With regard to the results of Class I HLA typing, none of the patients with trimethoprim-sulfamethoxazole-induced FDE were positive for HLA-A30 B13 Cw6 haplotype, or HLA-B55 antigen.

DISCUSSION

To date, there have been no studies specifically focusing on FDE in the elderly population. Thus, our 23-year clinical experience presented herein may contribute to shed light on the characteristic features of FDE in this special age group with unique challenges and needs.

Table 2: Characteristics of elderly patients with and without bullous fixed drug eruption

Characteristics of the patients	Type of fixed drug eruption		
	Bullous n=10	Non-bullous n=4	p-value
Age (years), mean±SD	66.4±5.3	65.5±11	*0.311**
Male/female, n (%)	3/7 (30/70)	2/2 (50/50)	*0.580***
Atopy, n (%)	1 (10)	1 (25)	*0.505***

SD: Standard deviation, *Mann-Whitney U test, **Fisher's exact test

Table 3: Localization and causative drugs of FDE in elderly patients

Characteristics	**Total n=14**
Distribution of FDE lesions	Skin only (9), skin and mucosa (4), mucosa only (1)
Skin lesions (n=13)	
***Localization (n)**	**Causative drug (n)**
Trunk and extremities (7)	Ornidazole (2)/dipyrone (1)/trimethoprim-sulfamethoxazole (1)/ piroxicam (1)/indomethacin (1)/dimenhydrinate (1)
Hands (7)	Trimethoprim-sulfamethoxazole (3)/naproxen (2)/piroxicam (1)/ornidazole (1)
Feet (4)	Trimethoprim-sulfamethoxazole (3)/ornidazole (1)
Lips (2)	Trimethoprim-sulfamethoxazole (1)/naproxen (1)
Neck (1)	Ornidazole (1)
Specific pattern (n)	**Causative drug (n)**
Finger/toe webs (5)	Trimethoprim-sulfamethoxazole (3)/naproxen (1)/dipyrone (1)
Vermillon border (2)	Trimethoprim-sulfamethoxazole (1)/naproxen (1)
Nasal philtrum (1)	Dipyrone (1)
Eyelid (1)	Trimethoprim-sulfamethoxazole (1)
Inner eye canthus (1)	Dipyrone (1)
Genital mucosal lesions (n=3)	
Localization (n)	**Causative drug (n)**
Penis (2)	Trimethoprim-sulfamethoxazole (2)
Vulva (1)	Dimenhydrinate (1)
Oral mucosal lesions (n=2)	
Localization (n)	**Causative drug (n)**
Buccal mucosa (1)	Naproxen (1)
Buccal mucosa and hard palate and tongue (1)	Piroxicam (1)

*The total number is >13 as more than one skin site was involved in most of the patients.

Our study is the first to show that FDE, although mainly reported in young adults, is also a common type of drug eruption among the elderly. The share of elderly patients was nearly 8% (n=14/182) in our study. We assume that this value may further increase in the near future, as the older population continues to grow in Turkey as well. Moreover, although FDE usually has no gender preference, a female predominance in our study (n=9/14; 64.3%) as well as in previously reported elderly cases (n=3/4; 75%) was remarkable (7, 10-12).

Trimethoprim-sulfamethoxazole (42.9%) and NSAIDs (35.7%) were the most common causative drugs of FDE in our elderly patients. Although trimethoprim-sulfamethoxazole was the main culprit in the earlier years of the study, it was superseded by NSAIDs since the year 2004, following the decline in trimethoprim-sulfamethoxazole use in Turkey, as previously reported (9). The high prevalence of NSAIDs-induced FDE was not surprising, as NSAIDs are among the most commonly prescribed medications for the management of pain and inflammation in this age group (15). A study based on data from the Norwegian Prescription Database showed that 7.3% of all elderly individuals aged >60 years filled at least one NSAID prescription in a one-year period (16).

In our experience, ornidazole was a remarkable novel FDE inducer since the year 2011, in concordance with the findings of a previous study from our clinic (9). The prevalence of ornidazole-induced FDE in the present series was 14.3% (n=2). Nevertheless, it is important to note that it was responsible for 66.7% of all generalized FDE (GFDE) cases, including the case of generalized bullous FDE (GBFDE) accompanied by atypical purpuric plaques. Ornidazole-induced FDE has been mainly reported from India and Turkey since the year 2005, reflecting its broad use in these countries for genitourinary infections and intestinal amoebiasis, respectively (9, 17-22). Compared with these cases, our patients (aged 77 and 82 years) were the oldest ones. Although ornidazole is a relatively newer 5-nitroimidazole derivative with a high safety profile, our experience along with the literature data indicate that it is an emerging inducer of FDE and should be used with caution, especially in elderly patients.

Polypharmacy is one of the biggest challenges in the identification of the causative drugs of FDE among the

elderly. Nevertheless, its definition is still a matter of debate. According to the World Health Organization (2019), polypharmacy is often defined as the routine use of five or more medications, including over-the-counter, prescription, and/or traditional and complementary medicines (23). A recent comprehensive review demonstrated that the prevalence of polypharmacy in older adults ranged from 4% to over 96.5% across various studies, according to the definition, age group, healthcare setting, and region (24). Yet, all studies included in that review showed an increasing prevalence of polypharmacy in older adults over time (24). In the present study, on the other hand, half of the patients (n=7) were using ≥5 medications and 21.4% of the patients (n=3) even ≥10 medications (including both the regularly used drugs for comorbidities and intermittently used drugs for infections, pain management, etc.). Interestingly, in all patients, FDE was induced by intermittently taken antibiotics, NSAIDs, or dimenhydrinate. We believe that this is an important finding which may guide clinicians when conducting a medication review. Intermittent use of prescribed or over-the-counter medications should always be questioned in elderly patients with suspected FDE.

In the present study, FDE lesions were mostly located on the trunk and extremities (50%) with a frequent acral (hands and/or feet) involvement. On the other hand, involvement of genital (21.4%) and oral mucosa (14.3%) was relatively low when compared with the results of previous studies from our clinic on FDE patients including younger adults (25, 26). All patients were suffering from disturbing pruritus, accompanied by a burning sensation in most of them. Xerosis and other age-related changes in skin could also have contributed to severe pruritus in these patients (27). Most patients had a disease duration of ≥1 year at the time of diagnosis, and experienced recurrent attacks which induced an increase in the number and/or size of FDE lesions in half of the patients. Taken together, these findings highlight the importance of early diagnosis of FDE to prevent further increase in disease severity, and to preserve the quality of life in elderly patients.

As a striking finding, most patients in this study had BFDE. Bullae had been present since the first attack in the majority, and developed during the subsequent attacks in two patients. BFDE is a rare and severe type of FDE that clinically presents with localized or generalized blisters or erosions overlying plaque lesions on the skin (28). The frequency of BFDE ranged from 29.6% to 36.6% across various studies including heterogeneous age groups from Tunisia, India, and Korea, whereas it was 50.8% in a nationwide retrospective multicentric study from France (28-32). Compared with these literature data, our study presents the highest frequency (71.4%) of BFDE, raising the question of whether the bullous type is a more common clinical subtype among the elderly. Further prospective large studies on elderly patients with FDE are warranted to confirm this potential association between BFDE and older age.

Diagnosing FDE is usually straightforward due to its unique clinical properties. Site-specific recurrence of the lesions is the diagnostic hallmark of FDE. However, bullous type, particularly GBFDE, may pose a big diagnostic challenge in elderly patients as it may mimic severe and potentially life-threatening drug reactions, such as Stevens-Johnson syndrome and toxic epidermal necrolysis, and autoimmune bullous diseases, such as pemphigus vulgaris and bullous pemphigoid (28, 33). To date, four elderly cases with FDE have been reported in literature (10-12). They presented almost exclusively with GBFDE, except a 75-year old male with multiple plaque lesions due to ivermectin (10). GBFDE was induced by the influenza vaccine in a 67-year-old female, whereas metamizole was the causative drug in two female patients, aged 83 and 91 years, respectively (11, 12). These data along with our findings highlight that FDE may present with bullous or generalized bullous lesions even in the late elderly. Thus, awareness of this clinical heterogeneity of FDE remains crucial in preventing misdiagnosis which may lead to recurrent attacks, irrelevant investigations, and overtreatments in this fragile age group.

Evaluation of elderly patients with FDE has special difficulties. First of all, obtaining a comprehensive medication history may be challenging in this age group, due to the relatively high prevalence of self-medication (including over-to-counter or herbal medications), polypharmacy, recall problems, and cognitive disorders, ranging from mild impairment to severe dementia (4-6). In certain circumstances, it may be helpful to ask the patients to bring in all the medications they regularly or intermittently take, and review the labels (5). Moreover, the use of drug provocation tests has some challenges in the elderly. Although topical provocation testing offers a safer alternative method than systemic provocation, it is also a less reliable diagnostic tool due to false-negative results (7). As elderly people have an age-related decline in immune functions, termed immunosenescence, caution should be exerted when interpreting the negative test results in these patients (4, 30). On the other hand, overpenetration of the drugs through the skin due to age-related skin atrophy may induce irritant and false-positive test reactions (4). Systemic (oral) drug provocation testing is the most reliable method of diagnosing FDE and establishing the causative drugs (7). Nevertheless, according to the European Network on Drug Allergy (ENDA) guidelines, it should not be performed in patients with comorbidities such as cardiac, hepatic, renal, or other diseases, where exposure might provoke a situation that is beyond medical control (35). This is particularly important for elderly patients with multicomorbidity and frailty which develops as a consequence of an age-related

decline in many physiological systems (36). Frailty refers to an enhanced vulnerability to poor resolution of homeostasis after a stressor event, which increases the risk of negative health-related outcomes (36, 37). Therefore, oral drug provocation tests should be performed with special caution in these patients, following a careful systemic evaluation and assessment of the risk-benefit ratio (4). In our long-term clinical experience, on the other hand, oral provocation tests were performed in all patients aged between 60 and 82 years, without any complications. Importantly, topical provocation testing was not able to identify the causative drug in a majority of patients, who were then diagnosed according to the positive results of oral provocation tests with the same drugs. Our findings indicated that, when performed carefully in selected patients by experienced clinicians, systemic provocation testing was a safe method in establishing the causative drugs of FDE in this age group.

The management of elderly patients with FDE requires a multidisciplinary approach. Dermatologists and clinicians from other specialties should collaborate to prevent further attacks and develop alternative treatment schedules which do not include the implicated drugs. Moreover, both patients and their caregivers should be informed and counseled on avoidance of the culprit medications to increase adherence.

The main limitation of this study was its retrospective nature.

CONCLUSION

The present study is the first to investigate the characteristic features of FDE in elderly patients, to the best of our knowledge. Our findings highlight that FDE, although mainly reported in young adults, might also affect the elderly. FDE was induced by intermittently taken drugs in all patients, half of whom had a high burden of polypharmacy. Trimethoprim-sulfamethoxazole and NSAIDs were the most common causative drugs, while ornidazole was a remarkable novel FDE inducer since the year 2011. As a striking finding, a great majority of patients had BFDE raising the question of whether the bullous type is a more common clinical phenotype in this age group. BFDE, particularly when generalized, may pose a big diagnostic challenge as it may clinically mimic severe and potentially life-threatening drug reactions, such as Stevens-Johnson syndrome and toxic epidermal necrolysis, and autoimmune bullous diseases, such as pemphigus vulgaris and bullous pemphigoid.

Evaluation of elderly patients with FDE has unique challenges such as polypharmacy, multimorbidity, recall problems, cognitive disorders, frailty, and immunosenescence. Therefore, awareness of the clinical heterogeneity, causative drugs, and diagnostic challenges of FDE remains crucial in preventing delayed diagnosis or misdiagnosis which may lead to recurrent attacks, irrelevant investigations, overtreatments, and further impairment of quality of life in this fragile patient population.

Ethics Committee Approval: This study was approved by the Ethical Committee of the Istanbul University, Istanbul Faculty of Medicine (Date: 18.03.2021, No: 139758).

Informed Consent: Written or verbal consent was obtained from the participants.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- G.B.K., E.Ö.; Data Acquisition- G.B.K., E.Ö.; Data Analysis/Interpretation- G.B.K., E.Ö.; Drafting Manuscript- G.B.K., E.Ö.; Critical Revision of Manuscript- G.B.K., E.Ö.; Final Approval and Accountability- G.B.K., E.Ö.

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A POSSIBLE RELATIONSHIP BETWEEN SERUM HOMOCYSTEINE LEVEL AND IgA NEPHROPATHY IN CHILDREN

ÇOCUKLARDA SERUM HOMOSİSTEİN DÜZEYİ İLE IgA NEFROPATİSİ ARASINDAKİ OLASI İLİŞKİ

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ABSTRACT

Objective: The evidences from experimental and epidemiological studies suggests that elevated serum homocysteine levels may lead to renal injury and may be a significant risk factor for the development of chronic kidney disease. The aim of this study was to investigate a possible relationship between serum homocysteine level and crescent formation in children with IgA nephropathy and Henoch-Schonlein purpura nephritis.

Material and Methods: A total of 31 patients diagnosed as biopsy proven IgA nephropathy and Henoch-Schonlein purpura nephritis and idiopathic crescentic glomerulonephritis in three Pediatric Nephrology centers within the last five years and 25 healthy controls were enrolled in the study.

Results: Homocysteine levels of patients were higher than the upper limit of normal value and also higher than the controls (p=0.0001). There was no significant difference between the patients with or without crescent formation regarding homocysteine levels (p>0.05). Presence or severity of proteinuria was not related to homocysteine levels (p>0.05).

Conclusion: Serum homocysteine levels are elevated in patients with IgA nephropathy and Henoch-Schonlein purpura nephritis.

ÖZET

Amaç: Deneysel ve epidemiyolojik çalışmalar, yüksek serum homosistein düzeylerinin renal hasara yol açabileceğini ve kronik böbrek hastalığı gelişimi için önemli bir risk faktörü olabileceğini göstermektedir. Çalışmamızın amacı IgA nefropatisi ve Henoch-Schönlein purpura nefriti olan hastalarda serum homosistein düzeyleri ile kresent oluşumu arasında bir ilişki olup olmadığını belirlemektir.

Gereç ve Yöntemler: Üç pediatrik nefroloji merkezinde son beş yılda biyopsi ile IgA nefropatisi, Henoch-Schönlein purpura nefriti ve idiopatik kresentik glomerulonefrit tanısı alan 31 hasta ve 25 sağlıklı kontrol çalışmaya alındı.

Bulgular: Hastaların homosistein düzeyleri normal değerin üst sınırından ve kontrollerden de daha yüksekti (p=0,0001). Biyopside kresent görülen ve görülmeyen hastalar arasında da homosistein düzeyleri açısından anlamlı fark yoktu (p>0,05). Proteinüri varlığı ve şiddeti ile homosistein düzeyleri arasında ilişki saptanmadı (p>0,05).

Sonuç: Serum homosistein düzeyleri IgA nefropatisi ve Henoch-Schonlein purpura nefriti olan hastalarda yüksek bulunmuştur. Sonuçlarımız, bu hasta grubunda yüksek serum homosistein

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Our results suggest that elevated serum homocysteine levels may be related to segmental glomerulosclerosis in these patient groups.

Keywords: IgA nephropathy, homocysteine, crescent

düzeylerinin segmental glomerüloskleroz ile ilişkili olabileceğini düşündürmektedir.

Anahtar Kelimeler: IgA nefropatisi, homosistein, kresent

INTRODUCTION

IgA nephropathy (IgAN), one of the most common glomerulonephritis in adults and children, may progress to end-stage renal disease (ESRD) in 20-50% of patients within 20 years (1-3). Although IgAN usually has a slow progression course, acute deterioration in renal function due to crescent formation may occur during the course of the disease (3, 4). It has been reported that crescent formation in IgAN may influence renal outcomes and aggressive immunosuppressive treatment may be required if the number of crescents involves >50% of the glomeruli in a renal biopsy (5). The Kidney Disease Improving Global Outcomes (KDIGO) guidelines recommend that Henoch-Schonlein Purpura Nephritis (HSPN) should be treated the same as IgAN because it has the same histopathologic features as IgAN in kidney biopsies (5). Crescent formation in glomeruli begins with the emergence of ruptures of glomerular capillary walls (6). These ruptures permit coagulation factors and cells including monocytes and lymphocytes to enter the Bowman's space, which results in crescent formation (6). There is some evidence to suggest that elevated concentration of serum homocysteine (Hcy) leads to endothelial cell injury, which results in stimulation of the coagulation system and resistance of anticoagulation activity on the endothelial surface (7, 8). Moreover, it has been reported that hyperhomocysteinemia causes glomerular and tubulointerstitial damage in several ways, such as increasing oxidative stress, stimulating monocyte chemoattractant protein-1 (MCP-1) expression, and nuclear factor kappa B activation (NF-B) (9-14).

We observed hyperhomocysteinemia in two patients with IgAN and crescent formation in their renal biopsy. Using observations from these two patients, we hypothesized that crescent formation may be related to hyperhomocysteinemia in IgAN. The aim of the study was to investigate whether a relationship exists between serum Hcy level and crescent formation in children with IgAN and HSPN.

MATERIALS AND METHODS

Thirty-one patients with renal biopsy-proven IgAN (n=17), HSPN (n=12), and idiopathic crescentic glomerulonephritis (ICGN; n=2) in three pediatric nephrology centers were enrolled in the study. The control group was consisted of 25 age matched healthy children without medical history of any chronic disease or renal disorder.

This study was approved by the Ethical Committee of Istanbul University Istanbul Faculty of Medicine (Date: 27.05.2013, No: 640). Informed consent was obtained from the parents of all participants. A physical examination was performed, and the patients' medical history and clinical findings were recorded at the time of the sampling.

Baseline investigations including serum urea, creatinine, electrolytes, lipids, total protein, albumin, Hcy, urinalysis and 24-hour urinary protein were performed in the patient group. Moreover, serum levels of vitamin B12 and folic acid, thyroid function tests, methylenetetrahydrofolate reductase *(MTHFR)* 677 and *MTHFR* 1298 genetic mutation analysis were also performed because hyperhomocysteinemia may be caused by deficiency of folic acid and vitamin B12, hypothyroidism or decreased enzyme activity of *MTHFR* due to genetic mutations. A physical examination was performed and serum urea, creatinine, Hcy, B12 and urinalysis were performed in the control group.

IgAN, HSPN, and ICGN were diagnosed based on clinical, laboratory and immunehistopathologic findings. Mesangial hypercellularity with predominant IgA deposition in the mesangium and/or capillary wall was described as IgAN in patients without systemic involvement. The MEST score was evaluated as mesangial hypercellularity (M0, M1), endocapillary hypercellularity (E0, E1), segmental glomerulosclerosis (S0, S1) and tubular atrophy/interstitial fibrosis (T0, T1), in accordance with the Oxford classification (4). Presence or absence of crescents was recorded. Henoch-Schonlein Purpura was diagnosed using the EULAR/PReS consensus criteria (15). Extracapillary proliferation of cells in the Bowman's space with more than two cell layers was described as cellular crescent (16). Idiopathic crescents in more than 50% of glomeruli on renal biopsies were described as ICGN.

Hypertension was defined as average clinic measured systolic and/or diastolic blood pressure ≥95th percentile on the basis of age, sex and height percentiles (17).

The mean estimated glomerular filtration rate (eGFR) was calculated according to the Schwartz formula (18). Hematuria was defined as five or more red blood cells per high power field in a urine specimen (19). Proteinuria was determined as a positive dipstick reading of ≥1+, urinary excretion ≥4 mg/m^2/per hour in urine over a period of 24 hours (19).

Venous blood samples were collected in tubes from the antecubital vein, followed by overnight fasting. The tubes were centrifuged at 2000 relative centrifugal force (RCF) (10 minutes) to remove the serum. The blood and serum samples were analyzed within an hour. Serum glucose, urea, creatinine, total cholesterol, HDL cholesterol, triglyceride, total protein, albumin, thyrotropin (TSH), free triiodothyronine (T3), free thyroxine (T4), and other biochemical parameters were determined using a Beckman Coulter AU5800 Clinical Chemistry, Dxl 800 Immunoassay Auto-Analyzer and commercial kits (Beckman Coulter, CA, USA). Urine protein levels were measured using Siemens BNII nephelometric system (Siemens Healthcare Diagnostics, USA) using reagents and protocols provided by the manufacturer. Serum Hcy, vitamin B12, and folic acid were determined using an Immulite 2000 chemiluminescence auto-analyzer and commercial kits (Siemens, USA). Hyperhomocysteinemia was defined as serum levels of Hcy >12µg/L (20). Deficiency of vitamin B12 was defined as <200 pg/mL and deficiency of folic acid was defined as <3 ng/mL (21, 22).

Genomic deoxyribonucleic acid (DNA), was isolated from peripheral blood leukocytes using the High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Mannheim, Germany) for C677T and A1298C of *MTHFR* gene mutations. Detection of gene mutations was achieved using rapid capillary PCR with melting curve analysis using fluorescence-labeled hybridization probes in a Light Cycler (Roche Diagnostics GmbH, Mannheim, Germany).

Statistical analysis in this study was performed with the package program Number Cruncher Statistical System (NCSS) 2007 Statistical Software (Utah, USA). Patients mentioned in the background section were excluded from statistical analyses because of their low eGFR to provide group homogeneity. In addition to standard descriptive statistical calculations (mean, standard deviation, median and IQR), the Kruskal-Wallis test was used in the comparison of groups, the Mann-Whitney U test was used in the comparison of two groups, and the Chi-square test was performed to evaluate qualitative data. Statistical significance level was established at $p<0.05$.

RESULTS

The median age of the patients and controls were 11.6 years (range: 3.53-19.37 years) and 12.60 years (range: 9.53-14.84), respectively. There was no significant difference between patient and control groups regarding age and gender distribution (p=0.145 and p=0.186; respectively). The median follow-up duration of the patients was 61.5 months (range: 3-365 months). The patient's characteristics are given in Table 1.

Mean serum Hcy level was significantly higher in the patients than in the controls (p=0.0001) (Table 2, Figure 1). Hcy levels were not significantly different in the patients with IgAN and HSPN (Table 2). The presence or severity of proteinuria were not related to Hcy levels (p>0.05) (Table 2).

Table 1: Characteristics of the study group

Patients characteristics	n (%)
Total	31 (100)
Histopathologic diagnosis	
IgA nephropathy	17 (54.8)
Henoch-Schonlein nephritis	12 (38.7)
Idiopathic crescentic glomerulonephritis	2 (6.5)
Presence of crescent	**11 (35.5)**
IgA nephropathy	3 (27.3)
Henoch-Schonlein nephritis	6 (54.5)
Idiopathic crescentic Glomerulonephritis	2 (18.2)
Presenting symptoms	
Macroscopic hematuria	12 (38.7)
Hypertension	6 (19.3)
Rash	12 (38.7)
Arthritis/arthralgia	9 (29)
Proteinuria	17 (54.8)
Nephrotic proteinuria	3 (9.6)
Deterioration of kidney function	4 (12.9)
Actual situation of the patients	
Microscopic hematuria	9 (29)
Non-nephrotic proteinuria	18 (58)
Hypoalbuminemia	0 (0)
Hypertension	2 (6.4)
Hyperlipidemia	7 (22.5)
Deterioration of kidney functions	1 (3.2)

Eleven (35.5%) of the patients had crescent formation in renal biopsy. Crescents existed in ≥50% of glomeruli in two patients with ICGN, in one patient with IgAN, and in one patient with HSPN. Other patients with IgAN and HSPN had crescents in ≤25% of glomeruli. There was no significant difference between the patients with and without crescent formation regarding Hcy levels (p=0.17) (Table 2).

According to the MEST score, 40.7% of the patients were assessed as M1, 48.1% as E1, 22.2% as S1, and 7.4% as T1. Serum Hcy level was significantly higher in patients who were assessed as S1 (29.36±11.99 µg/L) than in those assessed as S0 (19.74±4.15µg/L) with regard to MEST score (p=0.009) although other parameters of the score were not related to Hcy level (p>0.05).

As an interesting observation, Hcy levels were higher than the upper limit of normal values in all our patients (Figure 1). Mean vitamin B12 level was significantly lower in the patient group than in the controls (238.77±127.24 vs 378.88±69.63; p=0.0001)(Table 2). Vitamin B12 level was lower than 200 ng/mL in 12 patients (38.7%) in our

Table 2: Serum homocysteine, folic acid, and vitamin B12 levels in controls and patients according to histopathology and proteinuria

	Homocysteine mean±SD (µg/L)	Vitamin B12 mean±SD (pg/mL)	Folic acid mean±SD (ng/mL)
Patients (n=29)	23.18±8.66	238.77±127.24	7.52±2.78
Controls (n=25)	8.88±1.79	378.88±69.63	8,01±1.46
p	0.0001	0.0001	0.427
Histopathologic diagnosis			
IgAN[a] (n=17)	22.67±8.49	222±69.88	8.87±3.74
HSPN[b] (n=12)	21.34±5.44	249.33±191.52	7.52±2.78
ICGN[c] (n=2)	38.65±15.76	318±41.01	9.05±0.40
p	0.790	0.413	0.376
Presence of crescent			
Crescent (+) (n=11)	26.71±12.16	261±168.08	7.69±2.39
Crescent (-) (n=20)	21.25±5.43	226.55±105.38	8.73±3.69
p	0.173	0.695	0.536
Presence of proteinuria			
Proteinuria (+) (n=18)	22.56±8.41	218.56±102.02	8.24±4.03
Proteinuria (-) (n=13)	24.06±9.28	266.77±160.07	8.52±1.98
p	0.575	0.378	0.328
Severity of proteinuria			
<150 mg/24h (n=13)	24.06±9.28	266.77±160.07	8.52±1.98
150-500 mg/24h (n=10)	20.26±3.59	198.5±41.26	7.16±1.99
500-1000 mg/24h (n=8)	25.44±11.75	243.63±147.64	9.59±5.53
p	0.480	0.625	0.424

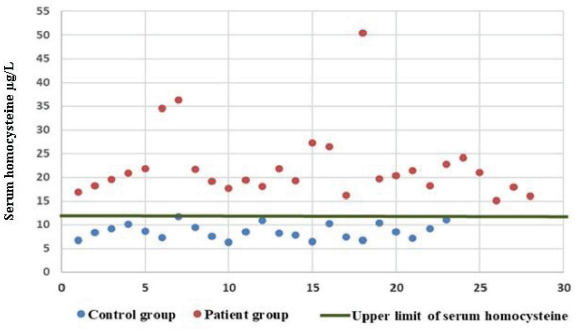

Figure 1: Serum homocysteine levels of the controls and patients

study groups. There was no significant difference between patients who had normal vitamin B12 levels and those who had low vitamin B12 in terms of crescent formation (p=0.641). Serum folic acid level and thyroid function tests were within the normal range in all our patients. The frequency of 677CC, 677CT, and 677TT genotypes was 32.2%, 61.2%, and 6.4%, respectively. The results for the 1298AA, 1298AC, and 1298CC genotypes were 19.3%, 77.4%, and 3.2%, respectively.

DISCUSSION

Crescent formation may occur at any time during the course of IgAN and HSPN. We previously observed crescents in two patients with both IgAN and hyperhomocysteinemia in our outpatient clinic. It has been reported that elevation of serum Hcy level has many harmful effects on the endothelium (23). Some of these effects are activation of coagulation and inhibition of fibrinolysis, increase of vascular tonus, and stimulation of oxidative stress (23). The effects of Hcy have also been demonstrated in glomeruli via some experimental studies (11, 13, 14). Hence, we expected that serum Hcy levels might be higher in patients with crescents than in those without among children with IgAN and HSPN. On the contrary, Hcy levels were not different between patients with or without crescents in their glomeruli in our study group.

Interestingly, all of our patients had elevated serum Hcy levels. Therefore, we evaluated the well-known risk factors for hyperhomocysteinemia such as hypothyroidism, vitamin B12 and folic acid deficiency, and *MTHFR* gene polymorphisms (24). TT polymorphism of *MTHFR* C677T was found to be associated with decreased activity of *MTHFR*, which results in higher Hcy and lower folic acid levels. Also, it has been reported that the CC variant of *MTHFR* A1298C might be associated with lower enzyme activity, but results in the literature are less conclusive than for C677T (25-27). As possible causes of hyperhomocysteinemia, vitamin B12 deficiency was found in 12 patients (38.7%) in our study group. Moreover, TT polymorphisms were detected

in two patients and CC polymorphism in one patient. However, serum Hcy levels were also found elevated in the remaining 16 patients who did not have features that may predispose to hyperhomocysteinemia. We speculate that hyperhomocysteinemia may be related to IgAN itself because there was no other reason for hyperhomocysteinemia in these patients.

Endothelial cells have an important role in the maintenance of vascular structure integrity in glomeruli (28, 29). It has been considered that endothelial cells are related to the progression of IgAN. Kusano T et al. evaluated the number of glomerular endothelial cells in patients with IgAN by examining the number of nuclei of CD34+ glomerular endothelial cells in renal biopsies (6). The authors demonstrated that the loss of CD34+ endothelial cells was associated with glomerular necrosis, segmental and global sclerosis in IgAN (6). Also, there were fewer CD34+ endothelial cells found in glomeruli with chronic lesions than in normal glomeruli in the biopsy samples (6). Moreover, hyperhomocysteinemia may aggravate endothelial damage and sclerotic modification in glomeruli (11, 13, 30). According to Oxford Classifications, segmental glomerulosclerosis was related to the poor prognosis in IgAN (4). Hcy levels were significantly higher in patients with segmental sclerosis in their kidney biopsies in our study group, which suggests that hyperhomocysteinemia may be related to sclerosis and chronic changes in glomeruli and consequently poor prognosis in IgAN.

Proteinuria is another important factor that is known to impact the prognosis of patients with IgAN (31). Thus, the relationship between the serum Hcy levels and proteinuria was evaluated in our study. According to our results, there were no differences between the presence or absence of proteinuria and between the different levels of proteinuria in patients with IgAN in terms of serum Hcy levels. In our previous study, we evaluated whether there was Hcy elevation in idiopathic nephrotic syndrome in children. The serum Hcy levels were not higher in nephrotic children than in the control group (32). Kong et al. demonstrated that hyperhomocysteinemia was an independent risk factor of decreased eGFR in the elderly population, although there was no relationship between proteinuria and elevated serum Hcy levels in their study (33). On the contrary, Shuwei et al. reported that hyperhomocsyteinemia was associated with lower eGFR and also initial proteinuria in patients with IgAN (34). Although these studies had different results about the relationship between serum Hcy levels and proteinuria, they concluded that hyperhomocysteinemia was related to decreasing in eGFR and poor prognosis.

Our study had some limitations. One of these limitations was that our patients were not in the acute phase of the illness. The mean duration between kidney biopsy and blood sampling for the study was 35 months. We believe that further studies in larger groups of patients in the acute phase of the illness may reveal a relationship between these pathologic prognostic factors. The second limitation was our relatively small sample size.

In conclusion, we demonstrated that serum Hcy levels are elevated in patients with IgAN and HSPN. Our results suggest that elevated serum Hcy levels may be related to segmental glomerulosclerosis in these patient groups.

Ethics Committee Approval: This study was approved by the Clinical Research Ethical Committee of the Istanbul University, Istanbul Faculty of Medicine (Date: 27.05.2013, No: 640).

Informed Consent: Written consent was obtained from the participants.

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THE USE OF CONTRACEPTIVE METHOD PATTERNS: EVALUATION AT FAMILY HEALTH CENTERS*

KONTRASEPTİF YÖNTEM KULLANIM DURUMU: AİLE SAĞLIĞI MERKEZİNDE DEĞERLENDİRME

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ABSTRACT

Objective: Family planning is one of key responsibilities of family physicians; providing birth control methods, and ensuring its practical application remains important for maternal and child health. The aim of this study is to determine the contraception methods used by the individuals who applied to Family Health Centers (FHC) and to reveal the relationship between family planning methods chosen.

Material and Methods: A descriptive research method was adopted for this study using the follow-up records of the FHCs. One thousand two hundred thirty-two follow-up records in total were accessed between March 2018 and December 2018, and SPSS 21.0 was used for data analysis.

Results: Mean ranks provide evidence that age scores were higher for the users of tubal ligation, and this group had significantly more children than the users of combined oral contraceptives ($p<0.001$), condom ($p<0.001$) and intrauterine device ($p=0.043$). When all the follow-ups were evaluated, it was seen that the number of people who did not use contraceptive methods was high.

Conclusion: Family planning and sexual education are associated with the availability and sustainability of resources that are crucial for healthcare. It remains important to provide Family Planning counseling to those who do not use any contraceptive method during the follow-up.

Keywords: Family planning, women's health, birth control, family medicine

ÖZET

Amaç: Aile planlaması, aile hekimlerinin temel sorumluluklarından biridir; doğum kontrol yöntemlerinin sağlanması ve pratik uygulamasının sağlanması anne ve çocuk sağlığı için önemini korumaktadır. Bu çalışmanın amacı, Aile Sağlığı Merkezlerine (ASM) başvuran bireylerin kullandığı kontrasepsiyon yöntemlerini belirlemek ve seçilen aile planlaması yöntemleri arasındaki ilişkiyi ortaya çıkarmaktır.

Gereç ve Yöntemler: Bu çalışma için ASM'lerin izlem kayıtları kullanılarak tanımlayıcı bir araştırma yöntemi benimsenmiştir. Mart 2018 ve Aralık 2018 tarihleri arasında toplam 1232 takip kaydına erişilmiş ve veri analizi için SPSS 21,0 kullanılmıştır.

Bulgular: Tüp ligasyonu kullananların yaş ortalamaları daha yüksekti ve bu grup kombine oral kontraseptif ($p<0,001$), prezervatif ($p<0,001$) ve intrauterin araç ($p=0,043$) kullananlardan daha fazla çocuk sahibiydi. Tüm izlemler değerlendirildiğinde kontraseptif yöntem kullanmayanların fazla olduğu görüldü.

Sonuç: Aile planlaması ve cinsel eğitim, sağlık hizmetleri için çok önemli olan kaynakların mevcudiyeti ve sürdürülebilirliği ile ilişkilidir. Herhangi bir kontraseptif yöntem kullanmayanlara izlemler sırasında Aile Planlaması danışmanlığı verilmesi önemini korumaktadır.

Anahtar Kelimeler: Aile planlaması, kadın sağlığı, doğum kontrolü, aile hekimliği

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INTRODUCTION

Equity should be one of the principles of all nations' health systems. "Health for all" is a family physician principle adopted at the Astana Declaration last year. The Astana Declaration recognizes that many people need better access to healthcare, particularly the poor, and states that it is "unacceptable that inequity in health and disparities in health outcomes persist" (1).

The United Nations Third-World Women's Conference in Nairobi was an important point about "equality, development and peace" and "employment, health and education" (2). In 1994, the World Health Organization (WHO) prepared the "Mother-baby package" with UNDP, UNICEF, UNFPA, World Bank, and some of the governments and universities. In this way, organizations declared that reducing the number of unwanted pregnancies is one of the key points for making motherhood safe (3). These were some of the milestones for women's health (4).

Contraception method choices are different all over the world. Biological, psychosocial, and cultural determinants in the regulation of fertility affect the use and selection of contraceptives. Among adolescent and young adults in Finland, the first option for adolescents is condoms, backed-up by emergency contraception; and later hormonal contraceptives in a longer, mutually monogamous relationship. Condoms and hormonal contraception combined can be well recommended for adolescents as dual protection. Long-acting reversible contraception (LARC), including both intrauterine contraception and implants, are safe and highly effective, and thus well suited for adolescents (5). The research among 15-49 year-old women in Nepal has reported that oral contraceptives (OCs) were the most preferred method. The second method was injections, but after a one-year education, the choices changed to injections first and condoms second. Women's fear of their partners also affected the choice of contraceptive methods. The most striking outcome of this research is the women who feared their partners were more likely to choose female sterilization than condoms. Therefore, more education and reduction of the fear of partners could change contraceptive behaviors (6). Yusuf et al. researched intimate partner violence (IPV) versus knowledge and use of contraception methods in the African region. They reported no significant difference between the victims of IPV compared to non-victims, for not only the level of knowledge, but also the actual usage of contraception. This study also revealed, *"If a woman knew or used traditional and folkloric methods, traditional method took priority"* (7). In Bangladesh, 15,699 married women were evaluated, and it was reported that rural women used contraceptive methods less than urban women. Religious teachings also affect the usage of contraceptive methods (8). Another point is that women with higher autonomy have higher rates of contraceptive use (9).

Although contraceptive pills for men are in the process of development, condoms, coitus interruptus (CI), and vasectomy are the methods currently used. In the United States, OCs are the most popular reversible contraceptive method, while the usage rates of condom and OCs increased due to greater gender equity (10, 11).

The first-year failure rate of OCs in USA ranges from 3% to 27% (10). According to research, for women living in poverty and relying on a partner-dependent method (such as the condom or CI), failure rates are greater (12).

The first family planning initiatives in Turkey were implemented in 1965. This program was designed to give education for promotion of birth control methods and the family planning (13). "Mother and Childcare and Family Panning Centers" are giving service for couples and families while this care is the responsibility of "Family Health Centers".

In 1974, a sexual health education program for couples was implemented in the rural part of the Ankara region (33 villages situated 20-50 km northwest of Ankara). The structured education had two steps: one to one, and group programs. This initiative was effective, and the education programs were repeated in other primary care health centers (14).

On May 27, 1983, the No. 2827 "Population Planning Code" was implemented, and in the same year in November, new abortion rules were also published in Turkey. Until this date, many women died because of "self-induced abortion". Due to this regulation, free healthcare services for abortion were provided by the state (15). In 2013, because of the sexual health education programs and services at family healthcare services, and Mother and Childcare and Family Planning Centers, the rates of modern contraceptive methods rose to 47%; until the early 2000s, CI had been the most preferred method among Turkish couples (16).

MATERIALS AND METHODS

A descriptive research method was adopted for this study using the follow-up records of the Family Health Centers (FHC) in Izmir. According to the sample formula, the minimum number of participants to be reached with 80% power and 95% confidence interval was calculated as 384. One thousand two hundred thirty-two follow-up records were accessed in total between March 2018 and December 2018, and SPSS 22.0 was used for data analysis. The sample was from different settlements of the city, so that different demographic and health characteristics could be presented (Table 1).

Table 1: Demographic and health information of the participants

Variable	n	%	M	SD	Min.	Max.
Age	896		31.65	9.366	15	49
Marital status	253					
Single	137	54.2				
Married	100	39.5				
Divorced	16	6.3				
Maternity	533	59.5			0	9
Live birth	492	54.9			0	7
Stillbirth	10	27.1			0	2
Miscarriage	148	38.5			0	5
Self-induced abortion	58	31.1			0	4
Therapeutic abortion	6	26.8			0	2
Contraceptive use/non-use	1216					
Combined oral contraceptives	56	4.6				
Intrauterine device	124	10.2				
Condom	228	18.8				
Tubal ligation	66	5.4				
Coitus interrupts	91	7.5				
Non-use of contraceptive methods	651	53.5				

RESULTS

The age of the participants ranged from 15 to 49 (M=31.65, SD=9.366). Of the sample, 137 (54.2%) were single, 100 (39.9%) were married, and 16 (6.3%) were divorced. As displayed in Table 2 and Table 3, due to the non-normal distribution of the tested variables, Kruskal-Wallis H tests were used to compare the mean differences of age, and the numbers of FHC visits, maternities, live births, stillbirths, children, miscarriages, self-induced abortions, therapeutic abortions and congenital anomalies between the users of CI, combined OCs, condoms, intrauterine devices and tubal ligation, and the non-users of contraceptive methods. Dunn's pairwise tests as post hoc tests, and Bonferroni corrections were performed for the six-paired groups. Also, a chi-square analysis was run to investigate the relationship between marital status and the use and the non-use of contraceptive methods. However, 61 percent of the cells had an expected count less than 5, which violated one of the chi-square test assumptions. Therefore, the chi-square analysis was not reported.

The mean ranks of age scores were 362 for the non-use of contraceptive methods, 460 for the use of combined OCs, 568 for the use of condom, 586 for the use of intrauterine device, 611 for the use of CI and 728 for the use of tubal ligation. Bonferroni corrections showed that there were significant differences between the non-use of contraceptive methods and the uses of condom, intrauterine device, CI, and tubal ligation. Regarding mean ranks, age scores were lower among those who did not use any contraceptive methods compared to those using the condom ($p<0.001$), intrauterine device ($p<0.001$), CI ($p<0.001$) and tubal ligation ($p<0.001$). Furthermore, there were statistical differences between the use of tubal ligation and the uses of combined OCs and condom. Mean ranks evidenced that age scores were higher for the users of tubal ligation than the users of combined OCs ($p<0.001$) and condom ($p=0.006$).

Family health center visit scores' mean ranks were 509 for the use of condom, 594 for the use of intrauterine device, 631 for the non-use of contraceptive methods, 684 for the use of tubal ligation, 689 for the use of CI and 742 for the use of combined OCs. Bonferroni corrections and mean ranks demonstrated that FHC visit scores were significantly lower for the condom users than the users of tubal ligation ($p=0.002$), CI ($p<0.001$), combined OCs ($p<0.001$) and the non-users of contraceptive methods ($p<0.001$).

The mean ranks of maternity scores were 336 for the non-use of contraceptive methods, 500 for the use of combined OCs, 594 for the use of condom, 643 for the use of intrauterine device, 647 for the use of CI and 767 for the use of tubal ligation. According to the Bonferroni corrections and mean ranks, maternity scores of the non-users

Table 2: Kruskal-Wallis H test ranks

Grouping variable	Combined oral contraceptives	Intrauterine device	Condom	Tubal ligation	Coitus interrupts	No use
Age						
N	32	78	194	41	42	525
Mean rank	460	586	568	728	611	362
Family health center visits						
N	56	126	231	66	91	662
Mean rank	742	594	509	684	689	631
Number of maternities						
N	32	78	194	41	42	525
Mean rank	500	643	594	767	647	336
Number of live births						
N	32	78	194	41	42	525
Mean rank	507	650	608	793	664	326
Number of children						
N	32	78	194	41	42	525
Mean rank	510	650	605	732	661	327
Number of miscarriages						
N	32	78	194	41	42	525
Mean rank	476	481	490	508	467	435
Number of self-induced abortions						
N	32	78	194	41	42	525
Mean rank	455	484	454	526	469	447
Number of congenital anomalies						
N	32	78	194	41	42	525
Mean rank	467	377	386	458	357	502
Number of stillbirths						
N	32	78	194	41	42	525
Mean rank	452	463	456	452	462	456
Number of therapeutic abortions						
N	32	78	194	41	42	525
Mean rank	454	454	458	454	454	457

were significantly lower than for those using combined OCs (p=0.006), condom (p<0.001), intrauterine device (p<0.001), CI (p<0.001) and tubal ligation (p<0.001). Maternity scores of the users of tubal ligation were even higher for those who used the methods of combined OCs (p<0.001) and condom (p=0.001).

Mean ranks of live births were 326 for the non-use of contraceptive methods, 507 for combined OCs, 608 for condom, 650 for intrauterine device, 664 for CI and 793 for tubal ligation. According to the Bonferroni corrections and mean ranks, live births among the non-users of contraceptive methods were significantly lower

Table 3: Kruskal-Wallis H test statistics

Grouping variable	Chi-square	df	Asymp. sig.
Age	178.737	5	.000
Family health center visits	42.444	5	.000
Number of maternities	306.225	5	.000
Number of live births	371.782	5	.000
Number of children	365.738	5	.000
Number of miscarriages	22.005	5	.001
Number of self-induced abortions	23.531	5	.000
Number of congenital anomalies	69.071	5	.000
Number of stillbirths	3.112	5	.683
Number of therapeutic abortions	1.773	5	.880

than among users of combined OCs (p=0.001), condom (p<0.001), intrauterine device (p<0.001), CI (p<0.001) and tubal ligation (p<0.001). In addition, the users of tubal ligation had significantly more live births than the users of combined OCs (p<0.001), condom (p<0.001) and intrauterine device (p=0.043).

The mean ranks of children were 327 for the non-use of contraceptive methods, 510 for the use of combined OCs, 605 for the use of condom, 650 for the use of intrauterine device, 661 for the use of CI and 792 for the use of tubal ligation. Bonferroni corrections and mean ranks provided evidence that the numbers of children among the non-users of contraceptive methods were significantly lower than among users of combined OCs (p=0.001), condom (p<0.001), intrauterine device (p<0.001), CI (p<0.001) and tubal ligation (p<0.001). In addition, the users of tubal ligation had significantly more children than the users of combined OCs (p<0.001), condom (p<0.001) and intrauterine device (p=0.043).

Miscarriage mean ranks were 435 for the non-use of contraceptive methods, 467 for the use of CI, 476 for the use of combined OCs, 481 for the use of intrauterine device, 490 for the use of condom and 508 for the use of tubal ligation. Pairwise comparisons based on the Bonferroni corrections showed only one significant difference, that miscarriage scores among the users of condom were higher than the non-users of contraceptive methods (p=0.002).

Self-induced abortion mean ranks were 448 for the non-use of contraceptive method, 454 for the use of condom, 455 for the use of combined OCs, 469 for the use of CI and 526 for the use tubal ligation. According to Bonferroni corrections and mean ranks, self-induced abortion was higher among the users of tubal ligation than the condom users (p=0.004) and the non-users of contraceptive methods (p<0.001).

The mean ranks of congenital anomalies were 358 for the use of CI, 377 for the use of intrauterine device, 386 for the use of condom, 458 for the use of tubal ligation, 467 for the use of combined OCs, and 502 for the non-use of contraceptive methods. Bonferroni corrections and mean ranks evidenced that congenital anomalies were significantly higher among those who used no contraceptive methods than the users of CI (p<0.001), intrauterine device (p<0.001) and condom (p<0.001).

DISCUSSION

In Turkey, 26.5% of couples reported that they do use contraceptive methods, according to the data Turkish Population and Health Surveys (TNSA) 2013. According to the TNSA 2013 reports, the most preferred method is withdrawal/coitus interrupts (CI) (25.5%) and the second preferred method is IUDs (16.8%) and after that, condom (15.8%) (17). In research from India, Kovavisarach E et al. reported that 70% of the participants used "*coitus interruptus*" (CI), and is defined as a male contraceptive method where the penis is withdrawn before ejaculation and is not considered an effective form of contraception (18). In Bingöl, the most frequently used methods were CI (23.1%), IUD (21.5%), condom (19.8%) and OC (13.9%) (19). In 2007, Bozkurt et al. reported that 40.7% women used CI (while IUDs were the most common method, and condom, the third). In this study, younger women (between 17-30 years old) chose condom as a contraceptive method, while older ones (45 years and older) used IUDs. Nevertheless, CI is in the top three of the list (20). Kulczycki revealed this result as a "husband-wife agreement" but provided no evidence that this contraceptive method tends to be a more "*egalitarian mode of reproductive decision making*" (16). Another study in Diyarbakır reported that 42% of the women preferred to use a contraceptive method but were unable to, and 57% used CI (21). Thus, the ratios change over the age groups and according to location. In a study performed in Ankara, 65.2% of women use a birth control method. In our study, most of the women chose tubal ligation, OCs and /or IUDs (728, 480 and 586 respectively), in contrast to Tountas, who reported these methods as "limited" (22). This result should be analyzed as to whether this is a co-decision procedure structured by the couples, or whether women are following the choice of their sexual partners, because for 611 women, coitus interruptus was still used as a contraceptive method.

Ilhan et al. investigated the choices of women between 15-49 years old, and reported that IUDs were the most used method, while condom was the third (23). Our study

revealed the same results, with IUDs as the one of the top three methods. Oral contraceptives, condoms, and IUDs are available for free from FHCs. These results are associated with availability and sustainability, which are crucial for healthcare.

Those using the tubal ligation method were older than those using OCs. This could be because the older women had reached the planned number of children, unlike the younger ones (significantly, the maternity scores of the users of tubal ligation were even higher for those who used combined OCs methods ($p<0.001$) and condom ($p=0.001$).

In our study, age scores were significantly lower for those who did not use contraceptive methods. One of the main reasons for this result could be the desire to have a baby, or not being in a sexual relationship. Tountas et al. from Greece reported that adolescent participants must be considered more carefully (22). Both Tountas and our study revealed that the great majority of women followed their sexual partners' choices, particularly for condom or CI. In the study of Kokanalı et al. *coitus interruptus,* may have been the most chosen because it was the first choice of adolescents that have undergone voluntary termination. After the contraception methods education, they chose neither the rhythm method nor coitus interruptus (24). Sufficient and well-structured counseling about contraception is still the optimal option. Sexual education is needed not only for legally married women, but also for single women and for the most vulnerable, such as adolescents. Well-structured sexual education focused on sexual abuse, contraceptive methods and prevention of sexually transmitted diseases are needed in public health, and should consider the features of the community, and changes in families/sexual partners caused by migration, COVID-19, etc.

Self-induced abortion seems to be an ongoing problem affecting women and babies' health and lives. Further research is needed to find the root reasons. Family planning is also a socio-economic issue (13). Gumus et al. reported that pregnant women who describe a negative body image also described negative relations with their husbands. This finding was significant among low-income families, although in this group, 80.7% of women reported planning their pregnancy (25). Ending an unwanted pregnancy has also been subject to legislation. Not only the women's but also the "*legal*" husband's approval is needed (15). Care for the baby and the mother is an important responsibility of the state.

Some studies reported that the "educational status" could affect the "practiced contraceptive method" (4, 26, 27). For example, Doğru et al. has reported that for female university graduates, 69.2% use modern methods (28). Being employed could also affect the method. In 2013, The Turkish Demographic and Health Survey (TDHS) revealed that employed women preferred modern contraceptive methods over traditional (17). In various societies, the decision to choose the contraceptive method is made by the husband and/or mother-in-law, rather than the woman (4). This traditional situation has negative effects: if the woman needs to get the consent/approval of the decision-makers, the method is more likely to be traditional, or she could be forced into a method not suitable for her (29). In other words, pregnant women may not be able to choose their delivery method. "For decision making", the family physicians and nurses/midwives should be involved as well as the couples (30).

In our study, the results concerning miscarriage and condom usage are interesting: the only significant difference was that miscarriage scores were higher among the users of condom than the non-users of contraceptive methods ($p=0.002$). This raises a new research question; why miscarriages are more correlated with the usage of condoms than without any methods. Another research question is whether the couples are using this method properly. Self-induced abortion was significantly higher among the users of tubal ligation than the condom users ($p=0.004$), and the non-users of contraceptive methods ($p<0.001$). This shows that women and/or their physicians found an irreversible solution for this problem by choosing tubal ligation.

No couples used vasectomy as a contraceptive method, while tubal ligation was one of the most used. All over the world, vasectomy as a contraceptive method is less chosen than tubal ligation (31).

CONCLUSION

Every minute, 380 women worldwide become pregnant, and of these, half of them face unwanted or unplanned pregnancies, and suffer complications, have miscarriages, and dies. Differential failure risk due to ethnicity and socioeconomic factors could underlie this phenomenon. The Astana declaration recommends "health for all", and the need to reduce risks through family planning, improved sexual health counseling and education about contraception. Well-structured education programs are needed, not only for individuals and the general population, but also for healthcare workers.

The sociodemographic structure has changed due to the increased refugee population, migration, and globalization; therefore, sexual education of adolescents should be considered from this perspective.

All people have the right to access quality reproductive health services. Providing counseling on the contraceptive methods is among the duties of family physicians. To achieve this goal, primary care needs to take a patient-centered, people-oriented holistic approach with continuity of care.

One of the social determinants of health is the rate of the deaths of mother and newborn. Planned pregnancy with well-structured care is essential to achieve this goal. Appropriate contraceptive methods are also important points to women's health. As the family physicians are the gatekeepers, the discipline also includes "comprehensiveness, prevention, treatment and rehabilitation, and bio-psycho-social dimensions". Nevertheless, the care of family physicians in the follow-up of fertile women remains as the key-point.

Strengths

One of the strengths of the study was that the data were collected from primary care records. For this reason, the analysis of the data we obtained revealed the knowledge and attitudes of people who should receive services in the area of birth control methods. Based on these results, it provides foresight to provide the necessary information on this subject. In this regard, it has been stated which efforts should be made to reach the desired level of health.

Limitations

It is a limitation that the study is conducted only on patients' records who apply to a primary care facility. For this reason, the situation of those who do not apply to any health institution is unknown. For this, community-based household studies should be conducted. Although family physicians need to know all the characteristics of the registered population, today it is not possible to record these data in a healthy way since the number of registered patients per physician is high. Also, it is difficult for every physician to devote time to special topics and counseling.

It is important to find out to what effect women have access to methods that can be applied in terms of family planning. In addition, revealing women's thoughts on family planning through qualitative research can also make an important contribution. The lack of these qualitative data in our study is one of the limitations.

Ethics Committee Approval: This study was approved by the Ethical Committee of the Dokuz Eylul University (Date: 21.06.2018, No: 15-37).

Informed Consent: Written consent was obtained from the participants.

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AN EVALUATION OF CONTINUOUS, SELECTIVE ATTENTION, REASONING AND COLLISION TIME PREDICTION SKILLS IN SECURITY GUARDS

GÜVENLİK GÖREVLİLERİNDE SÜREKLİ, SEÇİCİ DİKKAT, MUHAKEME VE ÇARPIŞMA ZAMANI TAHMİN BECERİLERİNİN DEĞERLENDİRİLMESİ

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ABSTRACT

Objective: This study aims at investigating the success of the continuous and selective attention, reasoning and collision prediction time test batteries which were among the tests used in the psychotechnical evaluation of security guards in meeting standard norms.

Materials and Methods: One hundred twenty-three security guards (95 male, 28 female) working as security guards at the university and agreeing to participate in the study were included in the study. Each participant was administered the Continuous Attention Test, Selective Attention Test (SEDT), Reasoning Test (MT), Collision Time Test on the computer-aided system, respectively, and the scores obtained were recorded on the computer-aided system. Licensed evaluation system software developed by ALG Psikoteknik (https://www.algpsikoteknik.com) was used as the test system. Norm values were created from the data collected from 100% Turkish society. Norm values are values for drivers in the computer-aided system.

Results: From the test batteries applied, 99 participants (80.5%) in the continuous attention test, 117 participants (95.1%) in the selective attention test, 111 participants (90.2%) in the reasoning test and 73 participants (59.3%) in the collision time prediction test were successful. In three of the 4 test batteries applied, over 80% success was achieved and almost 60% success was provided in the collision time prediction test.

ÖZET

Amaç: Bu çalışmada güvenlik görevlilerinde, psikoteknik değerlendirmede kullanılan testlerden sürekli ve seçici dikkat, muhakeme ve çarpışma zaman tahmin test bataryalarının standart normları karşılamada olan başarılarının araştırılması amaçlanmıştır.

Gereç ve Yöntemler: Üniversitede güvenlik görevlisi olarak çalışan, çalışmaya katılmayı kabul eden 123 güvenlik görevlisi (95 erkek, 28 kadın) çalışma kapsamına dahil edildi. Her bir katılımcıya sırası ile bilgisayar destekli sistem üzerinde Sürekli Dikkat Testi (SÜDT), Seçici Dikkat Testi (SEDT), Muhakeme Testi (MT), Çarpışma Zamanı Testi uygulanarak alınan puanlar bilgisayar destekli sistem üzerinde kayıt edildi. Test sistemi olarak ALG psikoteknik (https://www.algpsikoteknik.com) tarafından geliştirilen lisanslı değerlendirme sistem yazılımı kullanıldı. Bilgisayar destekli sistemde norm değerleri, yüzde yüz Türk toplumundan toplanan verilerden oluşturulmuştur. Norm değerler sürücülere yönelik değerlerdir.

Bulgular: Uygulanan test bataryalarından; Sürekli dikkat testinde 99 katılımcı (%80,5), Seçici dikkat testinde 117 katılımcı (%95,1), Muhakeme testinde 111 katılımcı (%90,2), Çarpışma zamanı tahmin testinde ise 73 katılımcı (%59,3) başarılı olmuştur. Uygulanan 4 test bataryasının üç tanesinde; %80'in üzerinde başarı, çarpışma zamanı tahmin testinde ise %60'a yakın bir başarı elde edilmiştir.

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Conclusion: Creating norm values specific to security guards and applying psychotechnical evaluation test batteries are beneficial in evaluating psychological situations.

Keywords: Security guards, psychotechnical evaluation, test batteries

Sonuç: Güvenlik görevlilerine özgü norm değerlerinin oluşturulması ve psikoteknik değerlendirme test bataryalarının uygulanması psikolojik durumların değerlendirilmesinde fayda sağlamaktadır.

Anahtar Kelimeler: Güvenlik görevlileri, psikoteknik değerlendirme, test bataryaları

INTRODUCTION

Private security has been one of the sectors that has developed significantly since the concept of the public service began to change in Turkey. The sector consists of many sub-sectors that provide services to meet various types of security needs of the society (1). Today the need for security is increasing in parallel with the increase in the quality and quantity of the assets that individuals possess. If properly used, the private security guards will effectively meet individual security needs, provide savings on security services, reduce the burden of overall security and lay the groundwork for more convenient and efficient work in their areas. The fragmentary and variable structure of modern society which is associated with the perception of danger and threat increases the need for security and makes police and other private security services more important (2).

However, security guards are exposed to violent attacks by citizens during their duties (3). Moreover, employees in these professions cannot withdraw from their working environment, even if they are under serious threat because they are called upon for help, and they also have a great responsibility to maintain law and order in society. Therefore, the problem-solving skills and personality traits of security guards become important when it comes to intervening in an incident (4, 5). The term "*Problem*" is defined as the obstacles that an individual faces at any stage to achieve the intended goal. The problem, which is expressed as an obstacle faced by the individual, has certain characteristics which cause uncertainty in the human mind, which must be solved and which bother that individual (6). The effective power available to overcome a wide range of issues from everyday life to traumatic incidents is defined as problem solving. The problem solving stage begins with cognitive processes aimed at a goal. Problem-solving skills mediate individuals' ability to adapt effectively to the environment in which they live. While some problems have correct answers or clear solutions, others do not. In this case, interdisciplinary knowledge and multifaceted thinking are important in solving problems (7). Different approaches to problem solving have been defined here according to the characteristics of the problem and the person interested in the problem.

The traditional approach involves identifying the problem, analysing its causes, identifying alternative solutions, evaluating and applying solutions, and determining whether the problem has been solved (8). In addition to the solution method, the continuous and selective attention, reasoning and reaction time of the security guard against the incidents also occupy an important place. Timely intervention in events will prevent the growth of negative consequences. If organizations make wrong choices in recruitment, there will be undesirable consequences including waste of resources for the organization and unhappiness at work for the staff. Choosing the right staff for the right job will make a positive contribution to the organization in terms of ensuring job satisfaction, using creativity and being successful (9). This study aimed to investigate the achievements of security guards in meeting standard norms by using continuous and selective attention, reasoning and collision time estimation tests batteries which are among the psychotechnical evaluation tests.

MATERIALS AND METHODS

One hundred twenty-three security guards (95 male, 28 female) working as security guards at the university and agreeing to participate in the study were included in the study. The success rate of the test of the participants was accepted as 0.60 and the minimum sample size (Type I error 0.05, Type II error 0.20, 0.80 power) was calculated as 110 people with a 20% error margin. Tests, which were applied to the participants by means of a computer-aided system installed in a quiet room, which was closed to external stimuli as shown by the university security supervisor, were explained in detail by Public Health Medical Specialty assistants and implemented after preliminary tests were carried out. After practitioners were trained by a specialist organization and entitled to receive a certificate of participation, the implementation phase was conducted. After answering 13 questions with regard to demographic features, each participant was administered the Continuous Attention Test, Selective Attention Test (SEDT), Reasoning Test (MT), Collision Time Test on the computer-aided system, respectively, and the scores obtained were recorded on the computer-aided system. Licensed evaluation system software developed by ALG Psikoteknik (https://www.algpsikoteknik.com) was used as the test system. Norm values were created from the data collected from 100% Turkish society. There are 12 test batteries in the system that measure mental and psychomotor skills. Four of the 12 test batteries were

implemented with computer support that did not require a driver system. Ethical approval for the study was obtained from İstanbul University; İstanbul Faculty of Medicine; Clinical Research Ethics Committee (Date 19.08.2019, No: 1026). In statistical analysis, student's t-test and chi-square test were applied in independent groups. Statistical significance was accepted as $p<0.05$ and two-way.

TESTS CONDUCTED

CONTINUOUS ATTENTION TEST

Definition of the test

For this test, a specific traffic sign was selected as the target figure, and distractors were created that are quite similar to this sign. The target figure and the distractors were arranged in a matrix of 11x17. There are 50 target figures in this matrix consisting of a total of 186 distractors. The task of the participants is to quickly find and mark the same target shape at the top of the Matrix among the shapes in the Matrix. Attention in general means that among the many sets of internal and external stimulants, the critical one is selected for further analysis. In the working environment, they have to pay attention to many stimulants coming from different modalities such as visual or audio, to perceive them and to continue their safe behavior (10).

Purpose of the test

This test was developed to measure the ability of drivers to quickly and accurately identify and select a specific stimulant that is critical at the moment in the routine and monotonous flow of information so that they can demonstrate the necessary cognitive and motor skills in a traffic environment. It was also implemented to test the ability of security guards to quickly and accurately select a specific stimulant.

Overall Test Score Index: It is calculated by the formula [(Total Number of Correct Answers)/((Total Number of Skipped Responses) + (Total Number of Correct Answers) + (Total Number of Incorrect Answers) x100].

This index is a general score that shows the participant's level of continuous attention. The raw scores obtained by the participants from the test are compared with the norm values and evaluated to determine whether they received above the norm score.

SELECTIVE ATTENTION TEST (SEDT)

Definition of the test

Each question in this test consists of 6 traffic signs. Three of these signs are located on the upper row of a 3x3 Matrix, and 3 of them are located on the left side. Two of the traffic signs on the top and left are identical. The Test contains a total of 60 matrices. The participant must quickly find identical shapes, each located on the left and top of the Matrix, and show the intersection of these shapes by marking them on the screen with the index finger or a pen.

Purpose of the test

The traffic environment is a task that involves the selective attention of the driver in many cases so that he/she can demonstrate safe driving skills. For this reason, this test is aimed at evaluating the driver's ability to selectively pay attention to constantly changing environmental and road conditions so that they can demonstrate the necessary cognitive and motor skills in a traffic environment. In order to measure the selective attention of the driver in the test, an attempt is made to measure the ability to select similar ones from different stimulants and ignore others.

Correct Answer Percentage (Overall Index): [(Total Number of Correct Answers/Total Number of Questions) x100]

This index is a general score indicating the participant's selective attention level. The raw scores obtained by the participants from the test are compared with the norm values and evaluated to determine whether they received above the norm score.

REASONING TEST (MT)

Definition of the test

This test includes 50 multiple choice question items. The questions are sorted from easy to difficult. In each question, the participant is first asked to find the logical relationship between the two figures. Then, the participant must find and mark in which of the options another form of this relationship is presented. Each question has 4 options, and only one of them is the correct answer. The person has an answer time of 3 minutes for each question. The test is automatically terminated when the participant incorrectly answers or skips 2 questions in a row.

a) Colour: The change in the relationship between the two figures is based on color. In other words, the color of the first figure changes, resulting in the second figure.

b) Form: The change in the relationship between the two figures is based on the form. In other words, the form of the first figure changes, resulting in the second figure.

c) Quantity: The change in the relationship between the two figures is based on numbers. The second figure differs from the first figure in number or quantity.

d) Rotation: The change in the relationship between the two figures is based on rotation. The first figure or a part of the figure is rotated in various degrees, resulting in the second figure. When a participant gives an incorrect answer or skips a question twice in a row, the test automatically ends without moving on to other questions.

Purpose of the test
The main purpose of this test is to measure one's general reasoning ability using nonverbal geometric shapes, regardless of culture. In the test, the participant's task is to find and determine the relationship between the two figures and then find the correct answer by determining the figure containing the same rule among the figures presented in four options. The main purpose of the test is to measure the reasoning ability through matching, regardless of culture and knowledge of mathematics or arithmetic.

Correct Answer Percentage (Overall Index): [(Total Number of Correct Answers/Total Number of Questions) x100]

This index is a general score indicating the participant's selective attention level. The raw scores obtained by the participant from the test are compared with the norm values and evaluated to determine whether they received above the norm score (10).

COLLISION TIME TEST

Purpose of the test
The participant is asked to estimate both the collision time and the collision location of the objects.

Test features
The moment (duration) at which objects actually collide with each other is considered the center, and the interval from the moment the balls disappear on the screen until the moment when a 77-pixel time was added to the moment when they collide is determined as the response area. Pursuant to the end of this area, the interval from the point of collision (different for each item) until the moment after 1786 ms. is considered to be the late response area. If no response is given, it is passed to the next question when the balls arrive at the end of the late response area, and this question is considered to be skipped. The moment of collision, which is the center of the response area, is rated as "100" and the score decreases at a certain rate as you go from the center to the ends.

The extreme points of the response area and the late response area are "0" points. When calculating the location score, the sum of the distances of the point that the driver responds to for each question, in pixels, to the actual collision point, is divided by the number of questions that the driver responds to. Giving the response after 1056 pixels, which is the screen limit, causes the response to be considered skipped. After the driver specifies each prediction by tapping the screen, the time and location specified by the driver is automatically calculated. According to where this time and place coincide in the designated response area, the driver is given two separate scores for both time and location. Security guards are asked to predict both the collision time and the collision location of objects oscillating in two directions on the computer screen.

Evaluation of the results
The following measurements are taken regarding the results of the test. The Collision Time Prediction Score (Overall Index) is the collision location distance score (Overall Index), the number of late responses and the number of skipped responses. The Collision Time Prediction Score (Overall Index) is a general score that indicates the time of collision. By comparing the raw scores of the participant from the test with the norm values, it is evaluated whether they get above the norm score (10).

FINDINGS

A total of 123 security guards were included in the study, 95 men (77.2%) and 28 women (22.8%) who worked as security guards at the university and agreed to participate in the study. Distribution of age, working year and total working year by gender is given in Table 1. The marital status, working style and educational status of the individuals covered by the study are given in Table 2. According to the distribution of marital status, 34 were single (27.6%), 89 were married (72.4%), 107 (87%) were working in shifts, and the majority 105 (85.4%) were high school graduates.

Table 1: Distribution of age, year of work and total years of work of individuals covered within the scope of the study by gender

Gender		Age	Working year	Total working year
Male	Mean	35.24	9.28	13.76
	S. Deviation	4.71	3.57	5.70
	Median	35	10	14
	Minimum	24	2	3
	Maximum	49	16	33
Female	Mean	36.04	10.87	13.16
	S. Deviation	5.35	3.02	4.73
	Median	34.5	10	13.25
	Minimum	28	3.5	3.5
	Maximum	48	16	26
Total	Mean	35.42	9.648	13.63
	S. Deviation	4.8	3.51	5.48
	Median	35	10	14
	Minimum	24	2	3
	Maximum	49	16	33

Table 2: Marital status, working style and educational status of individuals covered by the study

Marital status	**Number**	**Percentage**
Single	34	27.6
Married	89	72.4
Total	123	100
Working style	**Number**	**Percentage**
Day	16	13
Shift	107	87
Total	123	100
Education status	**Number**	**Percentage**
Secondary school	6	4.9
High school	105	85.4
University	12	9.8
Total	123	100

The success status of the individuals covered by the study in psychotechnical test batteries is given in Table 3. Among the test batteries applied, 99 participants (80.5%) were successful in the Continuous Attention Test, 117 participants (95.1%) in the Selective Attention Test, 111 participants (90.2%) in Reasoning Test, and 73 participants (59.3%) in the Collision Time Prediction Test. In three of the four test batteries applied, more than 80% success was achieved, and almost 60% success was achieved in the Collision Time Prediction Test. Distribution of test results by gender is given in Table 4. The distribution of test results by gender was not found to be statistically significant. Distribution of test results by years of working is given in Table 5. The distribution of test results by years of working was not found to be statistically significant. Distribution of test results by education level is given in Table 6.

Table 3: Distribution of success status in psychotechnical test batteries of individuals covered by the study

Continuous attention	**Number**	**Percentage**
Adequate	***99***	***80.5***
Inadequate	24	19.5
Total	123	100
Selective attention	**Number**	**Percentage**
Adequate	***117***	***95.1***
Inadequate	6	4.9
Total	123	100
Reasoning	**Number**	**Percentage**
Adequate	***111***	***90.2***
Inadequate	12	9.8
Total	123	100
Collision time prediction	**Number**	**Percentage**
Adequate	***73***	***59.3***
Inadequate	50	40.7
Total	123	100

When the distributions of the applied tests according to the educational status of the participants were examined, only a statistically significant difference was found in the Reasoning Test (p=0.002). Distributions were not found to be statistically significant in continuous, selective attention and collision time testing.

DISCUSSION

The Psychotechnical method is defined as a behavioural measurement method that has certain characteristics and is created with the help of tests collected within a special system (11). Individuals' compliance with the profession includes factors such as measuring their potential abilities and qualifications required for work, as well as the perception, reasoning, creativity and psychomotor abilities of employees, as well as the measurement of their physical and mental strength (12). Even though the psychotechnical assessments are usually at the forefront for drivers, they have begun to be performed as competency assessments for train drivers, forklift drivers, crane operators, security guards, production staff, pilots and many business lines including people involved in other public transport and critical tasks (13).

In our study, the Continuous Attention Test, Selective Attention Test (SEDT), Reasoning Test (MT), Collision Time Test were applied to the security guards of the university on the computer-aided system, and the scores obtained were recorded on the computer-aided system. Among the test batteries applied, 99 participants (80.5%) were successful in the Continuous Attention Test, 117 participants (95.1%) in the Selective Attention Test, 111 participants (90.2%) in Reasoning Test, and 73 participants (59.3%) in the Collision Time Prediction Test. In three of the four test batteries applied, more than 80% success was achieved, and almost 60% success was achieved in the Collision Time Prediction Test. When success scores were evaluated by gender and duration of working, no statistically significant differences were found in terms of gender and duration of work.

When the distributions of the applied tests according to the educational status of the participants were examined, only a statistically significant difference was found in the reasoning test. The number of participants achieving adequate results in high school and college educated people is significantly higher than secondary

Table 4: Distribution of test results by gender

		Gender		Total	X^2	p
	Continuous attention	**Male**	**Female**		**0.695**	**0.404**
Adequate	Number	78	21	99		
	Percent	82.1	75.0	80.5		
Inadequate	Number	17	7	24		
	Percent	17.9	25.0	19.5		
Total	Number	95	28	123		
		Gender		**Total**		
	Selective attention	**Male**	**Female**		**0.401**	**0.527**
Adequate	Number	91	26	117		
	Percent	95.8	92.9	95.1		
Inadequate	Number	4	2	6		
	Percent	4.2	7.1	4.9		
Total	Number	95	28	123		
		Gender		**Total**		
	Reasoning	**Male**	**Female**		**0.038**	**0.846**
Adequate	Number	86	25	111		
	Percent	90.5	89.3	90.2		
Inadequate	Number	9	3	12		
	Percent	9.5	10.7	9.8		
Total	Number	95	28	123		
		Gender		**Total**		
	Collision time	**Male**	**Female**		**1.314**	**0.252**
Adequate	Number	59	14	73		
	Percent	62.1	50.0	59.3		
Inadequate	Number	36	14	50		
	Percent	37.9	50.0	40.7		
Total	Number	95	28	123		

Table 5: Distribution of test results by working year

Continuous attention	**Working years**						
	Mean	**S. Deviation**	**Median**	**Minimum**	**Maximum**	**t**	**p**
Inadequate	10.54	3.36	11	3	16	1.398	0.165
Adequate	9.43	3.53	10	2	16		
Selective attention	**Mean**	**S. Deviation**	**Median**	**Minimum**	**Maximum**		
Inadequate	11.83	2.86	12	8	15	1.574	0.118
Adequate	9.53	3.51	10	2	16		
Reasoning	**Mean**	**S. Deviation**	**Median**	**Minimum**	**Maximum**		
Inadequate	10.17	2.29	9.5	7	14	0.538	0.592
Adequate	9.59	3.62	10	2	16		
Collision time test	**Mean**	**S. Deviation**	**Median**	**Minimum**	**Maximum**		
Inadequate	9.81	3.19	10	3	14	0.407	0.684
Adequate	9.54	3.73	10	2	16		

Table 6: Distribution of test results by education Level

		Education level					
Continuous attention		**Sec. School**	**High School**	**University**	**Total**	**X^2**	**p**
Adequate	Number	5	87	7	99	4.15	0.125
	Percent.	83.3	82.8	58.3	80.5		
Selective attention							
Adequate	Number	6	99	12	117	1.08	0.582
	Percent.	100.0	94.3	100.0	95.1		
Reasoning test							
Adequate	Number	3	96	12	111	12.5	0.002
	Percent.	50.0	91.4	100.0	90.2		
Collision time test							
Adequate	Number	3	65	5	73	2.05	0.358
	Percent.	50.0	61.9	41.7	59.3		

school graduates. Since there were no previous studies on this subject, it was not possible to compare it with similar study results. The importance of personnel selection systems, which ensure that the right personnel are reached and the appropriate persons are brought into the institution, is better understood every day. The basis of personnel selection systems that will allow the selection of the right personnel is to recognize and define the work correctly (14). Difficulties and hardship in social life are especially defined as problems. Problems usually consist of uncertainties, situations where accuracy and authenticity cannot be achieved and troubles and relationships with difficulties (15). In their study on private security guards, which is a dangerous and risky profession, Balli et al. suggested that the "extrovert" personality trait explains the perception of private security guards (in management positions) as leaders (16). In professions where interpersonal relationships are intense, placing a person suitable for work will keep possible problems at a minimal level.

Limitations of the research

The fact that the study was carried out only with security guards in a university and the compulsory use of some of the psychotechnical evaluations can be considered as limitations.

The results of this study showed that security guards were sufficiently successful in the attention, reasoning, and collision time prediction tests which were set as standard. In addition to assessing their psychological status when necessary, we believe that it would be useful to conduct a psychotechnical assessment prior to hiring those who work in critical tasks with intensive public interaction and communication (17).

Ethics Committee Approval: This study was approved by the Clinical Research Ethical Committee of the Istanbul University, Istanbul Faculty of Medicine (Date: 19.08.2019 No: 1026).

Informed Consent: Written consent was obtained from the participants.

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Author Contributions: Conception/Design of Study- H.İ., E.E., G.Ö.; Data Acquisition- H.İ., E.E., N.Ş, Z.B.S., F.C.; Data Analysis/Interpretation- E.H.İ., G.Ö.; Drafting Manuscript- H.İ., G.Ö., F.C.; Critical Revision of Manuscript- H.İ., E.E., N.Ş., Z.B.S., G.Ö., F.C.; Final Approval and Accountability- H.İ., E.E., N.Ş., Z.B.S., G.Ö., F.C.

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İSTANBUL ÜNİVERSİTESİ YAYINEVİ

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SARS-COV-2 INFECTION AND THYROID DISEASES

SARS-COV-2 ENFEKSİYONU VE TİROİD HASTALIKLARI

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ABSTRACT

The severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) virus, was identified as the cause of a pandemic of respiratory illness in Wuhan, China one year ago. The Coronavirus disease 2019 (COVID-19), may cause mild disease with nonspecific signs and symptoms such as fever, cough, myalgia, and fatigue, or severe pneumonia with respiratory failure and sepsis. However, endocrinological manifestations are yet to be established, in patients with COVID-19. The effect of COVID-19 on thyroid function is unknown at this time. Evidence support that patients with COVID-19 who are followed up in intensive care units may develop thyroid dysfunction as a non-thyroidal illness syndrome. Until now, twenty-two cases with subacute thyroiditis and five cases with Graves' Diseases potentially associated with SARS-CoV-2 infection have been reported in literature. Physicians should be aware of possible relationships between thyroid dysfunction and COVID-19. This study aimed to review thyroid dysfunction in patients with COVID-19, and to overview thyroid diseases that are probably related to COVID-19.

Keywords: COVID-19, SARS-CoV-2, thyroid, non-thyroidal illness syndrome, subacute thyroiditis, Graves' disease

ÖZET

Şiddetli akut solunum sendromu-koronavirüs-2 (SARS-CoV-2) virüsü, bir yıl önce Çin'in Wuhan kentinde bir solunum yolu hastalığı salgınının nedeni olarak tanımlandı. Coronavirus hastalığı 2019 (COVID-19), ateş, öksürük, miyalji ve yorgunluk gibi spesifik olmayan bulgu ve semptomlarla hafif hastalığa veya solunum yetmezliği ve sepsisle birlikte şiddetli pnömoniye neden olabilir. Bununla birlikte, COVID-19 hastalarında endokrinolojik belirtiler henüz tanımlanmamıştır. Şu anda, COVID-19'un tiroid fonksiyonu üzerinde bir etkisinin olup olmadığı belirsizdir. Kanıtlar yoğun bakım ünitelerinde takip edilen COVID-19 hastalarının hasta ötiroid sendrom olarak tiroid disfonksiyonu geliştirebileceklerini desteklemektedir. Şimdiye kadar, literatürde potansiyel olarak SARS-CoV-2 enfeksiyonu ile ilişkili yirmi iki subakut tiroidit vakası ve beş Graves' hastalığı vakası bildirilmiştir. Hekimler, tiroid disfonksiyonu ile COVID-19 arasındaki olası ilişkilerin farkında olmalıdır. Bu derlemede, COVID-19 hastalarında tiroid disfonksiyonu ve muhtemelen COVID-19 ile ilişkili tiroid hastalıklarının gözden geçirilmesi amaçlanmıştır.

Anahtar Kelimeler: COVID-19, SARS-CoV-2, tiroid, non-tiroidal hastalık sendromu, subakut tiroidit, Graves' hastalığı

INTRODUCTION

Coronavirus disease 2019 (COVID-19), which is caused by the severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) virus, has already become a pandemic just a few months after it was first reported in China (1). The virus penetrates the body via upper respiratory mucous membranes and then spreads to the lungs. The majority of COVID-19 patients suffer from a mild to moderate illness (fever, cough, myalgia, fatigue) or viral pneumonia after an incubation period of 2–14 days (median five days). However, some patients experience serious disease characterized by respiratory failure, acute respiratory distress (ARDS), sepsis, myocarditis, and acute kidney injury (2). However, endocrine disorders have yet to be explicitly identified in patients with COVID-19. Researchers have looked into the possibility of thyroid dysfunction among the various extra-pulmonary manifestations. In this review, thyroid dysfunction in patients with COVID-19 and an of overview thyroid diseases probably related to COVID-19 were explored.

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SARS-CoV-2 and thyroid gland

The receptor-binding domain of SARS-CoV-2 uses host angiotensin-converting enzyme 2 (ACE2) for a fusion of viral and host cell membranes. The thyroid, pancreas, intestine, testis, ovary, adrenal glands, and pituitary are among the endocrine organs that express ACE2 (2, 3). Recently, intestine, testis, kidneys, heart, thyroid, and adipose tissue were shown to have the greatest levels of ACE2 expression. Only in males, ACE2 expression levels demonstrated strong positive associations with CD8+ T cell enrichment and interferon reaction markers in the thyroid gland (4).

In 2002, it was observed that the severe acute respiratory syndrome (SARS) epidemic caused some abnormalities in the thyroid function. Although SARS-CoV was isolated in endocrine organs including parathyroid, pituitary, pancreas, adrenal gland, it could not be detected in the thyroid, testis, ovary and uterus (5). Wei L et al. showed that thyroid glands in autopsies of five SARS cases were extensively affected by the disease with substantial injury to the follicular epithelial cells and the para-follicular cells (6). It was characterized by destruction of the follicular epithelium and desquamation of the epithelial cells into the follicular lumen.

They revealed presence of apoptosis by the TUNEL assay but no inflammatory infiltrate or features of cellular necrosis (6). However, Yao et al. evaluated pathological alterations in individuals who died of COVID-19 using minimally invasive autopsies from several organs. The thyroid follicular morphology was normal, although lymphocytic infiltration in the interstitium was seen (7). SARS-CoV-2 was not discovered in the thyroid gland by tissue immunohistochemistry or PCR (8, 9). By using direct molecular analysis of surgical samples of thyroid tissue, Rotondi et al. demonstrated that the ACE-2 receptor mRNA is expressed in thyroid follicular cells, making them a possible target for SARS-CoV-2 invasion (10).

COVID-19 and thyroid dysfunction including non-thyroidal illness syndrome

Evidence supports that patients with COVID-19 who are followed up in an intensive care unit may develop thyroid dysfunction as a non-thyroidal illness syndrome (NTIS). Despite the fact that the pathways causing the NTIS are complex, circulating cytokines are thought to be the primary mediators because of their various effects on the hypothalamic-pituitary-thyroid (HPT) axis, circulating thyroid hormone-binding proteins, and thyroid hormone peripheral metabolism (11). Recently, Lania et al. evaluated thyroid function tests and serum interleukin-6 (IL-6) values in 287 patients hospitalized for COVID-19 in non-intensive care units. In the regression analysis, thyrotoxicosis was detected in 58 patients (20.2%) (overt in 31 cases), and thyrotoxicosis was found to be significantly associated with higher IL-6. They concluded that COVID-19 may be attributed to a greater risk of thyrotoxicosis in a relationship with systemic immune activation caused by the SARS-CoV-2 infection (12). Chen et al. reported low tyroid sitimulating hormon (TSH) and total triiodothyronine (T3) levels during the course of their COVID-19 infection due to NTIS or the direct pituitary effect of the virus (13).

Müller et al. compared patients admitted to a high intensity of care unit (HICU) in 2020 because of COVID-19 (HICU-20 group), with those admitted to HICU (non-COVID-19) in 2019 (HICU-19 group) and patients with COVID-19 who were admitted to the low intensity of care units (LICU-20 group) (14). Thirteen (15%) of 85 patients in the HICU-20 group had thyrotoxicosis (defined as TSH 0.28 mIU/L and/or free thyroxine (FT4)>21.9 pmol/L), compared to one (1%) of 78 patients in the HICU-19 group (p=0.002) and one (2%) of 41 patients in the LICU-20 group (p=0.025). The number of males was higher in the HICU-20 group (nine [64%] men and five [36%] women; p=0.017), and they had higher C-reactive protein (CRP) levels and free thyroxine concentrations. After 55 days of follow-up, euthyroid status was maintained in 75% of patients (6/8) with 25% (2/8) of them developing hypothyroidism. They concluded their finding as a combination of thyrotoxicosis (possibly due to subacute thyroiditis) and NTIS (14). However, Kohoo et al. followed up 456 patients from 3 different London Hospitals with a clinical suspicion of COVID-19 (15). The majority of patients (86.6%) presenting with COVID-19 were euthyroid, with none presenting with overt thyrotoxicosis after excluding the potential interference of cortisol on TSH. Of the participants in 5.1% had subclinical hypothyroidism, 0.6% had overt hypothyroidism. Secondary hypothyroidism was suspected in eight patients (2.4%). TSH and FT4 levels were mildly reduced, which was consistent with an NTIS. Lui et al. showed that 13.1% had thyroid dysfunction among 191 patients with mild to moderate COVID-19. Ten patients had isolated low FT3, with normal TSH and FT4 levels, indicating a possible NTIS. Ten patients had isolated low TSH, indicating subclinical thyrotoxicosis related to thyroiditis, albeit autoimmunity was likely a factor in two of them. Another patient's subclinical hypothyroidism was almost certainly caused by autoimmune thyroiditis (16). Recently, abnormal thyroid function tests were observed in 62 patients (16.9%) in another study from this group. None of the patients showed overt thyrotoxicosis or hypothyroidism. NTIS was found in twenty-seven patients (7.4%). Five of the patients had pre-existing autoimmune thyroid conditions (17). Gao et al. evaluated thyroid function tests in patients with mild COVID-19, survivors, and non-survivors from COVID-19 (18). TSH and FT3 levels, but not FT4 levels, were significantly lower in patients with severe COVID-19 than those in patients with mild COVID-19.

Patients with severe COVID-19 had lower FT3 levels, which projected all-cause mortality. Also, Schwarz et al. reported that patients with a low FT3 (in the lowest tertile of FT3 values) had a significantly higher disease severity and increased mortality (40% mortality rate) compared with patients with a higher FT3 (5% mortality rate in the higher tertiles) (19). Campi et al. found that suppressed TSH levels were observed in 39% of patients at admission or during hospitalization and were related with low FT3 in half of the cases. They hypothesized that COVID-19 causes a combined effect in the hypothalamic-pituitary level and peripheral organs due to cytokine release (20).

In conclusion, numerous studies in COVID-19 patients reveal that the NTIS is the most often observed alteration in thyroid diseases.

Subacute thyroiditis

SAT is an inflammatory disease of the thyroid associated with painful thyroid enlargement. Anterior neck pain radiating to the jaw and ear, malaise, fatigue, myalgia, and arthralgia are typical symptoms of SAT. A mild to moderate fever is often seen, sometimes occasionally exceeding 40°C, rising especially at night. SAT clinic may reach its peak within 3 to 4 days and disappears within a week, but usually, the onset extends over 1 to 2 weeks and persists for 3 to 6 weeks (21). Clinic presentation of SAT and COVID-19 may be similar to each other in many aspects. SAT incidence is four times higher in women than in men and SAT is more frequent between ages 40-50 years (22). Several studies showed that susceptibility to the disease and recurrence risk are associated with human leukocyte antigens (HLA) mainly HLA-Bw35, but also HLAB67, HLA-B15/62, and HLA-Drw8 (23, 24). Previous viral infection (around 2–6 weeks earlier) caused by viruses including Coxsackie virus, Epstein-Barr virus, adenoviruses, influenza viruses, mumps, measles, primary human immunodeficiency virus infection is thought to be a trigger factor (25). SAT is defined by elevated erythrocyte sedimentation rate (ESR) and CRP level, typical ultrasound findings including inhomogeneous hypo-echogenic texture with diminished vascularity and laboratory markers of thyrotoxicosis. Symptomatic treatment includes nonsteroidal anti-inflammatory drugs (NSAIDs), glucocorticoids (21).

First, Brancatella et al. presented an 18-year-old woman with SAT diagnosis which occurred after 2 weeks of SARS-CoV-2 infection (26). Prednisone (25 mg/day as the starting dose) was given to the patient, thyroid function and inflammatory markers of the patient normalized in 40 days. Ippolito et al. reported a 69-year-old woman with COVID-19 during the recovery phase following back surgery. Previously, she had a non-toxic nodular goiter and she diagnosed with thyrotoxicosis during COVID-19. They considered SAT because the patient responded to steroids, not methimazole (27). Asfuroglu et al. reported a 41-year-old woman with SAT and they warned physicians should be aware of screening SAT patients for COVID-19 (28). Ruggeri et al. described a 43-year-old woman who developed SAT with thyrotoxicosis six weeks after SARS-COV-2 infection. Oral prednisone (25 mg/day as the starting dose) was given to the patient and progressive remission of symptoms and signs and euthyroid status was provided after four weeks (29). Brancatella et al. described an additional four patients with SAT after COVID-19 (30). Until now, 22 cases of SAT potentially associated with SARS-CoV-2 infection have been reported in literature (31-39). In a recent review, SAT was found more frequent in women than in men (18 women/4 men), patients with a mean age of 39±11 years during or after (21±11 days) an episode of COVID-19. These patients have mild symptoms and signs including fever, myalgia, asthenia, palpitations, weight loss, and anterior neck pain or asymptomatic. Most patients were treated with β-blockers, aspirin, glucocorticoids (prednisone 25–40 mg) gradually discontinued over an average of 3 or 4 weeks. Despite a short follow-up (35±12 days), euthyroid status was achieved after a short duration of subclinical hypothyroidism in most patients (40).

Graves' disease

Graves' disease (GD) is an autoimmune disorder caused by stimulating thyroid autoantibodies that results in thyroxine overproduction leading to hyperthyroidism. The etiology of GD is not clear. It has been suggested that different environmental conditions (i.e. infections, smoking, stress, radiation, medications, iodine, etc.) can trigger GD especially in genetically vulnerable individuals

The significance of stress in the development of hyperthyroidism in GD patients is still debated. In cross-sectional studies, stressful life events (SLE) have been shown to be more common in the months before the development of GD (47). Vita et al. evaluated the relationship of SLE with the onset and outcome of GD. Patients with SLE experienced at least one exacerbation or relapse prior to each exacerbation or relapse. The patients who experienced more exacerbation or relapse lived more SLE than the patients with remission (48). Previously, we showed that the number and impact of negative SLE in GD patients were higher when compared to healthy controls according to Life Experience Survey (49). Recently we have recommended methimazole and beta-blocker combination for initial therapy and considered dietary changes and RAI treatment unadvisable during the COVID-19 pandemic (50).

CONCLUSION

In patients who were severely affected during the course of COVID-19, changes in thyroid function may relate to NTIS but there may be a relation with a specific thyroid

disease after COVID-19. Thyroid dysfunction could be observed during and after COVID-19 and, therefore, it is expected that some new-onset or recurrent thyroid dysfunctions could be attributed to a recent SARS-CoV-2 infection. Physicians should be aware of possible relationships between thyroid dysfunction and COVID-19, which should be researched by prospective studies.

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İSTANBUL ÜNİVERSİTESİ YAYINEVİ

CASE REPORT - OLGU SUNUMU
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A CASE WITH SYSTEMIC LUPUS ERITHEMATOSUS MIMICKING COVID-19 PNEUMONIA*

COVID-19 PNÖMONİSİNİ TAKLİT EDEN SİSTEMİK LUPUS ERİTHEMATOSUS VAKASI

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ABSTRACT

Coronavirus-19 disease (COVID-19) is a worldwide health emergency which has a high mortality ratio. Diagnosis requires a positive quantitative real-time polymerase chain reaction (qRT-PCR) test however there are radiological findings strongly suggest the diagnosis of COVID-19. Here we reported a 63-year-old woman presented with cough and dyspnea and medical history of lung cancer and systemic lupus erythematosus (SLE). Chest computed tomography demonstrated widespread ground glass opacities in both lung fields that have been reported to be compatible with COVID-19 pneumonia. qRT-PCR test was negative for twice and radiological regression after hydroxychloroquine, azithromycin and piperacillin-tazobactam was not significant. Considering lung involvement of SLE methylprednisolone was initiated, symptoms and radiological findings improved. The underlying diseases may mimic the COVID-19 infection or the signs and symptoms of the disease may be seen together with COVID-19.

Keywords: COVID-19, pneumonia, systemic lupus erythematosus, differential diagnosis

ÖZET

Koronavirus-19 hastalığı (COVID-19), dünya genelinde yüksek mortalite oranına sahip sağlık acil durumudur. Radyolojik bulguların COVID-19 ile uyumlu olmasının yanında, kantitatif gerçek zamanlı polimeraz zincir reaksiyonu (qRT-PCR) testinin pozitifliği tanı için gereklidir. Bu çalışmada, 63 yaşında akciğer kanseri ve sistemik lupus eritematozus (SLE) öyküsü olan, öksürük ve nefes darlığı ile başvuran bir kadın hasta sunuldu. Toraks tomografisinde; COVID-19 pnömonisi ile uyumlu olan her iki akciğer alanlarında da yaygın buzlu cam dansiteleri saptandı. İki kez bakılan qRT-PCR testi negatif olarak raporlandı. Hidroksiklorokin, azitromisin ve piperasilin-tazobaktam tedavisi sonrası anlamlı bir radyolojik düzelme görülemedi. SLE'nin akciğer tutulumu olabileceği düşünülerek metilprednizolon başlanan hastanın semptomlarında ve radyolojik bulgularında iyileşme izlendi. Altta yatan hastalıklar COVID-19 enfeksiyonunu taklit edebilir veya hastalığın belirti ve bulguları COVID-19 ile birlikte görülebilir.

Anahtar Kelimeler: COVID-19, pnömoni, sistemik lupus eritematozus, ayırıcı tanı

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INTRODUCTION

Coronavirus-19 disease (COVID-19) was detected in Wuhan, China in December 2019 (1). Severe acute respiratory syndrome coronavirus-2 (SARS-Cov-2) which is an RNA virus is responsible for the condition (2). COVID-19 pneumonia particularly affects the lower lobes of the lung. Ground glass opacities (GGO) involving multiple lobes of the lung can be generally detected (3). The aim of this case report is to emphasize the importance of differential diagnosis in patients with underlying disease in pandemic era so as not to miss the lung involvement of the primary disease.

CASE

A 63-year-old woman was admitted to our inpatient clinic for cough and shortness of breath. She had a medical history of systemic lupus erythematosus for 11 years and left upper lobectomy for squamous cell lung cancer seven months ago. She was given carboplatin and paclitaxel chemotherapy regimen and had ongoing radiotherapy for bone metastasis on the second rib. She had a 40 pack year history of smoking. On admission, her oxygen saturation on room air was measured as 89 percent by pulse oximeter. Arterial blood sample was obtained and there was neither acidosis nor hypercapnia. It showed that partial pressure of oxygen (PaO2) was 54 mmHg which was consistent with hypoxemia and hypoxemic respiratory failure. Through administration of the high flow nasal cannula oxygen, oxygen saturation hardly reached 95 percent.

Chest X-ray showed reticulonodular opacities in both lung fields, elevated left hemidiaphragm due to left upper lobectomy (Figure 1). Previous radiological images of the patient demonstrated that there was mild lung involvement from lupus. Chest computed tomography (chest CT) was performed which revealed widespread ground glass opacities in both lung fields that have been reported to be consistent with COVID-19 pneumonia due to the pandemic (Figure 2a, 2b). Blood work showed normal white blood cell count and low lymphocyte count (0,3x10³/mL). The level of d-dimer was higher than 20 microgram/mL. Erythrocyte sedimentation rate (ESR) was slightly elevated. Lactate dehydrogenase enzyme level was extremely high at 923 U/L. C-reactive protein level was 81 mg/dl (laboratory upper limit is lower than 5 mg/dl).

Both laboratory findings and radiological evidence were compatible with COVID-19 pneumonia. Combined nasopharyngeal and oropharyngeal swab sample was taken to evaluate COVID-19 by qRT-PCR on 2 different occasions-subsequently on admission and after 24 hours. However, at the beginning of the pandemic, we could

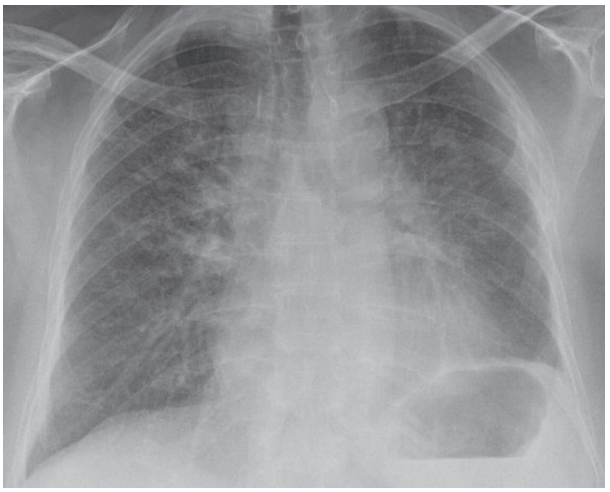

Figure 1: Chest radiography; reticulonodular opacities in both lung fields, elevated left hemidiaphragm

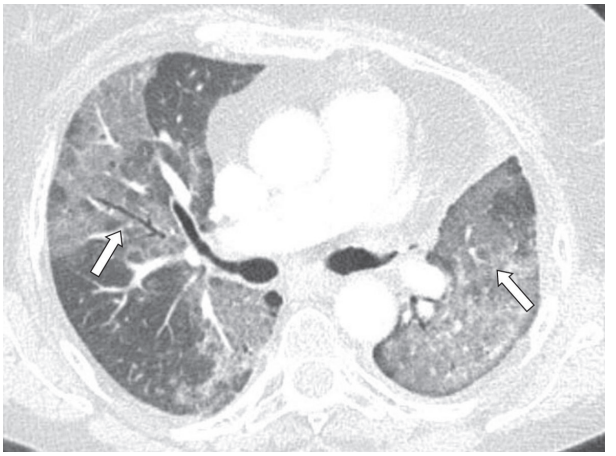

Figure 2a: Contrast enhanced tomography: diffuse ground glass opacities in both lung fields

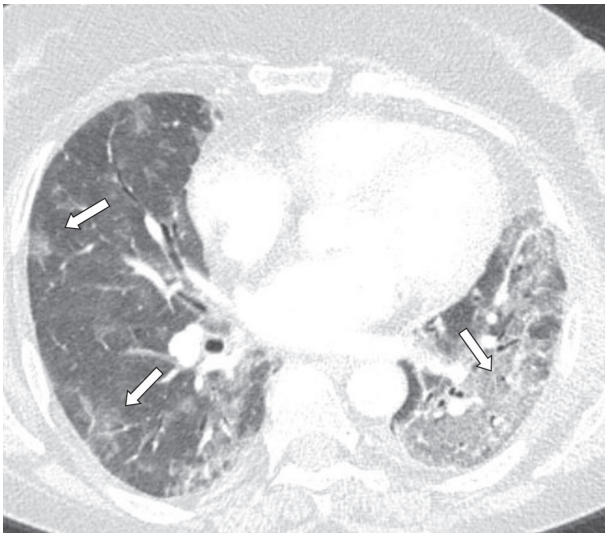

Figure 2b: Both diffuse and patchy ground glass opacities

only have the qRT-PCR results almost 24-36 hours later because of the limited number of PCR laboratories. The chest CT indicated the GGO might be related to viral pneumonia, other interstitial pneumonia types and lung involvement of lupus. Until the PCR results were known hydroxychloroquine, azithromycin, piperacillin-tazobactam and low molecular weight heparin was started. The COVID-19 qRT-PCR test results were both negative.

Although the above mentioned therapy was completed, our patient still needed oxygen supply through the high flow nasal cannula (flow: 60 L/dk, fraction of inspired oxygen: 50%) and also there was no significant radiological improvement. Dyspnea was worsening in spite of improving acute phase reactants. The patient was evaluated again after a 5-day-therapy considering history of her systemic lupus erythematosus (SLE) to ascertain whether the radiological findings could be related to SLE. According to this reassessment methylprednisolone 40 mg intravenously was started. She responded to the prednisolone therapy dramatically. She declared her dyspnea had improved after 3-day-prednisolone therapy. Afterwards she was discharged with a recommendation to continue prednisolone for a while until the next outpatient clinic check-up. Due to the fact that Oxygen requirement diminished gradually and oxygen saturation on room air was 92 percent through pulse oximeter, long-term oxygen therapy was not planned. Immunoglobulin M and G type antibodies against COVID-19 were also evaluated and were found negative on the 7th and 21st days of admission. Radiological findings disappeared after 6-month-prednisolon therapy (Figure 3). According to these findings and response to anti-inflammatory therapy patient was accepted as lung involvement due to SLE.

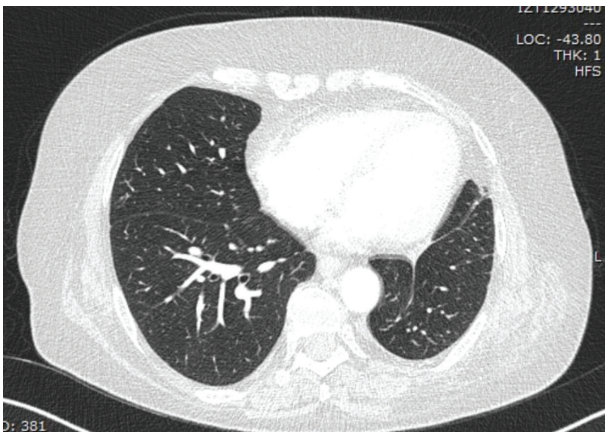

Figure 3: After 6-month-corticosteroid therapy; resolution of ground glass opacities

DISCUSSION

The coronavirus-19 disease was first identified in Wuhan, China at the end of 2019 (1). The causative agent of the disease is a RNA virus, called severe acute respiratory syndrome coronavirus-2 (SARS-Cov-2) (2). Typical chest computed tomography features of COVID-19 pneumonia are ground glass opacities (GGO), involving multiple lobes of the lung and notably the lower lobes (3). Pan et al. reported GGO in 85.7% of 63 COVID-19 RT-PCR test positive patients (4). Salehi et al. reported a systematic review and demonstrated that GGO was the most common CT findings of COVID-19 with 88.0% of 919 COVID-19 patients (5). In our case, due to the COVID-19 pandemic, we initially researched any clues of coronavirus infection, keeping in mind the pulmonary involvement from the underlying SLE. Since, both lupus pneumonitis and COVID-19 pneumonia could be characterized by fever, dyspnea, cough and hypoxia, it was a challenge to make differential diagnosis (6).

SLE is an autoimmune, multisystemic, chronic disease which can affect the lungs as well (7-11). Although pleurisy is common, incidence of interstitial pneumonia is lower in SLE. Lian et al. reported that ground glass opacities are the commonest pattern among interstitial involvement patterns of SLE on chest computed tomography (9).

Our patient was diagnosed as SLE 11 years ago. During the diagnosis, the patient had a positive anti-nuclear antibody (ANA) result (1/160, homogenous pattern) and anti-dsDNA antibodies were also positive. The complete blood count revealed mild lymphopenia (1000 cells/ml). At the time of diagnosis, on physical examination, the patient was afebrile with malar rash.

In our case it was a challenging predicament whether it was COVID-19 pneumonia or lung involvement from lupus. As it is well known, there is no particular treatment for interstitial pneumonia of SLE. Corticosteroids and other immunosuppressive agents are used for both SLE itself and lung complication (12, 13). Although the COVID-19 real time PCR was negative twice, hydroxychloroquine, azithromycin and piperacillin-tazobactam therapy were initiated. Because both ongoing chemotherapy and radiotherapy make the host more vulnerable to any kinds of infectious disease and causing elevated acute phase reactants, treatment was completed. Clinical or radiological responses were not good enough after the treatment and the patient still had dyspnea and cough. That made us re-evaluate the patient as pulmonary complication of SLE, and corticosteroid (CS) therapy was started. Through the CS therapy, the dyspnea, cough and clinical condition improved. The radiological findings significantly disappeared (Figure 3). Meanwhile Immunoglobulin M and G antibodies against COVID-19 became negative as well. Improvements of symptoms and radiological features after CS therapy dictates that progression of underlying SLE disease should also be considered even during pandemic.

Differential diagnosis of COVID-19 and other diseases is essential. Chen et al. reported a pulmonary contusion case having trauma history and GGO on chest CT resembling COVID-19 pneumonia that was spontaneously alleviated (14). It should be noted that COVID-19 may concomitantly superimpose on an underlying condition. Bekci et al. reported a 7-year-old boy with a history of trauma having both COVID-19 pneumonia with positive qRT-PCR and pulmonary contusion (15).

The aim of this case is to emphasize the differential diagnosis of COVID-19 pneumonia. In the pandemic period; it is reasonable to consider the diagnosis of COVID-19 in all patients whose clinical and/or radiological findings suggested disease. However, alternative diagnoses should also be kept in mind especially in patients with underlying diseases. Additionally, it should not be forgotten that COVID-19 and other infectious or non-infectious disease with pulmonary involvement may coexist.

CONCLUSION

Ground glass opacities on chest CT is a significant dilemma in the COVID-19 era. Real time PCR and laboratory findings can be helpful for differential diagnosis. It should be kept in mind that pulmonary involvement of any systemic condition, infectious diseases other than COVID-19 and trauma may mimic radiological findings of COVID-19 or may co-exist with COVID-19.

Informed Consent: Written consent was obtained from the participants.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- E.Ş., İ.B., S.A.B.; Data Acquisition- E.Ş., S.D.; Data Analysis/Interpretation- A.K., İ.B.; Drafting Manuscript- E.Ş., A.K.; Critical Revision of Manuscript- İ.B., S.D., S.A.B.; Approval and Accountability- S.A.B., S.D., A.K., İ.B.

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İSTANBUL ÜNİVERSİTESİ YAYINEVİ

CASE REPORT / OLGU SUNUMU
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EARLY-ONSET CENTRAL DIABETES INSIPIDUS IN A NEWBORN WITH HOLOPROSENCEPHALY

HOLOPROZENSEFALİ TANILI BİR YENİDOĞANDA ERKEN BAŞLANGIÇLI SANTRAL DİABETES İNSİPİDUS

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ABSTRACT

Holoprosencephaly is a complex brain malformation caused by the inability of the prosencephalon to divide to form the cerebral hemispheres. Central diabetes insipidus (CDI), as a result of a defect in vasopressin release, may be seen due to the abnormal hypothalamic infundibular region. CDI developing secondary to holoprosencephaly in the early neonatal period has rarely been reported in the literature. A case of early-onset CDI with holoprosencephaly and 13q deletion is presented.

Keywords: Holoprosencephaly, central diabetes insipidus, newborn, desmopressin

ÖZET

Holoprozensefali, prozensefalonun serebral hemisferleri oluşturmak için bölünmesi sırasındaki yetersizlikten kaynaklanan kompleks bir beyin malformasyonudur. Anormal hipotalamik infundibular bölge nedeniyle vazopressin salınımındaki kusura bağlı santral diabetes insipidus (SDİ) görülebilmektedir. Erken neonatal dönemde holoprozensefaliye ikincil gelişen SDİ vakası enderdir. Holoprozensefali ve 13q delesyonu olan erken başlangıçlı bir SDİ vakası sunulmuştur.

Anahtar Kelimeler: Holoprozensefali, santral diabetes insipidus, yenidoğan,

INTRODUCTION

Holoprosencephaly (HPE) is a complex brain malformation caused by the failure of the prosencephalon to divide between the 35th and 42nd days of embryonic life. Depending on the degree of septation deficiency, it can occur in three forms as alobar, semilobar and lobar. The incidence of alobar HPE has been reported to be 0.6-1.9 per 10,000 live births. Holoprosencephaly can either be isolated or accompany various syndromes. The prognosis depends on the severity of the brain and facial deformities along with the presence of related anomalies. Alobar HPE carries the worst prognosis among other prosencephalon division anomalies. Central diabetes insipidus (CDI) may be an accompanying feature due to a defect in vasopressin release as a result of abnormalities in the hypothalamic infundibular region (1, 2).

CASE PRESENTATION

A baby boy with a birth weight of 3,000 grams (AGA) was born to a 34-year-old $G_6P_4A_2$ mother at $39^{3/7}$ weeks of gestation. Antenatal ultrasonography (USG) showed that the fetus had HPE and corpus callosum agenesis. After birth the baby was admitted to the neonatal intensive care unit for further investigation and follow-up. The history of the mother revealed two previous pregnancies, one resulting in miscarriage at the 8th week and the other live birth with HPE who died at the age of 5.5 years. No genetic analysis had been performed for either the child who died with holoprosencephaly or the two miscarriages. The parents were first degree cousins. Microcephaly, slanted palpebral fissures, hypotelorism, clinodactyly in the fifth finger of the left hand and mild hypertonicity were detected on physical examination (Figure 1).

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Figure 1: External appearance of the patient

Cranial magnetic resonance imaging showed semilobar HPE with fusion in the thalami, agenesis of corpus callosum and interhemispheric fissure, fusion of lateral ventricles at the corpuscular level and dilatation in the fourth ventricle and cisterns (Figure 2).

In the first three days of life, the baby fed well and there was no pathological weight loss. However, on the 4th postnatal day, polyuria developed (urine output: 5.9 cc/kg/hour). Daily weight loss was high (5.4%/day). Serum biochemistry results were as follows: urea 40.8 mg/dL, creatinine 1.05 mg/dL, BUN 19.7 mg/dL, sodium 149 mEq/L and other electrolytes were normal. Blood gases revealed pH 7.31, pCO2 42 mmHg, HCO3 20.4 mEq/L, base deficit -2.1 mEq/L, lactate 1.3 mEq/L, and glucose 87 mg/dL. Sepsis markers were negative. Urinary sodium level was 14 mEq L, urine density was 1,002 while serum osmolarity was 325 mOsm/L. Blood cortisol level

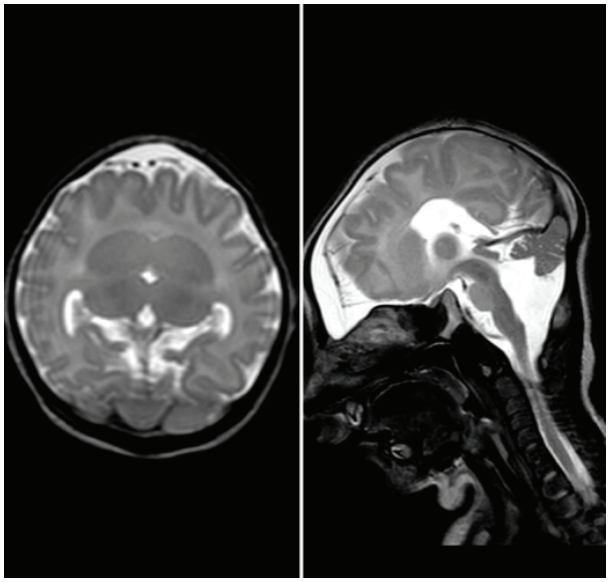

Figure 2: Cranial magnetic resonance imaging sections (holoprosencephaly and corpus callosum agenesis)

(6.26 mcg/dL), renin activity (4.02 ng/mL/hour), aldosterone level (80.7 ng/dL) and thyroid function tests (thyroid stimulating hormon (TSH) 5.21 pmol/L, free thyroxine (T4) 25.1 mIU/L) were normal. Although enteral feeding and parenteral fluid support was gradually increased to a total of 250 cc/kg/day, polyuria continued and serum sodium levels increased (156 mEq/L) on the postnatal 5th day. Oral desmopressin 1.2 µg/day was started and the dose was increased to 2.4 µg/day according to the clinical findings and laboratory results of the patient. On the 3rd day of the treatment, blood sodium levels and urine output returned to normal values (Table 1). Parenteral fluid support was gradually decreased while oral intake was increased. On the postnatal 9th day, parenteral fluids were discontinued completely. The genetic test results showed a deletion in the long arm on the chromosome 13. On the postnatal 11th day, the baby started to gain weight and his laboratory values and urine output were normal. The patient, who did not need parenteral fluid support and was fed with breast milk, was discharged with oral desmopressin treatment (2.4 µg/day)

DISCUSSION

Holoprosencephaly (HPE) is the most common malformation of the prosencephalon, which cannot be separated into two hemispheres and ventricles. It is a midline growth and separation defect characterized by severe malformations of the face and brain, often resulting in intrauterine death. Approximately 25-50% of individuals with HPE have a chromosomal abnormality. Chromosomal abnormalities are nonspecific and either numeric or structural, and can involve any chromosome (3). The region on chromosome 13q contains the *ZIC2*

Table 1: Follow-up during hospitalization

Days of life	1	2	3	4	5	6	7	8	9	10	11
Total fluid (mL/kg/day)	Breastfeeding	Breastfeeding	Breastfeeding	150 (Breastfeeding and parenteral fluid)	200 (Breastfeeding and parenteral fluid)	250 (Breastfeeding and parenteral fluid)	200 (Breastfeeding and parenteral fluid)	180 (Breastfeeding and parenteral fluid)	Breastfeeding	Breastfeeding	Breastfeeding
Daily weight difference (%)	-1.2	-2.5	-3.1	-5.4	-2.1	-1.5	+3.6	+3.4	+2.9	+1.4	+0.9
Serum sodium (mEq/L)	-	-	146	149	156	151	144	142	140	138	139
Serum osmolarity (mOsm/L)	-	-	-	325	332	304	297	289	278	280	-
Diuresis (cc/kg/hour)	-	-	-	5.9	5.4	4.1	2.3	2.0	2.5	2.1	2.2
Urine osmolarity (mOsm/L)	-	-	-	79	75	108	197	264	262	256	-
Urine density	-	-	-	1002	1002	1003	1009	1011	1011	1010	1011
Spot urine sodium (mEq/L)	-	-	-	14	10	19	17	15	18	16	-
Oral desmopressin dose (µg/day)	-	-	-	-	1.2	2.4	2.4	2.4	2.4	2.4	2.4

gene. The *ZIC2* transcription factor is one of the most commonly mutated genes in HPE probands (4). The genetic test results of our case showed a deletion in the long arm on the chromosome 13. However as genetic analysis had not been performed for either the child who died with holoprosencephaly or the two miscarriages, nor the parents, we could not comment on the inheritance model of HPE seen in this index case.

Central diabetes insipidus (CDI) is a disease characterized by hypernatremia, hyposthenuria and polyuria that develops due to a deficiency of antidiuretic hormone (ADH) secretion from the posterior pituitary. CDI in the neonatal period generally develops as a complication of intrauterine and perinatal diseases (5). Asphyxia, severe infections, intracranial hemorrhage, trauma, hereditary mutations of the gene encoding arginine vasopressin and central nervous system malformations, may be the etiologic factors underlying neonatal CDI. In some rare cases no etiology can be found (6, 7). In a study conducted with 12 babies diagnosed with CDI during a ten-year period, it was reported that 10 babies had central nervous system malformations whereas only one patient had semilobar holoprosencephaly and partial corpus callosum agenesis similar to our patient (8). In another study including 23 babies diagnosed with CDI between the ages of 1 day and 9 months, holoprosencephaly was found in 5 babies (9). Other studies reported perinatal asphyxia, intraventricular bleeding and idiopathic cases (10, 13). Considering the age at diagnosis of CDI in the literature, it was reported that CDI developed in the early neonatal period in two infants in one study and at the postnatal 90th day in another study (8, 9). In another case report, CDI developed on the postnatal 30th day in a baby diagnosed with holoprosencephaly (14). The age of the development of CDI was also in the late neonatal period in some other reports (11, 12). In our patient, intracranial hemorrhage, neonatal sepsis and meningitis were excluded, and CDI was accepted as secondary to HPE. Looking at the relationship between CDI and other endocrinopathies, no additional endocrinopathy was reported in some cases (9, 15), while anterior pituitary insufficiency accompanied HPE in other cases (8). In our patient, thyroid function tests (TSH, fT_4) and blood cortisol levels were normal excluding additional endocrinopathies. A standard treatment for neonatal CDI has not been defined in the literature and the management of these cases is difficult. It has been reported that intranasal desmopressin treatment causes hypernatremia and wide fluctuations in antidiuretic effects due to irregular nasal absorption in babies while oral desmopressin treatment has more positive results

than the intranasal route (11, 16, 17). Oral desmopressin treatment was preferred in our patient whose normal sodium levels were achieved with appropriate feeding and fluid support as needed.

In conclusion, although HPE is an uncommon condition, it should be kept in mind that hypernatremic dehydration may develop due to CDI in these patients, even in the early neonatal period. There is no predictive factor for the development of this condition to date, and close follow-up and management should be done accordingly.

Informed Consent: Written consent was obtained from the participants.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- M.T.A., A.Ç., Z.İ.; Data Acquisition- M.T.A.; Data Analysis/Interpretation- M.T.A., A.Ç., Z.İ.; Drafting Manuscript- M.T.A., A.Ç., Z.İ.; Critical Revision of Manuscript- Z.İ.,A.Ç.; Approval and Accountability- M.T.A., A.Ç., Z.İ.

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ERUPTIVE XANTHOMA: A MARKER OF HYPERTRIGLYCERIDEMIA

BİR HİPERTRİGLİSERİDEMİ BULGUSU: ERÜPTİF KSANTOM

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ABSTRACT

Eruptive xanthomas are benign lesions which are found in extensor surfaces of the extremities and are often associated with hypertriglyceridemia and/or uncontrolled diabetes. In this case report, we present a patient with hypertriglyceridemia, type 2 diabetes mellitus and eruptive xanthomas who recovers fully after treatment. A 37-year-old male patient presented to our clinic with reddish yellow lesions on his elbows. His body mass index was 30 kg/m^2 and his laboratory results showed high serum triglyceride and glucose levels (triglyceride 6548 mg/dL, glucose 245 mg/dL), his hemoglobin A1c was 11.2%. Although eruptive xanthomas have a benign nature, they are associated with disease which often need lifelong treatment.

Keywords: Eruptive xanthoma, hypertriglyceridemia, type 2 diabetes

ÖZET

Erüptif ksantomlar, ekstremitelerin ekstansör yüzlerinde bulunan ve sıklıkla hipertrigliseridemi ve/veya kontrolsüz diyabet ile ilişkili iyi huylu lezyonlardır. Bu olgu sunumunda hipertrigliseridemi, tip 2 diyabet ve erüptif ksantomları olan ve tedaviden sonra tamamen iyileşen bir hastayı sunuyoruz. 37 yaşında erkek hasta, dirseklerinde kırmızımsı sarı renkli lezyonlar ile kliniğimize başvurdu. Vücut kitle indeksi 30 kg/m^2 ve laboratuvar sonuçlarında yüksek trigliserid ve glukoz seviyeleri (trigliserid 6548 mg/dL, glukoz 245 mg/dL) ile birlikte hemoglobin A1c % 11,2 idi. Erüptif ksantomlar iyi huylu olmalarına rağmen, genellikle ömür boyu tedavi gerektiren hastalıklarla ilişkilidirler.

Anahtar Kelimeler: Erüptif ksantom, hipertrigliseridemi, tip 2 diyabet

INTRODUCTION

Eruptive xanthomas are benign lesions which are often associated with hypertriglyceridemia and/or uncontrolled diabetes (1). They can be the first symptom of a metabolic disorder or they can accompany an already deteriorating condition (2). It is seen with an estimated prevalence of 18 cases in 100 000 inhabitants and it is pathognomonic for patients with triglyceride levels >992 mg/dL (11.2 mmol/L) (3). Lesions are usually found in extensor surfaces of the extremities or buttocks in some patients (4). In most cases lesions disappear after treatment of underlying metabolic condition. In this case report we present a patient with hypertriglyceridemia, type 2 diabetes and eruptive xanthomas who recovers fully after treatment.

CASE PRESENTATION

A 37-year-old male patient presented to our clinic with reddish yellow lesions on his elbows (Figure 1). He described them to be pruritic and present for more than a month. He described no fever or joint pain. He denied abdominal pain, visual changes, recent changes in medications, or contact with anyone with similar symptoms. He had no history of smoking, alcohol or drug use. Family history revealed type 2 diabetes mellitus in second degree relatives and there was no history of hypertriglyceridemia in the patient's family. Patient complained of polyuria, polydipsia and weight loss of 18 kilograms in the last 3 months. His body mass index was 30 kg/m^2 and waist circumference was 103.5 cm. His vital signs were in normal

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range. There were multiple 2-5 mm sized eruptive xanthomas on both elbows and there was a palmar xanthoma on fourth finger of right hand along with the skinfold line (Figure 2). Punch biopsy revealed keratinized multilayered squamous epithelium and underneath it there were foamy macrophages below dermis compatible with eruptive xanthoma (Figure 3). Laboratory results showed high triglyceride and glucose levels (triglyceride 6548 mg/dL, total cholesterol 842 mg/dL, LDL-cholesterol 261 mg/dL, HDL-cholesterol 38 mg/dL, glucose 245 mg/dL), his hemoglobin A1c was 11.2%. Other than these parameters his laboratory work up was normal (Table 1). Patient was started on hypocaloric diet, metformin 2x1000 mg, insulin glargine 1x14 units, fenofibrate 267 mg 1x1 and omega-3 2.5 grams/day. After one month his triglyceride levels resumed to normal values and his xanthomas faded. The patient provided informed consent for publication.

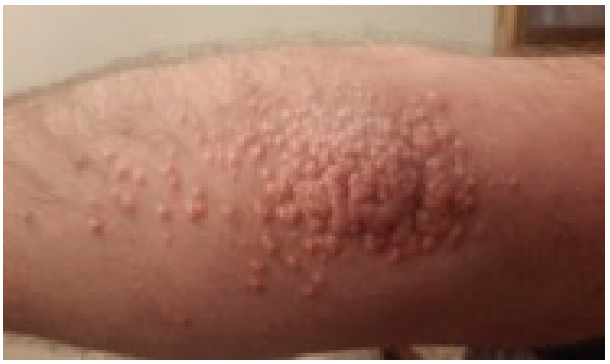

Figure 1: Eruptive xanthoma

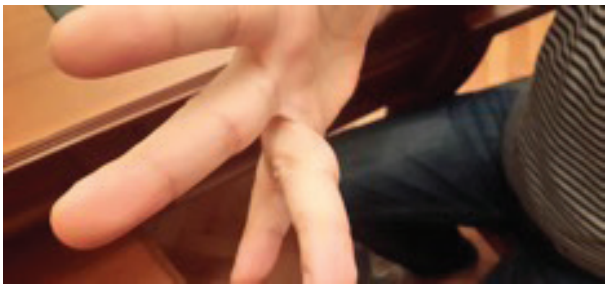

Figure 2: Palmar xanthoma

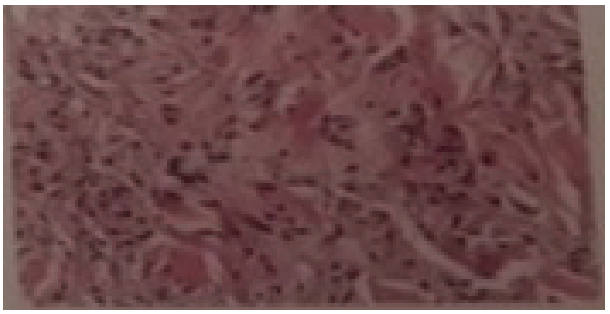

Figure 3: Pathology image

Table 1: Laboratory findings

Laboratory parameters	Results	Reference values
Fasting Blood Glucose (mg/dL)	245	70-100
Hba1c (%)	11.8	4.5-5.7
Fasting Insulin (μIU/mL)	16.6	0-25
Total cholesterol (mg/dL)	842	0-200
Triglyceride (mg/dL)	6548	0-150
HDL-cholesterol (mg/dL)	38	40-60
LDL-cholesterol (mg/dL)	261	0-100
Creatinine (mg/dL)	0.9	0.7-1.2
Uric acid (mg/dL)	5	2.4-5.7
Sodium (mEq/L)	138	135-145
Potassium (mEq/L)	4.16	3.5-5.3
Amilase (U/L)	42	28-110
Lipase (U/L)	37	13-60
Aspartate aminotransferase (U/L)	22	10-35
Alanine aminotransferase (U/L)	35	0-35
Gamma glutamyl transferase (U/L)	51	15-85
Alkaline Phosphatase (U/L)	84	30-105
Thyroid-stimulating hormone (μIU/mL)	1.6	0.27-4.2
Free thyroxine (ng/dL)	1.12	0.93-1.7

DISCUSSION

There are five types of xanthomas; eruptive xanthomas, tuberous/tendinous xanthoma, flat xanthomas, verrucous xanthomas, and xanthelasma (5). Eruptive xanthomas are skin lesions which are a manifestation of hypertriglyceridemia and with keratinized multilayered squamous epithelium and foamy macrophages under the dermis in their pathology. Differential diagnosis of eruptive xanthomas includes Langerhans cell histiocytosis, disseminated granuloma annulare, non-Langerhans cell histiocytosis (xanthoma disseminatum, the micronodular form of juvenile xanthogranuloma), and generalized eruptive histiocytoma.

On the other hand, tendon xanthomas are closely associated with familial hypercholesterolemia and coronary heart disease (6). Xanthelasma are small, soft and yellowish lesions on the eyelids and are often bilateral. They can present in case of hyperlipidemia however in some cases there are no lipid disorders (7). Nevertheless Christoffersen et al. has reported an association between xanthelasma and coronary heart disease independent of conventional cardiac risk factors (8).

Detection of eruptive xanthomas should lead to an early investigation of triglyceride, glucose, thyroid stimulating hormone and creatinine levels because they can be the telltale lesion of diabetes, hypothyroidism or chronic kidney disease. Because hypertriglyceridemia is associated with acute pancreatitis in some cases these lesions can help physicians diagnose the precise cause of abdominal pain in emergency units (9). Our patient did not have a secondary disease that could be the underlying cause of hyperlipidemia other than type 2 diabetes mellitus. He didn't have a family history of hyperlipidemia. Patient had high triglyceride and low HDL cholesterol levels. Although it seemed his lipid profile was compatible with type 1 hyperlipidemia of Frederickson classification because he also had high LDL levels, it was hard to distinguish from type 4 and type 5 hyperlipoproteinemia (10). His VLDL level couldn't be calculated due to high triglyceride levels and unlike his LDL, his VLDL was not measured directly. However, because he also had type 2 diabetes in an early age, it is plausible to think an early atherosclerotic disease associated with type 4 or type 5 hyperlipoproteinemia can also be present in this patient. Type 2 diabetes and high LDL are known atherosclerotic risk factors whereas the impact of hypertriglyceridemia on coronary heart disease has not been laid as clear as other classic risk factors. Lee et al. has suggested Apo B/A1 ratio to identify cardiovascular risk more definitely in a patient with type 1 diabetes and hypertriglyceridemia (7). To the best of our knowledge, unlike tendon xanthomas, there are no reports of an association between eruptive xanthomas and cardiovascular disease to this date.

Studies have shown deleterious effects of non-esterified (free) fatty acids (NEFAs) on beta cell function (11). They induce nitric oxide mediated apoptosis and cause loss of beta cell function (12). Therefore, hyperlipidemia should be avoided in these patients and they should be ordered a strict diet to preserve beta cell function and to decrease fat deposit in visceral organs. Treatment of hyperlipidemia starts with diet and physical activity. Pharmacological treatment is added often in moderate and severe cases of hypertriglyceridemia. These treatments include fenofibrates, omega-3 fatty acids, niacin, insulin in patients with diabetes and triglyceride apheresis or plasmapheresis in patients with severe hypertriglyceridemia (13, 14). Volanesorsen is a new drug developed by antisense technology with promising results in patients with familial chylomicronemia syndrome (15).

CONCLUSION

In conclusion, although eruptive xanthomas have a benign nature, they are associated with disease s which often need lifelong treatment. Therefore, every physician should be aware of the appearance and disease burden of eruptive xanthomas.

Informed Consent: Written consent was obtained from the participants.

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