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**Acta Medica Alanya 2021;5(2)**
**Contents/ *İçindekiler***

**EDITORIAL/ *EDİTÖRYAL***



**RESEARCH ARTICLE/ *ARAŞTIRMA MAKALESİ***

**CASE REPORT/ *OLGU SUNUMU***



**REVİEW/ *DERLEME***

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EDITORIAL

EDİTÖRYAL

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# Transcranial Magnetic stimulation in Psychiatry: Past, Current and Future

## Psikiyatride Transkraniyal Manyetik Uyarım: Geçmiş, Şimdi ve Gelecek

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**ABSTRACT**

Transcranial Magnetic Stimulation (rTMS) is a novel non-invasive neuromodulation method applied via a coil to the skull surface of the patient stimulating relevant brain regions. Replicating data suggest the therapeutic role of repetitive transcranial magnetic stimulation (rTMS) in many psychiatric diseases though there are limited human neuroprotective data. Here we aimed to evaluate the therapeutic role of rTMS from a multifaceted perspective, including its effects on the neuroplasticity and neuroprotection processes. As a conclusion, rTMS seems to offer a potential for neuroprotective therapy.

Keywords: Transcranial Magnetic Stimulation; Neuroprotection; Psychiatric Diseases; Neuroplasticity

**ÖZ**

Transkraniyal Manyetik Uyarım (TMU), hastanın kafatası yüzeyine bir bobin aracılığıyla uygulanarak ilgili beyin bölgelerini uyaran yeni bir non-invaziv nöromodülasyon yöntemidir.insan çalışmalarında nöroprotektif etkisine dair kanıtlar sınırlı olsa da, yinelenen veriler, birçok psikiyatrik bozuklukta TMU' nın terapötik rol oynadığını düşündürmektedir. Bu yazıda nöroplastisite ve nöroproteksiyonun süreçler üzerindeki etkileri de dahil olmak üzere, çok yönlü bir bakış açısıyla TMU'nın terapötik etkisini araştırdık. Sonuç olarak, TMU, nöroprotektif tedavi için bir potansiyel sunuyor gibi görünmektedir.

Anahtar Kelimeler: Transkraniyal Manyetik Uyarım; Nöroproteksiyon; Psikiyatrik Hastalıklar; Nöroplastisite

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## Introduction

Historically, the development of the TMS is has been shown to relate with the discovery of higher brain functions involving the unique topographical organization ability of the brain1. Magnetic stimulation, in other words, transcranial magnetic stimulation (TMS), is applied via a coil to the skull surface of the patient. Faraday firstly discovered the mechanism of TMS based on a simple principle of electromagnetic stimulation: The magnetic field derived from the electrical energy may, in turn, induce a secondary electric field that stimulates relevant brain regions. With modern transcranial magnetic stimulation development, TMS was a preferred method in many neurology and psychiatric disciplines1.

Basics and Clinical Applications

Since 2008 r T MS is an FDA (Food and drug administration) approved anti-depressant therapy in the United States. Furthermore, TMS has also been suggested to treat several other psychiatric diseases such as mania, OCD and schizophrenia. Therapeutically, there are two basic forms of TMS applications. The single-pulse TMS is used primarily for scientific and diagnostic purposes, while the repetitive TMS ( rTMS ), consisting of repetitive pulses, is used mainly for therapeutic purposes. Also, different frequencies of rTMS have divergent effects. For instance, High-frequency rTMS ( > 5 Hz) increases the cortical excitability , while lower frequencies ( < 5 Hz) decrease the motor excitability2. As mentioned above, rTMS is a preferred therapeutic method in the psychiatry discipline, especially when it comes to depression. The major advantage of this novel method is that it is not painful and does not require anaesthesia like other invasive stimulation methods, such as electro-convulsive therapy2,3. Furthermore, TMS does not alter consciousness, and technically it can penetrate the scalp, skull and brain cortex without inducing seizure activity1,2. Shortly, TMS is a safe, non-invasive and well-tolerated therapy method with very mild side effects, including mild headaches responding well to simple analgesics.

Alternative Mechanisms

Although the mechanism of its action seems mechanistic, many effects could not be simply explained with its stimulation effect on the cortex. For instance, many experimental studies have revealed that TMS might exert a neuroprotective and neuroplasticity inducing effect in addition to its anti-depressant effects3,4,5. For instance, rtMS (at high frequencies) induced neuronal plasticity similar to many anti-depressants suggesting that rTMS's anti-depressant effects might also involve restoring the impaired neuroplasticity process in depression6. Further neurochemistry and neurobiology studies elucidating the underlying mechanisms of the TMS finally showed that TMS might not only change the electrical activity but also lead to some critical changes in critical anti-depressant and neuroprotective molecules7. For instance, in several animal and human studies, BDNF, a well-known pro-cognitive, anti-depressant and neuroprotective molecule, is increased after rTMS6,7. Since no cognitive side effects are described with rTMS, this opened a new window of the therapeutic role of ATMs in neurodegenerative diseases such as Alzheimer's and Parkinson's Disease8. Considering the role of neurodegeneration also in some psychiatric diseases, rTMS might be a novel tool with its additional neuroprotective effect. Although there are no conclusive human studies in this respect, animal data, including many psychiatric neurodegeneration models, are rapidly replicating6,7,8.

**Conclusion:** TMS is a novel tool for many neurological and psychiatric diseases. Beyond the time and location of stimulation, TMS can also modulate brain chemistry and network activity, offering the potential for neuroprotective therapy.

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RESEARCH ARTICLE

ARAŞTIRMA

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# Preliminary Study About A Significant and Treatable Cause of Epileptic Encephalopathy: GRIN2D Mutation

## Epileptik Ensefalopatinin Önemli ve Tedavi Edilebilir Bir Nedeni Hakkında Ön Çalışma: GRIN2D Mutasyonu

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## ABSTRACT

**Aim:** The *GRIN2D* gene mutation causes severe forms of epileptic encephalopathy. NMDAR antagonists and magnesium sulfate could be useful as adjunctive therapy to control seizures in individuals with *GRIN2D* encephalopathy. The aim of this study was to describe the clinical features and treatment options of *GRIN2D* encephalopathy.
**Methods:** Patients followed up with epileptic encephalopathy in our pediatric neurology clinic were investigated for genetic etiology using next-generation sequencing (NGS)-based tests. Patients with the *GRIN2D* mutation were overviewed for clinical and genetic characteristics.
**Results:** A total of 53 patients were screened and *GRIN2D* mutations (c.3684_3685insGA, c.3248_3254del, c.1579G>T, c.47_49del) were detected in four patients. Occipital epileptic activity was frequently detected among our patients. Three patients received memantine treatment for intractable epilepsy and remained seizure-free.
**Conclusion:** *GRIN2D* encephalopathy is a treatable epileptic encephalopathy, and its recognition is important in terms of outcomes. Occipital epilepsy is generally benign, but developmental and epileptic encephalopathies such as *GRIN2D* encephalopathy should be considered in the presence of concomitant developmental delay.

Keywords: developmental delay; epileptic encephalopathy; *GRIN2D*; memantine; NMDA

## ÖZ

**Amaç:** *GRIN2D* gen mutasyonu, ağır epileptik ensefalopatiye neden olur. NMDAR antagonistleri ve magnezyum, *GRIN2D* ensefalopatili bireylerde nöbetleri kontrol etmek için faydalı bir tedavi seçeneği olabilir. Bu çalışmanın amacı *GRIN2D* ensefalopatisinin klinik özellikleri ile tedavi seçeneklerini tanımlamaktır.
**Yöntemler:** Çocuk nöroloji kliniğimizde epileptik ensefalopati ile izlenen hastalar genetik etiyoloji açısından yeni nesil dizileme yöntemi tabanlı testler ile incelendi. *GRIN2D* mutasyonu olan hastalar klinik ve genetik özellikler açısından değerlendirildi.
**Bulgular:** Toplam 53 hasta tarandı. 4 hastada *GRIN2D* mutasyonları (c.3684_3685insGA, c.3248_3254del, c.1579G>T, c.47_49del) tespit edildi. Hastalarımızda oksipital epileptik aktivite sıklıkla tespit edildi. 3 hastaya inatçı epilepsi için memantin tedavisi başlandı ve bu hastalar nöbetsiz olarak takip edilmekteler.
**Sonuç:** *GRIN2D* ensefalopati, tedavi edilebilir bir epileptik ensefalopatidir ve hastanın sağkalımı açısından tanınması önemlidir. Oksipital epilepsi genellikle iyi huyludur, ancak eşlik eden gelişimsel gecikme varlığında *GRIN2D* ensefalopatisi gibi gelişimsel ve epileptik ensefalopatiler akla gelmelidir.

Anahtar Sözcükler: epileptik ensefalopati, gelişme geriliği, *GRIN2D*, memantin, NMDA

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## INTRODUCTION

Glutamate is the main excitatory neurotransmitter of the central nervous system (CNS). Glutamate takes an important part in many basic neuronal functions and CNS processes, such as learning, memory, and synaptic plasticity. [1] N-methyl- D-aspartate receptors (NMDAR) are voltage-dependent ionotropic glutamate receptors and tetrameric assemblies containing GluN1 and GluN2 subunits. Four independent genes (*GRIN2A, GRIN2B, GRIN2C,* and *GRIN2D*) encode GluN2A-D subunits.[2] NMDAR activity is crucial for neurodevelopment, synaptogenesis, general cognition, spatial learning, locomotion, and memory formation. NMDAR mutations (*GRIN1* [MIM: 138249], *GRIN2A* [MIM: 138253], *GRIN2B* [MIM: 138252], *GRIN2C* [MIM: 138254], and *GRIN2D* [MIM: 602717]) are associated with variable neurologic diseases, including schizophrenia, intellectual disabilities, autism, epilepsy, and attention-deficit/hyperactivity disorder.[3] Epileptic encephalopathies manifest with intractable seizures and neurodevelopmental disabilities and have monogenic disorders as part of the various etiologies. Children with epileptic encephalopathy have a shortened life expectancy, and most patients experience little to no relief from seizures with anti-epileptic drug (AED) treatment. In light of technological advances, genetic analysis has provided for establishing the precise genetic etiology in individuals.[4] Validation of genetic etiologies and specific disease mechanisms make personalized therapeutic regimens possible. Genetic variations of the autosomal dominant inherited *GRIN2D* gene mutation cause severe forms of epileptic encephalopathy, which manifest early during infantile or adolescent development. NMDAR mutations cause greatly increased current flow through mutant-GluN2A-containing NMDARs, leading to the excessive excitatory drive, thereby inducing seizure activity and/or excitotoxicity.[5] Memantine is a noncompetitive antagonist of the NMDA-type glutamate receptor. It interacts with the Mg+2 binding site of the channel to prevent excessive activation while sparing normal function.[6] NMDAR antagonists and magnesium sulfate might be useful adjunctive therapy to control seizures in individuals with *GRIN2D* encephalopathy.[4] Here, we report distinct clinical, genomic, and therapeutic features of four patients with *GRIN2D* encephalopathy with different variants of the *GRIN2D* gene.

## METHODS

Patients who were followed up with epileptic encephalopathy in the pediatric neurology clinic of Antalya Training and Research Hospital and had a genetic diagnosis were retrospectively screened. The genetic diagnosis was made by investigating the most common actionable genes of epileptic encephalopathy using next-generation sequencing (NGS)-based tests (Table 1).[7] Each variant was evaluated according to the American College of Medical Genetics and Genomics (ACMG) criteria. [8] The information about genetic examinations of the patients was given in details in the cases section.

Epileptic encephalopathy is defined as an electroclinical syndrome associated with a high probability of encephalopathic features that present or worsen after the onset of epilepsy by the International League Against Epilepsy (ILAE) Commission on Classification and Terminology.[9]

The aim of this study was to describe the clinical features and treatment options of *GRIN2D* encephalopathy. Electroencephalography (EEG), magnetic resonance imaging (MRI), and metabolic screening tests were performed for all patients with *GRIN2D* mutations. Only the clinical and genetic characteristics of patients with *GRIN2D* encephalopathy were reviewed and presented in this study. Written informed consent was obtained from all parents of the children, which was approved by Antalya Research and Training Hospital Ethics Committee (Date: 04.03.2021 number: 1/44).

## RESULTS

Patients:

A total of 53 patients with epileptic encephalopathy were screened using NGS-based tests. A genetic mutation associated with epileptic encephalopathy was detected in 33 patients (62,2%). Novel variants in *GRIN2D* were detected in four patients (7,5%) and none of these variants was reported in the literature before. By using the ACMG criteria, we classified *GRIN2D* variants as pathogenic or likely pathogenic. The clinical and genetic

characteristics and treatment regimens of patients with *GRIN2D* encephalopathy which is rare and treatable are presented as case reports below.

Case 1

The first patient was a 3-year-old girl, who presented to our clinic with status epilepticus at age 2.5 years. She was previously followed with intractable epilepsy, global developmental delay, and static encephalopathy. Her prenatal period was uneventful, and she was born at term as the first child from nonconsanguineous parents. She had no previous neurologic medical history in her family. She had migratory focal clonic seizures in the first week of life. Later, hypotonia and eye flutter accompanied the seizures. Phenobarbital, levetiracetam, and topiramate were given for the seizures, respectively. She was unresponsive to treatment, had status epilepticus, and was admitted to the pediatric intensive care unit (PICU) at age 3 months. Her seizures were managed with midazolam and thiopental infusions. Carbamazepine and clonazepam were added to treatment instead of phenobarbital. She was admitted to the PICU three more times for status epilepticus until the age of 2 years. Valproic acid, lamotrigine, sulthiame, and clobazam were also added to treatment but she was unresponsive.

A physical examination revealed normal deep tendon reflexes, strabismus, bilateral ocular flutter, truncal ataxia, and mild hypotonia. She had no dysmorphic features or pathologic reflexes. She was able to hold her head at the age of 8 months and sit unsupported at the age of 14 months. She could not walk or talk.

The patient's first EEG showed multifocal epileptic anomaly and remained similar until the age of 1 year. Metabolic studies: ammonia, plasma, and urine amino acid analysis, long-chained fatty acid analysis, urine organic acid analysis, lactate, pyruvate, tandem mass spectrometry, vitamin B12, and folic acid were within normal limits. Her brain MRI and MR spectroscopy were normal.

At age 2.5 years, she had status epilepticus and was admitted to the PICU, when we first evaluated her for a pediatric neurology consultation. Her recent treatment regimen was valproic acid, lamotrigine, sulthiame, and clobazam. Her seizures continued as a multifocal clonic type while she was receiving midazolam and thiopental infusions. An EEG study during status epilepticus showed ictal epileptic discharges in the bilateral parietooccipital region. Ketamine infusion was initiated, and her seizures stopped. The second EEG study after the ketamine infusion showed interictal parietooccipital epileptic activity. Clinical exome sequencing (CES) was conducted after the ketamine response. A novel, heterozygous *GRIN2D* c.3684_3685insGA variant was detected. A *GRIN2D* c.3684_3685insGA (NM_000836.4, p.Pro1229AspfsTer290T) variant was evaluated according to the ACMG criteria, and this variant was classified as pathogenic because it was a null variant (frame-shift) and in gene *GRIN2D*, for which loss-of-function is a known mechanism of disease (gnomAD Loss-of-Function Observed/ Expected = 0.0529 is less than 0.763), associated with developmental and epileptic encephalopathy 46 (PVS1 criteria), not found in gnomAD genomes (PM2 criteria), compatible with the patient's clinic (PP4 criteria). Oral memantine (0.5 mg/kg/day) and magnesium sulfate therapies were initiated. Her seizure frequency and severity reduced gradually. In the last visit, the patient was seizure-free for 4 months, could continue her daily life, and her neuromotor development was improved. The latest EEG study was normal (Figure 1). She has been able to walk and talk with simple words since the age of 33 months (Table 2).

Case 2

Patient 2 was a 4.5-year-old girl who presented with neonatal seizures. Her prenatal period was uneventful, and she was born at term as the second child from nonconsanguineous parents. She had no previous neurologic medical history in her family. Her first seizure was focal onset motor clonic type on the 16th day of life. The first EEG study was normal. She was seizure-free for 2 months after phenobarbital therapy. She had flexor spasm seizures at 3 months, and the EEG study revealed hypsarrythmia. Adrenocorticotropic hormone (ACTH) and pyridoxine treatments were initiated and levetiracetam was added to treatment later. She had neuromotor developmental delay; she was able to raise her head at age 9 months and sit unsupported at the age of 26 months. She could not walk or talk.

**Table 1. List of actionable genes**

| Actionable genes | HGNC Approved Gene Symbol | Phenotypes #MIM number |
|---|---|---|
| Alpha-2B-Adrenergic Receptor | *ADRA2B* | 607876 |
| Aldehyde Dehydrogenase 7 Family, Member A1 | *ALDH7A1* | 266100 |
| Folate Receptor, Alpha | *FOLR1* | 613068 |
| Guanidinoacetate Methyltransferase | *GAMT* | 612736 |
| L-Arginine:Glycine Amidinotransferase | *GATM* | 612718, 134600 |
| Potassium Channel, Voltage-gated, Kqt-Like Subfamily, Member 2 | *KCNQ2* | 613720, 121200 |
| Potassium Channel, Voltage-Gated, Kqt-Like Subfamily, Member 3 | *KCNQ3* | 121201 |
| Potassium Channel, Subfamily T, Member 1 | *KCNT1* | 614959, 615005 |
| Methyl-Cpg-binding Protein 2 | *MECP2* | 300496, 300673, 300260, 300055, 312750 |
| Pyridoxamine 5-Prime-Phosphate Oxidase | *PNPO* | 610090 |
| Polymerase, DNA, Gamma | *POLG* | 203700, 613662, 607459, 157640, 258450 |
| Proline-Rich Transmembrane Protein 2 | *PRRT2* | 602066, 128200, 605751 |
| Quinoid Dihydropteridine Reductase | *QDPR* | 261630 |
| Sodium Voltage-gated Channel, Alpha Subunit 1 | *SCN1A* | 607208, 604403, 609634 |
| Sodium Voltage-gated Channel, Alpha Subunit 2 | *SCN2A* | 613721, 618924, 607745 |
| Sodium Voltage-gated Channel, Alpha Subunit 8 | *SCN8A* | 618364, 614306, 614558, 617080 |
| Solute Carrier Family 19 (Thiamine Transporter), Member 3 | *SLC19A3* | 607483 |
| Solute Carrier Family 2 (Facilitated Glucose Transporter), Member 1 | *SLC2A1* | 614847, 606777, 612126, 608885 |
| Solute Carrier Family 6 (Neurotransmitter Transporter, Creatine), Member 8 | *SLC6A8* | 300352 |
| Syntaxin-binding Protein 1 | *STXBP1* | 612164 |
| Tsc Complex Subunit 1 | *TSC1* | 607341, 606690, 191100 |
| Tsc Complex Subunit 2 | *TSC2* | 607341, 606690, 613254 |
| Glutamate Receptor, Ionotropic, N-Methyl-D-Aspartate, Subunit 2A | *GRIN2A* | 245570 |
| Pyridoxal Phosphate-binding Protein | *PLPBP* | 617290 |
| Glutamate Receptor, Ionotropic, N-Methyl-D-Aspartate, Subunit 2D | *GRIN2D* | 617162 |

The term "actionable genes" is used to indicate clinical applicability based on evidence and to describe the genes that are most effective in curing, preventing and/or delaying clinical disease. In our center, the "actionable genes" list includes genes that are statistically common and associated with specific FDA-approved drug response to help patients with epilepsy to receive appropriate treatment faster. These actionable genes were filtered out by Medical Genetics Department in our center.

**Table 2. Phenotype and variant summary of GRIN2D encephalopathy patients. PVL: Periventricular leukomalacia, AEDs: antiepileptic drugs, LVT: levetiracetam, VPA: valproic acid, CBZ: carbamazepine,**

| Patient number | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Age on-set | 1 wk | 16 days | 4 years old | 16 days |
| Current Age (years)/Sex | 3/F | 4/F | 18/M | 7.5/M |
| *GRIN2D* variants | *GRIN2D* c.3684_3685insGA | *GRIN2D* c.3248_3254del | *GRIN2D* c.1579G>T | *GRIN2D* c.47_49del |
| EEG | Parietooccipital epileptic activity | Parieto-occipital epileptic activity | Bilateral temporal epileptic activity | Bilateral temporoparietal epileptic activity |
| MRI | Normal | PVL | Normal | Normal |
| Response to AEDs | No response to other AEDs, seizure-free with memantine and MgSO4 | No response to LVT and VPA, seizure-free with memantine | Mild response with CBZ, LTG, LVT and memantine | Seizure free with VPA |
| Age on-set | 1 week | 16 days | 4 years | 16 days |
| Develop-mental delay and other neurological features | Hold her head at 8 mo and sit unsupported at 14 mo. She can ataxic walk and talk with simple words since 33 mo. Occuler flutter. | Raise her head at 9 mo, sit unsupported at 26 mo. Cannot walk or talk. Poor attention to surroundings, strabismus, hypertonicity, hyperactive deep tendon reflexes. | Mild mental retardation | Delayed speech development |

LTG: lamotrigine.

In a physical examination, she had poor attention to surroundings, strabismus, hypertonicity, and hyperactive deep tendon reflexes. She had no dysmorphic features or pathologic reflexes.

Metabolic studies: ammonia, plasma, and urine amino acid analysis, long-chained fatty acid analysis, urine organic acid analysis, lactate, pyruvate, tandem mass spectrometry, vitamin B12, and folic acid were within normal limits. Brain MRI revealed periventricular leukomalacia and MR spectroscopy was normal. An epilepsy panel performed by using NGS analysis revealed a novel, heterozygous *GRIN2D* c.3248_3254del variant. The *GRIN2D* c.3248_3254del (NM_000836.4, p.Gly1083GlufsTer433) variant was evaluated according to the ACMG criteria, and this variant was classified as pathogenic because the variant is a null variant (frame-shift), and in gene *GRIN2D*, for which loss-of-function is a known mechanism of disease (gnomAD Loss-of-Function Observed/ Expected = 0.0529 is less than 0.763), associated with developmental and epileptic encephalopathy 46 (PVS1 criteria), not found in gnomAD genomes (PM2 criteria), compatible with the patient's clinic (PP4 criteria). No other new therapies were initiated after the genetic analysis because her seizures had been under control with levetiracetam for 2 years, but follow-up EEG studies revealed parietooccipital epileptic activity. She had migratory focal clonic seizures and ocular flutter at the age of 3.5 years. Her seizures were prolonged and refractory, she had status epilepticus and was admitted to the PICU. Valproic acid was added to her antiepileptic treatment. An EEG study showed interictal epileptic activity in the parietooccipital region (Figure 2). On follow-up, her seizures continued as the multifocal clonic-type and ocular flutter. Memantine (0.5 mg/kg/day) was added to the treatment, seizures remained under control, and valproic acid and levetiracetam treatments were stopped gradually. She has been seizure-free for 8 months and the latest EEG was normal (Table 2).

Case 3

Patient 3 was an 18-year-old boy who was born at term to nonconsanguineous parents. His neonatal period was uneventful and his neuromotor development was appropriate, only his speech development was delayed. He presented for medical attention with a right focal motor clonic seizure following sudden visual loss at age 4 years. A EEG study showed bilateral occipital epileptic activity. Levetiracetam was initiated after the first seizure and oxcarbazepine was added to treatment later because of continuing multifocal clonic seizures. Multifocal clonic seizures and bilateral occipital epileptic activity remained for almost 4 years. At the age of 8 years, atonic seizures presented, and EEG studies showed bilateral temporal epileptic activity. Valproic acid, clobazam, carbamazepine, lamotrigine, rufinamide, lacosamide, sulthiame, primidone, zonisamide, topiramate were tried for treatment. Metabolic studies: ammonia, plasma, and urine amino acid analysis, long-chained fatty acid analysis, urine organic acid analysis, lactate, pyruvate, tandem mass spectrometry, vitamin B12, and folic acid were within normal limits. Brain MR and MR spectroscopy were normal. He presented to our clinic at around the age of 15 years. The only pathologic finding was mild mental retardation on physical examination. His intelligence quotient (IQ) score was 80 (IQ, Stanford Binet Intelligence Scales, 5th Edition). By that time, his seizure frequency was 4-5 times per week and the seizure types were atonic and multifocal clonic. On follow-up, his treatment was rearranged as carbamazepine, levetiracetam, and lamotrigine, and an EEG study showed bilateral frontotemporal epileptic activity. He was diagnosed as having non-Hodgkin lymphoma at the age of 16 years; therefore, he did not continue regular follow-ups for epilepsy for 1.5 years. After full remission of non-Hodgkin lymphoma, whole-exome sequencing (WES) analysis was performed and a novel, heterozygous *GRIN*2D c.1579G>T variant was detected. The *GRIN2D* c.1579G>T (NM_000836.4, p.Glu527Ter) variant was evaluated according to the ACMG criteria and classified as pathogenic because it was a null variant (frame-shift), and in gene *GRIN2D*, for which loss-of-function is a known mechanism of disease (gnomAD Loss-of-Function Observed/ Expected = 0.0529 is less than 0.763), associated with developmental and epileptic encephalopathy 46 (PVS1 criteria), not found in gnomAD genomes (PM2 criteria), compatible with the patient's clinic (PP4 criteria). Memantine (0.5 mg/kg/day) was initiated. The latest EEG study showed bilateral

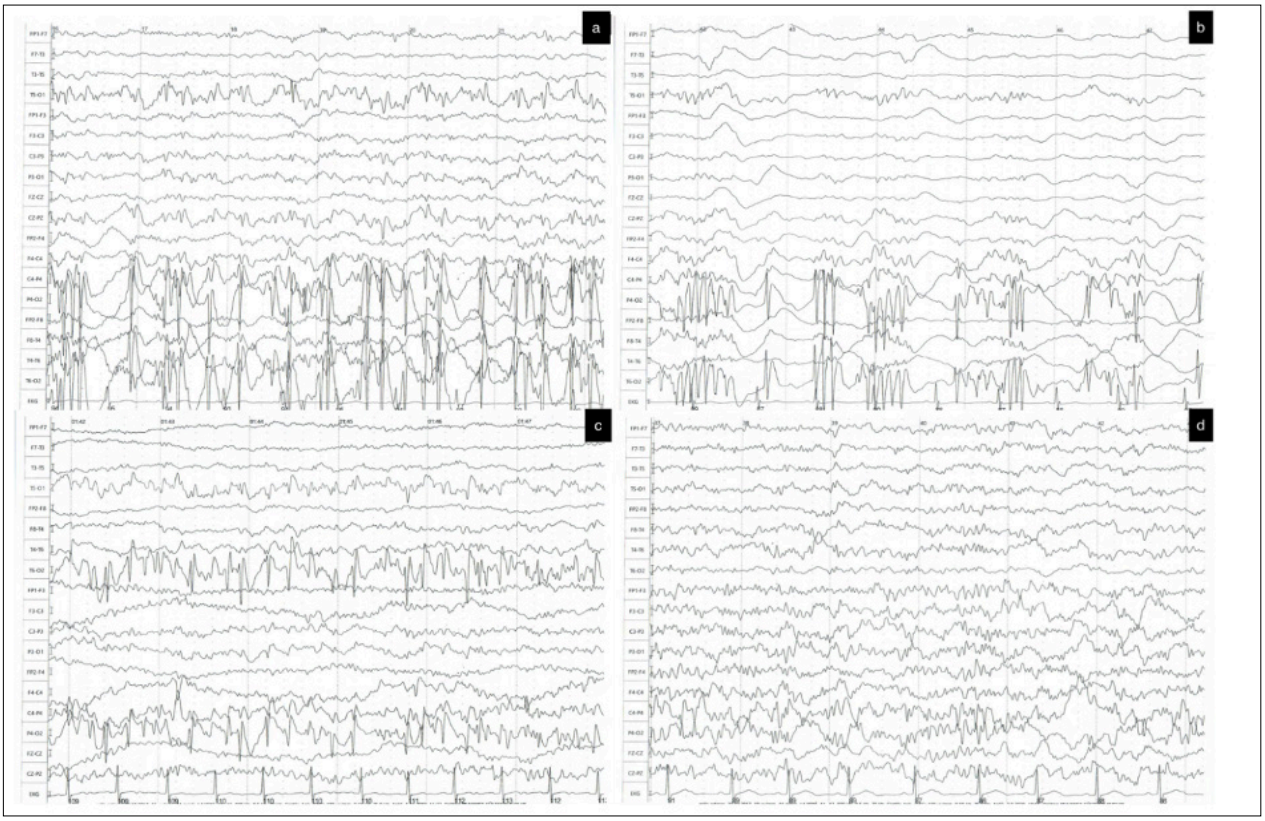


**Figure 1. EEG pattern changes of patient 1: a) Convulsive status epilepticus, constant right parietooccipital epileptic activity. b)Epileptic activity after thiopental administration. c)Epileptic activity after ketamine administration. d)Normal EEG pattern after memantine treatment**

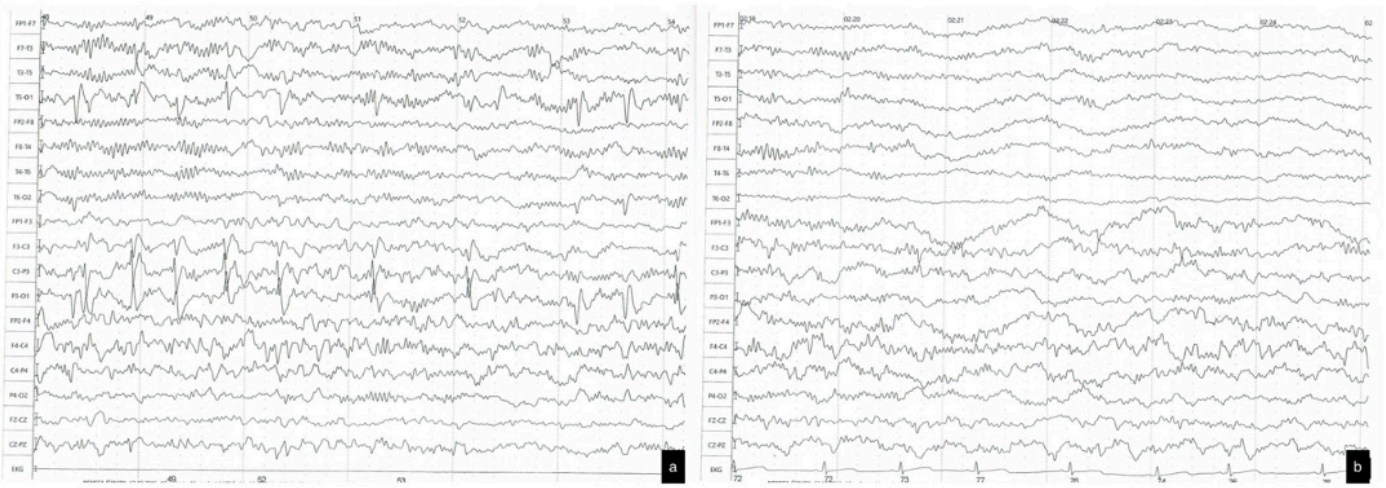


**Figure 2. EEG pattern changes of patient 2: a) Intractable epilepsy left parietooccipital epileptic activity. b)Normal EEG findings after memantine treatment.**

temporal epileptic activity. His seizure frequency was reduced to one in four or five months (Table 2).

Case 4

Patient 4 was a 7.5-year-old boy who was born at term. His prenatal period was uneventful. He was the third child of nonconsanguineous healthy parents, and his two elder brothers were healthy. Mental retardation was seen in two members of the extended family. His first seizure was multifocal clonic type and occurred on the 16th day of life. The first EEG study was normal. Phenobarbital was initiated as anticonvulsant therapy. He had a second multifocal clonic seizure at age 6 months and levetiracetam was added to his therapy. By the age of 8 months, he had the third seizure, the same as in previous seizures. Anticonvulsant therapy was rearranged as a higher dose of levetiracetam monotherapy until 1 year of age

when he experienced agitation and valproic acid monotherapy was initiated. All EEG studies were normal until age 3.5 years. He had a tonic seizure, and parietooccipital epileptic activity was detected on an EEG study for the first time.

He could sit unsupported and walk independently at the appropriate age, but his speech development was delayed. A physical examination was normal except for language skills.

Metabolic studies: ammonia, plasma, and urine amino acid analysis, long-chained fatty acid analysis, urine organic acid analysis, lactate, pyruvate, tandem mass spectrometry, vitamin B12, and folic acid were within normal limits. Brain MRI and MR spectroscopy were normal.

The patient presented to or clinic at age 4.5 years. His anticonvulsant therapy at that time was valproic acid and an EEG study showed parietooccipital epileptic activity. He had seizures once per year for 2 years. WES analysis was conducted and a novel, heterozygous *GRIN2D* c.58_60delCTG variant was detected. The *GRIN2D* (NM_000836.4, p.Leu20del) variant was evaluated according to the ACMG criteria and classified as likely pathogenic because it was located in a mutational hot spot and/or critical and well-established functional domain (e.g., the active site of an enzyme) without benign variation (PM1 criteria), not found in gnomAD genomes (PM2 criteria), protein length changes as a result of in-frame deletions/insertions in a non-repeat region or stop-loss variants (PM4 criteria), and compatible with the patient's clinic (PP4 criteria).

Follow-up EEG studies showed bilateral temporoparietal epileptic activity. Memantine was not initiated, and anticonvulsant therapy remained as valproic acid because his seizure frequency was low. His last seizure was 6 months ago. He has been receiving speech therapy rehabilitation (Table 2).

## DISCUSSION

Developmental and epileptic encephalopathies (DEEs) represent a clinically and genetically heterogeneous group of age-dependent neurologic disorders characterized by the onset of refractory seizures in infancy or early childhood. Affected individuals have delayed psychomotor development or developmental regression, particularly after the onset of seizures. DEE incorporates the previous grouping of "early infantile epileptic encephalopathies (EIEE)." There are 89 described DEEs in the literature and the phenotype is also observed in other genetic disorders, including GLUT1 deficiency syndrome, glycine encephalopathy, Aicardi-Goutières syndrome, and in males with *MECP2* mutations. [10] *NMDAR* mutations are associated with DEEs; DEE27 is caused by a mutation in the *GRIN2B* gene, DEE46 is caused by a mutation in the *GRIN2D* gene.[3,4,11]

We described four patients with DEE and de novo variants in the *GRIN2D* gene. Age at onset of epileptic encephalopathy is generally before 2 years of age.[12] Camp et al. reported a median age of onset of 6.5 months for *GRIN2D* DEE.[3] Three of our patients had seizures within the first month of life and patient 3 had their first seizure rather late, at age 4 years.

Early studies of *NMDAR* genes were about schizophrenia and autism spectrum disorder. In 2011, Tarabeux et al. showed neuropsychiatric disease's relation to *GRIN1*, *GRIN2A*, *GRIN2B*, and *GRIN2D* families.[1] Camp et al. found that 31% of patients displayed autistic-like features and behaviors accompanying epilepsy.[3] None of our patients had autistic-like features or behaviors. Developmental delay and intellectual disability are part of the clinical characteristics, but affected individuals show varying severity, as in our patients; patient 3 was independent in his daily life, but patient 2 was bedbound.

In epileptic encephalopathies, particularly spasm, and tonic, clonic, myoclonic, atonic, atypical absence seizures can be seen in infancy. Over time, changes can be seen between these seizure types.[10,13] Multifocal clonic seizures and spasms are prominent in *GRIN2D* encephalopathy.[3] Multifocal clonic seizures were more common in our patients. With age, as in other epileptic encephalopathies, our patients also developed atonic, tonic, and atypical absence seizures. In *GRIN2D* encephalopathy, unlike other epileptic encephalopathies, ocular flutter, tongue movement, and body tremor, which occur out of

seizures and increase during seizures, have been reported.[3]

Hypsarrhythmia and multifocal epileptiform abnormalities are prominent EEG patterns for epileptic encephalopathies in infancy. EEG patterns can change into multifocal/focal epileptiform abnormalities, Lennox-Gastaut syndrome, and epileptic encephalopathy with continuous spike-and-wave during sleep (CSWS) over time.[13,14] A limited case series about *GRIN2D* encephalopathy revealed a multifocal epileptiform abnormality, hypsarrhythmia, focal spike-wave activity, and paroxysmal fast activity patterns on EEG.[3] One of our patients had a multifocal epileptiform abnormality and one had a hypsarrhythmia pattern in infancy. Different from the literature, two patients had normal EEG patterns in infancy. Patients 1 and 2 developed bilateral parietooccipital epileptiform activity and patient 3 developed bilateral occipital epileptiform activity later in life. Occipital epileptiform activity is mainly seen in childhood epilepsy with occipital paroxysm (CEOP) and CEOP is classified as benign childhood epilepsy. Li et al. reported a poor prognosis of occipital epileptiform activity in *GRIN2D* encephalopathy.[4] Although cerebral atrophy, cerebellar atrophy, and cortical atrophy were frequently mentioned in previous studies, no specific MRI findings were reported in *GRIN2D* mutation.[3,15]

Specific treatment options are available for some of the genetic epileptic encephalopathies; pyridoxine dependent epilepsy (PDE) caused by an *ALDH7A1* genetic defect (PDE-*ALDH7A1*), Menkes disease, pyridox(am)ine-5-phosphate oxidase (PNPO) deficiency, cobalamin G deficiency, severe methylenetetrahydrofolate reductase (MTHFR) deficiency, glucose transporter 1 (GLUT1) deficiency, glycine encephalopathy, and pyruvate dehydrogenase complex (PDHC) deficiency, uridine responsive CAD deficiency, cerebral folate deficiency, creatinine deficiency syndrome, DEND syndrome, serine biosynthesis defect, biotinidase deficiency, nonketotic hyperglycinemia, KCNQ2 encephalopathy, KCNT1-related epilepsy, and BH4 deficiency (Table 1).[16–18] Memantine is prominently used in cognitive impairments such as Alzheimer's disease.[19] With its effect on NMDARs, studies have been conducted for the use of memantine as an AED in GRIN mutations.[4–6,20] Memantine was used in patient 1 with refractory status, patient 2 with intractable seizures, and patient 3 with frequent seizures. Both patients 1 and 2 remained seizure-free, and the seizure frequency of patient 3 was reduced.

Limitations of the study

Our study has several limitations, mainly related to the study population size. The number of patients meeting the inclusion criteria was small because it was a single center study. In our opinion, it would be more effective to identify new variants and define the phenotype-genotype characteristics of these variants in a multi-center study.

## CONCLUSION

Occipital epilepsy is usually benign, but with developmental delay, epileptic encephalopathy must be considered. Genetic studies are important for epileptic encephalopathy because specific therapies for targeted gene mutations are available for some epileptic encephalopathies. Genotype-phenotype correlation studies are necessary to gain more information about the subject.

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RESEARCH ARTICLE

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# The relationship between the changes in inflammatory parameters and response to treatment in major depression patients starting antidepressant treatment

## Antidepresan tedavi başlanan major depresyon hastalarında inflamatuar parametrelerdeki değişikliklerin tedavi yanıtı ile ilişkisi

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## ABSTRACT

**Aim:** Changes in inflammatory parameters such as cytokines, stress hormones and C reactive protein that occur in depression, are important in understanding the pathophysiology of depression and developing new treatment approaches. The main purpose of this study was to determine the changes in inflammatory markers in patients with major depression, before and after antidepressant treatment, as well as to determine the effect of antidepressant treatment types on these changes.

**Methods:** This study was a single center, retrospective study. According to the retrospective records of the last five years in the psychiatry outpatient clinic of Alanya Alaaddin Keykubat University Training and Research Hospital, the patients diagnosed with Major Depressive Disorder (MDD), started on single antidepressant treatment for the first time and who used antidepressant treatment for at least 6-8 weeks, were included in the study. Patients whose Hamilton Depression Rating Scale (HDRS), complete blood count, C reactive protein and cortisol values were reached from the system during MDD treatment, constituted the sample of the study.

**Results:** In the present study, after the antidepressant treatment, while HDRS scores decreased significantly in patients with Major Depression compared to before treatment (p<0,001), no significant correlation was found between the changes in inflammatory parameters and the response to treatment (p>0,05). This condition was independent of the type of antidepressant used in the treatment (p>0,05 in the SSRI treatment group, p>0,05 in the SNRI treatment group). In addition, it was observed that the decrease in depression scores was not associated with the type of antidepressant (p=0,001, in the SSRI treatment group, p=0,005, in the SNRI treatment group).

**Conclusion:** Results to support the inflammatory hypothesis in Major Depressive Disorder were not conclusive in this study. Considering that the pathophysiology of depression is quite complex, it could be argued that a single group of blood tests may not be sufficient to explain the link between inflammation and depression. Considering all the limitations of the study, a future a prospective study to prove the inflammatory hypothesis in MDD, including the detailed blood, BOS tests, along with more comprehensive neuroimaging parameters on the brain pathways, might provide more effective results.

Keywords: major depressive disorder, inflammation, inflammatory parameters, antidepressant treatment, antidepressant type, treatment response

## ÖZ

**Amaç:** Depresyonda meydana gelen sitokinler, stres hormonları ve C reaktif protein gibi inflamatuvar parametrelerin değişimi, depresyonun patofizyolojisinin anlaşılması ve yeni tedavi yaklaşımlarının geliştirilmesi açısından önem taşımaktadır. Bu çalışmanın temel amacı, major depresyon hastalarında antidepresan tedavi öncesi ve sonrasında inflamatuvar belirteçlerde nasıl bir değişiklik olduğunu tespit etmek ve antidepresan tedavi türünün bu değişiklikler üzerindeki etkisini belirlemektir.

**Yöntem:** Tek merkezli, retrospektif olarak gerçekleştirilen çalışmada, Alanya Alaaddin Keykubat Üniversitesi Eğitim ve Araştırma Hastanesi psikiyatri polikliniğinde son beş yıla ait geriye dönük incelenen kayıtlara göre, Majör Depresif Bozukluk (MDB) tanısı konulup, ilk kez tekli antidepresan tedavisine başlanan ve en az 6-8 hafta antidepresan tedavi kullanmış, tedavi öncesi ve sonrası Hamilton Depresyon Derecelendirme Ölçeği (HDDÖ), tam kan sayımı, C reaktif protein, kortizol değerlerine ulaşılabilen hastalar çalışmanın örneklemini oluşturmuştur.

**Bulgular:** Çalışmamızda, antidepresan tedavi sonrası, HDDÖ puanları Majör Depresyonlu hastalarda tedavi öncesine göre anlamlı olarak azalırken (p<0,001), inflamatuar parametrelerdeki değişiklikler ile tedaviye yanıt arasında anlamlı bir ilişki belirlenmemiştir (p>0,05). Bu durum, tedavide kullanılan antidepresan ilaç türünden bağımsızdır (SSRI tedavi grubunda p>0,05, SNRI tedavi grubunda p>0,05). Ayrıca depresyon puanlarındaki azalmanın da, antidepresan ilaç türü (SSRI-SNRI) ile ilişkili olmadığı belirlenmiştir (SSRI tedavi grubunda, p=0,001, SNRI tedavi grubunda, p=0,005).

**Sonuç:** Çalışmamızda, MDB'de inflamatuar hipotezi destekleyecek sonuçlar belirlenmemiştir. Depresyonun patofizyolojisinin oldukça karmaşık olduğu düşünüldüğünde, inflamasyon ve depresyon arasındaki bağlantıyı açıklamak için yalnızca bir grup kan testinin yeterli olmayabileceği düşünülebilir. Çalışmamızdaki tüm sınırlılıklar göz önünde bulundurulduğunda, gelecekte daha kapsamlı kan, BOS testleri ile beraber beyin yolaklarına ilişkin nörogörüntüleme parametrelerini de içeren prospektif çalışmalar, MDB'de inflamatuar hipotezi kanıtlamak için daha etkili sonuçlar sağlayabilir.

Anahtar sözcükler: major depresif bozukluk, inflamasyon, inflamatuar parametreler, antidepresan tedavi, antidepresan türü, tedavi yanıtı

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## INTRODUCTION

According to the World Health Organization, depression is the leading cause of disability and worldwide, in excess of 350 million people are affected [1]. Major Depressive Disorder (MDD) is the most severe among the depressive disorder subgroups and it is conventionally treated with antidepressants. Despite numerous treatment options, complete remission cannot be achieved in many patients with major depression [1]. In recent years, researchers have focused on identifying the reasons for the unresponsiveness of MDD therapy. The inflammation and inflammatory responses play a role in depression and its pharmacological treatment. Psychosocial stress reveals an immune response that ends in inflammation [2]. Increased inflammation increases the probability of developing depression [3]. This hypothesis has been based on the idea that chronic inflammation may contribute to serotonergic, noradrenergic and dopaminergic dysfunction [4].

Historically, the "monoamine-depletion hypothesis" pointed to an imbalance, principally between serotonergic and noradrenergic neurotransmission, however this suggestion offers no explanation why current antidepressants are not helpful for a number of patients. It has been suggested that depression relapses and lack of therapeutic benefits from antidepressants might be associated with overall activation of the inflammatory response [5]. Therefore, immune dysregulation or chronic inflammation might be present in resistance to therapy of MDD [6]. In this context, cerebrospinal fluid (CSF) and inflammatory blood markers have been detected. Increased levels of pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), interleukin-1 beta (IL-1 β), and C reactive protein (CRP) are found in MDD patients [7-11].

Previous studies with small samples showed that the neutrophil lymphocyte ratio (NLR), a common biomarker for systemic inflammatory condition, is associated with MDD and that NLR is significantly increased in Major Depression patients, compared to healthy controls [12]. In a study by Kasama et al., high NLR levels were found to be related with increased oxidative stress and inflammatory cytokines [13]. However, the relationship between major depression and inflammation remains problematic, notwithstanding the fact that a great proportion of patients show no significant inflammation sign [14].

In light of the existing data suggesting a possible link between inflammation and depression, the main purpose of this study was to determine the changes in inflammatory markers in patients with major depression, before and after antidepressant treatment, and to determine the effects of antidepressant treatment types on these changes. Studies in this field are important in understanding the pathophysiology of depression and developing new treatment approaches.

## MATERIAL AND METHODS

### Ethics committee approval

Ethics committee approval of the study was obtained from Alanya Alaaddin Keykubat University Faculty of Medicine Clinical Research Ethics Committee, with the decision number 20-26 dated 18.06.2020.

### Sample Selection and Study Design

This study is a single-center, retrospective study. Routine tests such as hemograms are performed for patients who are referred to the psychiatry outpatient clinic by other departments, or who will be prescribed medication for the first time and are repeated in the controls, as a result of possible side effect of the drug. Following a retrospective examination of the records of the last five years from the psychiatry outpatient clinic of Alanya Alaaddin Keykubat University (ALKU) Training and Research Hospital (TRH), the patients diagnosed with Major Depressive Disorder (MDD), who were started on a single antidepressant treatment for the first time and used antidepressant treatment for at least 6-8 weeks, were included in the study. The records of a total of twenty-four MDD patients, three males and twenty-one females, between the ages of 18-41, were examined for the purpose of the study. Patients whose Hamilton Depression Rating Scale (HDRS), C reactive protein (CRP), cortisol, and complete blood count [Lymphocyte, neutrophil, platelet, neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), red cell

distribution width (RDW), immunoglobulin (IG)] values were reached during MDD treatment, constituted the sample of the study.

Patients with a history of autoimmune disease that may affect inflammatory parameters, a history of hematologic diseases, chronic inflammatory diseases, leukocyte value suggestive of infection, those using antidepressant drugs and those with diagnoses of alcohol or substance addiction, mental retardation and organic brain disease, were all excluded from the study.

Despite diligent efforts, differences may be found in some characteristics in independent groups. In biological subjects, including human patients, the variations in traits such as height, body weight and other factors, are very pronounced. As a result of these disadvantages of working with independent groups, the present study was designed as a "before-after" type: the inflammatory values of patients with major depressive disorder, before and after antidepressant treatment, were compared. The patients' antidepressant agents (SSRI or SNRI), age and gender were also recorded.

Data Collection Tools

Hamilton Depression Rating Scale (HDRS): HDRS administered by the clinician, aims to measure the severity of depressive symptoms. It was developed by Hamilton et al. in 1960 [15]. The Turkish validity and reliability study conducted by Akdemir et al. in 1996 [16].

Statistical Analysis

Number, minimum, maximum and median values were used as descriptive statistical methods in the evaluation of the data. The Related-Samples Wilcoxon Signed Ranks Test was used to compare the variables forming two dependent groups. The data was evaluated with the SPSS 22,0 statistical program and the level of $p \leq 0,05$ was accepted for statistical significance [17].

## RESULTS

The inflammatory parameter values and Hamilton Depression Rating Scale scores of the Major Depressive Disorder patients, before and after antidepressant treatment, as well as their sociodemographic characteristics are shown in Table 1. The study sample consisted of 24 Major Depressive Disorder patients, aged between 18 and 41. The median age of the MDD patients group was 33,50 consisting of 21 female and 3 male. While 13 patients were on SSRI therapy, 11 patients received SNRI therapy. The comparison of inflammatory parameter values and depression scores of MDD patients, again before and after antidepressant treatment, is also shown in Table 1. The median of the CRP level was 0,100 mg / L. before the antidepressant treatment, it was 0,200 mg / L. after the treatment ($p = 0,074$). The median of the cortisol level was 8,08 mcg / dL. before the antidepressant treatment, it was 7,47 mcg / dL. after the treatment ($p = 0,511$). The median of the Neutrophil level was 4625 -10^3/uL. before the antidepressant treatment, it was 4280 -10^3/uL. after the treatment ($p = 0,511$). The median of the Lymphocyte level was 2570 -10^3/uL. before the antidepressant treatment, it was 2180 -10^3/uL. after the treatment ($p = 0,284$). The median of the Platelet level was 283000 -10^3/uL. before the antidepressant treatment, it was 279000 -10^3/uL. after the treatment ($p = 0,148$). The median of the RDW-CV was 13,50%, before the antidepressant treatment, it was 13,30% after the treatment ($p = 0,807$). The median of the RDW-SD was 39,35%, before the antidepressant treatment, it was 39,35% after the treatment ($p = 0,807$). The median of the IG was 0,025 -10^3/uL before the antidepressant treatment, it was 0,020 -10^3/uL. after the treatment ($p = 0,761$). The median of the Neutrophil-Lymphocyte ratio was 2,12 before the antidepressant treatment, it was 1,87 after the treatment ($p = 0,932$). The median of the Platelet-Lymphocyte ratio was 123,44 before the antidepressant treatment, it was 120,22 after the treatment. ($p = 0,549$).

The median of the Hamilton Depression Rating Scale points was 20,00 before the antidepressant treatment, and it was 8,00 after the treatment ($p < 0,001$, Related-Samples Wilcoxon Signed Ranks Test, Table 1).

In the study, two groups were created according to the antidepressant treatment types: SSRI treatment group and SNRI treatment group. The comparison of inflammatory parameter values and depression scores of Major Depressive Disorder patients before and after antidepressant

treatment in the SSRI treatment group, is shown in Table 2. In the SSRI treatment group: The median of the CRP level was 0,100 mg / L. before the antidepressant treatment, it was 0,100 mg / L. after the treatment (p = 0,527). The median of the cortisol level was 9,00 mcg / dL. before the antidepressant treatment, it was 7,56 mcg / dL. after the treatment (p = 0,345). The median of the Neutrophil level was 4730 -10^3/uL. before the antidepressant treatment, it was 4170 -10^3/ uL. after the treatment (p = 0,221). The median of the Lymphocyte level was 2590 -10^3/uL. before the antidepressant treatment, it was 2140 -10^3/ uL. after the treatment (p = 0,101). The median of the Platelet level was 293000 -10^3/uL. before the antidepressant treatment, it was 277000 -10^3/uL. after the treatment (p = 0,158). The median of the RDW-CV was 12,70%, before the antidepressant treatment, it was 13,00% after the treatment (p = 0,582). The median of the RDW-SD was 41,10%, before the antidepressant treatment, it was 39,20% after the treatment (p = 0,347). The median of the IG was 0,020 -10^3/uL before the antidepressant treatment, it was 0,020 -10^3/ uL. after the treatment (p = 0,357). The median of the Neutrophil-Lymphocyte ratio was 2,15 before the antidepressant treatment, it was 1,89 after the treatment (p = 0,889). The median of the Platelet-Lymphocyte ratio was 105,39 before the antidepressant treatment, it was 132,63 after the treatment (p = 0,701).

The median of the Hamilton Depression Rating Scale points was 21,00 before the antidepressant treatment, and it was 9,00 after the treatment in the SSRI treatment group (p = 0,001, Related-Samples Wilcoxon Signed Ranks Test, Table 2).

The comparison of inflammatory parameter values and depression scores of Major Depressive Disorder patients before and after antidepressant treatment in the SNRI treatment group, is shown in Table 3. In the SNRI treatment group: The median of the CRP level was 0,100 mg / L. before the antidepressant treatment, it was 0,300 mg / L. after the treatment (p = 0,068). The median of the cortisol level was 7,62 mcg / dL. before the antidepressant treatment, it was 7,38 mcg / dL. after the treatment (p = 0,859). The median of the Neutrophil level was 4520 -10^3/uL. before the antidepressant treatment, it was 4660 -10^3/ uL. after the treatment (p = 0,722). The median of the Lymphocyte level was 2550 -10^3/uL. before the antidepressant treatment, it was 2270 -10^3/ uL. after the treatment (p = 0,824). The median of the Platelet level was 271000 -10^3/uL. before the antidepressant treatment, it was 281000 -10^3/uL. after the treatment (p = 0,328). The median of the RDW-CV was 13,70%, before the antidepressant treatment, it was 13,70% after the treatment (p = 0,327). The median of the RDW-SD was 38,80%, before the antidepressant treatment, it was 39,40% after the treatment (p = 0,534). The median of the IG was 0,030 -10^3/uL. before the antidepressant treatment, it was 0,020 -10^3/ uL. after the treatment (p = 0,119). The median of the Neutrophil-Lymphocyte ratio was 2,12 before the antidepressant treatment, it was 1,53 after the treatment (p = 0,965). The median of the Platelet-Lymphocyte ratio was 131,60 before the antidepressant treatment, it was 105,15 after the treatment (p = 0,286).

The median of the Hamilton Depression Rating Scale points was 20,00 before the antidepressant treatment, and it was 7,00 after the treatment in the SNRI treatment group (p = 0,005, Related-Samples Wilcoxon Signed Ranks Test, Table 3).

## DISCUSSION

In this study, it was aimed to evaluate the changes in inflammatory markers along with depression scores during the treatment of Major Depressive Disorder and to determine the effect of antidepressant type on these changes. Although it was found that Hamilton Depression Rating Scale scores decreased significantly after antidepressant treatment in patients with Major Depression, there was no significant relationship between inflammatory parameters and treatment response. This condition was independent of the type of antidepressant used in the treatment (SSRI or SNRI). In addition, it was observed that the decrease in depression scores was not associated with the type of antidepressant (SSRI or SNRI).

There are inconsistencies in studies investigating changes in inflammation in Major Depressive Disorder in the literature. Many studies show the significant association between depression and inflammation [18-22]. A recent cumulative meta-

**Table 1. Sociodemographic characteristics, inflammatory parameter values and Hamilton Depression Rating Scale scores of Major Depressive Disorder patients and the comparison of inflammatory parameter values and depression scores before and after antidepressant treatment**

| | | Min. | Max. | N / | Z | p |
|---|---|---|---|---|---|---|
| Median | | | | | | |
| N: 24 | Age | 18 | 41 | 33,50 | | |
| Gender | Female | | | 21 | | |
| | Male | | | 3 | | |
| AD Treatment Type | SSRI | | | 13 | | |
| | SNRI | | | 11 | | |
| HDRS Score | Before Treat. | 12 | 27 | 20,00 | -4,205 | <0,001 |
| | After Treat. | 4 | 20 | 8,00 | | |
| CRP (mg/L.) | Before Treat. | 0,00 | 1,3 | 0,100 | -1,788 | 0,074 |
| | After Treat. | 0,00 | 1,7 | 0,200 | | |
| Cortisol (mcg/dL.) | Before Treat. | 3,73 | 20,84 | 8,08 | -0,657 | 0,511 |
| | After Treat. | 3,63 | 23,32 | 7,47 | | |
| Platelet (10^3/uL.) | Before Treat. | 207000 | 460000 | 283000 | -1,445 | 0,148 |
| | After Treat. | 192000 | 378000 | 279000 | | |
| Lymphocyte (10^3/uL.) | Before Treat. | 1490 | 3420 | 2570 | -1,071 | 0,284 |
| | After Treat. | 1390 | 3530 | 2180 | | |
| Neutrophil (10^3/uL.) | Before Treat. | 1980 | 7960 | 4625 | -0,657 | 0,511 |
| | After Treat. | 1940 | 8410 | 4280 | | |
| RDW-CV (%) | Before Treat. | 11,8 | 24,6 | 13,50 | -0,244 | 0,807 |
| | After Treat. | 12,0 | 22,3 | 13,30 | | |
| RDW-SD (%) | Before Treat. | 34,5 | 62,3 | 39,35 | -0,304 | 0,807 |
| | After Treat. | 33,8 | 48,7 | 39,35 | | |
| IG (10^3/ uL.) | Before Treat. | 0,01 | 0,17 | 0,025 | -1,740 | 0,761 |
| | After Treat. | 0,01 | 0,15 | 0,020 | | |
| Neutrophil-Lymphocyte Ratio | Before Treat. | 1,15 | 3,82 | 2,12 | -0,086 | 0,932 |
| | After Treat. | 0,98 | 4,26 | 1,87 | | |
| Platelet-Lymphocyte Ratio | Before Treat. | 65,78 | 239,13 | 123,44 | -0,600 | 0,549 |
| | After Treat. | 75,35 | 209,52 | 120,22 | | |

**N: Number of samples, SD: Standard Deviation, Min: Minimum, Max: Maximum, Treat: Treatment, AD: Antidepressant, SSRI: Selective Serotonin Reuptake Inhibitor, SNRI: Serotonin and Noradrenaline Reuptake Inhibitor, CRP: C-reactive protein, HDRS: Hamilton Depression Rating Scale, Ig: Immunoglobulin, RDW: Red cell distribution width, CV: Coefficient of Variation, p: Statistical significance for Wilcoxon Signed Ranks Test, p ≤ 0,05**

**Table 2. The comparison of inflammatory parameter values and depression scores of Major Depressive Disorder patients before and after antidepressant treatment in the SSRI treatment group**

| | | Min. | Max. | N / | Z | p |
|---|---|---|---|---|---|---|
| | Median | | | | | |
| N: 13 | Age | 18 | 41 | 27,00 | | |
| Gender | Female | | | 11 | | |
| | Male | | | 2 | | |
| HDRS Score | Before Treat. | 12 | 26 | 21,00 | -3,192 | 0,001 |
| | After Treat. | 4 | 15 | 9,00 | | |
| CRP (mg/L.) | Before Treat. | 0,00 | 0,8 | 0,100 | -0,632 | 0,527 |
| | After Treat. | 0,00 | 1,1 | 0,100 | | |
| Cortisol (mcg/dL.) | Before Treat. | 4,77 | 20,84 | 9,00 | -0,943 | 0,345 |
| | After Treat. | 4,31 | 17,08 | 7,56 | | |
| Platelet (10^3/uL.) | Before Treat. | 207000 | 443000 | 293000 | -1,412 | 0,158 |
| | After Treat. | 192000 | 378000 | 277000 | | |
| Lymphocyte (10^3/uL.) | Before Treat. | 1490 | 2970 | 2590 | -1,642 | 0,101 |
| | After Treat. | 1390 | 2850 | 2140 | | |
| Neutrophil (10^3/uL.) | Before Treat. | 1980 | 7960 | 4730 | -1,223 | 0,221 |
| | After Treat. | 1940 | 7640 | 4170 | | |
| RDW-CV (%) | Before Treat. | 12,0 | 24,6 | 12,70 | -0,550 | 0,582 |
| | After Treat. | 12,0 | 17,5 | 13,00 | | |
| RDW-SD (%) | Before Treat. | 36,2 | 62,3 | 41,10 | -0,941 | 0,347 |
| | After Treat. | 34,8 | 48,7 | 39,20 | | |
| IG (10^3/ uL.) | Before Treat. | 0,01 | 0,04 | 0,020 | -0,921 | 0,357 |
| | After Treat. | 0,01 | 0,05 | 0,020 | | |
| Neutrophil-Lymphocyte Ratio | Before Treat. | 1,19 | 3,82 | 2,15 | -0,140 | 0,889 |
| | After Treat. | 0,98 | 3,32 | 1,89 | | |
| Platelet-Lymphocyte Ratio | Before Treat. | 78,98 | 239,13 | 105,39 | -0,384 | 0,701 |
| | After Treat. | 93,08 | 209,52 | 132,63 | | |

**N: Number of samples, SD: Standard Deviation, Min: Minimum, Max: Maximum, Treat: Treatment, SSRI: Selective Serotonin Reuptake Inhibitor, CRP: C-reactive protein, HDRS: Hamilton Depression Rating Scale, Ig: Immunoglobulin, RDW: Red cell distribution width, CV: Coefficient of Variation, p: Statistical significance for Wilcoxon Signed Ranks Test, p ≤ 0,05**

**Table 3. The comparison of inflammatory parameter values and depression scores of Major Depressive Disorder patients before and after antidepressant treatment in the SNRI treatment group**

| | | Min. | Max. | N / Median | Z | p |
|---|---|---|---|---|---|---|
| N: 11 | Age | 20 | 40 | 34,00 | | |
| Gender | Female | | | 10 | | |
| | Male | | | 1 | | |
| HDRS Score | Before Treat. | 13 | 27 | 20,00 | -2,805 | 0,005 |
| | After Treat. | 4 | 20 | 7,00 | | |
| CRP (mg/L.) | Before Treat. | 0,00 | 1,3 | 0,100 | -1,823 | 0,068 |
| | After Treat. | 0,00 | 1,7 | 0,300 | | |
| Cortisol (mcg/dL.) | Before Treat. | 3,73 | 16,98 | 7,62 | -0,178 | 0,859 |
| | After Treat. | 3,63 | 23,32 | 7,38 | | |
| Platelet (10^3/uL.) | Before Treat. | 209000 | 460000 | 271000 | -0,979 | 0,328 |
| | After Treat. | 201000 | 378000 | 281000 | | |
| Lymphocyte (10^3/uL.) | Before Treat. | 1680 | 3420 | 2550 | -0,222 | 0,824 |
| | After Treat. | 1970 | 3530 | 2270 | | |
| Neutrophil (10^3/uL.) | Before Treat. | 2110 | 6310 | 4520 | -0,356 | 0,722 |
| | After Treat. | 2890 | 8410 | 4660 | | |
| RDW-CV (%) | Before Treat. | 11,8 | 20,1 | 13,70 | -0,981 | 0,327 |
| | After Treat. | 12,2 | 22,3 | 13,70 | | |
| RDW-SD (%) | Before Treat. | 34,5 | 43,2 | 38,80 | -0,622 | 0,534 |
| | After Treat. | 33,8 | 47,7 | 39,40 | | |
| IG (10^3/uL.) | Before Treat. | 0,01 | 0,17 | 0,030 | -1,561 | 0,119 |
| | After Treat. | 0,01 | 0,15 | 0,020 | | |
| Neutrophil-Lymphocyte Ratio | Before Treat. | 1,15 | 3,07 | 2,12 | -0,044 | 0,965 |
| | After Treat. | 1,19 | 4,26 | 1,53 | | |
| Platelet-Lymphocyte Ratio | Before Treat. | 65,78 | 171,37 | 131,60 | -1,067 | 0,286 |
| | After Treat. | 75,35 | 166,51 | 105,15 | | |

**N: Number of samples, SD: Standard Deviation, Min: Minimum, Max: Maximum, Treat: Treatment, SNRI: Serotonin and Noradrenaline Reuptake Inhibitor, CRP: C-reactive protein, HDRS: Hamilton Depression Rating Scale, Ig: Immunoglobulin, RDW: Red cell distribution width, CV: Coefficient of Variation, p: Statistical significance for Wilcoxon Signed Ranks Test, p ≤ 0,05**

analysis has been shown that C reactive protein, IL-1, and IL-6 were positively associated with depression [23]. On the other hand, there are also many studies that did not find a relationship between inflammation and depression [24-26].

In the present study, changes in C reactive protein (CRP), a reliable marker of systemic inflammation during the MDD therapy were not associated with the response to treatment. In both the SSRI treatment group and the SNRI treatment group, there was no significant relationship between CRP levels and depression scores. In the study conducted by Uher et al., it was stated that the initial CRP levels could be an important factor in the choice of antidepressant treatment [27]. Individuals with low baseline CRP levels (< 1 mg. / L) have been shown to respond better to serotonergic antidepressants, and individuals with higher baseline CRP (> 1 mg. / L.) to noradrenergic antidepressants. The small sample size of the present study has not only prevented group formation according to the CRP levels, but may also have influenced findings of a significant relationship between CRP and different antidepressant types.

Studies investigating the changes in Neutrophil-Lymphocyte ratio (NLR) and Platelet-Lymphocyte ratio during MDD therapy have reported conflicting results. In the present study, changes in the NLR during antidepressant treatment were not associated with the response to treatment. In line with this study, no significant differences were found between major depression patients and healthy controls in terms of complete blood count levels, including NLR during MDD therapy, in a study carried out by Maes et al. [25]. On the other hand, Demircan et al.'s study

showed that compared to normal controls, NLR levels were accompanied by relief of depressive symptoms during the antidepressant treatment in MDD patients [20]. In a study investigating the relationship between NLR and geriatric depression in the elderly population in China, it was shown that increased NLR is associated with depression in young and middle-aged Chinese adult females. However, in the same study, the relationship of increased NLR with depressive symptoms in males could not be determined [28]. Changes in platelets, which are fractions produced by megakaryocytes, remain unclear in MDD. In the present study, changes in the Platelet-Lymphocyte ratio (PLR) during antidepressant treatment were also not associated with the response to treatment. Contrary to our results, in the study of Cai et al., the MDD group showed a significantly higher PLR compared to healthy controls [12]. The small sample size of the present study may be one of the factors that affected the findings of a significant relationship between NLR and PLR, with the response to treatment and depression scores. On the other hand, considering that the pathophysiology of depression is quite complex, it could be argued that a single group of blood tests may not be sufficient to explain the link between inflammation and depression.

In the literature, inconsistent results were found in studies investigating the relationship between antidepressant treatment response and depression severity with cortisol, one of the hypothalamus-pituitary-adrenal (HPA) axis hormones secreted in response to stress. Some studies have found significant changes in serum cortisol levels, before and after antidepressant therapy [29]. In the present study there was no relationship between the changes in cortisol levels and treatment response during MDD therapy. Consistent with this study, the findings by Alenko et al. did not show an association between serum cortisol levels and treatment response to SSRI [30]. There are many parameters in the measurement of cortisol and other stress hormones that are very difficult to control, such as sample collection time and exercise. The fact that this study was retrospective may have resulted in deficiencies in the details that may affect cortisol levels, therefore a significant result may not be found in the relationship between these and antidepressant treatment response.

In general, the present study did not reveal a relationship between changes in inflammatory parameters and antidepressant treatment response, as well as the type of antidepressant used in the treatment. In line with these results, a recent work using network psychometrics to examine the existing data from the database of the Netherlands Study of Depression and Anxiety, found no direct link between depression and inflammation. Nevertheless, this study has revealed important insights into the effects of individual symptoms and lifestyle factors [24]. Conflicting results have been obtained in studies conducted to understand the effects of anti-inflammatory and antidepressant agents on depression and inflammation. In a randomized study performed by Raison et al., with treatment-resistant depression patients, Anti-Tumor Necrosis Factor (TNF) infliximab or a saltwater placebo revealed no differences in terms of their antidepressant effect. Nevertheless, the inflammation group did respond to infliximab [26]. Many biological studies suggest that inflammatory mediators might have divergent effects on brain regions with different functions such as motivation, reward, fear, anxiety and mood regulation. However, the idea that anti-inflammation therapy might relieve depression symptoms has not been consistently demonstrated with clinical studies [31, 32].

Limitations of the Study:

Despite the interesting findings, the present study had some limitations. First, the sample size available was small. Second, this study only examined some specific inflammation related hematological parameters, C-reactive protein and cortisol. It may be useful if more sensitive inflammatory markers such as highly sensitive CRP or interleukins, were used to investigate the relationship between depression and inflammation, namely since those inflammatory markers could also reveal the subclinical (low grade) inflammation. Third, there was no healthy control group used to allow further comparison. Finally, the present study was designed as a retrospective study and as with all non-prospective designs, a causal relationship cannot be established with the results that have been obtained.

## CONCLUSION

In conclusion, our understanding of the immunology underlying the inflammation in depression is limited to a small number of human studies [33]. For instance, despite the question being asked repeatedly, there yet remains no explicit knowledge about the percentage of patients suffering with depression, in whom inflammation plays a pivotal role. Results to support the inflammatory hypothesis in Major Depressive Disorder were not conclusive in this study. Considering all the limitations in the study and in order to prove the inflammatory hypothesis in Major Depressive Disorder, a future prospective study including detailed blood, BOS tests, along with more comprehensive neuroimaging parameters on the brain pathways, might provide more conclusive results.

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# Evaluation of the Effect of Cryotherapy in Limbal Conjunctival Autograft Technique in Pterygium Surgery

## Pterjium Cerrahisinde Limbal Konjonktival Otogreft Tekniğinde Kriyoterapinin Etkinliğinin Değerlendirilmesi

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## ABSTRACT

**Aim:** To compare the complication and recurrence rates in patients undergoing limbal conjunctival autograft with or without adjuvant cryotherapy for primary or recurrent pterygia.

**Methods:** All information about the cases operated between January 2014 and January 2019 was scanned from the electronic database. Fifty-three eyes undergoing limbal conjunctival autograft combined with cryotherapy were classified as Group 1, and 47 eyes undergoing only limbal conjunctival autograft were classified as Group 2. Any development of new fibrovascular tissue from the limbus to the cornea after surgery was considered a recurrence. Any complications occurring during and after surgery were noted.

**Results:** In Group 1, 45 of the 53 patients had primary and eight had recurrent pterygia. In Group 2, 41 of the 47 patients had primary and six had recurrent pterygia. Both groups were similar in terms of age and gender (p=0.880 and p=0.835, respectively). The mean follow-up period was 27.0±8.6 months in Group 1 and 28.7±7.8 months in Group 2 (p=0.287). No recurrences were observed during the follow-up period in Group 1, while the recurrence rate in Group 2 was 10.6% (p=0.02). All relapses occurred within the first year. No complications were observed during surgery in either group. Graft oedema was observed in the early post-operative period in four patients in Group 1 and three patients in Group 2.

**Conclusion:** Limbal conjunctival graft technique combined with cryotherapy is a successful and reliable method in the treatment of primary and recurrent pterygium.

Keywords: cryotherapy, limbal conjunctival autograft, pterygium

## ÖZ

**Amaç:** Primer veya tekrarlayan pterjiumda adjuvan kriyoterapi ile birlikte veya tek başına limbal konjonktival otogreft uygulanan hastalarda komplikasyon ve nüks oranlarını karşılaştırmak.

**Metot:** Ocak 2014 ile Ocak 2019 tarihleri arasında ameliyat edilen vakalarla ilgili tüm bilgiler elektronik veri tabanından tarandı. Kriyoterapi ile kombine olarak limbal konjonktival otogreft uygulanan 53 göz Grup 1, sadece limbal konjonktival otogreft uygulanan 47 göz Grup 2 olarak sınıflandırıldı. Ameliyattan sonra limbustan korneaya herhangi bir yeni fibrovasküler doku gelişimi nüks olarak kabul edildi. Ameliyat sırasında ve sonrasında ortaya çıkan herhangi bir komplikasyon not edildi.

**Bulgular:** Grup 1'de 53 hastanın 45'inde primer ve sekizinde tekrarlayan pterjium vardı. Grup 2'de 47 hastanın 41'inde primer ve altısında tekrarlayan pterjium vardı. Her iki grup yaş ve cinsiyet açısından benzerdi (p=0.880 ve p=0.835; sırasıyla). Ortalama takip süresi Grup 1'de 27.0±8.6 ay, Grup 2'de 28.7±7.8 aydı (p=0.287). Grup 1'de takip döneminde nüks görülmezken, Grup 2'de nüks oranı % 10,6 idi. (p=0.02). Tüm nüksler ilk yıl içinde gerçekleşti. Her iki grupta da ameliyat sırasında herhangi bir komplikasyon görülmedi. Grup 1'de 4, Grup 2'de 3 hastada erken postoperatif dönemde greft ödemi görüldü.

**Sonuç:** Kriyoterapi ile kombine edilerek yapılan limbal konjonktival greft tekniği primer ve rekürren pterjium tedavisinde başarılı ve güvenilir bir yöntemdir.

Anahtar Kelimeler: kriyoterapi, limbal konjonktival otogreft, pterjiyum

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## INTRODUCTION

A pterygium is a conjunctival pathology with an unclear aetiology, manifesting as fibrovascular proliferation of the bulbar conjunctiva towards the cornea. Although it has a good prognosis, surgical removal is recommended if it extends to the visual axis, which may threaten visual acuity and cause astigmatism or frequent inflammation and discomfort [1].

Many surgical techniques, such as bare sclera, conjunctival autograft, limbal conjunctival autograft, amniotic membrane, and conjunctival trans positional flap, have been described in pterygium surgery [1]. The most important problem after surgery is recurrence. Today, conjunctival autograft and limbal conjunctival autograft are considered the most effective techniques [1].

However, even after conjunctival autograft surgery, studies have reported recurrence rates ranging between 3.3% and 14.6% [2-4]. Therefore, various adjuvant therapies, including mitomycin-C, 5-fluorouracil, anti-vascular endothelial growth factor, radiation, and cryotherapy, have been recommended to prevent recurrence [5,6]. In addition, it has been reported that the use of topical cyclosporine before and after surgery reduces the development of recurrence [7].

Cryotherapy as an adjuvant treatment was first described in a case series by Fraunfelder in 2008 [6]. The purpose of cryotherapy is to induce ice crystal formation in intracellular and extracellular areas, thereby causing organelle damage, local vasoconstriction, thrombosis, and cell death by ischemia and immune mechanisms, by freezing living tissue [8,9]. Fraunfelder suggested that cryotherapy could reduce the recurrence rate by preventing abnormal cell proliferation after primary pterygium surgery [6].

The aim of this study, therefore, was to compare the recurrence and complication rates in primary and recurrent pterygium cases undergoing limbal conjunctival autograft with and without adjuvant cryotherapy.

## MATERIAL AND METHOD

The study included 100 eyes of 100 patients of the same ethnic origin who underwent surgery for primary or recurrent pterygia between January 2014 and January 2019. Fifty-three patients who undergo limbal conjunctival autograft combined with cryotherapy were classified as Group 1, and 47 patients who underwent only limbal conjunctival autograft were classified as Group 2. Ocular surface diseases, keratoconjunctivitis sicca, dry eye, ocular pemphigoid, limbal stem cell deficiency, previous ocular surgery and systemic diseases and drugs that affect ocular surface were excluded from the study. Patients who had glaucoma or suspected malignancy and findings consistent with malignancy on post-operative histopathological examination and patients with a follow-up period of less than one year were also excluded. Pterygium grading was performed in all patients. All study participants signed informed consent forms prior to surgery. This study was conducted in accordance with the principles of the Declaration of Helsinki. Ethics approval was obtained from the Diyarbakır Gazi Yaşargil Research and Training Hospital ethic committee.

### Surgical Method

Before surgery, local anaesthesia was induced with proparacaine hydrochloride drops, and local site cleaning was performed with 10% povidone iodine solution. The ocular surface was washed with 5% povidone iodine. After three minutes, the eye was irrigated with saline solution. Conjunctival local anaesthesia was induced by a subconjunctival injection of lidocaine containing 1 ml of 2% epinephrine in the pterygium area. The pterygium body was separated from the underlying conjunctiva by blunt dissection. The pterygium head was separated from the cornea towards the limbus using a blunt-tip knife. The procedures followed thus far were identical in both groups.

In Group 2, immediately after these procedures, graft tissue of the same size as the conjunctival defect obtained after excision of pterygium tissue was prepared from the superior temporal conjunctiva of the same eye to the limbal area. Care was taken to completely separate the graft from the Tenon tissue. After the limbus part of the graft was placed to coincide with the limbus part of the open sclera, the graft was sutured to the conjunctiva one by one with vicryl 8-0 suture.

In Group 1, cryotherapy was performed using a

Cryo-S Electric II (Metrum Cryoflex, Poland) device after excision of the pterygium tissue. The Cryo-S Electric is a cryosurgery device that uses nitrous oxide (N2O) as a cooling medium. N2O expands in the cryoprobe, producing a temperature as low as −89°C. The cryotherapy procedure was performed by contacting an approximately 3-mm cryoprobe with the corneoscleral limbus for one–two seconds (fig 1). The procedure was applied one or more times using a double freeze-thaw technique depending on the size of the lesion, with intervals of approximately 30 seconds between freeze applications. Cryotherapy was then also applied to the margins of the rest of the conjunctiva (fig 2). After that, using the same technique as in Group 2, a limbal conjunctival autograft taken from the superior temporal conjunctiva was placed in the open sclera and sutured to the conjunctiva one by one with vicryl 8-0 suture. In both groups, the pterygium tissue removed was sent for histopathological examination.

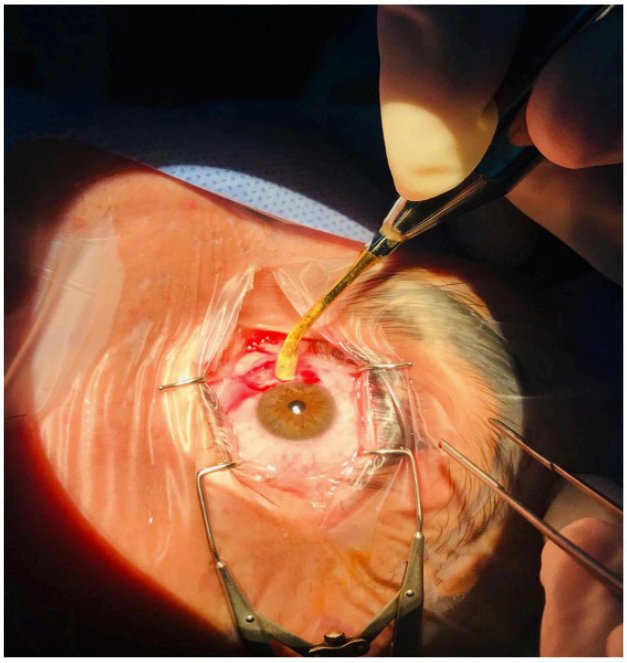

Figure 1. Contacting the cryoprobe to the corneoscleral limbus.

All eyes were kept closed for 24 hours by applying a tight bandage with an antibiotic ointment. Moxifloxacin 4×1 and topical corticosteroid fluorometholone 3×1 were applied fifteen days after surgery. Preservative-free artificial eye drops, polyvinyl alcohol povidone 6×1, and antibiotic ointment oxytetracycline 2×1 were used for one month after surgery. All cases were followed up for at least one year to monitor recurrences and complications. Any fibrovascular tissue development from the limbus to the cornea was considered a recurrence.

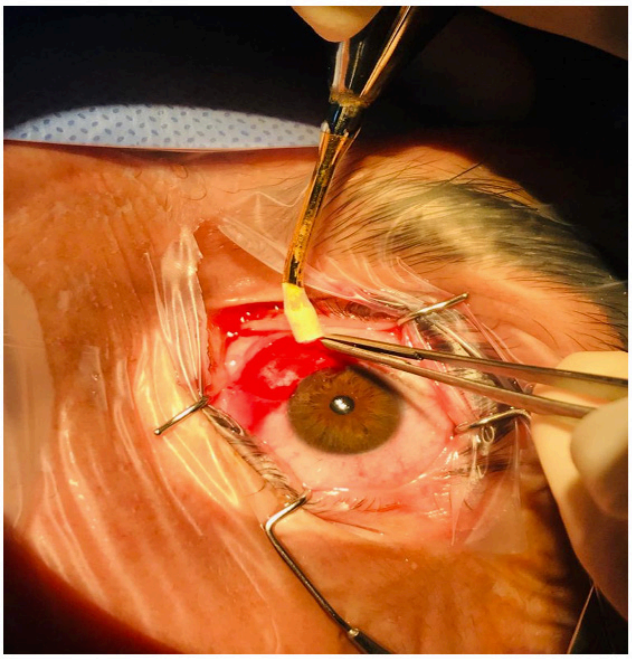

Figure 2. Cryotherapy application to the remaining conjunctival tissue.

## Statistical Analysis

Statistical analysis was performed using SPSS 20.0 (Statistical Package for the Social Sciences, IBM, NY, USA). The normality of data distribution was checked with the Kolmogorov-Smirnov test. Comparisons between the two groups were performed with the independent t-test, Pearson's chi-square test, or Fisher's exact test. Descriptive statistics were expressed as means ± standard deviations (minimum-maximum) for numerical variables and as numbers and percentages for categorical variables. A value of $p < 0.05$ was considered statistically significant.

## RESULTS

The groups were similar in terms of age and gender ($p = 0.880$ and $p = 0.835$, respectively; Table 1). In Group 1, 45 of the 53 patients had primary and eight had recurrent pterygia. In Group 2, 41 of the 47 patients had primary and six had recurrent pterygia. There was no statistical difference between groups in pterygium grading ($p = 0.879$). In all cases, histopathological examination was consistent with pterygium.

**Table 1. Distribution of demographic and clinical characteristics in the two groups**

| Variable | Group 1 (n = 53) | Group 2 (n = 47) | P |
|---|---|---|---|
| Age (years), mean ± SD | 47.4 ± 14.8 | 47.0 ± 13.3 | 0.880* |
| Gender (female/male) | 17/36 | 16/31 | 0.835† |
| Follow-up period (months), mean ± SD | 27.0 ± 8.6 | 28.7 ± 7.8 | 0.287* |
| Recurrences, n (%) | 0 (0) | 5 (10.6) | 0.02‡ |

Group 1: limbal conjunctival autograft combined with cryotherapy
Group 2: limbal conjunctival autograft only
*Independent t-test; †Pearson's chi-square test; ‡Fisher's exact test
SD: standard deviation

The mean follow-up time was 27.0 ± 8.6 months (12–42 months) in Group 1 and 28.7 ± 7.8 months (13–40 months) in Group 2 (p = 0.287). No recurrences were observed during the follow-up period in Group 1. In Group 2, recurrences were observed in five (10.6%) patients (p = 0.02), of whom two had primary and three had recurrent pterygia. The median recurrence time was five months (three–nine months). None of these patients accepted a second surgical procedure.

No complications were observed during surgery in either group. Graft oedema was observed in the early post-operative period in four cases in Group 1 and three cases in Group 2. In all cases, it was relieved by intensive topical steroid therapy.

## DISCUSSION

Cryotherapy induces cell death by six therapeutic mechanisms, including cryodestruction, cryoadhesion, cryoextraction, cryostripping, and cryoobliteration [10,11]. Today, it is widely and safely used in many medical fields, such as gynaecology, dermatology, proctology, phlebology, and ophthalmology.

Cryotherapy was first used in ophthalmology by Bietti in 1933 for the treatment of retinal hollows [12]. It was later used for the treatment of many retinal diseases, such as retinal detachment, retinopathy of prematurity, and retinoblastoma, glaucoma, conjunctival diseases, and benign and malignant lesions in the eyelid [13-18].

When cryotherapy is mentioned in the treatment of conjunctival diseases, the first thing that comes to mind is malignant lesions in the conjunctiva. After surgical excision of the conjunctival malignant lesion, small tumour residues at the surgical margins causing recurrence are eliminated by cryotherapy applied to the surgical margins. Although pterygium is considered a degenerative disease of the conjunctiva, pterygium tissue may exhibit tumour-like properties [19].

Based on these tumour-like properties of the pterygium, Fraunfelder suggested that cryotherapy could be used as an adjuvant treatment to prevent recurrence after pterygium surgery, claiming that it can directly induce cytotoxicity and apoptosis in proliferating epithelial stem cells, which are believed to cause recurrences [6]. He also suggested that the elimination of the microvascular structure in pterygium tissue with a rich vascular structure by cryoobliteration may be effective [6]. Fraunfelder first presented a case series of 24 patients, 18 of whom had primary and six had recurrent pterygia, undergoing liquid nitrogen cryotherapy after surgical excision of pterygium tissue [18]. After excision, the author made a 2-mm liquid nitrogen cryoprobe contact with the corneoscleral limbus several times (using a double freeze-thaw technique) for approximately one second. He then sutured the conjunctiva primarily over the limbus. In the primary pterygium group, within a median follow-up of 24.5 months, one case reported a recurrence nine months after surgery (recurrence rate: 5.5%). In the recurrent pterygium group, the median follow-up period was 27 months, and four of six patients reported recurrences after an average of 3.3 months. The author recommended liquid nitrogen cryotherapy as adjuvant to primary pterygium surgery but reported that it was unsuccessful in recurrent pterygium treatment [18]. However, he performed surgical excision and cryotherapy for a second time in a patient in the recurrent pterygium group who developed a recurrence and observed no recurrence after the second procedure [18].

In our study, no recurrence was observed during the follow-up period in any patients with primary or recurrent pterygia undergoing cryotherapy. However, a few differences between our and Fraunfelder's study should be noted. First, Fraunfelder preferred to close the conjunctiva by suturing following primary excision after cryotherapy, whereas in this study, we applied cryotherapy combined with limbal conjunctival

autograft surgery. Primary excision is a simple and fast surgical procedure, but degenerative cells may proliferate and lead to a recurrence as a result of insufficient excision of subconjunctival fibrovascular tissue [20]. In limbal conjunctival graft surgery, it is assumed that limbal stem cells can provide faster healing of the wound site and reconstruct it anatomically [20]. Furthermore, in this study, we compared recurrence rates between patients undergoing limbal conjunctival autograft and cryotherapy and patients undergoing limbal conjunctival autograft only. Although it has been reported that limbal conjunctival graft application decreases the recurrence rates after pterygium surgery, the literature suggests that it does not eliminate the risk [2-4]. In our study, the recurrence rate was 10.6%. The fact that no recurrences were observed in Group 1 demonstrates the effectiveness of cryotherapy as an adjuvant treatment.

Another difference between our and Fraunfelder's study is that the latter used liquid nitrogen as a cryogen, whereas we used N2O. Various cryogens are used in cryosurgery. In ophthalmology, freon (−29.8°C to −40.8°C), N2O (−89°C), and solid carbon dioxide (−79°C) are preferred [18]. Liquid nitrogen (−196°C) has a very low boiling point and causes cell destruction by inducing very fast freezing. Because of this property, liquid nitrogen cryotherapy is mostly used in the treatment of eye and eyelid tumours [17,21]. N2O cryotherapy is also successfully used for the same purpose [22,23]. However, the biological effects of the various cryogens on ocular tissues are not fully known [18]. Our results show that N2O cryotherapy is as successful as liquid nitrogen cryotherapy as a therapy adjuvant to pterygium surgery.

A third difference between our and Fraunfelder's study is the area where cryotherapy was applied. Based on the assumption that recurrences are caused by epithelial stem cells in the limbal region, Fraunfelder applied cryotherapy only to the corneoscleral limbal region [24]. In our study, in addition to the corneoscleral limbus, cryotherapy was applied to the margins of the remaining conjunctiva after primary excision. Thus, degenerated fibrovascular cells at the conjunctival surgical margin were eliminated. We believe that this technique can be effective in preventing recurrence even in recurrent pterygium cases.

In terms of complications during and after surgery, the results were similar in the two groups. No complications occurred during surgery in either group, and the only complication in the early post-operative period was graft oedema.

Limitations

The main limitation of our study is that it is a retrospective study and has a short follow-up period. We included cases with a follow-up period of at least one year, as it is known that most recurrences after pterygium surgery are seen within the first year [25]. This is consistent with the fact that the five recurrence cases in our study were seen in the first year and were all cases where only limbal conjunctival grafts had been applied.

## CONCLUSION

In conclusion, we believe that cryotherapy can be used as an effective treatment adjuvant to limbal conjunctival graft in both primary and recurrence pterygium cases. Moreover, with the right technique, it appears to be a very reliable method in terms of complications. In future studies, longer follow-up periods to monitor patients for any recurrences are recommended.

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RESEARCH ARTICLE

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# Antibacterial effects of leukocyte and platelet-rich fibrin against Escherichia coli and Enterococcus faecalis

## Lökosit ve Trombositten Zengin Fibrinin Escherichia Coli'ye ve Enterococcus Faecalis'e Karşı Antibakteriyel Etkileri

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## ABSTRACT

**Aim:** The main goal of this research was to explore the in-vitro antibacterial characteristics of leukocyte and platelet-rich fibrin (L-PRF) against Escherichia coli (E. Coli) and Enterococcus faecalis (E. Faecalis).
**Materials and methods:** The study was conducted on 21 patients (10 females, 11 males, age range 21-32 years). L-PRF was prepared from the participants' own blood. Antibacterial activity of L-PRF against E. coli and E. faecalis ATCC standard strains was analyzed using the Kirby Bauer disk diffusion method.
**Results:** The inhibition zones with PRF had not been detected even though the results obtained using control discs were in accordance with the expectations.
**Conclusion:** L-PRF demonstrated no inhibition zone against E. coli and E. faecalis.

Key words: Antibacterial, Escherichia Coli, Enterococcus Faecalis, Leukocyte and Platelet-Rich Fibrin, postoperative infection.

## ÖZ

**Amaç:** Bu çalışmanın amacı, lökosit ve trombositten zengin fibrinin (TZF) Escherichia coli'ye (E. Coli) ve Enterococcus faecalis'e (E. Faecalis) karşı in vitro antibakteriyel etkilerini araştırmaktır.
**Gereç ve yöntemler:** Çalışma 21 hasta (10 kadın, 11 erkek, yaş aralığı 21-32 yaş) üzerinde yürütülmüştür. Katılımcılardan elde edilen kan örnekleri kullanılarak TZF hazırlanmıştır. TZF'nin E. coli ve E. faecalis ATCC standart suşlarına antibakteriyel aktivitesi Kirby Bauer disk difüzyon yöntemi kullanılarak test edilmiştir.
**Bulgular:** Beklenen sonuçlar kontrol diski ile elde edilmesine rağmen, TZF'nin değerlendirilen bakterilere karşı inhibisyon zonu göstermediği tespit edilmiştir
**Sonuç:** TZF, E.coli'ye ve E. faecalis'e karşı inhibisyon bölgesi göstermemiştir.

Anahtar Kelimeler: Antibakteriyel, Escherichia Coli, Enterococcus Faecalis, lökosit ve trombositten zengin fibrin, postoperatif enfeksiyon

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## INTRODUCTION

Platelet concentrates are utilized extensively in a wide variety of medical areas in order to promote tissue regeneration. They are classified into four groups based on their fibrin structure and leucocyte content, and all have their respective areas of use and various characteristics. Platelet-rich fibrin (PRF) is a second-generation platelet concentrate that has been proposed by Choukroun et al. In contrast to platelet-rich plasma (PRP), this procedure does not necessitate anticoagulant or bovine thrombin, making it easy and rapid to prepare [1,2]. In addition, due to its dense and mature three-dimensional architecture, L-PRF releases higher quantities of growth factors for longer periods than PRP [3,4]. In the literature, PRF has demonstrated promising results in enhancing bone regeneration, soft tissue maturation and wound healing [1,5-7].

Platelet concentrations could be divided into various categories based on their contents of leukocyte and fibrin; they are P-PRP (pure platelet rich plasma), L-PRP (Leucocyte and platelet rich plasma), pure platelet-rich fibrin (P-PRF) and leukocyte- and platelet-rich fibrin (L-PRF) [8]. L-PRF contains many more leucocytes than other concentrations. To obtain P-PRP, venous blood is collected in numerous small tubes and then centrifugation process is applied. After the PPGF (platelet poor in growth factors) has been removed, the left over PRGF (platelet rich in growth factors) is collected, and fibrin polymerization is triggered with 10% calcium chloride. The aim of this application is to avoid collecting the leucocytes. In contrast, to obtain L-PRF, venous blood is collected in dry glass tubes without anticoagulants and centrifugation process was applied at low speed. In this way, both platelets and leucocytes can be obtained with great efficiency [8].

In addition to all these properties, the antimicrobial properties of platelet concentrations have recently been addressed. The mechanisms behind the antibacterial characteristics of platelet concentrates are still not fully understood and remain to be explored [9]. The antimicrobial potential of platelet blood concentrations is based on the roles in host response of platelets and leukocytes. Platelets which are essential for tissue healing possess receptors that are critical in recognizing the viruses, bacteria and parasites, also affect bacteria through a mechanism that involves direct contact. Activated platelets release some bactericidal structures such as defensins, kinocidins, platelet factor 4 and synthesize hydrogen peroxide and reactive oxygen species which are toxic to bacteria [9]. Leukocytes, like platelets, exhibit antimicrobial activity in circulation. White blood cells maintain these properties through phagocytic activity and antimicrobial molecules demonstrate phagocytic activity; these constitute a rich source of anti-microbial substances including defensins, cathelicidins, lysozyme, myeloperoxidase [9].

There are many studies in literature with particular focus on the antibacterial effects of PRP [10-16]. However, few studies [11,13,17] have investigated the antibacterial effects of L-PRF. Postoperative infection is among the most critical and primary causes of disruption to wound healing and may have an adverse effect on surgical success. Hence, the prevention of bacterial contamination is a prerequisite for the success of the surgery. Although Escherichia coli (E. Coli) and Enterococcus faecalis (E. Faecalis) have an important place in postoperative infection, none of the previous studies focused on the antibacterial effects of L-PRF against E. coli and E. faecalis. For all these reasons, we aimed to investigate the antibacterial effects of L-PRF, which was prepared according to the Choukroun's technique, against standard strains of E. coli and E. faecalis.

## METHODOLOGY

### Study population

Blood specimens were obtained from 21 patients, in which 10 were female and 11 were male. The age range of the patients was 21–32 years. They were admitted to the Department of Periodontology of the Baskent University Faculty of Dentistry for periodontal treatment and they volunteered to participate in this study. All the study subjects were healthy (ASA 1–2). Smokers, patients who had taken antibiotics during the last six months or patients who underwent anticoagulant or immunosuppressive therapy, were excluded from this study. The written informed consents were obtained from all patients who were enrolled to

the study, which was conducted in accordance with the guidelines of the Helsinki Declaration for biomedical research involving human subjects and approved by the Ethics Board of Baskent University (approval no:15/12).

Blood collection and production of L-PRF

The blood specimens were handled and treated in accordance with the PRF protocol described previously using a PC-02 table centrifuge and kits (Process, Nice, France) for collection [2]. Venous blood was collected in 10 mL tubes lacking anticoagulants and centrifuged immediately at 2700 rpm for 12 minutes. Following centrifugation, PRF was separated from red corpuscle base utilizing tweezers and scissors. Then it was transferred to a PRF BOX (Process, Nice, France), and PRF membranes were obtained. Two membranes were obtained from each volunteer (Figure-1.

Bacterial strains

Standard strains of E. faecalis ATCC (American Type Culture Collection) 29212 and E. coli ATCC 25922 were used to evaluate the antibacterial effects of PRF.

Determination of antimicrobial activity

The antimicrobial activity for L-PRF was performed in a medical microbiology laboratory. The profiles of antimicrobial susceptibility were detected using the disc diffusion method, meeting the criteria of Clinical and Laboratory Standards Institute (CLSI). The isolates were stored at -20 °C until further usage. Prior to the test, the isolates were subcultured on blood agar and MacConkey agar in order to determine any possible contamination. The standard strains with 0.5 McFarland turbidity were plated on both Blood Agar Medium (BD, USA) and Mueller Hinton Agar (BD, USA). Mueller Hinton agar and blood agar were then used for in-vitro testing. An ampicillin disc (10 µg) was also used as a quality control. Following 24 and 48 hours of incubation at 37 oC, the diameter of the zones was measured visually and evaluated according to CLSI standards.

## RESULTS

The zone of inhibition of the control disc was concordant with CLSI standards. At both 24 and 48 hours of incubation, no inhibition zones were reported against the E. faecalis ATCC 29212 or E. coli ATCC 25922 standard strains (Figure-1 and Figure-2), (Table 1).

**Table 1: Demographic characteristics of patients and antimicrobial activity of L-PRF**

| | Age | Gender | Antimicrobial activity of L-PRF against the E. faecalis | Antimicrobial activity of L-PRF against the E. coli |
|---|---|---|---|---|
| P1 | 30 | Male | No | No |
| P2 | 26 | Male | No | No |
| P3 | 21 | Female | No | No |
| P4 | 22 | Male | No | No |
| P5 | 24 | Male | No | No |
| P6 | 21 | Female | No | No |
| P7 | 22 | Female | No | No |
| P8 | 22 | Female | No | No |
| P9 | 24 | Female | No | No |
| P10 | 26 | Male | No | No |
| P11 | 24 | Male | No | No |
| P12 | 25 | Male | No | No |
| P13 | 21 | Female | No | No |
| P14 | 26 | Male | No | No |
| P15 | 22 | Male | No | No |
| P16 | 22 | Male | No | No |
| P17 | 22 | Male | No | No |
| P18 | 25 | Female | No | No |
| P19 | 24 | Female | No | No |
| P20 | 32 | Female | No | No |
| P21 | 30 | Female | No | No |

P, patient; E. faecalis, Enterococcus faecalis; E. coli, Escherichia coli.

## DISCUSSION

Platelet concentrates have been used in the medical field [18] and in oral surgery with promising results. There is growing evidence that suggests that platelet concentrates not only slow bleeding but also contribute to protecting against infection [10]. The current research was conducted to investigate the antibacterial characteristics of L-PRF against E. faecalis and E. coli, which have been shown to be responsible for the postoperative infection [19,20], and the findings of this research indicated that L-PRF did not demonstrate any antibacterial activity against the selected bacteria.

The studies that have evaluated the antimicrobial

activities of different platelet concentrations have reported conflicting results [11,13]. For example, Badade et al. [13] scrutinized the antimicrobial activities of platelet-rich fibrin (PRF) and PRP against Porphyromonas gingivalis (P. gingivalis) and Aggregatibacter actinomycetemcomitans (A. actinomycetemcomitans) and reported that PRF demonstrated no antibacterial activity, whereas PRP was capable of inhibiting P. gingivalis at three–four days of incubation and A. actinomycetemcomitans at 48 h of incubation. When obtaining PRP, intravenous blood was procured in a blood collection tube coated with an anticoagulant, and 10% calcium chloride was used to activate PRP. It was concluded that the calcium chloride used for PRP activation may be responsible for the antibacterial activity of PRP.

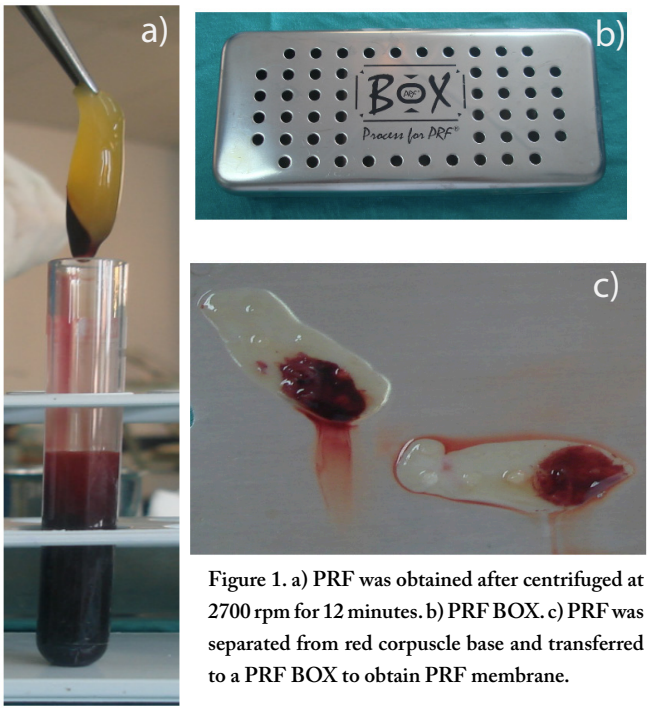


Figure 1. a) PRF was obtained after centrifuged at 2700 rpm for 12 minutes. b) PRF BOX. c) PRF was separated from red corpuscle base and transferred to a PRF BOX to obtain PRF membrane.

In another study Yang et al. tested four distinct plasma fractions (PRF, PPP, PDP, PRP) in terms of their antibacterial characteristics against P.gingivalis, A. actinomycetemcomitans and Fusobacterium nucleatum [11]. The authors found that all plasma concentrations, including PRF, inhibited bacterial growth, but when a comparison was made between the platelet concentrates, PRP was found to be the most effective antibacterial agent. Since Yang et al. used a different preparation protocol from our study to obtain PRF, a direct comparison with the present study was not possible. They obtained PRF from a fraction of PRP that was activated by calcium chloride, whereas in the present study, L-PRF was prepared by a protocol that is in conformity with the method proposed by Choukroun et al. [2].

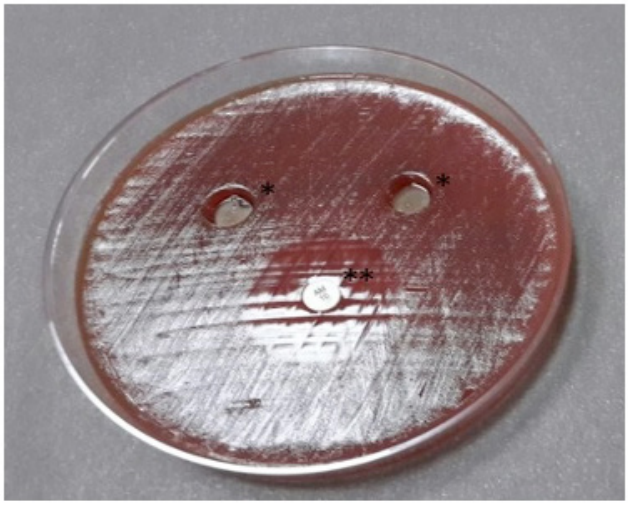

Figure 2. *Agar diffusion antibacterial testing of L-PRF against the E. faecalis, ** Agar diffusion antibacterial testing of control disc (ampicillin disc) for E. faecalis. (Blood Agar)

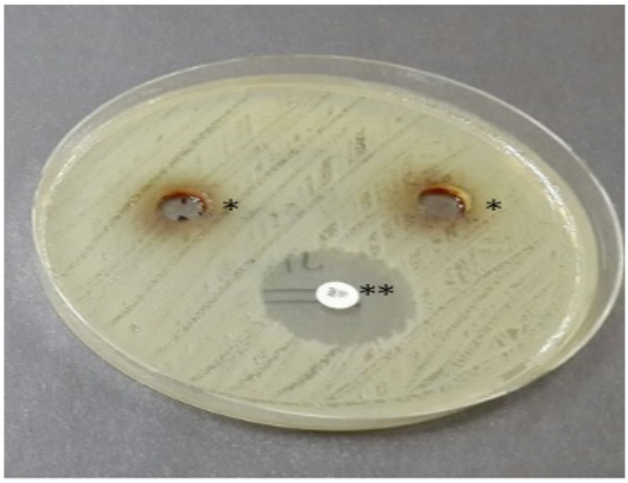

Figure 3. *Agar diffusion antibacterial testing of LPRF against the E. coli, ** Agar diffusion antibacterial testing of control (ampicillin disc) against the E. coli. (Mueller Hinton Agar)

Different results have been reported in a few studies [11,13,17] evaluating the antimicrobial effect of L-PRF. Among these, one study concluded that L-PRF had no antimicrobial properties [13], whereas in another, L-PRF showed the precise opposite [11,17]. In addition, there have been no study that have analyzed the antimicrobial effects of L-PRF on E. faecalis and E. coli. Therefore, the antibacterial effects of L-PRF against these were investigated in the present study to address the issue of the proven roles of these bacteria in postoperative infection

[19,20]. Although the literature does not address the antimicrobial action of PRF against E. coli and E. faecalis, the antimicrobial activities of other platelet concentrations against these have been evaluated in previous studies, with contradictory results [10,11,12,16,21,22,23]. Drago et al. [10], reported an antibacterial effect of PRP, whereas Bielecki et al. [21] and Li et al. [23], did not find out any antimicrobial effects of PRP against these bacteria.

Some authors have argued that antimicrobial activities of leukocytes increase the anti-inflammatory activity of platelet concentrations, although the antimicrobial effects of leucocytes that are removed from circulation when applied directly to the surgical field, are still open to question [9]. It has even been stated that leukocytes should be completely removed from their plasma concentrations, with suggestions that they may increase inflammatory response [24]. The reason for the lack of antimicrobial activity of L-PRF in this study may be a result of the large quantity of leucocytes in the contents and its tight fibrin structure, which may cause the trapping of several antimicrobial effect products. Further research is required to identify the antimicrobial efficacy of platelet concentrations.

Limitations: In-vitro antibacterial characteristics of leukocyte and platelet-rich fibrin (L-PRF) against Escherichia coli (E. Coli) and Enterococcus faecalis (E. Faecalis) was investigated in the present study. Evaluation of only these two bacteria can be considered as a limitation of this study.

Conclusion: In conclusion, L-PRF showed no inhibition zone against E. coli and E. faecalis.

Conflict of Interest: The author has no conflict of interest related to this article.

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# Alzheimer's Disease Mortality Trends in Turkey, 2009-2018

## Türkiye'de Alzheimer Hastalığına Bağlı Mortalite Eğilimleri, 2009-2018

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### ABSTRACT

**Aim:** World population has aged as a result of developments and improvements in the living conditions, care and health services. The most important risk factor of Alzheimer's disease is age, and with the aging of the population, both prevalence and mortality rates increase. The study aimed to determine the trend of mortality rates due to Alzheimer's disease over the years according to gender and age groups, between 2009-2018.

**Methods:** Alzheimer's disease-related data was obtained from the Turkish Statistical Institute death database. Age-standardized mortality rates were calculated through the direct method to the World Standard Population. Joinpoint Regression Analysis was used to estimate annual percentage change, average annual percentage change, and 95% confidence intervals.

**Results:** Over the period observed, more than 95 000 persons died due to Alzheimer's disease (37 961 males and 57 936 females). Joinpoint Regression Analysis indicated that the trend in Alzheimer's mortality rates a significant increase of 13.3% (95% CI: 10.6;16.2; p<0.001) every year from 2009 to 2015, and a non-significant decrease of 0.5% % (95% CI: -5.3;4.7; p=0.82), from 2015 to the end of the period. When evaluated according to gender, both in females and males, mortality rates showed a significant increase from 2009 to 2015. And then until the end of the period a non-significant decrease in males and a non-significant increase in females.

**Conclusion:** The findings from this study can provide information about current and future health planning and policy development in medicine, social work, public policy and public health.

Keywords: Alzheimer disease; mortality; trend; Joinpoint Regression

### ÖZ

**Amaç:** Dünyada, yaşam koşullarındaki iyileşmeler, bakım ve sağlık hizmetlerindeki gelişmelerin bir sonucu olarak nüfus yaşlanmaktadır. Alzheimer hastalığına ait en önemli risk faktörü yaştır ve nüfusun yaşlanmasıyla birlikte hem prevelans hem de mortalite oranları artmaktadır. Çalışmanın amacı, 2009-2018 yılları arasında Alzheimer hastalığına bağlı mortalite oranlarının cinsiyet ve yaş gruplarına göre yıllar itibari ile nasıl bir eğilim gösterdiğini belirlemektir.

**Metot:** Alzheimer hastalığı ile ilgili veriler Türkiye İstatistik Kurumu ölüm veri tabanından elde edildi. Yaşa göre standartlaştırılmış ölüm oranları, Dünya Standart Popülasyonuna göre doğrudan yöntem kullanılarak hesaplandı. Yıllık yüzde değişim, ortalama yıllık yüzde değişimini ve %95 güven aralıklarının (GA) tahmini için Joinpoint Regresyon Analizi'nden yararlanıldı.

**Bulgular:** Gözlemlenen dönemde Alzheimer hastalığından dolayı 95,000'den fazla kişi hayatını kaybetmiştir (37,961 erkek ve 57,936 kadın).

Joinpoint regresyon analizi, yaşa göre standartlaştırılmış Alzheimer ölüm oranı, 2009'dan 2015'e kadar her yıl %13.3'lük anlamlı bir artış (%95 GA: 10.6;16.2; p<0.001) ve 2015'den dönem sonuna kadar ise %0,5'lik anlamlı olmayan bir azalış (%95 GA: 5,3;4,7; p=0,82) göstermiştir. Cinsiyete göre değerlendirildiğinde ise hem kadınlarda hem de erkeklerde ölüm oranları 2009'dan 2015'e kadar önemli bir artış göstermiştir. Daha sonra dönem sonuna kadar erkeklerde önemsiz bir düşüş, kadınlarda ise önemsiz bir artış olmuştur.

**Sonuç:** Bu çalışmadan elde edilen bulgular tıpta, sosyal hizmetlerde, kamu politikasında ve halk sağlığında şimdiki ve gelecekteki sağlık planlaması ve politikalar geliştirme hakkında bilgi verebilir.

Anahtar Kelimeler: Alzheimer hastalığı; mortalite; eğilim; Joinpoint Regresyon

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## INTRODUCTION

Alzheimer's disease, a neurological disorder, is defined as the loss of memory and cognitive functions of the brain cells over time. Alzheimer's disease is considered the most common among dementia-caused disorders discomfort and constitutes 60-80% of cases [1]. The percentage of people with Alzheimer's dementia increases significantly with age [2,3]. In light of its characteristics as a non-physical and non-visible disease, Alzheimer's is often overlooked as a disease, resulting in insufficient research being performed on this subject, though in the past decade, a significant increase in public awareness in high-income countries has occurred, with the disease being increasingly discussed in the media [2]. Alzheimer's prevalence and mortality appear to increase with the aging of the population and the disease is now considered a growing Global public health problem [4]. All over the world, scientific and technological developments in the field of medicine, prevention of diseases, provision of early diagnosis and treatment, as well as the development of preventive health services, are among the principal reasons for the increase in the elderly population [5]. In European Union countries [6], France [7], Mexico [8], Brazil [9], as well as in Alzheimer's related mortality studies conducted in America [10], it is stated that the age-standardized mortality rates have increased over the years; in the 75 years age group, these increases are even higher. According to a study conducted in China, it was stated that there is a slight decrease in mortality rates in recent years [3]. The Turkish Neurological Society has reported some 300 000 Alzheimer's patients in Turkey. It is projected that as Turkey's current younger population will grow old, the disease will become one of the most prominent health problems over the next 30 to 40 years [11]. The purpose of our study of Alzheimer's disease in Turkey between the years 2009 and 2018, according to gender and age groups, was to determine the trend of the mortality rate (stable/increase/decrease) in this country.

## MATERIALS AND METHODS

Data Source

This population-based observational study covers ten years and was performed using data on gender and age of death associated with Alzheimer's disease, which was obtained from the Turkish Statistics Institute [12]. Annual data on the number of deaths due to Alzheimer's disease was analyses using code G30 in the 10th revision of the International Classification of Diseases (ICD). Age-standardized mortality rates (ASR) were determined using the direct standardization method to the WHO reference population [13]. Age-specific mortality rates were calculated for the age intervals from 0 to 80 and over a 10-year period. The results are not shown for the subgroups aged <45 years because fewer than five cases of Alzheimer's deaths occurred in any given year. In the analysis, subjects were age-grouped as 45 to 64, 65 to 74, 75 to 84 and ≥ 85 years.

Statistical analysis

Joinpoint Regression Analysis is a statistical method used to identify significant time points in the data set and model changing trends over time. This method has been widely used in epidemiological studies such as incidence, mortality or the survival of a population, to reveal any disease-related tendency. In such studies, the main goal is to give reliable estimates of incidence, mortality or survival rates, that provide information about current trends. The Joinpoint regression model is a piecewise regression model that characterizes the trend behavior in data, by defining the significant time points at which changes occur. The model with minimum error squares among the determined models, is the most suitable joinpoint regression model [14].

$$(x_1,y_1),\ldots.,(x_n,y_n),x_1\leq\cdots\leq x_n$$

including,

$$E[yIx]=\beta_0+\beta_1x+\delta_1(x-\tau_1)^+ +\cdots+\delta_k(x-\tau_k)^+$$

x: independent variable (time),

y: dependent variable,

$\beta_0,\beta_1,\delta_1,\delta_k$: regression coefficients,

$\tau_k$: unknown joinpoint,

$\delta_k$= differences in slope,

Annual Percent Change (APC), which is one of the significant concepts in joinpoint regression analysis, is used to describe the behavior of mortality trends. APC between $\tau_j$ ve $\tau_{(j+1)}$ is estimated as follows.

APC = $\{Exp(b_i) - 1\}x100$

One of the significant concepts in Joinpoint regression analysis, Average Annual Percentage Change (AAPC) is a summary measure of the trend in a predetermined fixed range. It is the weighted average of the APCs and is estimated as follows [15].

AAPC = $\left\{Exp\left(\frac{\sum w_i b_i}{\sum w_i}\right) - 1\right\}x100$

If there is no joinpoint in the model, APC and AAPC will be equal to each other. In the study, the death data of Alzheimer's disease, covering the period 2009-2018 and taken from the Turkstat database, were analyzed according to gender and age groups using the Joinpoint Regression Analysis Method. For each gender ASR, APC, AAPC values, and confidence intervals (CI) calculate [16]. In the analyzes, the Grid search method, which is suggested by Lerman, was used to predict change points. The Grid search method is applied to determine the number of possible breakpoints that can found in the data [17]. The Monte Carlo permutation and Bonferroni multiple comparison tests were used to test the determined breaking points [14]. Mortality trends from Alzheimer's were determined using the joinpoint regression software (4.6.0.0). The study was conducted in accordance with the ethical approval with the protocol number of 2019/11-341 from the Faculty of Medicine, Afyonkarahisar Health Science University.

## RESULTS

The number of deaths and female/male rates by gender by years are given in Table 1. The data from a total of 95 897 persons diagnosed with Alzheimer's disease in Turkey between the years 2009 to 2018, is also shown in Table 1. The proportion of those who lost their lives is 39.6% for males (37 961 individuals) and 60.4% for females (57 936 individuals). Considering the share of the number of people who died due to Alzheimer's in total deaths, it was 1.4 (Male: 1.0; Female: 1.8) in 2009 and 3.3 (Male: 2.3; Female: 4.5) in 2018. In view of the ratio of females to males in Table 1, it is seen that deaths are higher in females.

**Table 1. Number of deaths by gender and female / male ratio by years**

| Year | Deaths | Gender | | Female/Male Rate |
|---|---|---|---|---|
| | | Male | Female | |
| 2009 | 3870 | 1596 | 2274 | 1.42 |
| 2010 | 5333 | 2125 | 3208 | 1.51 |
| 2011 | 6301 | 2618 | 3683 | 1.41 |
| 2012 | 7696 | 3046 | 4650 | 1.53 |
| 2013 | 8984 | 3683 | 5301 | 1.44 |
| 2014 | 10382 | 4131 | 6251 | 1.51 |
| 2015 | 12279 | 4906 | 7373 | 1.50 |
| 2016 | 13269 | 5179 | 8090 | 1.56 |
| 2017 | 13831 | 5354 | 8477 | 1.58 |
| 2018 | 13952 | 5323 | 8629 | 1.62 |
| Total | 95897 | 37961 | 57936 | 1.53 |

Changes in the ASR rate and their comparison with the previous year are provided in Table 2. Looking at the percentage change in ASR, the maximum change occurred in 2010 with 21% for males and 32% for females. Both genders decreased in 2017 and 2018, compared to the previous year.

**Table 2. Distribution of Standardized Mortality Rates by Gender and percentage change in ASRs (Compared to the Previous Year). 2009-2018 (100 000).**

| Year | General ASR* | Gender | | ASR (% Change) | |
|---|---|---|---|---|---|
| | | Male | Female | Male | Female |
| 2009 | 4.77 | 5.05 | 4.57 | - | - |
| 2010 | 6.09 | 6.11 | 6.02 | 21 | 32 |
| 2011 | 6.83 | 7.34 | 6.45 | 20 | 7 |
| 2012 | 7.85 | 8.06 | 7.65 | 10 | 19 |
| 2013 | 8.69 | 9.36 | 8.22 | 16 | 7 |
| 2014 | 9.56 | 9.91 | 9.24 | 6 | 12 |
| 2015 | 10.74 | 11.29 | 10.34 | 14 | 12 |
| 2016 | 11.24 | 11.55 | 10.99 | 2 | 6 |
| 2017 | 11.21 | 11.38 | 11.01 | -1 | 0 |
| 2018 | 10.67 | 10.59 | 10.63 | -7 | -3 |

*ASR: Age-standardized mortality rates

Table 3 shows the results of the Joinpoint Regression Analysis (AAPC, APC and Cis, for each joinpoint point and gender). As can be seen from Table 3, when evaluated in general, the model with a single joinpoint is the best. Age-standardized Alzheimer's mortality rates showed a significant increase of 13.3% every year, from 2009 to 2015. Although there is an annual decrease of 0.5% from 2015 to the end of the period, this decrease is not statistically significant.

**Table 3. Annual percentage change and average annual percentage change by gender and age groups (2009-2018); Joinpoint Regression Analysis results.**

| | AAPC(95% CI) (2009-2018) | Trend 1 | | Trend 2 | |
|---|---|---|---|---|---|
| | | Period | APC (95% CI) | Period | APC (95% CI) |
| **Male** | | | | | |
| **45-64** | 2.4 (5.7;10.3) (p=0.12) | - | - | - | - |
| **65-74** | 2.9 (0.6;5.1) (p=0.01) | 2009-2014 | 8.1 (4.4;11.8) (p<0.05) | 2014-2018 | -3.3(-7.8;1.6) (p=0.14) |
| **75-84** | 7.9 (3.2;12.7) (p<0.05) | 2009-2015 | 14.5 (9.1;20.2) (p<0.05) | 2015-2018 | -4.3(-17.1;10.4) (p=0.46) |
| **85+** | 11.7 (8.6;14.9) (p<0.05) | 2009-2015 | 19.3 (15.7;23.1) (p<0.05) | 2015-2018 | -2.2 (-10.8;7.3) (p=0.57) |
| **Total (Male)** | 8.0 (5.7;10.3) (p<0.05) | 2009-2015 | 13.6 (10.2;17.1) (p<0.05) | 2015-2018 | -2.4 (-7.8;3.3) (p=0.32) |
| **Female** | | | | | |
| **45-64** | 4.7 (0.4;9.2) (p=0.04) | - | - | - | - |
| **65-74** | 3.3 (1.1;5.6) (p=0.01) | - | - | - | - |
| **75-84** | 7.3 (3.5;11.4) (p<0.05) | 2009-2015 | 12.4 (7.9;17.1) (p<0.05) | 2015-2018 | -2.1 (-13.2;10.3) (p=0.66) |
| **85+** | 12.6 (9.7;15.5) (p<0.05) | 2009-2015 | 18.4 (15.2;21.8) (p<0.05) | 2015-2018 | 1.8 (-6.3;10.7) (p=0.60) |
| **Total (Female)** | 8.9 (7.1;10.8) (p<0.05) | 2009-2015 | 13.1 (10.61;15.7) (p<0.05) | 2015-2018 | 1.0 (-4.0;6.2) (p=0.65) |
| **Total** | **8.5 (6.6; 10.5) (p<0.05)** | **2009-2015** | **13.3 (10.6;16.2) (p<0.05)** | **2015-2018** | **-0.5 (-5.3;4.7) (p=0.82)** |

**CI: Confidence Interval; AAPC: Average Annual Percent Change; APC: Annual Percent Change**

When evaluated according to genders, it is seen that the model with a single joinpoint was the best significant model for both males and females. Age-standardized Alzheimer's mortality rates showed a significant increase of 13.6% in males and 13.1% in females, every year from 2009 to 2015. Although an annual decrease of 2.4% in males and an increase of 1.0% in females, this was not statistically significant (2015 to the end of the period). Joinpoint Regression Analysis results regarding age-specific mortality rates for each gender given in Table 3.

In males, a similar structure was observed in all age groups except for the 45-64 age group. There was a non-significant increasing trend during the period specifically in the 45-64 age group. In the 65-74 age group, there was a continuous significant increase (2.9%; p = 0.01) until 2014, and after 2014 there were decreases, although insignificant. There was a similar trend in the 75-84 and 85+ age groups. In both age groups, there was a significant increase until 2015, and after 2015, there were non-significant decreases. The model with zero joinpoints provided the best model for females in the 45-64 and 65-74 age groups. In both age groups, a significant increase was observed during the period. The single-join point model was the best model for the 75-84 and 85+ age groups. In both age groups, 2015 was determined as the joinpoint. In the 75-84 age group, a continuous significant increase (12.4%, p <0.05) occurred until 2015, while decreases observed in the annual percentage change from 2015 to the end of the period. In the 85+ age group, the increase continued somewhat after 2015, but this increase was not significant (p = 0.60).

## DISCUSSION

According to the results obtained from the study, significant increases were observed in all female age groups throughout the period, while considering the age groups. In males, a significant increase was observed only in the age group 65 and over. The study by Niu et al. covering 28 European Union countries, stated that the death rates from Alzheimer's disease increased eight times in males and 12 times in females, between 1985-2004 [6]. A mortality study conducted in the United States emphasized that the mortality rates standardized by age have increased over the years, from 16.5 in 1999, to 25.4 in 2014 (per 100 000 people), and that chronic diseases and diabetes may cause this increase in Alzheimer's mortality [7,9]. In another study, it was stated that there is a strong relationship between the decrease in the incidence of dementia and preventive measures for better control and treatment of vascular risk factors [18]. A study conducted in China covering the years of 2009-2015, stated that the mortality rates of Alzheimer's disease increased in 2009-2010, and then there was a decrease until the end of the period [3]. A study by Teixeira et al. in Brazil found an annual increase of 8.4% for females aged 60 to 79, and 7.7% for males, 15.5% for females, and 14% for males aged 80 and over [9]. Likewise, a study in France stated that mortality increased in the group above 80 years old [7]. Taylor et al., for their part, reported that the mortality rate in females is higher than in male, and the mortality rate increases with age, especially in the group above 75 years old [10]. A study in Mexico stated that there is an increase in Alzheimer's mortality trends in males and females over the age of 40 [8]. A study by Moschetti et al. reported that the age-standardized mortality rates increased from 45.3 in 1999 to 50.0 in 2008 [19].

In females as well as males, the influence of longevity, biological differences, differences in cognitive performance and gendered social roles and opportunities, can differentially affect the risk and progression of Alzheimer's disease [20]. One reason why females living with Alzheimer's are found in higher numbers than males is likely that females live longer on average than males, and that older age is the biggest risk factor for Alzheimer's. Life expectancy at birth for females in Turkey (80.7 in 2018) males (75.3 in 2018) is estimated to be longer by TurkStat [12]. This may partially explain the observed differences between the genders.

Population aging can be suspected as the primary reason for the increase in the number of people living with Alzheimer's disease. Therefore, it is reasonable to believe that the disease burden caused by Alzheimer's disease will increase rapidly in the coming years. Escalating research on risk factors for Alzheimer's disease and its preclinical stage supports the development of Alzheimer's disease prevention programs, that will have a significant impact on public health by delaying the onset of Alzheimer's disease, even if only by a few years. Also, it is stressed that identifying individuals at risk of developing Alzheimer's disease may be the key to the success of further intervention studies [21].

Limitations of the Study

We had to use a short time period in our study: mortality data prior to 2009 was not included in the study, since it only covered provincial and district centers. In addition, death certificate data may be subject to potential miscoding and misclassification. Despite these limitations, this data provided the most comprehensive assessment of cause-specific Alzheimer's disease mortality rates at the national level.

Conclusion and recommendations

This study has the distinction of being the first comprehensive study examining trends in mortality in Alzheimer’s disease in Turkey. In providing a portrayal of the extent of the disease in this country, it emphasizes the offers important new evidence for the planning of future public health policy for the benefit of the elderly in our society. According to the results obtained from this study, Alzheimer's total mortality shows a negative trend in Turkey during the ten years that were observed. Furthermore, there are some insignificant decreases, in both females and males, after 2015. However, according to World Health Organization Data, by the year 2050 Turkey will be one of four countries in the world with the highest incidence of Alzheimer's disease. At present, an estimated 400 000 Alzheimer's patients exist in Turkey, with a disease that is known to be more widespread than reported and which is evolving in an aging of the population. In this context, it is necessary to increase the awareness of the disease in our society and to find solutions to the problem of prevention and care. Alzheimer's disease primarily affects the elderly and there is presently no effective treatment to stop or slow the progression of the disease. An intensification of the illness among the elderly population in the coming years, combined with increasing life expectancy and citizens concerned with care for the elderly, will become an important concern for the Turkish economy. The decline in mortality rates in recent years in the world and a desire to actively strive for better prevention and treatment of diseases associated with Alzheimer's, can be connected to create greater awareness in society. Various health and social services should provide support for Alzheimer's and dementia patients, and projects should be produced by non-profit associations, non-governmental organizations, private organizations, municipalities and governments, to raise public awareness on dementia and Alzheimer's disease. The principal goal is to make such institutions widespread throughout the world.

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# The Relationship of Inflammatory Indicators and Metabolic Syndrome with Gonarthrotic Cartilage Degeneration: A Novel Glance

## Gonartrotik Kıkırdak Dejenerasyonunun Metabolik Sendrom ve İnflamatuar İndikatörler İle İlişkisi: Yeni Bir Bakış

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## ABSTRACT

**Objective:** The combination of a number of metabolic abnormalities such as high body mass index (BMI), central obesity, low high-density lipoprotein (HDL), high triglycerides, high blood pressure, and hyperglycemia is defined as metabolic syndrome (MetS). This study aimed to clarify the effect of metabolic syndrome components on joint degeneration and investigate the relationship between systemic inflammatory response and end-stage osteoarthritis clinical course.

**Material and Methods:** Fifty-seven patients, who underwent total knee arthroplasty due to primary knee osteoarthritis, were classified according to metabolic syndrome diagnosis criteria. Their medial and lateral tibial plateau specimens were graded histopathologically according to Osteoarthritis Research Society International scoring system (OARSI).

**Results:** 33 patients were performed right total arthroplasty (57.9%), 24 were performed left (42.1%). The mean age was 68.46 ± 6.88 (range 57 to 85). The mean BMI value was 30.31 ± 5.26 (range 20.2 to 48). According to the International Diabetes Foundation (IDF) 2005 metabolic syndrome (MetS) diagnostic criteria; 31.5% (n = 18) of the patients did not have MetS, while 68.4% (n = 39) had. There was no statistically significant relationship between tibial plateau OARSI scores and metabolic syndrome (p> 0.05). Besides, these OARSI scores and the operation side, hypertension, and BMI had no statistically significant relationship (p> 0.05).

**Conclusions:** Metabolic syndrome components may play a role in initiating the osteoarthritic process via adipokines, but we could not identify certain effects of pro-inflammatory mediator components on tibial plateau cartilage degeneration with histopathological scores till end-stage arthritic progress.

Keywords: Knee, Osteoarthritis, Metabolic Syndrome, Cartilage Degeneration, Inflammatory Mediators

## ÖZ

**Amaç:** Yüksek vücut kitle indeksi (BMI), merkezi obezite, yüksek yoğunluklu lipoprotein (HDL) seviyesi düşüklüğü, yüksek trigliserid, yüksek tansiyon ve hiperglisemi gibi bir dizi metabolik anormalliğin kombinasyonu metabolik sendrom (MetS) olarak tanımlanır. Bu çalışma metabolik sendrom bileşenlerinin eklem dejenerasyonuna etkisini ve sistemik iltihabi cevapla son evre osteoartritin klinik gidişatı arasındaki ilişkiyi araştırmayı amaçlamaktadır.

**Material ve Metod:** Primer diz osteoartriti sebebiyle total diz artroplastisi uygulanan elliyedi hasta, metabolik sendrom tanı kriterlerine göre sınıflandırıldı ve Uluslarasarası Osteoartrit Araştırma Grubu (Osteoarthritis Research Society International - OARSI) skorlama sistemine göre medial ve lateral tibial plato örnekleri histopatolojik olarak evrelendi.

**Bulgular:** 33 hastaya sağ (% 57.9), 24 hastaya sol (% 42.1) total diz artroplastisi uygulandı. Ortalama yaş 68.46 ± 6.88 idi (57-85). Ortalama BMI değeri 30.31 ± 5.26 idi (20.2 - 48). Uluslarası Diabet Kuruluşu'nun (International Diabetes Foundation - IDF) 2005 metabolik sendrom tanı kriterlerine göre; % 31.5 (n = 18) hastada metabolik sendrom yokken, % 68.4 (n = 39) hastada vardı. Tibial plato OARSI skorlarıyla metabolik sendrom tanı kriterleri arasında istatistiksel anlamlı bir ilişki bulunamadı (p> 0.05). Ayrıca OARSI skorlarıyla opere taraf, hipertansiyon ve BMI arasında da istatistiksel anlamlı bir ilişki yoktu (p> 0.05).

**Sonuç:** Metabolik sendrom bileşenleri adipokinler yoluyla osteoartrit gelişimini başlatıcı etkiye sahip olabilse de, tibia plato kıkırdağı dejenerasyonu ile proinflamatuar mediatörler arasında artritik sürecin son evresine kadar devam eden bir ilişki tespit edemedik.

Anahtar kelimeler: Diz, Osteoartrit, Metabolik Sendrom, Kıkırdak Dejenerasyonu, İnflamatuar Mediatör

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## INTRODUCTION

Osteoarthritis (OA) is a disease of diarthrodial (synovial) joints characterized by clinical pain and functional limitation, osteophytes and narrowing in the joint space, and histopathological changes in cartilage and bone density. In the pathogenesis of OA, increases in pro-inflammatory cytokines, especially IL-1, TNF, and IL-6 in the synovial fluid and synovial membrane, have been shown to play essential roles. Inflammatory cytokines play essential roles in activating neutrophils, lymphocytes, and platelets, which can also affect themselves [1]. Levels of acute-phase protein, and other findings, such as anemia, thrombocytosis, and leukocytosis, may vary in response to inflammation. In knee OA, different intraarticular injections have been shown to reduce lipid peroxidation in synovial fluid [2]. The World Health Organization (WHO) defined weight classifications based on the Body Mass Index (BMI = kg / m2) over 20 years old: below 18.5: underweight, 18.5–24.9: normal weight, 25.0–29.9: pre-obesity, 30.0–34.9: obesity class I, 35.0–39.9: obesity class II, above 40: obesity class III.

The combination of several metabolic abnormalities such as low high-density lipoprotein (HDL) levels, central obesity, high triglyceride levels, high blood pressure, and hyperglycemia is defined as metabolic syndrome (MetS) [3]. The International Diabetes Foundation (IDF) declared MetS diagnostic criteria in 2005 [4]. According to these criteria, BMI> 30kg / m² and central obesity (waist circumference; male ≥ 94 cm, female ≥ 80 cm), and patients with any two of the following four factors were considered as MetS: 1) Triglycerides ≥ 150 mg / dL. 2) Low HDL (in men <40 mg / dL in women <50 mg / dL) or specific treatment for lipid abnormality. 3) Hypertension (systolic blood pressure ≥ 130 or diastolic blood pressure ≥ 85 mm Hg or previously diagnosed and treated high blood pressure). 4) High fasting plasma glucose (FPG) ≥ 100 mg / dL or previously diagnosed type 2 diabetes. So multiple risk factors that predispose to OA, can easily be found collectively in MetS [5].

The coincidence of OA and obesity, especially in weight-bearing knee joints, is well-known [6] and may predict increased chronic mechanical stress as a risk factor. However, in cartilage explant experiments, the application of excessive mechanical stress led to the release of pro-inflammatory cytokines and mediators that promoted disruption, causing joint inflammation and cartilage matrix destruction [7]. There has been increasing evidence that synovium's inflammatory and destructive response plays a significant role in OA [8]. However, it is still unclear to what extent the inflammation and immune response effectively initiate the joint's destructive process [9].

We hypothesized that the MetS criteria might not be effective in degeneration until the end-stage of osteoarthritis development. This study aimed to histopathologically investigate the effect of MetS components on joint degeneration and the relationship between systemic inflammatory response and end-stage OA clinical course.

## MATERIAL AND METHODS

This study was initiated after obtaining approval from the local Ethics Committee (decision number: 2018/164). Fifty-seven patients diagnosed with primary gonarthrosis and underwent total knee arthroplasty between 2018 and 2019 were included in the study after obtaining informed consent forms and meeting inclusion criteria. Patients' height, weight, waist circumference, blood pressure measurements, and biochemical data were retrospectively obtained from the hospital archive. Biochemical analyses, white blood cell count (WBC), platelet count (PC), lymphocyte count (LC), and C-reactive protein (CRP) had been measured from each patient's intravenous blood sample by standard laboratory methods.

Tibial proximal cut samples during the arthroplasty procedure were sent to the medical pathology laboratory in 10% formaldehyde. After macroscopic examination, medial and lateral tibial plateaus were cut horizontally, and they were decalcified with 20% formic acid. Samples were sliced to 3 µm thick sections with a microtome after routine follow-up. The sections taken were stained with Hematoxylin and Eosin (H&E) and examined microscopically. The cartilage degeneration observed in tibia samples was scored using the Osteoarthritis Research Society International (OARSI) scoring like Pritzker, K.P. et al. described [10]. Scoring is based on microscopic examination

and consists of 6 grades accordingly (Figure 1 a-f).

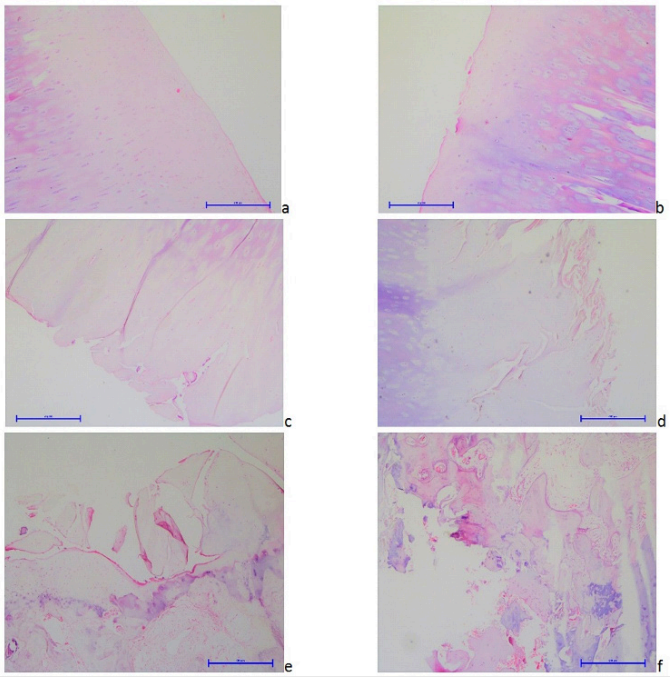

Figure 1: Figure shows microscopic examination of medial tibial plateau degeneration and consists of 6 grades accordingly Osteoarthritis Research Society International (OARSI) scoring. a. Slight superficial irregularity and fibrillation- Grade 1, b. Focal fibrillation progressing from superficial to medium zone - Grade 2, c. Vertical fissures progressing to middle shingles- Grade 3, d. Cartilage matrix loss, erosion- Grade 4, e. Microfractures extending to the bone - Grade5, f. Micro forties, repair tissue- Grade 6. Hematoxylin and Eosin (H&E) staining

Based on WHO categorization, Body Mass Index (BMI) was calculated for each patient. All patients included in the study were evaluated according to the IDF 2005 MetS diagnostic criteria and divided into two groups: MetS (-) and MetS (+) which were compared in this present study.

Patients who presented active infection, malignancy, chronic gastrointestinal diseases, patients using any drugs with potential effects on the gastrointestinal or coagulation system, patients with secondary gonarthrosis were excluded from the study. That was because of the altered response of inflammatory cytokines and difficulty in diagnosing MetS.

Statistical Analysis: NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA) program was used for statistical analysis. While evaluating the study data, descriptive statistical methods (Mean, Standard Deviation, Median, Frequency, Rate, Minimum, Maximum) and the distribution of the data were evaluated with the Shapiro-Wilk Test. ANOVA test was used for group comparisons of three or more quantitative data with normal distribution, and the Kruskal-Wallis test was used for comparison of three or more groups with no normal distribution. The Student-T test was used to compare two groups with a normal distribution of quantitative data, and the Mann-Whitney U test was used to compare two groups without normal distribution. Chi-square was used to determine the relationship between qualitative data. Significance was evaluated at $p < 0.05$ levels.

## RESULTS

Right total arthroplasty was performed in 57.9% (n = 33), and left total arthroplasty was performed in 42.1% (n = 24) of the 57 patients included in the study. The age value ranged from 57 to 85, with an average of 68.46 ± 6.88. Other data of the patients were given in Table 1.

Table 1. Demographic and inflammatory data of patients.

| | Mean±SD | Min-Max (Median) |
|---|---|---|
| Age | 68,46±6,88 | 57-85 (67) |
| Body Mass Index | 30,31±5,26 | 20,2-48 (29,6) |
| Waist Circumferance | 96,65±15,33 | 70-142 (96) |
| Fasting Plasma Glucose | 121,26±46,35 | 86-388 (108) |
| HDL | 53,07±12,45 | 30-87 (50) |
| LDL | 128,19±36,47 | 52-206 (128) |
| Total Collesterol | 211,88±37,49 | 129-289 (214) |
| Neutrophyle/ Lymphocyte | 2,62±2,11 | 1,2-15,16 (2) |
| Platelet/Lymphocyte | 140,72±59,68 | 55-350 (126) |
| C-reactive Protein | 5,88±4,1 | 3-22 (4) |

BMI value ranged from 20.2 to 48, with an average of 30.31 ± 5.26. While 10.5% (n = 6) of the patients were pre-obese, 40.4% (n = 23) were obesity class 1, 42.1% (n = 24) obesity class 2 and 7% ( n = 4) obesity class 3. 36.8% (n = 21) of the patients were hypertensive, 63.2% (n = 36) were not. According to the IDF 2005 MetS diagnostic criteria; 31.5% (n = 18) of the patients did not have MetS, while 68.4% (n = 39) had.

Although most of the patients had MetS, there was no statistically significant relationship between tibial plateau cartilage degeneration scores and the presence of MetS ($p > 0.05$). Thus, there was no statistically significant difference in end-stage knee OA whether or not having MetS. When the

relationship between cartilage degeneration scores and other parameters (surgery side, hypertension, and BMI scores) was evaluated, no statistically significant correlation was found (p> 0.05).

Degeneration was higher in the medial tibia plateau (p <0.05). That is because varus gonarthrosis can be seen more frequently than valgus gonarthrosis among the population. No statistically significant relationship was found when medial tibial plateau degeneration was compared with other parameters (p> 0.05) (Table 2).

Table 2. Comparison of medial tibial plateau degeneration and other parameters

| Parameter | OARSI score | n | Mean±SD | Min-Max (Median) | p |
|---|---|---|---|---|---|
| LDL | ≤ 3 | 18 | 134,17±39,14 | 62-206 (135) | [a]0,406 |
| | > 3 | 39 | 125,44±35,36 | 52-191 (128) | |
| Total Collesterol | ≤ 3 | 18 | 217,83±41,02 | 146-289 (218,5) | [a]0,420 |
| | > 3 | 39 | 209,13±35,98 | 129-278 (212) | |
| Waist Circumferance | ≤ 3 | 18 | 94,61±18,45 | 70-142 (89) | [b]0,253 |
| | > 3 | 39 | 97,59±13,83 | 71-136 (97) | |
| Fasting Plasma Glucose | ≤ 3 | 18 | 123,61±67,12 | 94-388 (107,5) | [b]0,503 |
| | > 3 | 39 | 120,18±33,86 | 86-234 (109) | |
| HDL | ≤ 3 | 18 | 56,11±13,93 | 39-87 (54) | [b]0,319 |
| | > 3 | 39 | 51,67±11,62 | 30-81 (50) | |
| Neutrphyle/ Lymphocyte | ≤ 3 | 18 | 2,89±3,24 | 1,2-15,16 (1,91) | [b]0,424 |
| | > 3 | 39 | 2,49±1,35 | 1,2-8,75 (2,17) | |
| Platelet/ Lymphocyte | ≤ 3 | 18 | 154,78±72,83 | 71-350 (131,5) | [b]0,405 |
| | > 3 | 39 | 134,23±52,34 | 55-295 (125) | |
| CRP | ≤ 3 | 18 | 6,58±5,64 | 3-22 (4) | [a]0,892 |
| | | 39 | 5,56±3,2 | 3-15 (4) | |

[a]Student T Testi [b]MannWhitney U Testi

## DISCUSSION

In this study, degeneration in synovial cartilage was evaluated histopathologically in end-stage knee OA patients who underwent total knee arthroplasty; and the relationship between MetS and systemic inflammatory response parameters was investigated. There have been studies about comparing incidence or symptom severity between MetS and OA, but we compared both tibial plateau specimens histopathologically with MetS. Our findings showed that there was no significant relationship between the cartilage degeneration seen in OA patients and MetS components and systemic inflammatory markers. However, the statistically significant difference between medial and lateral tibial plateau cartilage degeneration (independent of BMI) reminded the deviation of the mechanical axis medially in varus knees in terms of altering load balance.

OA is characterized by pathological inflammation associated with localized cartilage loss and remodeling of the bone adjacent to the cartilage. OA involves a slow and effective repair process. However, in some people, this situation cannot be compensated and results in symptomatic OA as a result of recurrent traumas or inadequate repair mechanisms, so this may explain why the disease comes with a wide range of clinical conditions in different patients, even in the same patient in different joints.

The frequency of hand OA in obese and pre-obese individuals has been shown to be a 2-fold increase in risk independent of excessive mechanical stress [11]. If it cannot be fully explained by excessive mechanical stress, then the relationship between obesity and hand OA reveals a systemic link between the two conditions mediated by adipokines [12]. Therefore, increased adiposity may help the progression of low-grade systemic inflammation, which also induces an increase in osteoarthritic inflammation.

The adipose tissue in obese individuals is known to secrete adipokines (leptin, adiponectin) and pro-inflammatory cytokines (TNFα, IL-6, or IL-1), which interact with each other and are also associated with OA [13]. These adipokines are involved in immune and inflammatory response processes as well as the regulation of glucose and adipocyte metabolism [14]. So they can play roles in both weight-bearing and non-weight-

bearing joints. These cytokines can also be released from certain cells, such as chondrocytes and synoviocytes. Leptin and visfatin have been well described in the osteoarthritic joint [15]. The production of these adipokines has been shown to be similar to chondrocyte activation accompanied by mechanical stress and pro-inflammatory cytokines in vitro [16].

The exact definition of MetS is variable because more than one criterion has been developed [17]. Nevertheless, OA may be associated with MetS or its components [18]. The prevalence of MetS in OA is higher than in those without OA (59 % vs. 23 %). MetS in OA is associated with worse pain and functional scores and advanced radiographic changes [19]. Furthermore, histological and immunohistochemical investigations revealed that the fat stored in the joints of patients with MetS and OA, and the fat on an experimental OA model had different secretion activity of the pro-inflammatory adipokines through adipocytes in the synovial membrane, infrapatellar fat pad, and abdominal fat. In the vast NHANES III cohort study conducted in the general population of America, the prevalence of MetS observed in patients with OA was increased even when adjusted according to age and BMI [20], and a similar Japanese study supported this thought [21]. So we believe having MetS components baseline may affect initiating the arthritic process through several immune and inflammatory ways. But the question is: does this relationship last till the end stages of OA?

Konstari et al. stated that the number of MetS components or any individual component did not predict an increased risk of knee OA. They only found that elevated plasma fasting glucose was associated with a reduced risk of incident knee OA in their 32-year follow-up study [22]. Our histopathological results also showed that MetS might not affect OA progress in the long term.

Only overweighting may not be a risk factor for cartilage degeneration. Disruption of joint homeostasis and OA progress is clearly and crucially associated with adipokines. However, the interaction between the adipokine network, especially the interplay between inflammatory paths and mechanical and metabolic processes in the cartilage and bone disorders, still remains unclear. Evidently, further investigations are needed to elucidate the intimate mechanisms regulating peripheral and central adipokines activity, which may also be advantageous for future treatment of OA [23]. Nevertheless, this present study showed that end-stage OA needs more explanation of several reason interactions.

Among the major limitations of our study, the vast majority of patients were female patients with varus gonarthrosis. Moreover, of course, statistical data may become more reliable with larger series of cases. However, this study reminded us that initiation of OA has different paths to lead end-stage of the disease.

**Conclusion:** Nevertheless, overweighting is as important as the mechanical loading axis in the onset of weight-bearing joint OA. MetS and its components have certain effects via adipokines at the beginning of the arthritic process. However, we could not show this effect in the long-term until the end-stage histopathologically.

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# Comparison between R2CHA2DS2-VASc Score and CHA2DS2-VASc Score to Predict Acute Stent Thrombosis in Patients with After Primary Percutaneous Coronary Intervention

## Primer Perkütan Koroner Girişim Yapılan Hastalarda Akut Stent Trombozunu Öngörmek İçin R2CHA2DS2-VASc Skoru ile CHA2DS2-VASc Skorlarının Karşılaştırılması

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## ABSTRACT

**Aim:** Acute stent thrombosis (AST) is an important complication resulting from sudden vascular occlusion after stent implantation, especially in patients with ST-segment elevation myocardial infarction (STEMI). It occurs in about 1% of the patients after primary percutaneous coronary intervention. The CHA2DS2-VASc score is easily applied in daily practice and the components of this score are similar to common risk factors of the AST. Chronic renal disease has a hypercoagulable state and this condition is associated with an increased risk of AST in STEMI. Since the CHA2DS2-VASc score is insufficient to assess the risk of AST in patients with renal dysfunction, we aimed to investigate the prognostic significance of the modified score, R2CHA2DS2-VASc score in patients with AST.
**Methods:**This cross-sectional study retrospectively included 56 patients withAST and 1493 patients without AST after STEMI. The CHA2DS2-VASc and R2CHA2DS2-VASc scores were compared between the two groups.
**Results:**The median CHA2DS2-VASc and R2CHA2DS2-VASc scores were significantly higher in the AST group (P <0.001, P <0.001, respectively). The R2CHA2DS2-VASc score ≥2 was used as a predictor of the AST with a sensitivity of 65% and specificity of 89%.
**Conclusions:** The R2CHA2DS2-VASc score is a simple, cheap, and easily accessible score that can predict AST.

Keywords: Acute stent thrombosis, CHA2DS2-VASc score, R2CHA2DS2-VASc score, ST- segment elevation myocardial infarction.

## ÖZ

**Amaç:** Akut stent trombozu (AST), özellikle ST segment yükselmesi olan miyokard infarktüsü (STYMI) hastalarında stent yerleştirildikten sonra ani damar tıkanıklığı sonucu oluşan önemli bir komplikasyondur. Primer perkütan koroner girişim sonrası hastaların yaklaşık% 1'inde ortaya çıkar. CHA2DS2-VASc skoru günlük pratikte kolayca uygulanır ve bu skorun bileşenleri AST'nin risk faktörlerine benzer. Kronik böbrek hastalığında pıhtılaşmaya meyilli bir duruma yol açabilir ve bu durum STYMI' da artmış AST riski ile ilişkilidir. CHA2DS2-VASc skoru renal disfonksiyonlu hastalarda yetersiz olduğundan AST'li hastalarda modifiye skor olan R2CHA2DS2-VASc skorunun prognostik önemini araştırmayı amaçladık.
**Yöntem:** STYMI sonrası 56 AST' li hasta ve AST'siz 1493 hasta retrospektif olarak bu kesitsel çalışmaya dahil edildi. CHA2DS2-VASc skoru ve R2CHA2DS2-VASc skoru iki grup arasında karşılaştırıldı.
**Bulgular:** Medyan CHA2DS2-VASc skoru ve medyan R2CHA2DS2-VASc skoru AST grubunda anlamlı olarak daha yüksekti (P <0.001, P <0.001). R2CHA2DS2-VASc skoru ≥2 iken %65 duyarlılık ve % 89 özgüllük ile AST' nin bir prediktörü olarak kullanılabilir.
**Sonuç:** R2CHA2DS2-VASc skoru, AST' yi tahmin edebilen basit, ucuz ve kolay erişilebilir bir skorlamadır.

Anahtar Kelimeler: Akut stent trombozu, CHA2DS2-VASc skoru, R2CHA2DS2-VASc skoru, ST segment yükselmeli miyokard enfarktüsü.

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## INTRODUCTION

Acute stent thrombosis (AST) is a high mortality complication of stent implantation, especially in acute coronary syndrome [1]. Previous studies have shown that AST resulting in acute coronary syndrome has an incidence of up to 1% following coronary stent implantation [2].

Many factors play a role in the pathophysiology of AST, but the exact mechanism is not fully understood. First and foremost of these factors are device-related factors such as stent design, material andsurface coating [3]. Another category includes procedural factors such as stent malposition, undersize stenting, and suboptimal antithrombotic therapy [4]. Another important category relates to a lesion or patient-specific factors such as vessel size, lesion length, acute coronary syndrome, presence of thrombus, plaque features, advanced age, left ventricular ejection fraction, peripheral artery disease, kidney failure, and diabetes mellitus [5].

The CHA2DS2-VASc (congestive heart failure, hypertension, age, diabetes mellitus, stroke, vascular disease, age, sex) score is easily applied in daily practice to predict thromboembolic risk in patients with atrial fibrillation (AF). The components of this score are related to atherosclerosis, vascular spasm and microvascular dysfunction similar to common risk factors of stent thrombosis [6]. Moreover, it has been shown to be a predictor of adverse outcomes after acute coronary syndrome [7]. R2CHA2DS2-VASc (renal failure in addition to CHA2DS2-VASc) is another scoring system that includes 2 points for renal failure (stage 2 or greater) [8].

In this study, we evaluated whether the R2CHA2DS2-VAScscore is useful as a simple tool for predicting the AST among patients with ST-segment elevated myocardial infarction (STEMI) who have undergone primary percutaneous coronary intervention (pPCI).

## MATERIAL AND METHODS

### Study population

This retrospective analytic,cross-sectional study used the data of 1549 consecutive patients from March 2017 to May 2020 who were admitted to our cardiovascular center with a diagnosis of acute STEMI and underwent pPCI. Acute STEMI was diagnosed when patients had symptoms of acute myocardial infarction and new ST-segment elevation in at least two contiguous leads of ≥0.2 mV in men or ≥0.15 mV in women in leads V2 to V3 and/or of ≥1 mm (0.1 mV) in other contiguous leads or new left bundle branch block, later confirmed by increases in troponin levels [9]. Patients with AF, malignancy, no-reflow (postprocedural thrombolysis in myocardial infarction flow grade <3), residual thrombus, stenosis >50% proximal or distal to the culprit lesion left untreated, intraprocedural stent thrombosis, persistent dissection, and stent undersizing and those who underwent coronary bifurcation intervention were excluded. Bedside 12-lead electrocardiography and routine blood tests were obtained from all admitted patients. Bedside echocardiography was also performed for the patients. Data from participants were analyzed retrospectively. Ethics approval was obtained from Gazi Osmanpasa University ethical committee with date: 17.08.2020, number: 20-KAEK-157. All procedures in this study were carried out in accordance with the 1975 Declaration of Helsinki, updated in 2013.

### Coronary angiography and pPCI

All patients underwent coronary angiography using a standard technique. First, they were administered 300 mg of acetylsalicylic acid. Ticagrelor of 180 mg was given to patients before undergoing coronary angiography. The patients received 100 IU / kg unfractionated heparin as a bolus.Stenting of the infarct-related artery was successfully completed in all patients immediately after the coronary angiography. Thrombus aspiration was applied in patients with high thrombus burden according to the operator's choice.

Tirofiban was used according to the discretion of the operator. Tirofiban infusion (0.15 mg/kg/min) was given to selected patients with no contraindications or tendency for bleeding. Other necessary medications such as beta-blockers, angiotensin-converting enzyme inhibitors,and statins were given according to relevant European Society of Cardiology guidelines.

### Definitions

Stent thrombosis occuring within the first 24 hours after stent implantation was defined as acute stent thrombosis. Academic Research Consortium criteria were used to detect stent thrombosis: presence of a thrombus originating from the instent or 5 mm proximal and distal of the stent, and presence of at least one of following: (a) acute ischemic symptoms at rest, (b) recent ischemic electrocardiographic changes suggestive of acute ischemia, (c) typical rise and fall in cardiac biomarkers [10].

The CHA2DS2-VASc score was calculated for each patient using the data available in the patient files recorded during hospitalization. According to the CHA2DS2-VASc scoring system, patients were given 1 point for congestive heart failure (signs/symptoms of heart failure and ejection fraction<40%), hypertension (taking anti-hypertensive medicine or systolic and diastolic blood pressure ≥140/90 mmHg), diabetes mellitus (defined as a fasting blood glucose level>126 mg/dL or blood glucose level ≥200 mg/dL or using anti-diabetic drugs), history of vascular disease (peripheral artery disease defined as stenosis of at least 50% in noncoronary artery circulation), age 65-74 years, female sex and 2 points for age 75 years or older and previous stroke or transient ischemic attack [11]. Data on race were also collected to determine the estimated glomerular filtration rate (GFR) using the Chronic Kidney Disease Epidemiology (CKD-EPI) creatinine equation [12]. The R2CHA2DS2-VASc score was calculated using the CHA2DS2-VASc scoring system with an additional 2 points assigned for renal failure, which was defined as a calculated GFR less than 60 from the CDK-EPI equation.

Statistical Analysis

Statistics Quantitative variables were defined as mean value ± standard deviation, and qualitative variables were defined as frequency and percentage. The Kolmogorov-Smirnov test was used to evaluate whether the distribution of continuous variables was normal. Categorical and continuous variables were analyzed using the chi-square test and independent-samples t-test, respectively. Multivariate logistic regression analyses were performed to determine the independent predictors. Variables that could be a predictor of stent thrombosis with a significant P value were entered into multivariate analysis. The results of univariate and multivariate regression analyses were presented as odds ratio with a 95% confidence interval (CI). Cutoff values of CHA2DS2-VASc and R2CHA2DS2-VASc scores with the highest sensitivity and specificity were calculated by nonparametric receiver–operating characteristics (ROC) curve analysis. Significance levels were demonstrated by P values. A P value <0.5 was accepted as statistically significant. The statistical analyses were performed with SPSS 18.0 for Windows (SPSS Inc, Chicago, Illinois).

## RESULTS

A total of 1549 patients (448 female (28.9%), mean age: 57±11.5 years) of whom 1493 patientswereinthe control group and 56 patients were in the AST group were included in this study. Demographic, clinical and angiographic data of the patients are listed in Table 1. Age, sex, smoking, diabetes mellitus were similar between the groups with AST and without AST. The median CHA2DS2-VASc score was significantly higher in the stent thrombosis group compared to control group (2[3]vs 1[2],P <0.001). Also, more importantly, the median R2CHA2DS2-VASc score was significantly higher in the AST group compared to the control group (3[4]vs1[2],P<0.001). Moreover, all components of the CHA2DS2-VASc score were statistically significantly different between the two groups including LV ejection fraction (%), which was significantly lower in the AST group (39.5 ± 7.2% vs 34.1 ± 7.8%, P<0.001). However, hypertension (41.0 % vs 34.6%, P =0.043), age between 65 and 74 (9.3% vs 5.3%, P<0.001), diabetes mellitus (42.8% vs 36.0%, P=0.114), previous stroke/transient ischemic attack (7.1%vs 1%, P<0.001), vascular disease (25% vs 13.4%, P=0.001), age 75 (14.1% vs 5.7%, P=0 .002), and female gender (28.0% vs 29.0%, P=0.702) were significantly higher in the AST group. Patients with the AST had significantly lower mean GFR (44.1 ± 6.9 mL/min/1.73 m2 vs 64.2 ± 14.2 mL/min/1.73 m2, P <0.001 ).

There was no significant difference between two cohorts in terms of duration from symptom initiation to pPCI (148.2 [123.4] min vs 148.6 [124.2] min, P=0.286). Regarding angiographic

findings, higher stent length (28 ±15 mm vs. 24 ±10 mm P< 0.001) and lower stent diameter (2.75 ± 0.5 mm vs. 2.75 ± 0.25 mm, P =0.092) were associated with AST.

**Table 1: Demographic, Clinical, and Angiographic Features of the Patients.**

| Variables | Control n=1493 | Stent trombosis, n= 56 | P value |
|---|---|---|---|
| Age, years, mean | 57.5 ±10 | 56.5 ±13 | 0.356 |
| Female gender, n (%) | 433 (29.0) | 15 (28.0) | 0.702 |
| Smoking, n (%) | 469 (31.4) | 17 (30.3) | 0.789 |
| Diabetes mellitus, n (%) | 537 (36.0) | 24 (42.8) | 0.114 |
| Hypertension, n (%) | 503 (34.6) | 23 (41.0) | 0.043 |
| History of stroke/TIA, n (%) | 15 (1) | 4 (7.1) | <0.001 |
| Vascular disease, n (%) | 200 (13.4) | 14 (25.0) | 0.001 |
| Previous MI | 148 (9.9) | 8 (14.2) | 0.496 |
| Peripheral arterial disease | 33 (2.2) | 7 (12.5) | <0.001 |
| Previous by-pass surgery | 30 (2.0) | 4 (7.1) | 0.001 |
| Total cholesterol, mg/dL | 183 ± 41 | 189 ± 42 | 0.626 |
| LDL-C, mg/dL | 113 ± 31 | 119 ± 36 | 0.294 |
| Triglycerides, mg/dL | 139 ± 62 | 152 ± 104 | 0.282 |
| Killip class >1, n (%) | 96 (6.4) | 4 (7.1) | 0.451 |
| LV ejection fraction, (%) | 39.5 ± 7.2 | 34.1 ± 7.8 | <0.001 |
| Serum creatinine, mg/dL | 1.06 ± 0.14 | 1.28 ± 0.72 | <0.001 |
| Chronic renal failure, n (%) | 131 (8.8) | 13 (23.2) | <0.001 |
| GFR, mL/min/1.73 m2 | 64.2± 14.2 | 44.1± 6.9 | <0.001 |
| CHA2DS2 -VASc score, median | 1 [2] | 2 [3] | <0.001 |
| R2CHA2DS2-VASc score, median | 1 [2] | 3 [4] | <0.001 |
| MI type, n(%) | | | <0.001 |
| Anterior | 632 (42.3) | 34 (60.7) | |
| Nonanterior | 861 (57.7) | 22 (39.3) | |
| Anemia, n (%) | 197 (13.2) | 14 (25.0) | 0.096 |
| Drugelutingstent, n(%) | 1061 (71.1) | 40 (71.4) | 0.806 |
| StentingwithoutPTCA, n (%) | 597 (40.0) | 21 (37.5) | 0.562 |
| Stent length,(mm) median | 24 ± 10 | 28 ± 15 | <0.001 |
| Stent diameter, (mm), median | 2.75 ± 0.25 | 2.75 ± 0.5 | 0.092 |
| Tirofiban infusion, n (%) | 696 (46.6) | 37 (66) | <0.001 |
| Thrombus aspiration, n (%) | 100 (6.7) | 6 (10.7) | 0.264 |
| Time to PCI, (min), median | 148.2 [123.4] | 169.4 [116.8] | 0.286 |
| In-hospital mortality, n (%) | 20 (0.7) | 17 (30.3) | <0.0001 |

GFR,Glomerular filtration rate; LV, left ventricular; LDL, low density lipoprotein; MI, myocardial infarction; PCI, primary percutaneous intervention; PTCA, percutaneous transluminal coronary angioplasty; TIA, transient ischemic attack.

Variables with a significant P value in the descriptive analysis were entered into univariate and multivariate regression analysis to determine potential risk factors of stent thrombosis. The results of this analysis are illustrated in Table 2. Individual components of the R2CHA2DS2-VASc score as a risk factor of the stent thrombosis were not entered in this analysis to avoid multicollinearity.

**Table 2. Univariate and multivariate regression analysis of predictors of stent thrombosis in the study population**

| Variables | Unadjusted OR(95% CI) | P value | Adjusted OR (95% CI) | P value |
|---|---|---|---|---|
| CHA2DS2-VASc score | 1.72 (1.46-2.01) | <0.001 | 1.56 (1.41-1.80) | <0.001 |
| R2CHA2DS2-VASc score | 2.84(1.96-3.41) | <0.001 | 3.26 (2.80-4.32) | <0.001 |
| GFR | 1.84 (1.21-2.60) | <0.001 | 2.24 (2.08-3.23) | <0.001 |
| Stent length | 1.44 (1.23-1.71) | <0.001 | 1.41 (1.39-1.72) | <0.001 |
| MI type | 1.90 (1.25-2.76) | 0.001 | 1.71 (1.17-2.5) | 0.1 |

CI, confidence interval; GFR, glomerular filtration rate; MI, myocardial infarction; OR, odds ratio.

The results from the multivariate logistic regression analysis showed that GFR (odds ratio [OR]: 2.24, 95% CI: 2.08-3.23, P<0.001), CHA2DS2-VASc score(OR: 1.56, 95% CI:1.41-1.80, P<0.001) and R2CHA2DS2-VASc score (OR:3.26, 95% CI: 2.80-4.32, P<0.001) were significant independent predictors. Then, we performed a ROC analysis as depicted in Figure 1 for evaluating the cutoff values of CHA2DS2-VASc and R2CHA2DS2-VASc scores in predicting the AST. Our study showed that CHA2DS2-VASc score ≥1 could be used as a predictor of the stent thrombosis in patients presenting with STEMI with a sensitivity of 45% and specificity of 75%, area under the curve: 0.654and 95% CI :0.58-0.74 whereas the R2CHA2DS2-VASc score ≥2 could be used as a predictor of the stent thrombosis in patients presenting with STEMI with a sensitivity of 65% and specificity of 89%, area under curve: 0.820 and 95% CI:0.77-0.89.

## DISCUSSION

This present study highlighted that in patients with STEMI, renal failure was associated with increased risk of AST. The findings revealed that the R2CHA2DS2-VASc score was a predictor of stent thrombosis after pPCI in patients with

STEMI. Actually both scoring systems were predictive but the sensitivity and specificity of R2CHA2DS2-VASc score was found to be greater in predicting stent thrombosis in patients with STEMI. AST is an abrupt vessel closure. Stent thrombosis is an important complication of stent implantation because it causes acute coronary syndrome. The incidence of AST after pPCI is approximately four times higher than after elective stent implantation [13]. It is known that in patients with AST, the incidence of cardiogenic shock increases and thus mortality also increases, so it has serious consequences. In randomized studies, the incidence of AST ranged from approximately 1.4% to 3.4% [14,15]. Numerous studies of AST formation have demonstrated the involvement of multiple risk factors such as diabetes mellitus, chronic kidney failure, stenting in acute or elective conditions, factors associated with lesions, number of affected vessels, total stent length, and presence of calcification [15-19]. Previous studies have shown various estimators of the stent thrombosis [19]. As confirmed by the findings of this study, lesion length >20 mm, and lower stent diameter were demonstrated to be the predictors of stent thrombosis. Also, GFR could predict AST. However, the need to use to a simple and fast scoring system for stent thrombosis risk classification in STEMI patients who are candidates for pPCI, compels the physician to choose the best treatment strategy. Since the underlying thromboembolic event mechanisms are similar in stent thrombosis and AF, the relationship between CHA2DS2-VASc score, which is a thromboembolic risk marker for AF, and stent thrombosis has been studied in the literature [19]. However, at the same time, patients with chronic renal disease have a hypercoagulable state and this condition is associated with an increased risk of stent thrombosis [18].

Several studies showed that renal dysfunction as an independent predictor of stent thrombosis in STEMI, but these studies did not include the GFR parameter [20]. In this study, GFR was also found to be significantly lower in the stent thrombosis group. Since the CHA2DS2-VASc score was insufficient to indicate the risk of thromboembolism in patients with renal dysfunction, we used the modified R2CHA2DS2-VASc score in this study.

The CHA2DS2-VASc score is a set of risk factors for thromboembolism and stroke, as suggested by the present guidelines for use as a proven predictor of thromboembolic events in patients with AF [21]. Stroke and transient ischemic attack might occur due to nonatherosclerotic vascular pathologies, as well as thromboembolism and atherosclerosis [19]. Abnormal vascular function was recommended as a stroke mediator [22].

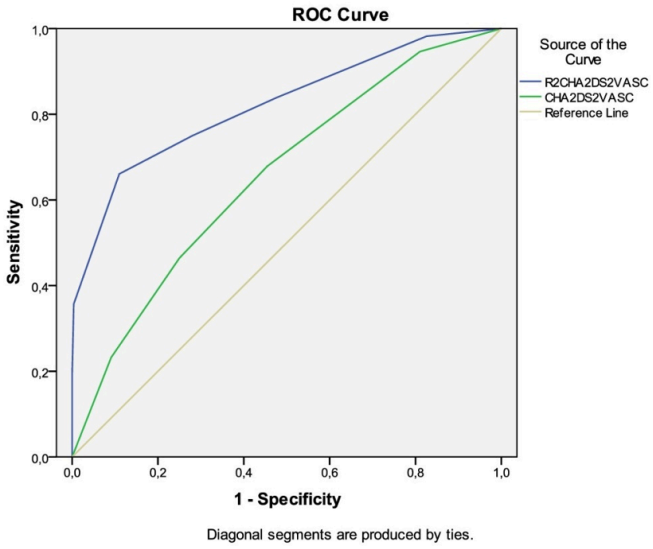


Figure-1: Receiver-operating curve analysis of CHA2DS2-VASc and R2CHA2DS2-VASc scores for predicting acute stent thrombosis

A significant positive correlation existed between the CHA2DS2-VASc and R2CHA2DS2-VASc scores.This should not be a surprise, as the two scoring systems shared many of the same scoring components and were similar to the weights of each component. This indicated the validity of using R2CHA2DS2-VASc score to estimate the risk of stent thrombosis. However, the addition of renal failure may not alter clinical decisions or outcomes because the CHA2DS2-VASc score has already been validated and is currently in use. It is important to try to control modifiable risk factors, such as renal function, because of the high mortality and morbidity associated with thromboembolism. Although most of the risk factors for CKD-EPI, AF and stroke are the same, renal failure may be a separate independent risk factor for thromboembolism. Piccini et al. tried to combine GFR with the CHADS2 score and created the R2CHADS2 score [23]. The GFR was calculated with the Cockcroft–Gault formula. In this study, CKD-EPI equation was used instead of

the Cockcroft–Gault formula for calculating GFR. It was because a previous study showed that the GFR-based scheme, R2CHADS2 , provided a significant improvement in the predictive ability for mortality risk in older patients with AF [24]. Huang et al. enrolled 3,295 patients with coronary artery disease (CAD) and found that R2CHADS2 had a comparable predictability to the Global Acute Coronary Events Registry score [25]. Compared to the CHA2DS2 score ,the R2CHA2DS2 score provided better discrimination for mortality. The results of this study showed that the R2CHA2DS2 score could be used to predict composite events for patients with CAD, and that the area under the curve of R2CHADS2 was statistically greater than the CHA2DS2 score. Decreased renal function was a critical in the prognosis of cardiovascular results in patients with CAD. Several potential mechanisms may explain these findings. Patients with reduced renal function often have consequences such as anemia, excessive volume burden, and oxidative stress that contribute to poor outcomes. In our study, the R2CHA2DS2-VASc score provided better discrimination for stent thrombosis than the CHA2DS2-VASc score.

Although the CHA2DS2-VASc score has already been tested in a previous study, the prognostic role of the modified R2CHA2DS2-VASc score in AST in patients with STEMI has not been addressed before. This study suggested that the R2CHA2DS2-VASc scoremight predict the risk of AST with reasonable efficacy for patients with STEMI better compared to the CHA2DS2-VASc score.

## Conclusion

The R2CHA2DS2-VASc score is an easily calculated and efficient index that can be considered a powerful and independent predictor of stent thrombosis in STEMI patients.This study suggested that,it could be a useful adjunct to standard tests in the diagnosis of stent thrombosis.

Limitations

Our study has several limitations. It was an observational, retrospective, single-center study with a relatively small number of patients. In this study, patients with non-STelevation myocardial infarction and unstable coronary artery disease were not included. In addition, patients with procedural complications that might cause AST were excluded from the study by careful examination of angiographic images, but intravascular imaging techniques such as intravascular ultrasound were not used. Therefore, future studies should include the aforementioned conditions and validate the findings of this study.

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# The relationship of gastrointestinal complications and ventilator related status with gastric residual volume in intensive care patients

## Yoğun Bakım Hastalarında İki Farklı Gastrik Rezidüel Volümün Gastrointestinal Komplikasyon ve Ventilatör İlişkili Durum İle İlişkisi

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## ABSTRACT

**Aim:** Our study aimed primarily to determine whether there was a relationship between total gastric residual volume (GRV) amounts and two different GRV thresholds and the development of gastrointestinal intolerance in patients on mechanical ventilation in the intensive care unit (ICU) and secondarily, to determine the effects of different GRV quantities on ventilator-related conditions (VAC).
**Methods:** Seventy patients above the age of 18 who were scheduled to be fed with enteral nutrition (EN) for at least three days, were divided into two groups including 35 patients according to GRV threshold values of 250 ml and 500 ml. The total amounts of GRV of the patients who did not exceed any of the two GRV thresholds during the follow-up period of 72 hours were recorded and calculated. For all patients, necessary data was recorded and high gastric residual volume rates (HGRV), times to reach target calories, mean GRV amounts, abdominal distension, vomiting, diarrhea, VAC and infection-related ventilator-related complications (IVAC) were all observed.
**Results:** Although there were statistically significant differences between the groups in terms of the HGRV rates and the HGRV rates exceeding the determined threshold values [p <0.05], there was no significant difference between the groups in terms of abdominal distension, vomiting, diarrhea, VAC and IVAC (p> 0.05).
**Conclusion:** The results of this study suggest that measuring the amount of GRV in intensive care patients fed by EN via the nasogastric tube in order to decide on gastrointestinal motility function and to reduce the complication rate, is not necessary.

Key Words: Enteral nutrition, complications, critical care

## ÖZ

**Amaç:** Çalışmamızın amacı, yoğun bakım ünitesinde solunumu mekanik ventilasyon ile sağlanan hastalarda, toplam gastrik kalıntı hacim miktarları ve iki farklı gastrik kalıntı hacmi eşiği ile gastrointestinal komplikasyon gelişimi arasında ilişki olup olmadığının tespiti, ikinci hedefimiz ise farklı gastrik kalıntı hacimlerinin, ventilatör ilişkili durumlar üzerindeki etkilerini belirlemektir.
**Metod:** Çalışmaya en az 3 gün enteral beslenme planlanan, 18 yaşın üzerindeki 70 adet yetişkin hasta dahil edildi. Birinci gruptaki 35 hastada gastrik kalıntı hacmi eşiği 250 ml, ikinci grupta ise 500 ml olarak belirlendi. İzlem süresi boyunca, belirlenen her iki gastrik kalıntı hacmi eşiğinden herhangi birini aşmamış olan hastaların, 72 saat boyunca kaydedilen gastrik kalıntı hacimlerinin toplam miktarı hesaplandı. Tüm hastaların yüksek gastrik kalıntı hacim oranları, hedef kaloriye ulaşma süreleri, ortalama mide kalıntı hacim miktarları, abdominal distansiyon, kusma, diyare, ventilatör ilişkili durum ve enfeksiyona bağlı ventilatör ilişkili komplikasyon oranları gözlendi.
**Bulgular:** Çalışmamızın sonunda, iki grup arasında yüksek gastrik kalıntı hacim oranları, belirlenen eşik değerini aşan yüksek gastrik kalıntı hacim oranları arasına, anlamlı bir fark oluşmasına rağmen (p<0.05), her iki grup arasında abdominal distansiyon, kusma, diyare, ventilatör ilişkili durum ve enfeksiyona bağlı ventilatör ilişkili komplikasyon açısından anlamlı bir fark yoktu. (p >0.05)
**Sonuç:** Bu sonuçlar, enteral yolla beslenen yoğun bakım hastalarında, gastrointestinal motiliteyi ölçmek ve komplikasyon oranını azaltmak için gastrik kalıntı hacim miktarlarının ölçülmesinin gerekli olmadığı düşündürmektedir.

Anahtar Kelimeler: Enteral beslenme, komplikasyonlar, yoğun bakım

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## INTRODUCTION

Nutrition is one of the important factors playing a role in decreasing morbidity and mortality rates, treating immunodeficiency and speeding up wound healing processes in intensive care patients [1]. In the instances where there are no contraindication, the physiological route of nutrition is enteral nutrition. However, gastrointestinal intolerance or dysfunction [vomiting, gastric distension, high gastric residual volume and diarrhea that may occur during enteral nutrition, may limit the practice of enteral nutrition [2]. Gastric residual volume (GRV) is defined as the volume of gastric juice pulled back by a syringe connected to the feeding tube, when aspirating the gastric contents [3]. The GRV measurement, which is one of the important markers of gastrointestinal function, is one of the standard procedures in care protocols of the patients receiving enteral nutrition and is still frequently used for the diagnosis of food intolerance [4]. The measurement of GRV for the evaluation of gastrointestinal dysfunction may also help the determination of intolerance to EN, during the onset and progression of EN [5].

In cases of high GRV resulting from delayed gastric emptying during enteral nutrition, foods accumulated in the stomach may accidentally pass into the trachea and pneumonia may occur as an result of this. Enteral nutrition is accepted as one of the risk factors for the development of ventilator-associated pneumonia (VAP), as well as respiratory failure, coma and depressed state of consciousness [6]. Because of these limitations, clinicians keep recommending the use of GRV measurements in the care of patients receiving enteral nutrition [7]. There is no ideal amount standardized by evidence-based medical practice for gastric residual volume to be used, for the measurement of gastric function during the nutrition of intensive care patients. However, volumes between 150 and 500 ml are generally used in practice and residual volumes are generally measured every six hours for the measurements of gastric residual volume, that are used as a marker of gastrointestinal intolerance [8].

Our study aimed to determine whether there was a relationship between total GRV amounts calculated based on the period of time to reach total calories in patients on mechanical ventilation in an intensive care unit, and gastrointestinal intolerance occurrence among patients with two different GRV thresholds. We also aimed to determine the results of this change on the incidence of ventilator-associated condition and different variables.

## MATERIAL AND METHOD

With the approval of Clinical Research Ethical Committee of Erciyes University Faculty of Medicine (2019/42), eighty patients over the age of 18 who were admitted to the intensive care unit due to pulmonary diseases, cerebrovascular diseases and large joint fractures, who were determined as patients requiring mechanical ventilation due to respiratory failure, coma, unconsciousness and hemodynamic stabilization, and who were scheduled to receive enteral feeding for at least three days, were included in the study. The entire study was carried out according to the principles of the Declaration of Helsinki. Our study plan was explained in detail to all the patients or patients' relatives and their informed consents for voluntary participation were obtained. The target calories for EN were not reached in 10 out of the 80 patients. Since the enteral nutrition was considered unsuccessful in these patients, additional feeding methods were used.

Those who stayed in intensive care unit for less than 3 days, those under the age of 18, those whose nutrition would stop for more than two hours for any reason, those with a gastrostomy or jejunostomy feeding tube, those who had non-functional bowel (ischemia, obstruction or anatomic conditions), those who had signs of generalized peritonitis and paralytic ileus, those who had severe diarrhea (>1000ml/day), those who were diagnosed with upper gastrointestinal bleeding and those who had morbid obesity (body mass index (BMI) >40 kg/m2), were all excluded from the study.

Group I (n = 35): measurements were carried out every 6 hours in the patient group whose GRV threshold was determined to be 250 ml and the amount of increase in the enteral feeding mixture after every six hours was 10 ml. The total amount of gastric residual volumes recorded for 72 hours in the patients whose GRV was under the threshold

value were calculated. Patients whose total GRV amount was up to 500 ml were also included in this group.

Group II (n = 35): measurements were carried out every 6 hours in the patient group whose GRV threshold was determined as 500 ml and the amount of increase in the enteral feeding mixture after every six hours was 10 ml. The total amount of gastric residual volumes recorded for 72 hours in the patients whose GRV was under the threshold value was calculated. Patients whose total GRV amount was more than 500 ml were also included in this group.

Patients' demographic data, comorbid diseases, sedative or inotropic drugs given, Acute Physiology and Chronic Health Evaluation II (APACHE II) scores and total days spent in intensive care unit, were recorded. A 12 fr size of nasogastric feeding tube was placed in all the patients and the position of nasogastric tube was confirmed with chest radiography and the method of auscultation through gastric insufflation of 15 ml of air with a syringe. The tubes were kept in the stomach during the feeding and measurement processes.

Patients were continuously fed by the same polymeric formula in which 1 cc was 1 calorie by using the kangaroo feeding pump Set (Abbot, Illinois, USA) which was regularly calibrated. Energy requirements of the patients were calculated by the Schofield formula. During nutrition and GRV measurements, the head of the bed was elevated 30–45º and Ramsay sedation score was kept at 3-6 by ensuring its compatibility with mechanical ventilator of the patients. Midazolam (Dormicum 15 mg/3ml Roche) 0.1 mg / kg / hour was administered for sedation. Opioids were not used. In cases where the sedation score was adequate, no additional sedation was given, whereas when it was inadequate, Midazolam was used to provide intravenous sedation, according to the clinical state of the patient, though the protocol of the sedation was not standardized. Gastrointestinal intolerance findings (vomiting, regurgitation abdominal distension and diarrhea) were monitored in the patients.

Nutrition was initiated at a rate of 20 ml/hour in both groups. In case of gastrointestinal intolerance, no EN was administered as long as the findings were still observed in the patients during nutrition intervals, until the next controls. If gastrointestinal intolerance was not found, planned increases were prepared in case GRV was under the threshold value. When GRV exceeded the threshold value, the nutrition continued at the last determined rate without any increase. When the patients whose nutrition was intermitted due to gastrointestinal intolerance were re-evaluated, the nutrition was initiated again at a rate of 20 ml/h if there was no finding of gastrointestinal intolerance, and the decisions on EN were made. According to the criteria used in the determination of gastrointestinal intolerance, clinical symptoms of gastrointestinal intolerance were as follows: abdominal distension: abdominal swelling felt by palpation and no bowel sounds; vomiting: orally ejected enteral formula; regurgitation: enteral formula in oral or nasal cavity; and diarrhea: watery stool for five times or more during 24-hour period or approximate stool volume equal to 2.000 ml/day or more.

All the patients were screened for ventilator-associated conditions and infection-related ventilator-associated complications. VAC was defined as the need for increase in daily minimum FiO2 at a rate of ≥20% or increase lasting two or more days in daily minimum PEEP (positive and expiratory pressure) at a rate of ≥3 cm H2O after a stability lasting for two or more days or followed by a decrease to daily minimum PEEP or daily minimum FiO2, in a patient who was connected to the mechanical ventilator. Infection-related ventilator-associated complications include possible infection markers at the same time with the onset of VAC in addition to positive radiographic findings. Infection-related ventilator-associated complications are defined as abnormal body temperature (>38 0C or 36 0C) or abnormal leukocyte count (4.000/mm3 or less or 12.000/mm3 or more) with the use of one or more than one antibiotic, which was initiated four or more days before and still used [9].

GRV measurements of the patients included in the study were recorded by group coordinators every six hours in both Group I and Group II, by evaluating the last states of the patients. Before the study started all the nurse practitioners were trained on infection control for the patients receiving enteral

nutrition, as the other factors were excluded and infection control had a vital importance.

Gastric residual volumes were measured in ml through nasogastric tube aspiration by a 50 ml syringe. The volume obtained from the patient was not returned but emptied. Required period of time to reach target EN rate, interval periods and their causes were noted. All the patients received EN for at least 72 hours. Patients who were discharged from intensive care unit before the 72 hour-length of stay were excluded from the study. According to the power analysis based on the study by Pinilla et al. [10], 34 patients were required in each group at 95% confidence interval and 80% statistical power with a mean ± standard deviation of 22±22 (mean ± SD) in the first implementation (GRV:200 ml) and 12±8 (mean ± SD) in the second implementation, in order to determine the difference between the durations of both implementations to reach the target calories.

Statistical analysis

Statistical analysis was carried out using the SPSS 22.0 Statistics Package of Social Sciences Software (IBM SPSS Statistics, Armonk, NY, USA). Continuous data was given as mean and standard deviation while categorical data was given as counts and percentiles. Differences between the groups in terms of independent variables were statistically evaluated. The results with p value under 0.05 were accepted as statistically significant. The Mann-Whitney U test was used in the evaluation of nonparametric data and the Chi-square and Fisher's exact tests were used in the evaluation of the difference between the groups.

## RESULTS

The study was initiated with 80 consecutive patients expected to remain in the intensive care unit for more than 3 days with mechanical ventilation support at the Niğde Education and Research Hospital.

Four of the patients were transferred to the ward before their follow-up period ended. Enteral nutrition was stopped and parenteral nutrition was initiated in one of the patients. In another patient, enteral nutrition was stopped as the result of a suspected cholecystitis and 4 of the patients were exitus. Out of 80 patients, 10 were excluded from the study due the reasons stated above and therefore the data of 70 patients were evaluated. There was no difference between the groups in terms of age, gender, causes of admission to the ICU, need for sedation, use of inotropes, APACHE 2 score and having a co-morbid disease (Table 1).

**Table 1: Patient characteristics**

| | Group I | Group II | P |
|---|---|---|---|
| Number of patients | 35 | 35 | |
| Age | 70 ± 18.8 | 75 ± 11.9 | 0.312 |
| Gender | | | 0.473 |
| Male | 15 (42.8%) | 18 (51.4%) | |
| Female | 20 (57.1%) | 17 (48.5%) | |
| Weight | 73 ± 11.4 | 76 ± 7.7 | 0.069 |
| Comorbid disease | | | 0.780 |
| SVH | 28.6% | 20.0% | |
| Heart lung disease | 42.9% | 48.6% | |
| Skeletal system disease | 11.4% | 8.6% | |
| Other | 17.1% | 22.9% | |
| APACHE II | 25.1 ± 4.6 | 26.4 ± 2.3 | 0.131 |
| Inotropic need | 45.7% | 57.1% | 0.339 |
| Sedation requirement | 48.6% | 45.7% | 0.811 |
| Additional disease | 65.6% | 68.7% | 0.799 |

**Values are expressed as n. Values for p <0.05 were considered as being statistically significant.**

In our study, mean total GRV was measured as 317.14 ± 127.39 ml in Group I while it was 598.86 ± 86.09 in Group II during the follow-up period of 3 days and a statistically significant difference was found between the two groups (p<0.001). While a high gastric residual volume was measured in 6 patients in Group I, no high GRV was monitored in Group II. No statistically significant difference was found between the groups in terms of the period of time to reach target calories, target calorie values and the length of stay in intensive care unit (Table 2). No statistically significant difference was found between Group I and Group II in terms of the abdominal distension, diarrhea, vomiting, rate of IVAC and rate of VAC (Table 3).

Table2: Obtained values

| | Group I | Group II | p |
|---|---|---|---|
| Patients with HGRV | 17.1% | 0.0% | 0.012 |
| Time until reaching target calories | 48.4 ± 19.3 | 53.4 ± 19.3 | 0.152 |
| Medium GRV | 317.1 ± 127.3 | 598.8 ± 86.0 | 0.001 |
| Target calories | 1673.1 ± 246.2 | 1708.2 ± 159.2 | 0.481 |
| Duration of stay | 19.6 ± 13.0 | 15.8 ± 10.2 | 0.205 |

GRV: gastric residual volume, HGRV: high gastric residual volume. Values are expressed as n. Values for p <0.05 were considered as statistically significant results.

Table 3: Rates of gastrointestinal complications

| | Group I | Group II | p |
|---|---|---|---|
| Patients with gastrointestinal complications | 45.5% | 54.5% | 0.607 |
| Patients with abdominal distension | 38.5% | 61.5% | 0.356 |
| Patients with diarrhea | 42.9% | 52.1% | 0.766 |
| Patients with vomiting | 37.5% | 62.5% | 0.710 |
| Patients with VAC | 45.5% | 54.5% | 0.743 |
| Patients with IVAC | 33.3% | 66.6% | 1.00 |

VAC: ventilator-associated condition. IVAC: infection-related ventilator-related complications. Values are expressed as n. Values for p <0.05 were considered as statistically significant results

## DISCUSSION

There exists some views asserting that gastrointestinal functions must be regularly measured when the evidence associated with negative results caused by the occurrence of gastrointestinal intolerance are considered in critical patients [11]. Using GRV in the determination of gastric intolerance in the patients receiving enteral nutrition is a method accepted as clinically routine and it has been included in the nutrition support algorithms in several intensive care units [12]. Currently, GRV is used for the measurement of gastrointestinal function in our own intensive care unit.

Targeted calories in enteral nutrition may generally be reached in 3 days, however, in some studies, periods between 3 days and 6 weeks have been observed [10, 13]. For the patients in our study group, the targeted period of time to reach the daily calorie intake was 3 days and no statistically significant difference was found between the groups, in terms of the period of time to reach target calories. These results were similar to those in the studies by Flesher et al. [14] and Pinilla et al. [10].

Montejo et al. suggested that a GRV value between 200 and 500 ml is a normal value since a threshold value of 500 ml during enteral nutrition implementation was not associated with gastrointestinal complications or adverse effects in the result variables. In this study, the threshold values of 200 ml and 500 ml were compared, and it was found that increasing the GRV threshold value in patients with mechanical ventilator was not associated with gastrointestinal complications [15]. The reason why we chose a threshold value of 500 ml in the second group of our study was that this value was determined as a clinical endpoint in literature. In our study, groups with GRV threshold value of 250 and 500 ml were compared. We could not find a statistically significant difference between the groups in terms of the period of time to reach target calories, regurgitation, aspiration, gastric distension and rate of VAC. These results were consistent with those in the study by Montejo et al [16].

It was reported that sedation might affect gastric drainage and indirectly, GRV [17]. This is the reason the Ramsay score was monitored and kept between 3 and 6 in all the patients included in this study. No statistically significant difference was found between the two groups in terms of sedation score. In our study, no statistically significant difference was found between GRV values of the patients using or not using sedative medication for sedation. It is recommended that the volume should be 10-20 ml when the nutrition is initiated, that it should be carefully increased by monitoring gastrointestinal symptoms and that it should not exceed the maximum energy of 20 kcal/kg, which is recommended for acute phases within 3 days. Two methods are available for the standardization of GRV measurement. The first is withdrawing gastric juice with a syringe and the second is performing this action with the

help of a drainage bag placed at the level of the stomach, and by monitoring the ejected volume in between 15 and 120 minutes [18]. In our study, we initiated enteral nutrition at a rate of 20 ml/hour and aimed to reach the determined energy target with separate Schofield formulas for each patient. While measuring GRV, we used the aspiration method, using a 50 ml syringe. The reason why we did not use free drainage system for GRV measurement was to avoid the adverse effects of nutrition intermittences needed for drainage measurement.

Williams et al. [19] have suggested returning the gastric aspirate withdrawn by the syringe to the patient. Buyukcoban et al. [20] did not return the gastric aspirate to the patient in order to avoid the adverse effects of bolus injection. We chose not to return the gastric aspirates.

Mc Clave et al. [8] compared GRV threshold values of 200 ml and 400 ml in terms of aspiration and regurgitation and found that high GRV did not increase the risk. In 2007, Desachy et al. [21] compared the vomiting rates of the patients whose GRV were >300 ml and <300 ml in their study, in which GRV threshold value was determined as 300 ml, and they found no significant difference. In our study, no significant difference was found between the vomiting rates of the patients whose threshold values were 250 ml and 500 ml. This result brought us to consider that the GRV amount was not critical in the rate of vomiting.

Bankhead et al. [22] found no relationship between high GRV and the probability of aspiration and related pneumonia. In a study by Reinger et al. [23] in 2013, the group with 200 ml GRV and the group with no GRV were compared through intermitting the nutrition, in case of gastrointestinal intolerance. The group in which no GRV was measured reached the target calories faster, but complications such as ventilator-associated pneumonia, infection or aspiration and lengths of stay in intensive care, were similar. Fogg et al. reported that personnel training, proper usage procedures and developed enteral nutrition protocols, decreased the level and incidence of bacterial contamination in enteral tube feeding [24]. Infection-related ventilator-associated complications occurred in none of our patients and no significant difference was found between the two groups in terms of VAC. Before the study started, all the nurse practitioners were trained on infection control for the patients receiving enteral nutrition, since the other factors were excluded, and infection control had a vital importance and would have affected our results.

In the evaluation based on total GRV amounts in our study, total GRV amounts were measured until target calories were reached and no statistically significant difference was found between the two groups in terms of gastrointestinal intolerance.

Although Elke et al. [25] reported that necessity for GRV measurements in units where nutrition implementations were carried out by an experienced nurse team and where standardized nutrition protocols and other safety criteria were implemented became a controversial topic. GRV is recommended to be used in particular in surgical intensive care patients and patients with an extremely severe condition. In our study, we could not find a statistically significant difference between the groups in terms of the period of time to reach target calories, regurgitation, aspiration, gastric distension and rate of VAC. Our results of standardized enteral nutrition protocol, based on two different GRV values implemented in our intensive care unit by experienced intensive care nurses, were investigated and these results were consistent with the views of Elke et al. [25]

Limitations of the study: In our study, GRV amounts of the patients were only measured for the period of reaching the target calories, not throughout their entire stay in the ICU.

**Conclusion:** Although a statistically significant difference was found between Group I for which 250 ml of GRV was based and Group II for which 500 ml of GRV based in terms of total GRV in our study, there was no difference in terms of occurrence of vomiting, diarrhea, regurgitation, abdominal distension, infection-related ventilator-associated complications and ventilator-associated condition. These results make the necessity of GRV measurement used to measure gastrointestinal motility in patients receiving enteral nutrition via nasogastric tube controversial; they suggest that the usage of enteral nutrition protocols standardized in intensive care units to

prevent gastrointestinal intolerance is crucial.

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# Ramelteon used to treat insomnia can reduce the occurrence of osteoporosis

## Uykusuzluğu tedavi etmek için kullanılan Ramelteon, osteoporoz oluşumunu azaltabilir

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## ABSTRACT

**Aim:** Melatonin promoted osteoblast differentiation and causes an increase in levels of markers of bone differentiation and proliferation. Ramelteon (RAMEL) activates melatonin receptors and binds to these receptors as non-selective. In this study, we investigated the preventive effects of the melatonin agonist RAMEL on osteoporosis by radiological, histological, and molecularly.
**Methods:** Groups 1: Control, Group 2: Osteoporosis: Overectomized group (OP), Group 3: OP + ramelteon 2 mg/kg, Group 4: OP + ramelteon 4 mg/kg. 24 animals underwent bilateral ovariectomy. RAMEL was administered orally once a day in the prophylactic treatment mode for 8 weeks, 6 weeks after ovariectomy.
**Results:** Fourteen weeks after ovariectomy, there was a significant reduction in femoral bone mineral density (BMD) (g/cm2) in the OP group compared to the control group. Compared to the OP group, RAMEL treatment significantly increased the BMD level ($p<0.05$). Bone matrix protein 2 (BMP2) and runt-related transcription factor 2 (RUNX2) mRNA levels were significantly lower in the OP group than in the control group ($p<0.05$). RUNX2 and BMP2 mRNA levels were significantly higher in the RAMEL treatment groups than in the OP group ($p<0.05$).
**Conclusion:** To take advantage of the peripheral effects of melatonin, RAMEL, a peripheral melatonin agonist, can be used to prevent osteoporosis.

Keywords: Ramelteon, melatonin, osteoporosis, bone

## ÖZ

**Amaç:** Melatonin osteoblast farklılaşmasını teşvik eder ve kemik farklılaşması ve proliferasyon belirteçlerinin düzeylerinde artışa neden olur. Ramelteon (RAMEL) melatonin reseptörlerini aktive eder ve bu reseptörlere seçici olmayan şekilde bağlanır. Bu çalışmada melatonin agonisti RAMEL' in osteoporoz üzerindeki önleyici etkileri radyolojik, histolojik ve moleküler olarak araştırıldı.
**Yöntem:** Grup 1: Kontrol, Grup 2: Osteoporoz: Over eksizyonu yapılan grup (OP), Grup 3: OP + ramelteon 2 mg/kg, Grup 4: OP + ramelteon 4 mg/kg. 24 hayvana, bilateral ovariektomi uygulandı. RAMEL, ovariektomiden 6 hafta sonra, 8 hafta boyunca profilaktik tedavi modunda günde bir kez oral yoldan uygulandı.
**Bulgular:** Ovariektomiden on dört hafta sonra, kontrol grubuna kıyasla OP grubunda femoral kemik mineral yoğunluğunda (g/cm2) anlamlı bir azalma oldu. OP grubu ile karşılaştırıldığında, RAMEL tedavisi BMD seviyesini önemli ölçüde artırdı ($p<0.05$). Bone matrix protein 2 (BMP2) ve runt-related transcription factor 2 (RUNX2) mRNA seviyeleri OP grubunda kontrol grubuna göre anlamlı derecede düşüktü ($p<0.05$). RUNX2 ve BMP2 mRNA seviyeleri, RAMEL tedavi gruplarında OP grubuna göre anlamlı olarak daha yüksekti ($p<0.05$).
**Sonuç:** Melatoninin periferik etkilerinden yararlanmak için, bir periferik melatonin agonisti olan RAMEL, osteoporozu önlemek için kullanılabilir.

Anahtar Kelimeler: Ramelteon, melatonin, osteoporoz, kemik

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## INTRODUCTION

Melatonin secretion during the night from the pineal gland has positive effects on bone in various aspects [1]. Melatonin promoted osteoblast differentiation and causes an increase in levels of markers of bone differentiation and proliferation, including increased synthesis of osteopontin, bone alkaline phosphatase, osteocalcin, a bone matrix protein 2 (BMP2) and runt-related transcription factor 2 (RUNX2) [2-5]. It has been shown that melatonin has a beneficial effect on osteoporosis after ovariectomy in rats [6] and promotes fracture healing [7].

Melatonin receptors (MT) have been identified in three subgroups. These receptors are MT1, MT2 and the recent MT3 [8]. MT1 and MT2 are membrane receptors and most effects of melatonin is mediated by these receptors [9,10] MT1 and MT2 expressions were seen in peripheral tissues and cells and contribute to several immune and vasomotor effects [11]. MT1 mainly mediates vasoconstriction, whereas MT2 mainly causes vasodilatation. However, tissue distributions and the roles of MT3 receptors are not yet fully understood. Several studies have shown that MT2 receptors are responsible for the effects of melatonin on the bone [2,12].

Ramelteon (RAMEL) activates both MT1 and MT2 melatonin receptors. In the United States, FDA approved it in 2005 for the treatment of insomnia and for individuals who have difficulty falling asleep [13]. RAMEL has a higher affinity for both receptor subtypes [13].

Many experimental studies in the current literature have shown several positive effects of melatonin in human bone proliferation and differentiation [2,14,15,16,17]. But long-term use of melatonin does not seem to be possible in the treatment of osteoporosis because of side effects resulting from hormonal stress on the chronological rhythm. Therefore, the current study investigated preventing osteoporosis using RAMEL, which was administered orally once daily in a preventive treatment mode for 8 weeks, 6 weeks after ovariectomy operation. To test this therapeutic strategy, improvement was assessed by Dual-energy X-ray absorptiometry (DEXA) and radiological analysis; in histopathology, hematoxylin-eosin (H&E) staining was evaluated, and molecularly, expression BMP2 and RUNX2 genes were measured with real-time PCR (Real-Time PCR).

## MATERIALS AND METHODS

### Animals

32 female albino Sprague-Dawley (10-12 weeks old) rats were provided from Atatürk University (ATADEM) Medical Experimental Research Center. The animals weighed were between 240 and 260 g and were divided into four equal groups. Experiments were performed according to the standard experimental procedures to keep normal temperature conditions (22 °C). Animal experiments were performed according to International Guidelines. This experiment was approved by the Institutional Animal Care and Use Committee of Ataturk University (2019/E.1900179336). During the experiment, the animals were kept under controlled light conditions (12 h light/dark cycle) and air-conditioned room conditions at 22 °C. Standard rat chow and tap water were provided ad libitum.

### Chemicals

Ramelteon (Ramelda©), Ketamine (Ketalar 500 mg/10 ml), xylazine (Basilazin %2), and Metamizole sodium (500 mg/ml) were purchased from Abdi Ibrahim, Pfizer, Biotek, Sanofi-Aventis, respectively.

### Surgical Procedures

The rats were given a diet of standard commercial rat pellets. Twenty-four animals underwent bilateral ovariectomy [18]. For this procedure, animals were anesthetized intraperitoneally with 80 mg/kg ketamine + 8 mg/kg xylazine. After making a longitudinal incision (0.5-1 cm) in the midline region of the lower abdomen, the ovaries were removed with a small peritoneal incision. Metamizole sodium was administered for postoperative pain control.

### Drug administration

After surgical procedures, the ovariectomized rats were randomly divided into the following three groups:

Group 1: Control: Sham-operated group

Group 2: OSTEOPOROSIS (OP); Ovariectomized control group

Group 3: Ovariectomized and 2 mg / kg ramelteon administered (in 1 ml dH2O) (RAMEL2)

Group 4: Ovariectomized and 4 mg / kg ramelteon administered (in 1 ml dH2O) (RAMEL4)

Drug administration was started 6 weeks after ovariectomy surgery. All the drugs were given orally once per day and for eight weeks.

Dual-energy X-ray absorptiometry (DEXA) estimations

Bone mineral density (BMD) was analyzed by DEXA using Discovery Wi (Hologic Inc., Bedford, MA, USA). The same investigator performed each measurement and all analysis were performed using the same GY region (ROI) window size.

Histopathological Analysis

Decalcification and Tissue Processing Procedure

Following the surgical procedure, femoral samples were kept in 10% formalin solution for fixation. The bones were kept for 7-10 days in 8% hydrochloric acid - 8% formic acid solution (mixed in equal volume) with a total volume of 10 times the tissue for decalcification. The decalcification solution was renewed every day and the distal end of the femur bones was perforated with a needle to check whether the decalcification process was over. To neutralize the decalcified bone tissues, soaking processes were applied in tapping water (30 minutes) - ammonium solution (5 drops / 100 ml - 30 minutes) and tapping water (12 hours), respectively. The proximal end of the femoral bones was cut with a lancet and placed in tissue cassettes for tissue processing. The tissue processing procedure was performed by passing the tissues through increasing alcohol (70% -80% -90%-absolute), xylol, and paraffin series, respectively, using the Leica TP 1050 device. Manually, using molten paraffin, tissues were formed into blocks and five sections were taken from each block onto positively charged slides with the aid of the Leica RM 2145 microtome device. Two of the sections were used for Hematoxylin Eosin (H&E) staining.

H&E Staining Procedure: Slides were kept in an EN 055 model incubator for 5 minutes at 60o and 30 minutes in xylene for fixation and deparaffinization. To hydrate the tissues, the sections were kept for three minutes in two series of xylol, decreasing alcohol series (96% -90% -80% -70% -50%) and under tapping water. The tissues were colored by hematoxylin staining (5 minutes), soaking in tapping water (5 minutes), eosin staining (2 minutes), and then the mounting process was applied with xylene, entellan and coverslip.

Monitoring and evaluation: H&E preparations were analyzed and recorded using an Olympus BH 40 light microscope and integrated camera. In the analysis stage of H&E stained preparations under a light microscope, the following criteria were taken into account: old bone mass; trabecula/ spicule thickness; alveolar volume, and new bone formation [20,21].

Molecular Studies

RNA extraction: 20 mg bone tissues were homogenized with Tissue Lyser II (Qiagen) using liquid nitrogen. Total RNA extracted according to the RNAeasy mini kit directions in QIAcube RNA isolation system. Total mRNA amounts were determined by using nanodrop spectrophotometry (EPOCH Take 3 Plate, Biotek) at 260/280 nm and then was stored at -80 ° C until the experiment.

cDNA Synthesis: High Capacity cDNA Reverse Transcription Kit was used for cDNA synthesis. 10µl RNA and 10 ul master mix were used in each reaction. After the reaction, the amount of cDNA was determined by nanodrop spectrophotometry (EPOCH Take3 Plate, Biotek) and stored at -20 °C until the experiment.

Reaction volume

10 µl total RNA 2 µl 10 X RT Random Primers

2 µl 10 X RT Buffer 1 Multil MultiScribe Reverse Transcriptase

0.8 µl 25 X dNTPs mix 4.2 µl diethylpyrocarbonate H2O.

Quantitative Determination of RUNX2 and BMP2mRNA Expressions

RUNX2 and BMP2 mRNA expression was performed by Real-Time PCR. Bactin was used as the reference gene. All procedures were performed using the Step One Plus Real-Time PCR System (Applied Biosystems). The following TaqMan® Gene Expression Assays for 200ng cDNA were continued by pipetting as described below for 40 cycles. Obtained Ct values were automatically converted into ΔΔCt [19].

Reaction Volume; 9 µl cDNA (200ng); 10 µl TaqMan Master Mix; 1 µl Assay

Statistical methods: One-way (ANOVA) post hoc Duncan's tests were performed to comparing between the groups in IBM SPSS 25.00 packet program. Means±standard deviation was used for analyses and P<0.05 was evaluated as statistically significant.

## RESULTS

BMD analyses: Forty-two days after the ovariectomy, femoral BMD (g/cm2) levels were significantly decreased in the OP group when compared to the control group (Fig. 1 and 2). When compared to the OP group, RAMEL administration significantly improved BMD levels in 4 mg/kg dose (p<0,05).

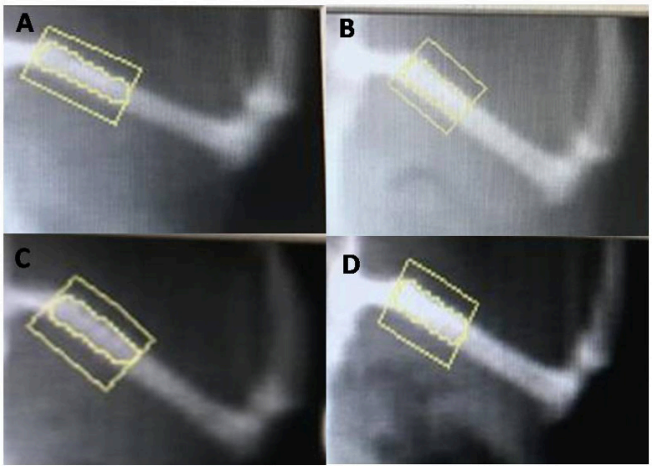


Figure 1: Left femoral bone DEXA images of SHAM, OSTEOPOROSIS, and RAMEL groups. A; SHAM, B; OSTEOPOROSIS, C; OP+RAMEL2, D; OP+RAMEL4. (Abbreviations: RAMEL: Ramelteon, OP: Osteoporosis, DEXA: Dual-energy X-ray absorptiometry)

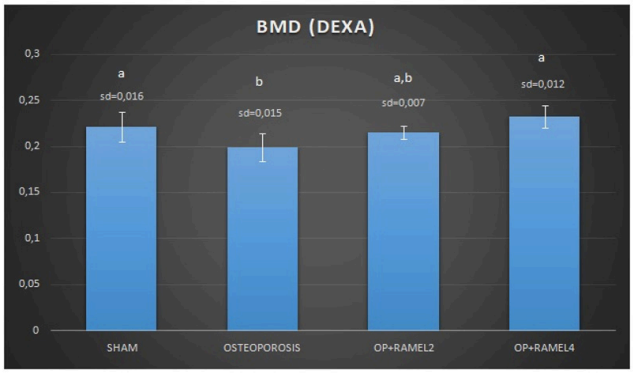


Figure 2: BMD measured with DEXA results of SHAM, OSTEOPOROSIS, and RAMEL groups. This means which have the same letter are not significantly different to the test of Duncan (p=0.05). Results are expressed as mean±SD. This means which have the same letter are not significantly different to the test of Duncan (p=0.05). Abbreviations: RAMEL: Ramelteon, OP: Osteoporosis, DEXA= Dual-energy X-ray absorptiometry, BMD=Bone mineral density, SD= Standard deviation

Real-Time PCR results

RUNX2 and BMP2 mRNA levels: Using Real-Time PCR, we investigated RUNX2 and BMP2mRNA expressions in the rats' femur bone tissue. RUNX2 and BMP2 mRNA levels were significantly lower in the OSTEOPOROSIS group when compared to the SHAM group (p<0.05) (Fig. 3 and 4). In contrast, RUNX2 and BMP2 mRNA levels were significantly higher in the RAMEL treatment groups when compared to the OSTEOPOROSIS group (p<0.05).

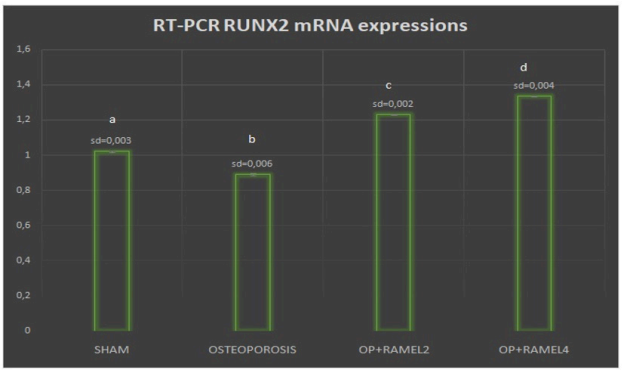


Figure 3: Relative mRNA expression levels of RUNX 2 in femur bone tissue of SHAM, OSTEOPOROSIS, and RAMEL groups. Expression of RUNX 2 was detected by quantitative Real-Time PCR analysis. The relative expression levels were calculated by the 2 (-ΔΔCT) method. β-Actin was used as the reference gene. Results are expressed as mean±SD. This means which have the same letter are not significantly different to the test of Duncan (p=0.05). (Abbreviations: RAMEL: Ramelteon, OP: Osteoporosis, SD= Standard deviation.)

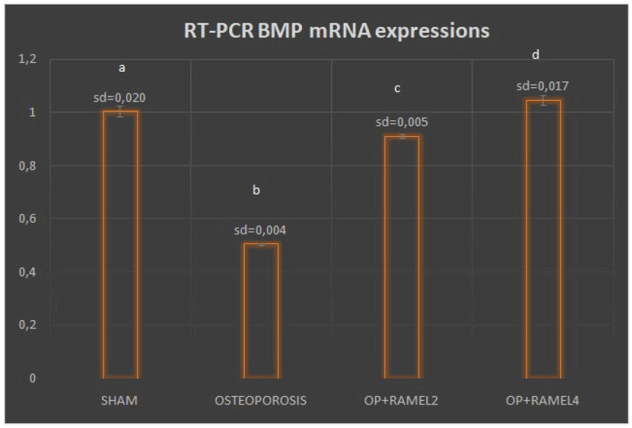


Figure 4: Relative mRNA expression levels of BMP in femur bone tissue of SHAM, OSTEOPOROSIS, and RAMEL groups. Expression of BMP was detected by quantitative Real-Time PCR analysis. The relative expression levels were calculated by the 2(−ΔΔCT) method. β-Actin was used as the reference gene. Results are expressed as mean±SD. This means which have the same letter are not significantly different to the test of Duncan (p=0.05). Abbreviations: RAMEL: Ramelteon, OP: Osteoporosis, SD= Standard deviation.

## Histopathology Results

H&E staining results: For evaluation, the frontal sections of the rat femur bones, the head and neck parts that are expected to be most affected by osteoporosis were analyzed.

SHAM: The general appearance of the compact (CB) and trabecular bone (TB) lines and bone marrow (BM) is normal. The thickness of the trabecular bone spicules, the dimensions of the alveolar spaces, and the integrity of the compact bone are similar to those of healthy animals (Fig. 5a).

OP: More observed total bone loss in trabecular bone compared with the sham group. Loss of bone tissue continuity has been observed in some parts of the trabecular bone due to extensive damage (triangles). There is increased osteoclast activity (curved arrow). (Fig. 5b)

OP + RAMEL2: New ossification areas draw attention in the cortical and trabecular bone line (arrowheads). The more intense eosinophilic staining of the bone tissue adjacent to the bone marrow, compared to the other parts, indicates that the osteoid accumulation in these areas is high. Callus tissues associated with the cortical bone have been identified. Compared to the OP + RAMEL4 group, it was evaluated that a low drug dose increased the ossification rate more (Fig. 5c).

OP + RAMEL4: Pale eosinophilic appearance indicates a low level of osteoid accumulation in new bone formation areas. It can be stated that the bone development rate is slower compared to the OP + RAMEL2 group. Although there are new ossification zones in the cortical and trabecular bone line (arrowheads), it was observed that bone damage due to osteopenia continued (triangles) (Fig. 5d).

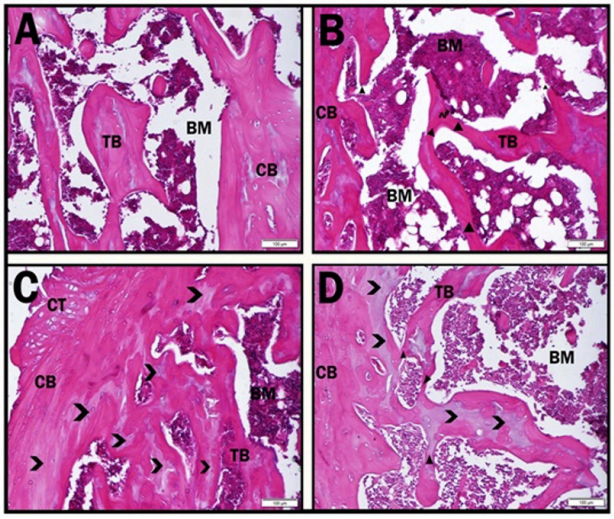


Figure 5. Effects of ramelteon in rats exposed to osteoporosis through ovariectomy. H&E staining findings in osteoporosis in femur bone tissue of SHAM, OSTEOPOROSIS, and RAMEL groups. A; SHAM, B; OSTEOPOROSIS, C; OP+RAMEL2, D; OP+RAMEL4. (Abbreviations: CB: Cortical bone, TB: Trabecular Bone, BM: Bone Marrow CT: Callus Tissue, Triangle: Possible bone resorption areas, Curved Arrow: Osteoclastic activity areas, Arrow Head: New bone formation areas, RAMEL: Ramelteon, OP: Osteoporosis)

To better understand the H&E staining findings, the above findings are summarized in the table below (tab 1) [20,21]. Osteoporosis leads to a decrease in old bone mass, consequently it should be taken into account that it causes a decrease in trabecular / spicule thickness and an increase in alveolar volume.

Table 1: H&E staining scores in femur bone tissue of SHAM, OSTEOPOROSIS, and BOSE groups.

| GROUPS | old bone mass | trabecula/ spicule thickness | alveolar volume | new bone formation |
|---|---|---|---|---|
| SHAM | +++ | ++ | ++ | + |
| OSTEOPOROSIS | ++ | + | +++ | + |
| OP+RAMEL2 | + | +++ | + | +++ |
| OP+RAMEL4 | ++ | ++ | ++ | ++ |

Grade 0: - (0% negative), Grade 1: +(0–33% mild positive), Grade 2: ++

(33–66% moderate positive), Grade 3: +++(66–100% severe positive). H&E: Hematoxylin-Eosin Staining, OP: Osteoporosis, RAMEL: Ramelteon

## DISCUSSION

Melatonin promotes osteogenesis by activating MT2, increasing bone alkaline phosphatase, osteocalcin, RUNX2, and BMP2 [5]. Melatonin has a beneficial effect on bone physiology at therapeutic doses and increased bone alkaline phosphatase and osteocalcin levels [7]. Bone alkaline phosphatase, osteocalcin and osteopontin are produced from osteoblasts and have critical roles in osteoblastic bone formation and play a role in the balance of calcium ions and bone mineralization. Melatonin has osteoblast-inducing effects by inducing RUNX2, inhibiting osteoclast formation and osteoclast differentiation [2]. Melatonin supports osteoblastic differentiation through the BMP signaling pathway [3]. Our study supports these studies in the literature.

We examined RUNX2 and BMP2 expressions [2,3]. We observed that the expressions of RUNX2 and BMP2 decreased in the OSTEOPOROSIS group compared to the SHAM group and increased with RAMEL treatment. We found that RAMEL, a peripheral melatonin agonist, increased RUNX2, and BMP2 in the RAMEL treatment groups compared to the OSTEOPOROSIS group, just like melatonin.

In histopathologic analyses, decreased bone mass and trabecula/spicule thickness and increased alveolar volume with OSTEOPOROSIS improved with RAMEL treatment. Old bone mass and new bone formation increased significantly in the RAMEL treatment groups compared to the OSTEOPOROSIS group. Especially new bone formation is evident in OP + RAMEL4 group.

Although the constructive effect of exogenous melatonin against osteoporosis is known, it is not possible to use it for a long period of time [6,15]. To take advantage of the peripheral effects of melatonin, RAMEL, a peripheral melatonin agonist, can be used to prevent osteoporosis. RAMEL may be the first-choice drug in patients with both insomnia and osteoporosis risk.

Limitations of the study: We had only two RAMEL treatment groups due to the small number of animals.

**Conclusion:** As a result of our study, the protective effect of RAMEL on osteoporosis formation was demonstrated by radiographic, histopathological and molecular analyzes, in the experimental osteoporosis model in rats. The protective effects of RAMEL in osteoporosis were enhanced by molecular analysis of RUNX2 and BMP2, and radiologically by DEXA (BMD). In conclusion, we suggest that RAMEL, a routinely approved drug, reduces the occurrence of osteoporosis. However, more clinical and experimental studies are needed to clarify the effects of RAMEL on osteoporosis in more detail.

**Conflict of Interest:** The author has no conflict of interest related to this article.

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# Investigation of compatibility between serological tests used in laboratory diagnosis of brucellosis

## Brusellozun Laboratuvar Tanısında Kullanılan Serolojik Testler Arasındaki Uyumluluğun Araştırılması

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## ABSTRACT

**Aim:** In the diagnosis of brucellosis, the production of microorganisms in blood or bone marrow culture is considered the gold standard. But it is not always possible to produce the microorganism. For this reason, serological tests are used to diagnose brucellosis. Rose Bengal test (RB), Standard Tube Agglutination test (STA), Coombs Test (CT) and 2-Mercaptoethanol (2-ME) tests are common methods. Immunocapture-agglutination test (ICA) and Brucella Coombs Gel test (BCGT) are tests that can detect blocking antibodies along with total antibodies. The aim of our study was to determine whether there is compatibility between the tests used in diagnosis and the ICA and BCGT tests.

**Material and Methods:** Serum samples were taken from patients with a preliminary diagnosis of brucellosis. RBT testing was performed primarily on all samples. Forty RBT positive and 40 RBT negative sera were included in the study. All serum samples were studied by STA, CT, 2-ME, ICA and BCGT methods. The compatibility between the tests were determined by using the kappa (κ) coefficient with the Cohen kappa analysis method.

**Results:** 28 of 40 patients with RBT positive were detected as positive with STA and 2-ME and 30 of them were positive with CT. BCGT and ICA test results were found positive in all RBT positive samples. All tests results were found to be negative in 40 RBT negative samples. Cohen Kappa analysis found that compliance between RBT and BCGT and ICA (Kappa 1.0 <0.001) was excellent. Compliance between STA, BCGT and ICA tests was found to be good (Kappa 0.7 p< 0.001). CT testing showed a very good level of compatibility between ICA and BCGT (Kappa 0.8 p<0.001). There is a very good compatibility between the 2ME test, ICA and BCGT tests (kappa 0.7 0.001), Compliance between BCGT and ICA was also found to be very good (Kappa 1.0, p<0.001).

**Conclusion:** According to these results, with the use of ICA and BCGT tests, both the number of tests will be reduced and the time to receive results will be reduced. But further studies are needed to determine the sensitivity and specificity of these tests based on culture results. But after that, we believe it can be used as a diagnostic test.

Keywords: Brucellosis, Standard Tube Agglutination test, Coombs Test, Brucella Coombs Gel test, Immunocapture-agglutination test

## ÖZ

**Amaç:** Bruselloz tanısında altın standart kan veya kemik iliği kültüründe mikroorganizmanın üretilmesi olarak kabul edilmektedir. Ancak mikroorganizmayı üretmek her zaman mümkün olmamaktadır. Bu nedenle Bruselloz tanısı için serolojik testler kullanılır. Rose Bengal testi (RB), Standart Tüp Aglütinasyon testi (STA), Coombs Testi (CT) ve 2-Merkaptoetanol (2-ME) testleri yaygın yöntemlerdir. İmmünokaptur-aglütinasyon testi (ICA) ve Brucella Coombs Jel testi (BCGT), toplam antikorlarla birlikte bloke edici antikorları tespit edebilen testlerdir. Çalışmamızın amacı tanıda kullanılan testler ile ICA ve BCGT testleri arasında uygunluk olup olmadığını belirlemektir.

**Gereç ve Yöntemler:** Bruselloz ön tanısı olan hastalardan serum örnekleri alındı. Öncelikle tüm numunelerde RBT testi çalışıldı. Çalışmaya 40 RBT pozitif ve 40 RBT negatif serum dahil edildi. Tüm serum örnekleri STA, CT, 2-ME, ICA ve BCGT yöntemleriyle çalışıldı. Testler arasındaki uyumluluk kappa (κ) katsayısı kohen kappa analiz yöntemi kullanılarak belirlendi.

**Bulgular:** RBT pozitif olan 40 hastanın 28'i STA ve 2-ME ile pozitif, 30'u BT ile pozitif bulundu. BCGT ve ICA test sonuçları tüm RBT pozitif örneklerde pozitif bulundu. Tüm test sonuçları 40 RBT negatif numunede negatif bulunmuştur. Kohen Kappa analizi, RBT ile BCGT ve ICA (Kappa 1.0, p <0.001) arasındaki uyumun mükemmel olduğu bulundu. STA, BCGT ve ICA testleri arasındaki uyumun ise iyi düzeyde olduğu belirlendi (Kappa 0.7, p <0.001). CT testi, ICA ve BCGT arasında çok iyi uyumluluk seviyesi gösterdi (Kappa 0.8, p <0.001). 2ME testi, ICA ve BCGT testleri arasında uyum çok iyi olarak değerlendirildi (kappa 0.7, p <0.001), BCGT ve ICA arasındaki uyumun da çok iyi düzeyde olduğu saptandı (Kappa 1.0, p <0.001).

**Sonuç:** Bu sonuçlara göre ICA ve BCGT testlerinin kullanılmasıyla hem test sayısı azalacak hem de sonuç alma süresi kısalacaktır. Ancak bu testlerin duyarlılığını ve özgüllüğünü kültür sonuçlarına göre belirlemek için daha fazla çalışmaya ihtiyaç vardır. Ancak ondan sonra, teşhis testi olarak kullanılabileceğine inanıyoruz.

Anahtar Kelimeler: Bruselloz, Standart Tüp Aglütinasyon testi, Coombs Testi, Brucella Coombs Jel testi, İmmünokaptur-aglütinasyon testi

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## INTRODUCTION

Brucellosis is a zoonosis that is caused by bacteria of the genus Brucella, which is common in humans and animals. The disease is often transmitted to people as a result of oral ingestion of contaminated food and contact with contaminated material [1,2].

Production of microorganisms in culture is the gold standard in brucellosis diagnosis. However, it is not always possible to produce the microorganism and for this reason, serological tests are used in the diagnosis of brucellosis. The Rose Bengal test (RBT), the standard tube agglutination test (STA), the Coombs test (CT) and the 2-merkapto ethanol test (2- ME) are often used in routine laboratories [1-3]. Aside from the RBT, the working time of these tests is long, the evaluation being carried out after 24 to 48 hours. Due to their specificity and sensitivity, which vary from one to the other, it is recommended that tests be used in combinations. For this reason, studies are continuing to develop simple, shorter-term diagnostic methods. Immuno-capture agglutination (ICA) and the Brucella Coombs Gel Tests (BCGT) are agglutination-based tests that can detect blocking antibodies as well as total antibodies. The ICA test results can be obtained in 24 to 48 hours, while the BCGT results are obtained on the same day and in as short as 2 hours [4,5]. By using these, the number of tests will be reduced, as well as the time to obtain results. It was aimed in this study to determine whether there is compliance between the ICA test and BCGT with the routinely used methods.

## MATERIAL AND METHODS

Serum samples of pre-diagnosed brucellosis patients sent to the microbiology unit of the TOTM Central Laboratory of Inonu University Faculty of Medicine, were included in the study. 40 RBT positive and 40 RBT negative samples were included in the study. In all samples, RBT (Seromed, Istanbul), STA (Seromed, Istanbul), 2-ME and CT (Seromed, Istanbul) tests were studied first. For RBT, one drop of antigen was dripped on one drop of patient serum. Agglutination after rotation was considered positive. For STA, serum samples were diluted to be final titer 1/1280. Antigen was added on it and incubated at 37 degrees. After 24 hours, the tubes were evaluated for agglutination. Titers 1/160 and above were considered significant. 2-ME test is a semi-quantitative tube agglutination test. 2-ME neutralizes IgM type antibodies. Afterwards, the IgG antibodies in the serum combine with the antigen and become agglutinated. CT is a semi-quantitative tube agglutination method. In this test, blocking antibodies bind with anti-human immunoglobulins added to the serum and a visible agglutination appears. Titers 1/160 and above were considered significant [1,2].

ICA (Vircell Brucella Capt test, Porque Technologica, Spain) consists of U-bottomed well strips coated with anti-human immunoglobulins. For this test, patients' serums were diluted. The antigen was added and incubated for 18-24 hours until agglutination occurs in the well strips. If there were no Brucella antibodies in the serum, the antigens appeared as blue dots and those that collapses to the bottom were considered negative. A homogeneous image attached to the inner surface of the well strips was evaluated as positive. BCGT (ODAK Brucella, Toprak Medical, Istanbul) is a method of agglutination that occurs in the wells containing gel matrix and coombs antibodies. The test was attempted according to the manufacturer's recommendations. At the end of the incubation, the results were evaluated visually. If there are no Brucella-specific antibodies, Brucella antigens appear pink at the bottom. If there is an antibody specific to Brucella, the antigen-antibody complex appears pink at the top of the gel [5]. The compatibility between the tests were determined using the Cohen Kappa analysis method and the Kappa (κ) coefficient (κ=0.61-0.80 good compatibility, κ=0.81-1.00 very good compatibility) [6]. Analysis were made by using the IBM SPSS Statistics for Windows version 25.0 (NY, USA).

## RESULTS

A total 28 of the 40 RBT positive patients were positive with STA and 2-ME, and 30 were positive with CT. BCGT and ICA test results were found as positive in all RBT positive samples. STA, CT, 2-ME, BCGT and ICA tests were also found to be negative in 40 serum samples that were RBT negative. Distribution of the test results

of 80 samples is given in the Table 1. Cohen Kappa analysis was performed to determine the compatibility between the tests. Compliance between ICA and BCGT with the RBT was very good (k 1.0 p<0.01). It was determined that compliance was good between the ICA and BCGT tests with the 2- ME test (k 0.7 p<0.01). Compliance between ICA and BCGT was observed to be good with the STA test (k 0.7 p<0.01). A very good level of compliance was found between ICA and BCGT tests with CT test (Kappa 0.8 p<0.01). Compliance between BCGT and ICA was also found to be very good (Kappa 1.0 p<0.001). Compliance between the tests (kappa coefficient) is given in the Table 2.

**Table 1. Distribution of the test results of 80 samples**

| TEST | Positive n(%) | Negative n(%) | Total n(%) |
|---|---|---|---|
| RBT | 40 (50) | 40(50) | 80(100.0) |
| STA | 28(35) | 52(65) | 80(100.0) |
| CT | 32(40) | 48(60) | 80(100.0) |
| 2-ME | 28(35) | 52(65) | 80(100.0) |
| BCGT | 40(50) | 40(50) | 80(100.0) |
| ICA | 40(50) | 40(50) | 80(100.0) |

**Table 2. Compliance between the tests (kappa coefficient)**

| | Kappa | p |
|---|---|---|
| RBT - BCGT | 1,0 | <0,001 |
| STA - BCGT | 0,7 | <0,001 |
| CT - BCGT | 0,8 | <0,001 |
| 2ME Test - BCGT | 0,7 | <0,001 |
| RBT- ICA | 1,0 | <0,001 |
| STA - ICA | 0,7 | <0,001 |
| CT- ICA | 0,8 | <0,001 |
| 2ME Test -ICA | 0,7 | <0,001 |
| BCGT - ICA | 1,0 | <0,001 |

BCGT: Brucella Coombs Gel Test, ICA: Immuno Capture Agglutination Test, CT: STA with Coombs, STA: Standard Tube Agglutination Test, 2ME: 2-Mercaptoethanol Test, RBT: Rose-Bengal Test. (κ=0,61-0,80 good compliance, κ=0,81-1,00 very good compliance)

## DISCUSSION

Culture, serology and molecular methods are used in laboratory diagnosis of brucellosis. A definitive diagnosis is done by isolating bacteria from blood or bone marrow and other tissues and body fluids. Production of bacteria in culture varies between 15-70% according to the period of disease, whether antibiotics are used or not [1, 2]. For this reason, serological tests are used in diagnosis. The sensitivity of the RBT has been reported at over 99%. It has been emphasized that it can be used as a screening test, and that positive results must be verified by other tests [1-3]. Total antibodies (IgG, IgM, IgA) are detected together in the STA test. Above 1/160 titters with clinical symptoms are considered positive. Demonstration of seroconversion is diagnostic. Because of the blocking antibodies found in chronic cases, CT testing should also be done and blocking antibodies should be revealed [5, 7-9].

ICA is a test that can be obtained within 18 to 24 hours based on immune capture and can detect blocking antibodies, along with total antibodies. It has been reported that it can be used to determine the activity of infection [4]. BCGT is a test produced in our country, based on agglutination, which can detect blocking antibodies along with total antibodies [5]. Ivrem and colleagues [10] reported a perfect match between the STA and CT (k 0.887) and between the CT and BCGT (k 0.977). Koroglu and colleagues [11] reported a very good level of compliance between the CT and ICA, between the CT and BCGT (k 0.844 p<0.001) and between the ICA and BCGT (k 0.802 p<0.001). Turk Dagı and colleagues [12] determined that the BCGT test had a similar performance to the serological tests used in the diagnosis and follow-up of brucellosis. Orduno and colleagues [13] reported very good performance between ICA and STA and good performance between ICA and CT. Kaya and colleagues [14] reported that BCGT can be used in diagnosis of brucellosis because it gives results in a short time and visual evaluation is facilitated. Gündem and colleagues [15] emphasized that ICA testing is an effective diagnostic method because it shows blocking antibodies. In our study, Cohen Kappa [6] analysis was used to determine compliance between the tests we use in routine and the BCGT and ICA tests. Good compatibility between STA and ICA (k 0.7 p<0.01), very good compatibility between CT and BCGT and ICA (k 0.8 p<0.01), very good compatibility between CT and BCGT and ICA (k 0.8 p<0.01) and compliance between BCGT and ICA was found to be very good (Kappa 1.0 p<0.001) (Table 2).

According to our results, performance values between tests are similar to those reported by other researchers. In the ICA method, the results are evaluated at the end of incubation for 18 to

24 hours. BCGT test results are received on the same day and in less than 2 hours. In our study, compliance between ICA and BCGT tests was also found to be very good. For this reason, ICA or BCGT tests will provide ease of operation, as well as shorten the analysis time, therefore it will also allow practitioners to begin treatment early. The BCGT test appears to be the primarily preferred test for diagnosis due to fact that the results can be obtained on the same day and in a time as short as 2 hours. However, together with the culture results, the sensitivity and specificity of the BCGT test should be determined and we believe it would be appropriate to use it as a routine test from now on.

**Conflict of Interest:** The author has no conflict of interest related to this article.

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# Ultrasound evaluation of the temporomandibular joint in healthy children and adolescents

## Sağlıklı Çocuklarda Temporomandibular Eklemin Ultrason ile Değerlendirilmesi

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## ABSTRACT

**Objectives:** The purpose of this study was to investigate normative values of the distance between the most lateral point of the articular capsule and the most lateral point of the mandibular condyle (LCCD), in children and adolescents. Since the disc is not always visible on ultrasound, LCCD measurement is a very practical indirect indicator for evaluating TMJ.

Methods: This prospective study evaluated 141 temporomandibular joints distance of 141 pediatric individuals with a median age of 9 years (5-13) for boys and 11 years (6.5-14) for girls, comprising 69 boys and 72 girls. LCCD measurements were made with the participants in the closed mouth position, and the relationship of these values with weight, height, age, gender and body mass index (BMI) was evaluated.

**Results:** Median LCCD values in age groups 2 and 3 were significantly higher than the age group 1 (p=0.001). There was no significant difference between median LCCD values in age group 2 and age group 3 (p=0.5). No significant difference was found among the median LCCD values of the males 1.5 mm (1.2-1.8) and females 1.4 mm (1.1-1.6).

**Conclusion:** LCDD values increased with age, but no significant difference shown among the genders. However, a negative relation was detected with BMI. This study provides the normative quantitative values of TMJ distance which could be a reference point for upcoming studies. US is a diagnostic method that can be used in the follow-up and screening of children with TMD risk and general population. Since it does not include radiation, it can be safely repeated.

Keywords: Children, temporomandibular joint, Ultrasonography.

## ÖZ

**Amaç:** Bu çalışmanın amacı, çocuklarda ve ergenlerde eklem kapsülünün lateral noktası ile mandibular kondilin en lateral noktası (LCCD) arasındaki mesafenin referans değerlerini belirlemektir. Disk ultrasonda her zaman görünmediğinden, LCCD ölçümü temporomandibular eklemi(TME) değerlendirmek için dolaylı bir gösterge olmakla beraber çok pratiktir.

**Yöntemler:** Prospektif çalışmamızda, medyan yaşı erkeklerde 9 (5-13) ve kızlarda 11 (6.5-14) olan, 69 erkek ve 72 kız olgunun toplamda 141 TME mesafesi değerlendirildi. Katılımcılar yaş gruplarına göre 3'e ayrıldı. Ultrasonda yapılan LCCD ölçümlerinin cinsiyet, yaş, boy, kilo, vücut kitle indeksi (VKİ) ile korelasyonı değerlendirildi.

**Bulgular:** Grup 2 ve 3'ün LCCD değeri grup 1'den anlamlı derecede yüksekti (p = 0,001). Ancak Grup 2 ve 3 arasında anlamlı fark yoktu (p = 0,5). LCCD değerleri erkeklerde 1,5 mm (1,2-1,8) ve kızlarda 1,4 mm (1,1-1,6) olarak bulundu.

**Sonuç:** Çalışmamızda LCDD değerleri yaşla birlikte artış göstermekle beraber cinsiyetler arası anlamlı fark saptanmadı. LCCD ile VKİ arasında negatif bir ilişki tespit edildi. Bu çalışma, gelecekteki çalışmalar için referans olabilecek TME mesafesinin normatif değerlerini sağlamaktadır. Ultrason, temporomandibular hastalık riski olan çocukların ve genel popülasyonun tarama ve takibinde kullanılabilecek bir tanı yöntemidir. Radyasyon içermediği için güvenle tekrarlanabilir.

Anahtar Kelimeler: Çocuk, Temporomandibular Eklem, Ultrasonografi

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## INTRODUCTION

The temporomandibular joint (TMJ) is one of the most commonly used joints in the human body and the only movable joint in the skull. Temporomandibular disorders (TMD) are characterized by pain and dysfunction involving the temporomandibular joint and masticator muscles. The incidence in children varies between 16-68% [1,2]. The TMJ disc has a biconcave shape with fibrocartilaginous structure like meniscus of the knee. The part of the disc that absorbs mechanical stress is the parts that adhere to the joint capsule [3]. One of the main causes of TMD is disc degeneration. Although many methods are used in TMJ imaging, no method has yet been agreed upon as a gold standard [4]. Magnetic resonance imaging (MRI) is the initial option for evaluation in TMJ dysfunction, thanks to its high soft tissue resolution. The main disadvantages of MRI are its non-availability in some centers, high cost and restricted use in patients with claustrophobia, a cardiac pacemaker or metallic prostheses [5]. Computed tomography (CT) can be used in assessment of underlying bone pathologies such as bone erosion, fractures and postoperative deformities [6]. Although the advantages of use are emphasized, it should not be forgotten that repetitive rounds increase radiation exposure when follow-ups are performed with CT, and alternative techniques should be used in these instances [7].

Ultrasound (US) is a cost-effective, non-invasive and quick examination technique that is accessible and ensures dynamic evaluation without ionizing radiation. Thus, it is a very beneficial screening tool for the assessment of TMJ and masticatory muscles, in particular with the pediatric age group [8]. The use of US plays an important role in screening and follow-up, especially in screening children with JRA or children at risk of temporomandibular joint disease (TMD). Therefore, we believe that determination of TMJ ultrasound properties and reference values will be useful.

In this study, LCCD values of children and adolescents were analyzed. The differences of this value between pre-school age, school age and adolescent groups were determined. In addition, we assessed changes based on sex, height, weight and body mass index (BMI).

## MATERIALS AND METHODS

Study subjects and design

In this study, we assessed 141 TMJ distances of 141 pediatric subjects with a median age of 9 years (5-13) for boys and 11 years (6.5-14) for girls, comprising 69 boys and 72 girls, from April to September 2020. Local ethics committee approval was obtained prior to initiation of the study and informed consent was obtained from the parents of the participants, just before the ultrasound examination. We included only healthy individuals: those without orofacial pain, TMJ pathology history or systemic disorders that might have an influence on the TMJ. Participants were first examined by the dentist and individuals with no dislocation findings upon physical examination and ultrasound, were included in the study. Some individuals were excluded, for the following reasons: refusal to participate, aged under 3, presence of systemic inflammatory arthritis, facial trauma, mandibular growth disturbance rheumatological diseases or prior surgery.

Participants were divided into 3 groups: 3-6 years (n = 47, preschool), 7-12 years (n = 47, school) and 13-17 years (n = 47, adolescents). Sociodemographic information, including gender, weight, height and BMI was recorded. BMI was calculated as BMI = weight (kg) / height (m2). The complicated structure of TMJ is shown schematically in Fig 1 [5]. The TMJ capsule is a membrane that connects to the disc and neck of the mandibular condyle.

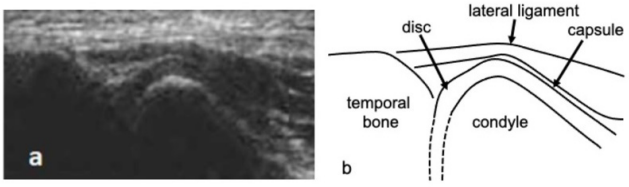


Fig 1. a) Parallel to Camper line US view of the TMJ. b) The broken lines match to the structures that are not able to be visualized. TMJ: temporomandibular joint

The fibrocartilage joint disc divides the joint space into two compartments; there are capsula articularis surrounding the TMJ and various supporting ligaments. The mandibular condyle,

glenoid fossa and joint cartilage are covered with a fibrous tissue. Between the condyle and the glenoid fossa there is a fibrous cartilaginous tissue. The joint is surrounded as a whole by a synovial membrane, above which is the joint capsule. US examination was completed using an AplioTM 500 Platinum ultrasonography device (Canon Medical Systems, Japan) with a 5-14 MHz high frequency linear probe and using the 'small parts' preset. The children were assessed in motionless supine position. Right TMJ measurements were used and the disc images were obtained with heads turned to the left side and closed mouth position. TMJ disc images were taken parallel to the camper plane (Fig 2). Ultrasound measurements were performed by the same pediatric radiologist with 15 years of ultrasound experience, repeated 3 times and the mean value of measurements were recorded. In each scan, the operator measured the distance between the most lateral point of the articular capsule and the most lateral point of the mandibular condyle. Since the disc was not seen directly on all ultrasound images, the LCCD was used as an indirect marker to state the disc situation.

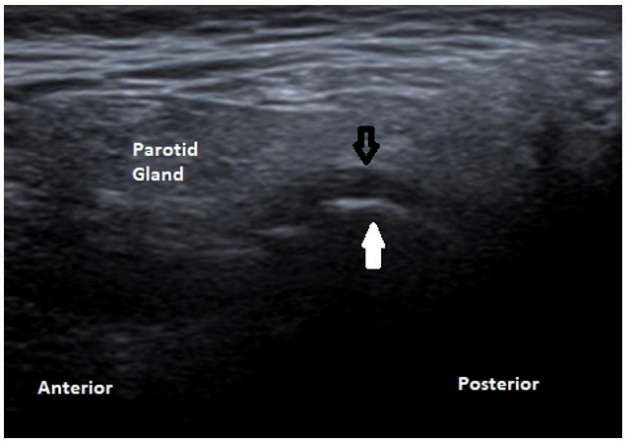


Fig 2. Extra-articular portion of the TMJ disc and articular capsule indicated between black and white arrow on the B-mode image. Hyperechoic line (Black arrow) running lateral and parallel to the lateral surface (White arrow) of the mandibular condyle, indicating the articular capsule. The distance between the articular capsule and the mandibular condyles' lateral surface showed between the apexes of the black and white arrow.

## Statistical Analysis

All the data was processed in Microsoft Office Excel and transferred to the SPSS (version 21.0, IBM Corp.) software for statistical analysis. The distribution of the data was evaluated with the Kolmogorov-Smirnov test, with close attention given to the skewness and kurtosis. Descriptive statistics of the data were demonstrated as median with interquartile range (IQR). Differences between median values of age, height, weight, BMI and LCCD parameters among the gender groups were compared using the Mann-Whitney U test. Differences between median values of age, height, weight, BMI and LCCD parameters among the three age groups were compared using the Kruskal-Wallis test. A comparison among two age groups was evaluated using the Mann-Whitney U t-test. Correlation analysis of the age, height, weight, BMI with LCCD parameters were tested with Spearman's correlation analysis. The scattered dot graphics were plotted for correlation of age and BMI parameters with LCCD values (Fig. 3a-b). Variables were studied at the 95% confidence interval with $p < 0.05$ accepted as statistically significant.

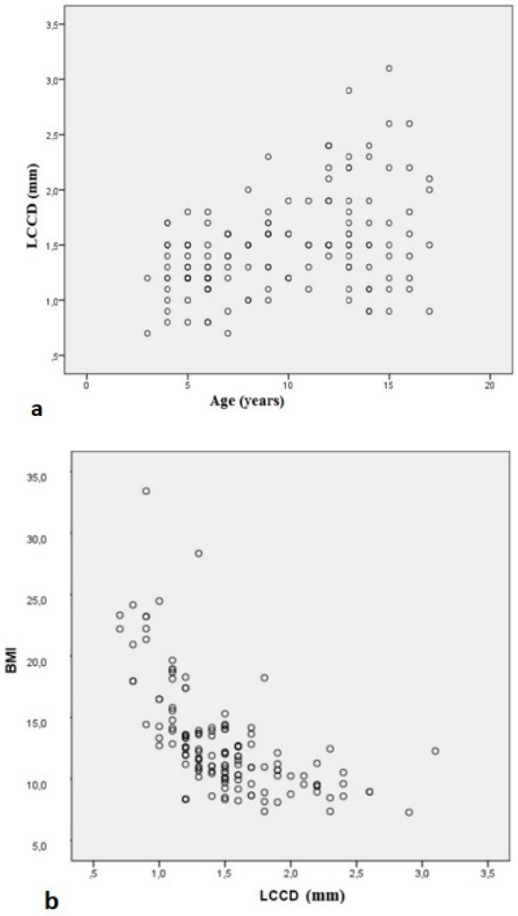


Fig 3a-b) The scattered dot graphics were plotted for correlation of age and BMI parameters with LCCD values

## RESULTS

Descriptive statistics of the age, height, weight, BMI and LCCD in gender and age groups are offered in Tables 1 and 2. No significant difference was found among the median ages of the males (9 (5-13)) years) and females (11 (6.5-14)) years) (p=0.06). No significant differences were found in median height, weight and BMI values of the males and females (p>0.05). No significant difference was found among the median LCCD values of the males (1.5 (1.2-1.8) mm) and females (1.4 (1.1-1.6)) mm) (p=0.11). There were statistically significant differences in median values of age, height and weight values, between each age group comparisons. Median BMI value in age group 3 was significantly higher than the median BMI values in age group 1 and age group 2, (p=0.049 and p=0.003, respectively). There was no significant difference between median BMI values in age group 1 and age group 2 (p=0.3). Median LCCD values in age groups 2 and 3 were significantly higher than the median LCCD value in age group 1 (p=0.001). There was no significant difference between median LCCD values in age group 2 and age group 3 (p=0.5).

Table 1. Descriptive statistics of age, height, weight, body mass index and LCCD parameters by gender groups

| Parameter | Descriptive statistics | | | p |
|---|---|---|---|---|
| | Median (Interquartile Range) | | | |
| | Girls (n:69 ) | Boys (n:72) | All(n:141) | |
| Age (years) | 11 (6.5-14) | 9 (5-13) | 9(6-13) | 0.06 |
| Height (cm) | 141 (119-156) | 128.5 (116-153) | 135(118-155) | 0.27 |
| Weight (kg) | 35 (22-50) | 27 (20-47) | 30(21-48) | 0.4 |
| BMI (kg/m2) | 12.26 (10.36-14.20) | 11.17 (9.55-13.58) | 11.88 (10.1-14.09) | 0.085 |
| LCCD (mm) | 1.4 (1.1-1.6) | 1.5 (1.2-1.8) | 1.5(1.2-1.7) | 0.11 |

p values by the Mann-Whitney U test.

Correlation analysis of age, height, weight and BMI values with LCCD parameters are found in Table 3. There was highly significant mild to moderate positive correlations of age (r=0.35), height (r=0.38) and weight (r=0.40) with LCCD values (p=0.001). There was highly significant moderate negative correlation of BMI (r=-0.68) with LCCD values (p=0.001).

Table 2. Descriptive statistics age, height, weight, body mass index and LCCD parameters by age groups

| | Group 1 | Group 2 | Group 3 | p | |
|---|---|---|---|---|---|
| | 3-6 years (n:47) Median (IQR) | 7-12 years (n:47) Median (IQR) | 13-17 years (n:47) Median (IQR) | | |
| Age (years) | 5 (4-6) | 9 (8-11) | 14 (13-15) | 0.001* | 1 vs 2: 0.001'<br>1 vs 3: 0.001'<br>2 vs 3: 0.001' |
| Height (cm) | 114 (108-119) | 135 (127-150) | 160 (152-163) | 0.001* | 1 vs 2: 0.001'<br>1 vs 3: 0.001'<br>2 vs 3: 0.001'<br>1 vs 2: 0.001'<br>1 vs 3: 0.001'<br>2 vs 3: 0.001' |
| Weight (kg) | 19 (17-23) | 30 (24-40) | 53 (47-61) | 0.001* | 1 vs 2: 0.001'<br>1 vs 3: 0.001'<br>2 vs 3: 0.001' |
| BMI (kg/m2) | 11.88 (10.08-13.59) | 11.09 (9.91-12.4) | 13.5 (10.25-18.13) | 0.002* | 1 vs 2: 0.3<br>1 vs 3: 0.049<br>2 vs 3: 0.003 |
| LCCD (mm) | 1.2 (1.1-1.5) | 1.5 (1.3-1.7) | 1.5 (1.2-2.1) | 0.001* | 1 vs 2: 0.001'<br>1 vs 3: 0.001'<br>2 vs 3: 0.5' |

P-values by the Kruskal Wallis* and Mann-Whitney U tests'
Bold p-values represent statistically significant results
IQR: Interquartile range

Table 3. Correlation results of the auxological parameters with LCCD

| Parameters | | p | R |
|---|---|---|---|
| LCCD | Age | 0.001 | 0.35 |
| | Height | 0.001 | 0.38 |
| | Weight | 0.001 | 0.40 |
| | BMI | 0.001 | -0.68 |

P-values by Spearman's correlation analysis.
Bold p-values represent statistically significant results

## DISCUSSION

TMD is usually caused by an anomalous relationship between the disc and neighboring articular structures. Although dislocation is most common towards the anterior, it can be in all directions [9]. TMJ problems containing the articular disc, capsule and muscles of mastication, occur with a variety of signs including pain, clicking and functional restriction [10]. Myofascial pain and regional tenderness are usual symptoms in TMJ diseases. But studies show that TMD may not cause any symptoms in childhood; in cases with symptoms, the diagnosis of head or ear pain is made by a pediatrician and otolaryngologist, and the actual diagnosis can be ignored [11].

There is a multifactorial etiology in the existence of TMD in children as well as in adults. Parafunctional habits such as trauma, malocclusion and bruxism, nail biting and finger sucking are among the main etiological causes of TMD in childhood [12, 13]. In order to prevent sustained orofacial diseases in adulthood, individuals who are at risk of TMD should undergo a routine examination of the TMJ. However, it is not practical to examine every child with an MRI or a CT scan. Long-term patient compliance is difficult to achieve and the need for sedation may cause problems in children while screening with MRI. CT is not appropriate as a screening method as a result of the ionizing radiation. Ultrasound can be used as a scanning method in children with head or ear pain, noise in the jaw and mandibular asymmetry, however use of this method can sometimes be difficult in the pediatric age group to achieve the examination. Therefore, it is important to determine a practical, easily measurable reproducible standard measurement parameter while evaluating TMD in ultrasound. In our study, by measuring the LCCD value in ultrasound, a fast and reliable preliminary evaluation is achieved in terms of TMD scanning.

We found the mean LCDD value in age group 1 (3-6 years old) to be 1.2 (1.1-1.5) mm, in group 2 (7-12 years) 1.5 (1.3-1.7) mm and in group 3 (13-17 years) 1.5 (1.2-2.1) mm. Median LCCD values in age groups 2 and 3 were significantly higher than the median LCCD value in age group 1. Although the LCDD value increased with age, there was no significant difference between the 7-12 and the 13-17 age groups. It has been suggested that indirect ultrasonographic signs can be used, due to the difficulty in observing the entire disc. For Hayashi et al., disc displacement should be suspected if the US reveals a distance between the articular capsule and the lateral surface of the mandibular condyle (LCCD) of 4 mm or more [14]. Our reference values can be used for quick control in ultrasound and in this study, no significant differences were determined in LCCD values between the sexes.

Otherwise, we found no relationship between height, weight and age with the LCCD values and these did not vary by gender. The effect of gender difference on TMD has been discussed extensively in the literature and while the signs and symptoms in childhood do not differ much by gender, it has been reported that symptoms are observed in young adult girls 1.5 to 2 times more compared to boys. This difference is possibly the result of pain sensitivity of girls and hormonal differences between the two genders [7, 15].

There was highly significant moderate negative correlation of BMI (r=-0.68) with LCCD values. An increase in LCCD value was accepted as significant as the secondary finding of internal derangement of the TMJ. However, its negative relationship with BMI may be secondary to a reduction in distance due to osteoarthritis. In a recent mouse model study, it was found that obesity could cause temporomandibular joint pathological changes and it was determined that both excessive compressive mechanical force and high fat diet induced obesity, caused TMJ osteoarthritis-like changes [16].

Destructive changes in the joint disc and synovial structures can be seen particularly in diseases that can show TMJ involvement, such as JIA, joint effusion, sclerosis, flattening of the joint condyle and inflammatory erosions. Pain is a rare symptom in children with TMJ involvement in JIA, therefore the focus should be on preventing mandibular growth disorders that may precede malocclusion and jaw dysfunction. US can also be used to monitor the progress of TMJ involvement and response to treatment [17].

In order to determine US reference values for TMJ and to create a guideline, multi-center studies with larger series in different age and disease groups are needed. The data of our study can be a source for these studies.

TMD usually develops due to rheumatological, degenerative changes and inflammatory processes. Familiarity with the evaluation of TMJ by US and knowing the value ranges of US parameters enables early identification of possible pathological differences. Coming studies should work on the connection among histopathological changes and US results in temporomandibular joint and TMD. Routine use of US will decrease the redundant procedures and rising the cost effectiveness.

The main limitation of our study is the limited number

of cases. Additionally, measurements were made only by one operator, which hinders assessment of inter- and intraobserver reliability. We did not have all of the laboratory information necessary to verify that the individuals were completely healthy. Participants were first examined by the dentist. Patients with no dislocation findings on physical examination and ultrasound were included in the study. Asymptomatic cases with disc displacement may have affected our results. Only the right TMJ of each patient was appraised and no comparisons were made among the sides.

**Conclusion:** US is a noninvasive diagnostic tool that can be used to evaluate TMJ and TMD in the pediatric age group. LCDD values increased with age, but no significant difference was shown between genders. However, a negative relation was detected with BMI. This study provides the normative quantitative values of TMJ distance which could be a reference point for upcoming studies. US is a diagnostic method that can be used in the follow-up and screening of children with TMD risk and general population and since it does not include radiation, it can be safely repeated.

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# Copy Number Variation Analysis in Turkish Patients with Congenital Bilateral Absence of Vas Deferens

## Konjenital Bilateral Vas Deferens Yokluğu Olan Türk Hastalarda Genomik Kopya Sayısı Varyasyonları Analizi

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## ABSTRACT

**Objective:** Congenital Bilateral Absence of the Vas Deferens (CBAVD) is a developmental abnormality that causes infertility in males. According to the literature, up to 88% of CBAVD cases have at least one pathogenic Cystic Fibrosis Transmembrane Conductance Regulator gene (*CFTR*) mutation. However, based on our previous data, this rate was 15.90% in Turkish patients with CBAVD. We aimed to identify genomic copy number variations (CNV) and candidate genomic regions which could related to the CBAVD in Turkish population.

**Methods:** CNV analysis was performed in 19 Turkish CBAVD patients normal karyotypes and a wild type *CFTR* genotype. We suggested that the *DAD1* gene may be a candidate gene related to CBAVD by reviewing online databases and analyzing CNV findings. Sanger sequencing of the *DAD1* gene exons was performed in 22 patients.

**Results:** We identified 11 CNVs that most likely related with the disease in nine of 19 (47.3%) patients. As the most common CNV, 14q11.2 deletions were detected in there (15.79%) of the patients. There was only *DAD1* gene in the sharing genomic region of two of the 14q11.2 deletions. No sequence variation was detected in the *DAD1* gene of the patients.

**Conclusion:** The 14q11.2 chromosomal region and the *DAD1* gene may be associated with CBAVD. Further studies are needed to indentify the contribution of CNVs and *DAD1* gene to CBAVD etiology.

Keywords: copy number variations; CBAVD; male infertility; *DAD1*

## ÖZ

**Amaç:** Konjenital Bilateral Vas Deferens Yokluğu (CBAVD), erkeklerde infertiliteye yol açan gelişimsel bir anomalidir. Literatüre göre CBAVD'li olguların %88'e kadarı en az bir Kistik Fibrozis Transmembran Regülatör geni (*CFTR*) mutasyonuna sahiptir. Ancak, daha önceki verilerimize göre CBAVD'li Türk hastalarda bu oran %15.90'dır. Çalışmamız kapsamında Türk popülasyonundaki CBAVD ile ilişkili olabilecek genomik kopya sayısı varyasyonları (CNV) ve aday genomik bölgelerin tanımlanması amaçlanmıştır.

**Yöntemler:** Normal karyotipe ve yabanıl tip *CFTR* genotipine sahip 19 Türk CBAVD'li hastanın CNV analizi gerçekleştirildi. CNV bulgularımızın analizi ve veritabanlarının taranması ile *DAD1* geni CBAVD ile ilişkili olabilecek potansiyel aday gen olarak tanımlandı. 22 CBAVD'li hastada da *DAD1* geni ekzonlarının dizi analizi Sanger dizileme metoduyla yapıldı.

**Bulgular:** 19 hastanın dokuzunda (%47.3) hastalıkla ilişkili olabilecek 11 adet CNV saptandı. En sık saptanan CNV olarak 14q11.2 delesyonu, hastaların üçünde (%15.79) belirlendi. 14q11.2 delesyonlarından ikisinin ortak varyant bölgesinde yalnızca *DAD1* geni lokalize idi. Dizi analizi ile, hastaların *DAD1* geninde herhangi bir dizi varyasyonu saptanmadı.

**Sonuç:** 14q11.2 kromozomal bölgesi ve *DAD1* geni CBAVD ile ilişkili olabilir. CNVlerin ve *DAD1* geninin CBAVD etiyolojisine katkısını tanımlamak için ileri çalışmalar gerekmektedir.

Anahtar Kelimeler: kopya sayısı varyasyonları; CBAVD; erkek infertilitesi, *DAD1*

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## INTRODUCTION

As a global health problem, infertility affects approximately 8-12% of all couples [1]. It has been estimated that 40-50% of all infertility cases is due to 'male factor' infertility and affects about 7% of all men [1]. Congenital structural anomalies of the male genital tract are contributing factors in male infertility. Numerical or structural chromosomal abnormalities, Y chromosome microdeletions, and Cystic Fibrosis Transmembrane Conductance Regulator (*CFTR*, NM_000492.3) gene mutations associated with congenital bilateral absence of vas deferens (CBAVD, OMIM 277180) are the most common genetic reasons of male infertility [2]. CBAVD accounts for 1-2% of all male infertility, which is a condition characterized by the blockade of the transportation of the spermatozoa from testicular or epididymal structures to the distal genital tract, resulting in azoospermia [3]. 88% of Caucasian, 78% of Non-Caucasian patients with CBAVD carry at least one *CFTR* mutation [4]. However, *CFTR* mutations were found to be less common in Turkish CBAVD patients [5-7]. We previously reported that only 15.90% had *CFTR* mutations [7]. The lower frequency of *CFTR* mutations among Turkish CBAVD cases, as well as differential mutation profiles, demonstrated the impact of geographical and ethnic backgrounds on CBAVD etiology [5, 7]. Besides *CFTR*, *ADGRG2* (Adhesion G protein-Coupled Receptor G2) gene variants were identified as a cause of CBAVD with a prevalence of 7.4% and 20% in French and Chinese cases, respectively [8, 9]. Recently, it was shown that, heterozygous *SLC9A3* deletions were found in 37.9% of the Taiwanese patients, and *SCNN1B* and *CA12* genes regulating CFTR protein levels were found to be associated with CBAVD in some patients without *CFTR* mutations [10, 11]. Nevertheless, these gene variations explain the disease in only a limited fraction of all CBAVD patients. The genetic structure of the present day Turkish people may not be similar with Europeans or Asians because of migrations from different geographical areas [12]. We hypothesized novel genes and/or genomic regions may be related to idiopathic CBAVD in Turkish people.

Genomic copy number variations (CNVs) can lead to disease by various molecular mechanisms including gene dosage effect, gene disruption, gene fusion, position effect and other effects on gene function [13]. To date, CNV analyses for idiopathic azoospermia from different ethnic backgrounds or populations have been performed in several studies [14, 15]. We hypothesise that CNVs might explain, at least partially, the unknown genetic etiology of CBAVD. Therefore, we aimed to identify the possible candidate genomic regions and genes which could cause CBAVD in Turkish patients not carrying *CFTR* mutations by performing whole genome CNV analysis. Here, we report that Defender Against Cell Death 1 (*DAD1*) localized at 14q11.2 in our cohort is a gene that may need to be investigated further in association with CBAVD. *DAD1* is known to be a negative regulator of apoptosis [16].

## MATERIALS AND METHODS

### Ethical approval and patients

This clinico-genetic study was conducted with the collaboration of Akdeniz University Faculty of Medicine, Izmir Katip Celebi University Faculty of Medicine, and Istanbul Sisli Memorial Hospital, Turkey. The study protocol was approved by the Akdeniz University Clinical Research Ethics Committee (approval No: 2014-# 383) under the recent version of the Helsinki Declaration. Informed consent was obtained from all subjects when they were enrolled in the study.

The patients who applied to the urology outpatient clinic due to primary infertility were evaluated. All of the patients had not used any birth control method for at least one year, they had no sexual dysfunction and no comorbidity. Twenty-two unrelated, adult Anatolia-originated Caucasian males with CBAVD were included in the study. All patients were azoospermic. CBAVD patients were determined by using physical examination, scrotal, transrectal ultrasound (USG), semen analysis, and biochemical tests. CBAVD cases with wild-type *CFTR* genotype, normal karyotype, without a Y chromosome microdeletion were included in this study. Renal aplasia was excluded by pelvic USG.

### Copy Number Variation Analysis

For molecular genetic analyses, patients' genomic DNA was isolated from peripheral blood by using a non-enzymatic method modified from Lahiri and

Nurnberger [17]. The quality of DNA samples was tested by agarose gel electrophoresis and by measuring the A260/A280 ratios on the Nanodrop 8000 Spectrophotometer (Thermoscientific Instruments, CA, USA). Nineteen DNA samples of sufficient quality for microarray were examined for whole-genome CNVs. Nine out of the 19 samples were profiled by using the Affymetrix CytoScan HD Array (Affymetrix, Santa Clara, CA, USA). Affymetrix CytoScan HD Array is a high-density platform containing more than 2.6 million-copy number markers with 750.000 SNP markers. Agilent ISCA 8x60K CGH Array (Agilent Technologies, Santa Clara, CA, USA), comprised of 60.000 oligonucleotide probes, was used for the rest of the samples. After the wet laboratory steps according to the manufacturer's instructions, data were analyzed and visualized with different software (Affymetrix Chromosome Analysis Suite 3.1, Agilent Cytogenomics 4.0.2.21.) depending on the platform utilized. In Affymetrix Chromosome Analysis Suite software to exclude false-positive CNVs, only deletions and duplications more than 100 Kbp in length that involved at least 25 and 50 consecutive probes respectively were considered real alterations. In Agilent Cytogenomics software at least 100 Kbp of DNA amplification or deletions including a minimum of 4 consecutive probes were reported as real copy number variations. Human genome hg19 assembly was used to map genomic coordinates, and CNVs were classified as pathogenic, variants of uncertain significance, likely benign, and benign following the latest ACMG guidelines [18]. To determine the pathogenicity of detected CNV regions; DGV (http://projects.tcag.ca/cgi-bin/variation/gbrowse/hg19/), DECIPHER (https://decipher.sanger.ac.uk/), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) databases were used. We excluded benign CNVs that had previously been reported to occur in healthy individuals frequently and reported CNVs possibly associated with a pathology.

Selection of the possible candidate gene for CBAVD

Overlapping CNV regions in at least two patients were determined by comparing the length of the shared sequence. The protein-coding gene/genes localized in the overlapping genomic region were hypothesized to be the potential candidate gene for CBAVD. The gene with a possible role in the development of the male genital tract was selected through a review of the literature (https://www.ncbi.nlm.nih.gov/pubmed) as well as databases on gene expression profiles (http://www.proteinatlas.org/), genotype–phenotype correlations (http://omim.org/, https://www.ncbi.nlm.nih.gov/gene), and animal models (http://www.informatics.jax.org/).

Sanger DNA sequencing for *DAD1*

Defender Against Cell Death 1 (*DAD1*) (NM_001344.4) was found to be a potential candidate gene associated with CBAVD by this study because of its localization in the overlapping genomic region of the common CNVs. Sanger sequencing was performed in all 22 CBAVD patients for all three exons and exon-intron boundaries of *DAD1*. The polymerase chain reaction (PCR) oligonucleotide primer sets are shown in Table 1. Amplification conditions consisted of an initial denaturation at 95 ℃ for 8 minutes, followed by 35 cycles at 95℃ for 30 seconds, 55℃ for 30 seconds, 72 ℃ for 30 seconds, and a final extension at 72 ℃ for 5 minutes. Each fragment was resolved by agarose gel electrophoresis. Aliquots of the samples were subjected to dideoxy sequencing on an ABI 3130XL Automated DNA Sequencer (Applied Biosystems, Foster City, CA, USA). NCBI Blast (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to analyze obtained sequences.

**Table 1: Primers used in Sanger sequencing of *DAD1***

| Exon | Forward primer (5'-> 3') | Reverse primer (5'-> 3') | $MgCl_2$ (mM) | Tm ℃ |
|---|---|---|---|---|
| 1 | GGCGGTGGT-CTGATATAGAGT | AGCTCATTG-ATAGGGCCATT | 1,4 | 55 |
| 2 | CCAGTCCCAT-CCAATTTCTC | TTTGGGGC-TATCTGGGTATC | 1,4 | 55 |
| 3 | ACAAGGAGG-CAGGTTCACAG | GATTTGGGG-CCCTGTCTCTA | 1,7 | 55 |

## RESULTS

Patients and clinical findings

The mean age of patients was 43 ± 12 years. All of the patients were primary infertile and azoospermic. On physical examination, the vas deferens of the patients were not palpable (both sides), and unilateral agenesis of vas deferens was confirmed radiologically. Their testicular volume

**Table 2 : CNV profiles of Turkish CBAVD cases**

| Case ID | CNV Type | Chr. Region | Coordinates (hg19) | Gain/ Loss | Size (Kbp) | Gene Quantity | Gene Involved | # times in DGV | # times in Decipher | Microarray |
|---|---|---|---|---|---|---|---|---|---|---|
| 8 | P | 15q13.2 q13.3 | chr15:30,954,76-32,509,926 | Loss | 1555 | 7 | *FAN1, MTMR10, TRPM1, MIR211, KLF13, OTUD7A, CHRNA7* | 1 (Multiple) | 1 (0) | Agilent ISCA 8x60K |
| 4 | VUS | 11q14.3 | chr11:89,474,817-89,895,988 | Gain | 421 | 10 | *TRIM49, MIR5692A1, TRIM53AP, TRIM64B, TRIM49D2P, TRIM49D1, TRIM64, TRIM49C, UBTFL1, NAALAD2* | 4 (Multiple) | 0 (0) | Affymetrix CytoScan HD |
| 18 | VUS | 14q11.2 | chr14:22,215,286-24,018,428 | Loss | 1803 | >30 | *DAD1, ABHD4, OXA1L, SLC7A7, MRPL52, MMP14, LRP10, REM2, RBM23, PRMT5, HAUS4, JUB, C14orf93, PSMB5, PSMB11, CDH24, ACIN1, 14orf119,CEBPE, SLC7A8...* | 0 (Multiple) | 0 (0) | Agilent ISCA 8x60K |
| 19 | VUS | 14q11.2 | chr14:22,299,149-23,061,615 | Loss | 762 | 1 | *DAD1* | 7 (Multiple) | 0 (0) | Agilent ISCA 8x60K |
| 8 | VUS | 15q13.3-q14 | chr15:32,942,601-34,627,075 | Gain | 1684 | 11 | *SCG5, GREM1, FMN1, RYR3, AVEN, CHRM5, C15orf24, PGBD4, C15orf29, TMEM85, SLC12A6* | 4 (Multiple) | 0 (0) | Agilent ISCA 8x60K |
| 15 | VUS | 17p13.3 | chr17:625,416-810,197 | Gain | 185 | 5 | *FAM57A, GEMIN4, DBIL5P, GLOD4, RNMTL1, NXN* | 13 (Multiple) | 0 (0) | Agilent ISCA 8x60K |
| 9 | VUS | 18p11.21 | chr18:14,194,813-14,851,736 | Gain | 657 | 6 | *ANKRD20A5, CYP4F35P, CXADRP3, POTEC, ANKRD30B, MIR3156-2* | 2 (Multiple) | 0 (0) | Affymetrix CytoScan HD |
| 4 | VUS | Xp22.33 | chrX:795,717-1,427,556 | Gain | 632 | 3 | *CRLF2, CSF2RA, MIR3690* | 3 (Multiple) | 0 (1) | Agilent ISCA 8x60K |
| 6 | VUS | Yq11.223 | chrY:25,296,950-26,066,972 | Gain | 770 | 2 | *DAZ1, DAZ2* | 3 (66) | 0 (3) | Agilent ISCA 8x60K |
| 1 | LB | 2q21.2 | chr2:132,696,181-133,136,747 | Gain | 441 | 2 | *ANKRD30BL, MIR663B* | 13 (Multiple) | 0 (0) | Affymetrix CytoScan HD |
| 7 | B | 14q11.2 | chr14:22,598,026-22,897,089 | Loss | 299 | 0 | - | 26 (Multiple) | 0 (0) | Agilent ISCA 8x60K |

**Abbreviations: P, pathogenic; VUS, variant of uncertain clinical significance; LB, likely benign; B, benign; Chr, Chromosome; Kbp, kilobase pair; DGV, Database of Genomic Variants. The number of CNVs in a very similar size is listed for both DGV and Decipher. The number of any size of CNVs overlapped any part of our CNV region is shown in parentheses.**

and penises were normal. However all patients are infertile and azoospermic, their ejaculate volumes, semen glucosidase and/or fructose levels and blood follicle stimulating hormone (FSH) levels (1.7-12mIU/ml) were normal. In addition, transrectal ultrasound (TRUS) demonstrated no other obstructive cause for azoospermia. There was no other abnormality accompanying CBAVD.

Genomic Copy Number Variations

In nine of the 19 patients (47.3%), we detected CNVs including a pathogenic (P) deletion, a likely benign (LB) variant of duplication and eight variants of uncertain significance (VUS) consisting of two deletions and six duplications. A benign (B) deletion (299 Kbp) in the 14q11.2 region containing no known genes was also detected in a different patient. We reported this benign variation as an exceptional due to its localization in a recurrent CNV region. The types and localization of these CNVs, minimum-maximum nucleotide numbers that show the borders in chromosomal DNA (based on hg19), length in base pairs, number of genes included in that region were listed in Table 2. All CNVs were detected in a heterozygous state. The average size of the CNVs was 837 Kbp. Duplications were more common than deletions (64% versus 36%).

In Case 8, we identified two CNVs, a pathogenic and a VUS on chromosome 15. Each of the other CNVs was found in different patients. The 15q13.2-q13.3 deletion in Case 8 was defined as pathogenic according to the ACMG criteria. This deletion was 1555 Kbp in length, for which a similar sized CNV present once in both DGV and DECIPHER CNV Syndromes. It overlapped with the genomic region associated with 15q13.3 microdeletion syndrome. Within the 15q13.2-q13.3 deletion, there were seven protein-coding genes (Table 2). We have had no data on whether the case 8 having the the clinical features of 15q13.3 microdeletion syndrome. There was no any other similar sized CNV present in DECIPHER for the other CNVs. When any sized CNV that overlaps our CNVs is considered, this was seen multiple times in DGV for most of all (66 times for the Yq11.223 duplication) and none in DECIPHER except the duplications of Xp22.33 (once) and Yq11.223 (three times) (Table 2).

Possible candidate gene and direct sequencing

As shown in the Table 2, deletions in the 14q11.2 chromosomal region was detected in 15.79% (n=3) of 19 patients. Software images of the 14q11.2 deletions are shown in Figures 1A, 1B and 1C. The overlapping genomic region of these three was 299 Kbp length where no known protein coding genes were localised. On the other hand, *DAD1* was the only gene localised in the overlapped region of two of the 14q11.2 deletions (Figure 2). DAD1 is a core subunit of the oligosaccharyltransferase (OST) complex catalyzes N-glycosylation of target proteins which is a key modification occurring in eukaryotes. Loss-of-function of the DAD1 protein triggers apoptosis [19]. Homozygous *Dad1* knockout mice models are embryonically lethal revealing essential role of the gene in embryological development [20]. DAD1 protein is expressed as medium level in male reproductive system tissues (https://www.proteinatlas.org/ENSG00000129562-DAD1/tissue). N-glycosylation, a common cotranslational modification, is the attachment of the core oligosaccharide to the asparagine residue of a protein by the OST. N-glycosylation defects cause impairments in the folding and stability of CFTR protein [21]. DAD1 is one of the globular modulators of OST stability thus affects directly OST dependent N-glycosylation [19]. Based on these results, *DAD1* gene coding a protein functioning in the posttranslational modification process of CFTR may lead to CBAVD in cases without *CFTR* mutations. Sequence analysis was performed to investigate if *DAD1* (NM_001344.4) single nucleotide variations contribute to CBAVD etiology. No variation was detected by Sanger sequencing of *DAD1* in our cohort of the study group.

## DISCUSSION

If any potentially pathogenic CNV is common in a cohort with a specific phenotype, that CNV region can be thought of as a candidate region for that physiological condition [22]. Within the scope of this study, we aimed to identify the most likely pathogenic CNVs that can cause a CBAVD phenotype. Genetic studies on etiology of CBAVD in Turkish population are very limited. This is the first study investigating CNVs and candidate

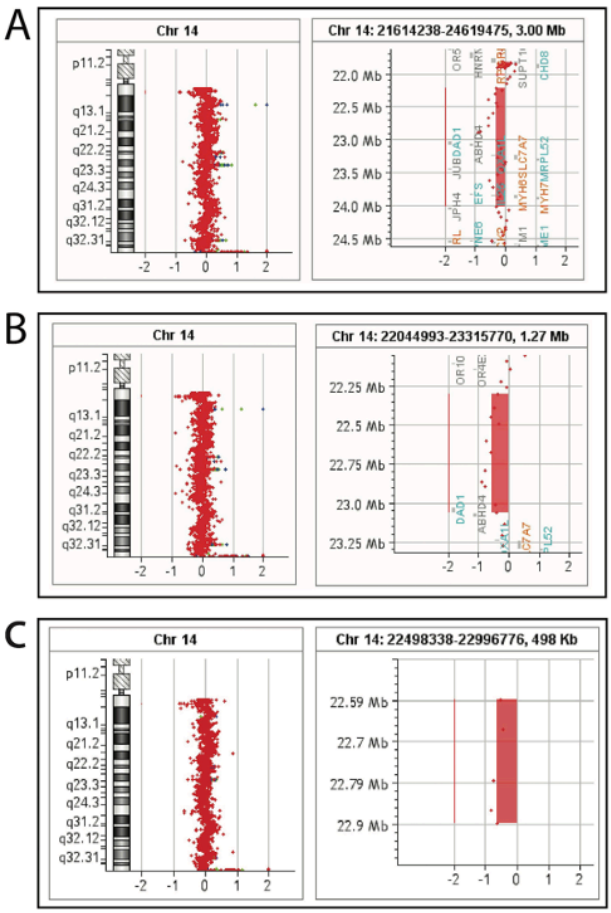


Figure 1: CNV analysis results of heterozygous 14q11.2 deletions. A) 1803 Kbp deleted region. B) 762 Kbp deleted region. C) 299 Kbp deleted region. The images show the log2 ratio of the reference versus patient DNA on the Y-axis and the position of each probe along the chromosome on the X-axis. Vertical red bars indicate the deleted genomic region. Images were produced using Agilent Cytogenomics 4.0.2.21 software.


Figure 2: Identification of reciprocal overlap in size for 14q11.2 deletions. Each colored bar (red, blue, green) represents CNV calls in cases 18, 19, and 7 respectively. Three CNV calls each shares a genomic region of 299 Kbp length at that locus. The overlapping region of two CNV calls is 762 Kbp in length and harbours the DAD1 gene which is marked with a red colored rectangle.

genomic regions or genes associated with CBAVD in Turkish cases. We reported large CNVs by using high-resolution array technologies without any other confirmation from an alternative method in the light of our previous studies [23, 24].

We evaluated the clinical findings of CBAVD patients. Despite the ejaculate volume and the fructose levels are usually low in CBAVD men [25]; we detected normal levels of ejaculate volumes and seminal glucosidase and/or fructose concentrations in our study group as we reported before [7]. Lin et al. also reported a case with CBAVD having normal semen parameters [26].

Additionally, Van der Ven K et al. and reported that *CFTR* mutations were variable and that *CFTR* mutations may cause reduced sperm quality without non-obstructive azoospermia or low fructose volume [27, 28]. Wedenoja et al. agreed with them [28]. Thus, *CFTR* mutations are related to variable semen parameters that may range from normal to subnormal semen parameters. We excluded CBAVD patients having *CFTR* mutations. They had no additional genital abnormalities that were seen on TRUS imaging. Moreover, it has been shown that CBAVD patients with renal aplasia did not have *CFTR* mutations [29]. Besides, the absence of unilateral vas deferens may be part of renal malformations. Gajbhiye et al. published that *CFTR* mutations could be detected with renal malformations [30]. However, we ruled out renal aplasia by using radiological evaluations, and we have not gotten any infertile patients with renal malformations. Nevertheless, we have one of the largest patient populations with *CFTR* mutations in our Turkish community and all genomic mutations were recorded.

The pathophysiology of CBAVD cannot be explained only by *CFTR* mutations, suggesting possible genetic heterogeneity. CNV studies to determine the underlying etiology of idiopathic CBAVD are very limited. To date, a study was done by Lee et al. investigating CNVs by using array CGH in Taiwanese CBAVD patients (n=8) in 2009. The researchers identified CNVs in the 3q26 chromosomal region in five of their cases and proposed that this region can be related to CBAVD. In addition, they also detected CNVs in a region including the *PANK2* gene that plays a role in the development of the urinary system in two of their cases. In our cohort, no CNV was detected in neither the 3q26 nor in the 20p12 region containing the *PANK2* gene. CNVs were found in the Yq11.223 region by both of the studies. While they detected a deletion in the Yq11.223, we determined an amplification [3]. The very low number of CNVs shared between our patients and Taiwanese patients may be due to ethnic and geographic differences between populations.

Despite the presence of apparently benign CNVs in the human genome, a large number of CNVs are involved in the etiology of various human diseases. Therefore, CNVs are an important source of mutation burden associated with human diseases. The disease phenotype occurs depending on the functions of the genes in the CNV region [22]. We searched the literature in terms of the possible relationship of genes localized in our CNV regions with the male reproductive system. However, we could not find a gene known to be directly related to the development of the male reproductive system. CBAVD exhibited variable inheritance patterns like autosomal recessive, autosomal dominant, and X-linked [25]. We found all CNVs present in heterozygous state. The significance of the heterozygous CNVs in the pathogenesis of CBAVD is unknown but suggests a possible inheritance model of autosomal dominant.

The most noteworthy findings in our study are the 14q11.2 (762 Kbp, 1803 Kbp, 299 Kbp) deletions in three of the cases. CNVs in the 14q11.2 region have also been identified in infertile men in other studies. Tüttelmann et al. performed CNV analysis in Caucasian patients with idiopathic severe oligozoospermia and Sertoli-cell-only syndrome (SCOS). They determined 14q11.2 deletions and duplications both in their patient and normozoospermic control group. All were 222.3 Kbp length and did not overlap with the ones we detected. In addition, the Yq11.223 duplication that they found in SCOS patients was also detected in our patient group [31]. Halder et al. revealed CNVs in Indian patients with testicular maturation arrest. They also found CNVs (smallest: ~ 207 Kbp, largest: ~ 219 Kbp) in the 14q11.2 region as gain or loss in 10 patients. These CNVs did not overlap with ours, they were located in the upstream region of the 14q11.2del segments we identified [32]. CNVs in Australian cases with azoospermia were investigated in another study. The researchers found heterozygous duplications as pathogenic CNVs in chromosomes 3 and 11 containing various genes that may be associated with male infertility. Incompatible with our findings, they detected a 14q11.2 deletion (~ 37 Kbp) in a case. This deletion overlapped with the 14q11.2 deletions of ours but it was smaller in length, did not contain any gene, thereby considering benign. It was located downstream of the *DAD1* gene [14]. In 2015, Dong et al. reported that three of 33 azoospermia cases had heterozygous CNVs in the 14q11.2 chromosomal region [15]. There was no overlapping region with the CNVs found

in this study and they were located about 2.6-2.7 Mb downstream of the *DAD1* gene. These results from different groups suggest the potential effects of 14q11.2 in male infertility. The 14q11.2 region was also associated with developmental delay, cognitive impairment, and similar minor anomalies [33]. In addition, it should be kept in mind that chromosome 14 has imprinted regions. Disruptions of the 14q11-q13 region may cause low birth weight and delayed growth, according to Kamnasaran and Cox [34]. Additionally, we detected 14q11.2 deletions containing the *DAD1* in two of the cases (Case 18 and Case19). The 14q11.2 deletion in Case 7 was spanning 136.7 Kbp downstream of the *DAD1* gene which coded on the minus strand of DNA. We can not exclude the regulatory role of this region on the *DAD1* gene, it should be further investigated. *DAD1* is expressed both at mRNA and protein levels in the male genital tract. Rockett et al. has published that mRNA levels of the *Dad1* gene can be decreased as a result of heat exposure in a heat-shocked testis mice model [35]. In addition, *DAD1* transcript is found to be down-regulated both in normozoospermic and asthenozoospermic groups when compared with the control group in a sperm transcriptome profiling study. As a result of these findings, it has been offered that apoptotic genes may play an important role in male infertility [36]. Although DAD1 is identified as an anti-apoptotic molecule that regulates programmed cell death negatively, it is one of the subunits of oligosaccharyltransferase (OST) that catalyzes the protein N-glycosylation process in eukaryotes [16, 19]. It has been thought that, as a common post-translational modification, N-glycosylation affects plasma membrane expression of glycoproteins by organizing the folding, targeting, and transportation through the endoplasmic reticulum (ER) [21]. It has been stated, that as a result of Glozman et al.'s study, N-glycans enhanced CFTR folding efficiency and the limited folding efficiency (30–40%) of the wild type CFTR in the ER, conceivably reflect inefficient posttranslational domain assembly [21]. Thus, it has been mentioned that an N-glycosylation defect reduces the cell surface expression of CFTR. According to Li et al., for the sperm capacitation, acrosome reaction, and sperm-oocyte fusion, wild-type CFTR protein is a necessity, and deficient CFTR protein expression may affect the sperm fertilizing capacity [37]. When we consider the relationship between CFTR and CBAVD as a part of OST, defects in the *DAD1* gene may cause an insufficient CFTR via the N-glycosylation process which may result in the CBAVD phenotype. As stated by Flannigan and Schlegel in 2017, there is an urgent need for specific genomic biomarkers in the diagnosis of CBAVD [38].

Limitation of Study: This study was a pilot study aimed to identify novel potential candidate genomic regions and/or genes related to CBAVD. Number of patients included to the study were relatively low. CNV analysis should be done in larger cohorts of CBAVD patients to identfy the candiate genomic regions first, and then the alternative biologic mechanisms associated with the disease pathophysiology.

## Conclusıon

Our study was the first study profiling CNVs in Turkish-Caucasian patients with CBAVD. The spectrum and frequency of nuclear gene mutations and copy number variations in Turkish patients with CBAVD are still largely unknown. These patients underscore the importance of comprehensive genomic analysis in Turkish-Caucasian patients. Using additional technologies and functional analyses will help us understand the mechanism of the disease and will highlight the interactions between phenotypes and genotypes. In the light of our data, we suggest that CNV and *DAD1* analysis should be done in a larger cohort of CBAVD patients to determine potential chromosomal regions and genes for CBAVD cases related to male infertility.

**Conflict of Interest:** The author has no conflict of interest related to this article.

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# Does Race Have a Role in The Effect of Fingerprint on Gender?

## Parmak İzinin Cinsiyete Etkisinde Irkların Rolü Varmı?

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## ABSTRACT

**Aim:** Fingerprint is one of the personal and reliable physical features. The fingerprint has an important role in forensics, and it is used in the detection of criminals and identity checks. Studies on different races have been conducted, but no interracial comparison has been found in the literature. In this study, we have investigated the effect of race on finger ridge using the same method on 5 different races.
**Methods:** In our study, the right thumb prints of 355 (148 female, 207 male) students aged 17-25 years in our school were taken with an ink pad (stamp) and recorded along with their ages, sexes, and races. Data were analysed by sex and race.
**Results:** In the comparison by sex, the finger ridge counts of male (11.94±2.08) and female (12.76±2.02) participants were found to be statistically significantly different ($p<0.001$). Participants were classified as Turkish, Arab, Russian, Turkmen, and African. The difference in finger ridge counts between Turkish-Africans was found to be statistically significant in the comparison by races ($p<0.05$).
**Conclusion:** Comparison of finger ridge counts by sex has been studied on many races, but each study has been conducted with a different method. In our study, we aimed to contribute to the literature by examining how the finger ridge counts of men and women differ in 5 different races by using the same method. Although the data we obtained are compatible with the literature for the Turkish race, there are differences for other races.

Keywords: Finger ridge density, Fingerprint, Sex determination, Turkish population

## ÖZ

**Giriş ve amaç:** Parmak izi, kişiye özgü ve güvenilir fiziksel özelliklerdendir. Adli vakalarda önemli bir görevi olan parmak izi, suçluların tespiti ve kimlik denetiminde kullanılmaktadır. Literatürde farklı ırklarda çalışmalar yapılmış; fakat ırklar arası bir karşılaştırmaya rastlanmamıştır. Biz bu çalışma ile 5 farklı ırk üzerinde aynı metodu kullanarak parmak izi tepe noktasına ırk faktörünün etkisini belirlemeye çalıştık.
**Method:** Çalışmamızda, okulumuzun 17-25 yaş aralığındaki 355 (148 kadın, 207 erkek) öğrencinin sağ başparmak izleri mürekkeple boyama yöntemi ile (ıstampa) alınmış, yaş-cinsiyet ve ırkları sorgulanarak kaydedilmiştir. Her kişinin parmak izi tepe sayısı Bayes yöntemi (Acree) ile hesaplanmıştır. Veriler cinsiyet ve ırka göre analiz edilmiştir.
**Bulgular:** Cinsiyetlere göre karşılaştırmada erkeklerin baş parmak halka sayısı (11.94±2.08) ve kadınların başparmak halka sayıları (12.76±2.02) istatistiksel olarak anlamlı derecede farklı bulunmuştur ($p<0.001$). Katılımcılar Türk, Arap, Rus, Türkmen ve Afrikalı olarak sınıflandırılmıştır. Irklara göre karşılaştırmada Türk-Afrikalılar arasında parmak halka sayısındaki fark istatistiksel olarak anlamlı bulunmuştur ($p<0.05$).
**Sonuç:** Sonuç olarak, birçok ırk üzerinde parmak izi tepe sayısının cinsiyetlere göre karşılaştırılması çalışılmış fakat her çalışma farklı metodla uygulanmıştır. Biz çalışmamızda 5 farklı ırkta kadın ve erkek cinsiyette tepe sayılarının nasıl değiştiğini aynı metodla inceleyerek literatüre katkı sağlamayı hedefledik. Elde ettiğimiz veriler Türk ırkı için literatür ile uyumlu olmakla beraber diğer ırklar için farklılıklar göstermektedir.

Anahtar Kelimeler: Parmak izi tepe yoğunluğu, Parmak izi, Cinsiyet belirleme, Türk popülasyonu

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## INTRODUCTION

Biometric characteristics such as fingerprint, face, ear, iris, retina, hand geometry, voice, signature, and gait are physical characteristics that are unique enough to be different for each person and are too reliable to be passed on from family [1]. Fingerprint is a valuable identification tool with its uniqueness, immutability, and classifiable features. It is estimated that there is 1 in 64 billion chance that the fingerprints of any two randomly selected people will be the same [2]. It is possible to argue that the same fingerprint did not occur twice throughout the history of humanity [3].

Fingerprints are formed between 13th and 19th weeks of pregnancy. Fingerprints do not develop on the skin, they occur due to the protrusions in the flesh under the skin [4]. Genetic and racial factors play a role in the formation of fingerprints. There is a possibility that genetic problems during the formation of fingerprints may also change the structure of fingerprints [5]. In addition to genetic factors, the mother's diet, chemical intake, hormone levels, and environmental radiation can also have an effect in fingerprint pattern [6]. The position of the fetus in the womb and the density of the amniotic fluid also affect the formation of the fingerprint [3, 6].

Fingerprints have a very important role in forensic cases, and they are successfully used in many areas from detection of criminals to identity checks. They have started to be used not only in criminal identification, but also in access control, user's computer login, polling, and similar applications. Fingerprints are frequently used in identification, as fingerprints do not change even as people age [3].

Fingerprint image is the image obtained by scanning or taking a picture of the fingerprint. When we look at a fingerprint image with the naked eye, the protruding areas are represented with a colour close to black, and the recessed areas are represented with a colour close to white. The near-black protruding areas are called the "ridges," and the near-white recessed areas are called the "valleys" [4].

The fingerprint minutiae are distinctive in fingerprint analyses. Identification is carried out by using the fact that the distance between these points is different for each person. In studies examining the finger ridges, it has been argued that the finger ridge count differs by sex, and sex estimation can be made using this characteristic [5].

The Bayesian method, which Acree used in his study with 400 people in a police station in the USA in 1999, has been generally accepted, and this method started to be used. With this method, Acree showed that fingerprints with 11 ridges/25mm2 or less in a given area may belong to a male individual, and those with 12 ridges/25mm2 or more may belong to a female individual [6].

In the literature review on determining sex from fingerprints, it was concluded that women had more finger ridges than men. So, sex determination could be made from fingerprints [1, 6-8]. Studies on different races, although limited in number, have been conducted, but no interracial comparison has been found in the literature. In this study, we have investigated the effect of race on finger ridge using the same method on 5 different races.

## MATERIALS AND METHODS

Our study started with the approval of Alanya Alaaddin Keykubat University Clinical Research Ethics Committee (Number: 2019:12-5). In our study, fingerprints of students at Alanya Alaaddin Keykubat University Faculty of Medicine and Dentistry Faculty and of foreign students at Turkish and Foreign Language Application and Research Center were used. The age, sex, and race information of the students between the ages of 17-25 years was inquired and recorded. The details of the study were explained in detail to each student, and an informed consent form was signed by the participants. Right thumb prints of the students were taken with an ink pad (stamp). This process was repeated three times, and the fingerprints were meticulously preserved.

In order to find the finger ridge count of each person, a method found by Acree was used. For this purpose, first of all, a square section of 5 mm x 5 mm in the upper left part of the right thumbprint, starting from the core, was extracted from the fingerprint. The lower right corner of the

square region taken from the right hand fingerprint sample shown in the figure is defined as the core point (Figure 1). The valleys and ridges in the 5 mm x 5 mm region obtained from the upper left part of this point were calculated.

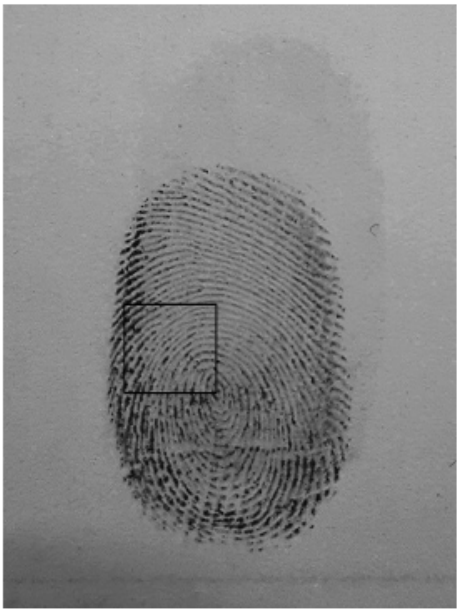

Figure 1: Core point and Bayerian Method

In the study, the sample size of 355 people who complied with the criteria determined as 1/1 sample also gives the sample size. Eighteen students were excluded from the research due to the predetermined criteria (absence of thumb, scarring, bandage due to temporary wounds, etc.). The demographic information (age, sex, nationality) of the participants in the study was recorded, and the loops on the thumb were counted. Data were analysed by sex and nationality.

Statistical analysis

In this study, the normal distribution was tested with skewness-kurtosis, histogram, Q-Q plots, standard deviation/mean, and Kolmogorov-Smirnov tests. Pearson correlation was used in the Independent Samples T-test correlation analysis in pairwise comparisons of normally distributed data. Kruskal-Wallis H test with Bonferroni correction was used in multiple comparisons of groups that were not normally distributed or had a small amount of data. In the statistical evaluation, $\alpha=0.05$ was accepted, and $p<\alpha$ was considered statistically significant. In this study, statistical analysis was carried out with IBM SPSS 22.00 package program.

## RESULTS

Of the participants in the study, 148 men and 207 women. In the comparison by sex, the thumb fingerprint loop count was found to be 11.94±2.08 for male participants, and it was found to be 12.76±2.02 for female participants. This difference is statistically significant ($p<0.001$) (Table 1).

Table 1: Comparison of the fingerprint loop count by sex

| Sex | N | Mean Loop Count | Sig.(p) |
|---|---|---|---|
| Male | 148 | 11.94±2.08 | 0.000 |
| Female | 207 | 12.76±2.02*** | |

Independent Samples t-test (*p<0.05,**p0.01,***p0.001), Data are represented as mean±standard deviation, Variables are shown as Mean±STD.

***The difference in the fingerprint loop count between the sexes is statistically significant (***p<0.001).

It is stated in the literature that the thumb fingerprint loop count is more than 12 for women and less than 12 for men. In order to test this information, frequency analysis between the sexes was carried out by making a cut-off value of 12. In the Chi-Square analysis, there was a difference between the sexes according to the cut-off value ($p<0.05$) (Table 2).

Table 2. Frequency Analysis by Sex

| Sex | Loop count below 12 | Loop count Above 12 | Total | Sig. (p) |
|---|---|---|---|---|
| Male<br>*Female | 60<br>62 | 88<br>145[a] | 148<br>20 | Value: 4.29<br>p:0.038 |

Pearson Chi-Square test (*p<0.05, **p0.01, ***p0.001)

a; The loop count of 12 or above is significantly higher in women than in men (p<0.05).

In our study, the demographic difference in the thumb fingerprint loop count was analysed by grouping the participants according to nationalities. Participants were classified as Turkish, Arab, Russian, Turkmen, and African. Due to the insufficient amount of data in some groups, the non-parametric Kruskal-Wallis H Test was used with Bonferroni correction in multi-group comparison. In the comparison by nationalities, the difference in the fingerprint loop count between Turkish-Africans was found to be statistically significant ($p<0.05$). There was no

significant difference between the other groups (p>0.05) (Table 3).

Table 3. Loop count analysis by nationality

| Nationality | N | Mean Loop Count Median (Min-Max) |
|---|---|---|
| Turkish | 269 | 12 (8-18) |
| Arab | 41 | 13 (9-17) |
| Russian | 15 | 13 (9-17) |
| Turkmen | 16 | 13.5 (9-17) |
| *Africana | 14 | 14 (11-16) |

(Min: Minimum; Max: Maximum), Kruskal-Wallis H Test post hoc Bonferroni test (*p<0.05, **p0.01, ***p0.001), Data are represented as Median (Min-Max), Variables are shown as Median (Min-Max)
a; The difference in the loop count between Africans and Turks is statistically significant (p<0.05)

## DISCUSSION

In order to distinguish fingerprints from each other, the fingerprint minutiae on each finger are examined. The frequency and arrangement of these fingerprint minutiae is different for each person. Since women have more detailed and specific fingerprints than men, when a certain area of the fingerprint is examined, it is seen that women have a higher ridge count than men. Accordingly, it can be stated that women's fingerprint images contain much more fingerprint minutiae [9]. Numerous studies have been conducted to examine the relationship between fingerprint and sex using these minutiae [10-14]. This information is used to save both time and energy in identifying the owner of the fingerprint by estimating their sex in legal cases and investigation processes [10].

According to the studies examining the relationship between fingerprint and sex in the literature, the average finger ridge count differs by sex and race, and the average finger ridge count of men is lower than that of women in any racial group [8, 10-17]. In our study, the average finger ridge count was found to be significantly different between sexes, the thumb fingerprint loop count was found to be 11.94±2.08 for men and 12.76±2.02 for women, and these values were found to be consistent with the findings in the literature.

In the study of Krishan K. et al., conducted with a total of 194 participants, 97 men and 97 women, aged between 18-25 years, a total of 1.940 fingerprints were taken, and the ridges in the epidermis in the upper left, upper right, and lower areas of these fingerprints were counted for each finger. Finger ridge densities were statistically compared by these three areas and by sex using t-test. The results showed that women have higher finger ridge density than men in all three areas. They reported that the finger ridge density in the upper right and upper left areas of the fingerprint had significantly higher values than the lower area [18].

In Acree's study on finger ridge on 100 African-American women, 100 African-American men and 100 Caucasian women, 100 Caucasian men with criminal records, it is reported that if the ridge count is 11 ridges/25 mm2 or below, the fingerprint is more likely to belong to a man, and if the ridge count is 12 ridges/25 mm2 or above, the fingerprint is more likely to belong to a woman [11].

Nayak et al.'s study on 200 people of Chinese origin (100 men and 100 women) and 100 people of Malaysian origin (50 men and 50 women) revealed that there were significant differences by sex in the finger ridge count. They reported that those with a finger ridge count of 12/25 mm2 or below were male, and those with a finger ridge count of 13/25 mm2 or above were female [12, 15].

Gutiérrez-Redomero et al. reported that the average finger ridge count in the population of 209 (99 male and 110 female) Caucasian Hispanic people was 17.91 in women and 16.23 in men [13]. Nithin et al. analysed fingerprints from 550 volunteers (275 male and 275 female) from the South Indian population, and reported that individuals with a finger ridge count of 13/25mm2 or below were most likely male, and those with 14/25 mm2 or above were most likely female [14].

In the study conducted by Oktem et al. on the Turkish population, the average finger ridge count taken from 206 students (118 female-88 male) was found to be 14.52 in females and 12.92 in males [8].

Eshak G.A. et al. conducted a study with 752 participants from Egypt (380 male, 372 female) in which they examined ridge count, square area, finger breadth and finally ridge density through

finger photographs, and reported that women had smaller finger breadth and square area but had more number of ridges and higher ridge density [19].

When the studies conducted around the world and the races used in these studies are considered, it is seen that the average finger ridge count of men is 12.68 ridges/25mm2, while the average finger ridge count of women is 14.88 ridges/25mm2. It is predicted that women have a higher finger ridge density than men. So, sex determination can be made using this characteristic. It has been seen that, in addition to the finger ridge density, the ratio of the ridge thickness to the valley thickness, the ridge count and the ridge width values can also be used in sex determination [10]. On the one hand, many studies in the literature are similar to our study, on the other hand, Mustanski et al. [20], Van Oel et al. [21], Ekanem et al. [22], and Wang et al. [23] reported higher finger ridge count in men than women.

As a result, the comparison of the finger ridge count by sex on many races has been studied, but not every study has used the same method. In this research we presented, we aimed to contribute to the literature with a different perspective by examining the differences in finger ridge count in women and men in 5 different races by the same researcher and the same method. Our study is a first in this respect. Although the results we obtained are compatible with the literature for the Turkish race, there are differences for other races.

In a conclusion; since our study is the first in this respect, it has some shortcomings. In particular, the inequality in the number of men and women and the low number of foreign racial fingerprints are among the limitations of our study. This study should be replicated with more people of a foreign race and by ensuring the same number of men and women.

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RESEARCH ARTICLE

ARAŞTIRMA

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# Comparing the outcomes of arthroscopic tenodesis versus tenotomy for the treatment of the long head of biceps tendon pathologies during supraspinatus tendon repair

## Supraspinatus Yırtığı Tedavisi Sırasında Biseps Uzun Başı Patolojilerinin Tedavisinde Artroskopik Tenodez Ve Tenotominin Sonuçlarının Karşılaştırılması

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## ABSTRACT

**Aim:** Long head of the biceps tendon pathologies are frequently accompanied by full-thickness rotator cuff tear. The purpose of this study was to compare functional scores, complication rates and time to return to work after tenotomy and tenodesis who underwent arthroscopic supraspinatus repair (ASR).

**Methods:** Overall, 129 patients who underwent ASR surgery were divided into 2 groups. Group 1 consisted of 62 patients who underwent biceps tenodesis and group 2 consisted of 67 patients who underwent biceps tenotomy. We evaluated demographic data, clinical findings, complications and American Shoulder and Elbow Surgeons, Constant Murley, Visual analogue scale and 36-item Short Form subscale scores.

**Results:** Mean follow-up time was 13.68±4.22 months. Mean postoperative and pre-operative-postoperative differences of ASES, CM, VAS and SF-36 subscale scores were not significantly different between the two groups. Popeye sign was positive for 13 (19.4%) patients in group 2, however, none of patients in group 1 was positive (p<0.001). Other complications were not significantly different between two groups.

**Conclusion:** The results show that arthroscopic biceps tenotomy and tenodesis are both viable treatments for proximal biceps tendon pathology, yielding similar clinical outcomes in the context of concomitant rotator cuff repair. Tenotomy can be chosen instead of tenodesis, which is technically more difficult and expensive.

Keywords: Biceps tendon, tenodesis, tenotomy, shoulder arthroscopy

## ÖZ

**Amaç:** Tam kat supraspinatus tendon yırtıkları sıklıkla biseps uzun baş tendonu patolojileri ile birlikte görülmektedir. Bu çalışmanın amacı artroskopik supraspinatus tamiri yapılan ve ek olarak biseps uzun başı tendon patolojisi nedeniyle tenodez ve tenotomi tedavisi yapılan hastaların klinik skor, komplikasyon oranı ve işe dönüş oran ve zamanlarını karşılaştırmaktır.

**Yöntemler:** 129 artroskopik rotator manşet onarımı yapılan hasta 2 gruba ayrıldı. 1. Grupta biseps tenodezi uygulanan 62 hasta, 2. grupta ise biseps tenotomisi uygulanan 67 hasta bulunmakta idi. Demografik ve klinik bulgular, komplikasyonlar, American Shoulder and Elbow Surgeons (ASES), Constant Murley (CM), Visual analogue scale (VAS) ve 36-item Short Form (SF-36) alt skorları değerlendirildi.

**Bulgular:** Ortalama takip süresi 13.68±4.22 ay idi. Ortalama ASES, CM, VAS ve SF-36 skorlarına bakıldığında gruplar arasında postoperatif ve preoperatif-postoperatif fark değerlerinde anlamlı fark bulunmadı. Popeye bulgusu 2. grupta 13 (%19,4) hastada pozitif iken 1. grupta hiçbir hastada görülmedi (p<0.001). Diğer kompikasyon oranlarında gruplar arasında anlamlı fark saptanmadı.

**Sonuç:** Artroskopik supraspinatus tamiri yapılan hastalarda, biseps tenodezi ve tenotomisi bisep uzun baş patolojilerinde klinik olarak iyi sonuç veren tedavi metodlarıdır. Teknik olarak daha zor ve maliyetli tenodez yerine tenotominin güvenle tercih edilebileceğini düşünmekteyiz.

Anahtar Kelimeler: Biseps tendonu, tenodez, tenotomi, omuz artroskopisi

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## INTRODUCTION

Rotator cuff (RC) rupture is the one of the most common causes of shoulder pain and disability [1]. Arthroscopic rotator cuff repair (ACRC) has experienced increasing popularity in the treatment of RC ruptures. Long head of the biceps tendon (LHBT) pathologies are frequently accompanying full-thickness rotator cuff tear (RCT) [2]. LHBT lesion may cause chronic pain and limitation of shoulder flexion after ARCR, when left untreated [3]. For this reason, routine intervention to the LHBT during ARCR is recommended [4].

Management of concomitant LHBT lesions with RCT remains controversial [3, 5]. The most popular treatment options are biceps tenotomy and tenodesis. Both options are reliable and useful to reduce pain and increase range of motion (ROM) [6]. Tenotomy is a less technically demanding, quicker and cheaper method, and the rehabilitation process is also accelerated. Muscle cramps, Popeye's deformity, and loss of supination strength are, however, major concerns after tenotomy; some authors favor tenodesis particularly in younger patients. The main advantages of tenodesis are maintaining normal muscle tension with lower cosmetic deformities and higher supination strength [2]. There are many studies to compare functional results following tenotomy and tenodesis. A recent meta-analysis report higher Constant Score after tenodesis [3] and cosmetic deformity is also less frequent after tenodesis [6]. On the other hand, many studies report similar functional results and complication rates [7]. In a recent randomized controlled study, 69 patients were evaluated and there were no significant difference in functional scores, life quality measures and arm strength. Popeye's deformity was in fact higher in patients received tenotomy [8].

The cost of treatment is an important concern after orthopedic procedures. The burden of the treatment consists of not only cost of implants, but also additional physiotherapy, prolonged time prior to returning to work, which also affect the cost of the treatment indirectly.

Some studies suggest tenodesis, however for long-term outcomes, some studies reported that there is no difference in terms of the clinical outcomes between it and tenotomy. There is in fact no consensus for the management of LHBT pathologies when performing ASR [9, 10]. The purpose of this study was therefore to compare functional scores, complication rates, and time to return to work after tenotomy and tenodesis who underwent ASR.

## MATERIALS AND METHODS

After institutional review board approval (Approval date-number: 02/06/2021, 2021-7/24), the medical records of 189 patients who underwent ARCR between February 2017 and December 2019, were evaluated. Of these, 70 patients were excluded because they either had a RC tear that was not repaired (n = 54) or had isolated subscapularis tendon repairs (n = 16); thus, 129 patients were included in the study. Patients were divided into two groups, either as having undergone biceps tenodesis (group 1) or tenotomy (group 2). The arthroscopic views of one patient from both groups are shown in Figure 1-2. Two different surgeon's patients were assessed, as two groups. One surgeon performs tenotomy for biceps pathologies with rotator cuff tear in his clinical practice and, the other one performs tenodesis.The study was conducted in accordance with the 1964 Declaration of Helsinki and its later updates.

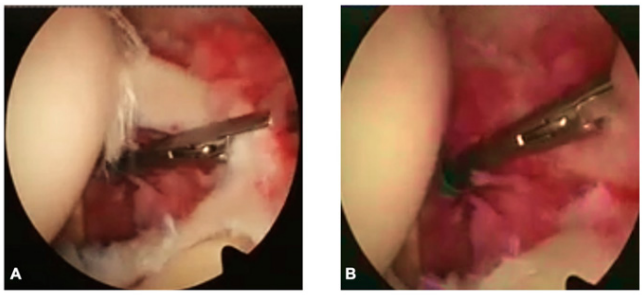


Figure 1: Arthroscopic view of a patient who underwent biceps tenotomy. A-before intervention, B-after intervention

Inclusion criteria of this study were patients aged > 18 years old, who underwent arthroscopic isolated anterosuperior, superior, and/or posterosuperior supraspinatus tendon repair with either biceps tenotomy or tenodesis and a minimum 6 month follow-up.

Excluded patients were those with symptomatic acromioclavicular arthritis, the presence of degenerative glenohumeral arthritis, frozen

shoulder, irreparable supraspinatus tendon tear, concomitant infraspinatus, teres minor and/or subscapularis tendon rupture, history of previous shoulder dislocation, patient under 18 years old, previous shoulder surgery from the affected side, follow-up period less than 6 months and ipsilateral neurological deficits.

All operations were performed in the beach chair position under general anesthesia. The standard posterior portal was used to examine the glenohumeral joint, the torn supraspinatus tendon was repaired with trans osseous equivalent double-row configuration. One surgeon performed a tenotomy, the other performed tenodesis for all their patients. The tenotomy was performed through the anterolateral portal with a radiofrequency probe from the most proximal side of the LHBT. Those patients who underwent biceps tenodesis, the LHBT was attached to bicipital groove with interference screw Bio composite tenodesis screw (Arthrex, USA). Subacromial decompression was performed to all patients, however acromioplasty was not routinely performed.

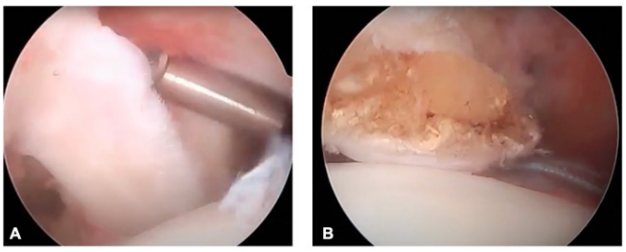


**Figure 2: Arthroscopic view of a patient who underwent biceps tenodesis. A-before intervention, B-after intervention**

Patients were held in velpeau bandage for four weeks postoperatively. Passive pendulum exercises and active elbow movement was immediately allowed. Active shoulder movements started after postoperative fourth week. Stretching was prohibited for 12 weeks. In patients who underwent tenodesis, biceps strengthening was started after 8th week.

Age, gender, body mass index, operated side, dominant side, number of comorbidities (classified as 0 and ≥1), tear chronicity (<3 months acute and >3 months chronic), tear size, subjective weakness that was mentioned by patient (present / absent) at last follow-up examination, surgery duration, return to work time and follow-up time, were all assessed. Tear size classification of full-thickness cuff ruptures were performed by assessing the magnetic resonance imaging (MRI) results according to the DeOrio and Cofield classification. Full-thickness tears of 1 cm were small, 1 - 3 cm were medium, 3 - 5 cm were large, and more than 5 cm were massive [11]. After tear size analysis, it was seen that it was homogenously distributed between groups (p=0.983). Intraoperative long head of biceps tendon injuries were noted and in group 2 rate of degenerative SLAP, lesions were significantly high; in group 1 the rate of partial LHBT ruptures were high (p<0.001). The mean age was significantly different between groups (52.77 vs 59.82, group 1 vs group 2) (p<0.001) (Table 1).

For the functional and quality of life evaluation of patients, pre- and postoperative American Shoulder and Elbow Surgeons (ASES) scores [12], Constant-Murley (CM) scores [13], Visual analogue scale (VAS) [14] and 36-item Short Form Health Survey (SF-36) [15] scores, were all evaluated.

The postoperative Yergason test was performed on all patients and anterior shoulder pain (ASP) was noted. Presence of Popeye signs and muscle cramps were recorded. In addition, complications (re-rupture and frozen shoulder) were recorded and compared with other outcomes.

Statistics: The mean, standard deviation, median, lowest and highest value, frequency and ratio were used in the presentation of descriptive statistics. The Shapiro-Wilk test was used for the evaluation of the distribution of variables. The Chi-Square test and Fischer exact tests were used in the comparison of independent qualitative data. The Mann-Whitney U test was used in the comparison of independent quantitative data. A P-value of < 0.05 was considered statistically significant. All statistical analysis was performed using IBM SPSS for Windows, version 22 (IBM corp., Armonk, NY).

## RESULTS

Table 1 presents the general demographics and disease-specific characteristics of the 129 patients included in this study (Table 1). When compared to group 1, group 2 had a significantly lower return to work time (p<0.001). Gender distribution was

**Table 1: Demographic and disease-specific characteristics of the patients**

| Variable | Entire Study Population | Group 1 | Group 2 | p |
|---|---|---|---|---|
| Patient number, n (%) | 129 (100) | 62 (48.1) | 67 (51.9) | 0.424 |
| Age, year, SD | 56.43±4.37 | 52.77±2.63 | 59.82±2.54 | <0.001 |
| Gender, n (%) | | | | |
| Female | 74 (57.4) | 42 (67.7) | 32 (47.8) | 022 |
| Male | 55 (42.6) | 20 (32.3) | 35 (52.2) | |
| BMI, kg/m2 | 27.34±2.47 | 27.12±2.45 | 27.55±2.50 | 0.334 |
| Injured side, n (%) | | | | |
| Right | 65 (50.4) | 30 (48.4) | 35 (52.2) | 0.662 |
| Left | 64 (49.6) | 32 (51.6) | 32 (47.8) | |
| Tear Chronicity, n (%) | | | | |
| Acute | 36 (27.9) | 22 (35.5) | 14 (20.9) | 0.065 |
| Chronic | 93 (72.1) | 40 (64.5) | 53 (79.1) | |
| Long head of biceps tendon injury, n (%) | | | | |
| partial rupture | 24 (18.6) | 18 (29) | 6 (9) | <0.001 |
| tenosynovitis | 58 (45) | 34 (54.8) | 24 (35.8) | |
| subluxation | 20 (15.5) | 10 (16.1) | 10 (14.9) | |
| degenerative slap | 24 (18.6) | 0 (0) | 24 (35.8) | |
| dislocation | 3 (2.3) | 0 (0) | 3 (4.5) | |
| Comorbidity, n (%) | | | | |
| yes | 28 (21.7) | 10 (16.1) | 18 (26.9) | 0.139 |
| no | 101 (78.3) | 52 (83.9) | 49 (73.1) | |
| Tear size, n (%) | | | | |
| Small | 30 (23.3) | 14 (22.6) | 16 (23.9) | 0.983 |
| Medium | 74 (57.4) | 36 (58.1) | 38 (56.7) | |
| Large | 25 (19.4) | 12 (19.4) | 13 (19.4) | |
| Dominant side, n (%) | | | | |
| Yes | 78 (60.5) | 36 (58.1) | 42 (62.7) | 0.592 |
| No | 51 (39.5) | 26 (41.9) | 25 (37.3) | |
| Surgery time, minutes, SD | 87.05±14.03 | 95.96±9.27 | 78.80±12.61 | 0.155 |
| Frozen Shoulder, n (%) | | | | |
| Yes | 8 (6.2) | 2 (3.2) | 6 (9) | 0.178 |
| No | 121 (93.8) | 60 (96.8) | 61 (91) | |
| Re-rupture, n (%) | | | | |
| Yes | 9 (7) | 4 (6.5) | 5 (7.5) | 0.822 |
| No | 120 (93) | 58 (93.5) | 62 (92.5) | |
| Return to work time, days, SD | 83.83±14.40 | 86.77±16.27 | 81.11±11.92 | <0.001 |
| Follow-up time, months, SD | 13.68±4.22 | 13.09±3.59 | 14.23±4.70 | 0.071 |

**Abbreviations: SD standard deviation, p<0.05 was defined as significant and defined bold**

significantly different between groups (p=0.022). In terms of LHBT injury, 34 (54.8%) patients had tenosynovitis in group 1, 24 (35.8%) had tenosynovitis and 24 (35.8%) had degenerative SLAP lesion in group 2 and the difference was significant (p<0.001). Other clinical findings and complications were not significantly different between two groups (p>0.05).

Table 2 presents the postoperative physical examination findings and complication rates compared between two groups. The Popeye sign was positive in 13 patients in group 2, however, none of patients in group 1 was positive (p<0.001).

Preoperative and postoperative ASES, CM, VAS and SF-36 subscale scores at final examination for all patients are shown in Table 3. Mean differences of the scores between preoperative and postoperative values were also defined. All postoperative clinical, quality of life subscales and VAS scores were not significantly different

Table 2: Postoperative clinical outcomes and complications

| Variable | Entire Study Population | Group 1 | Group 2 | P |
|---|---|---|---|---|
| Subjective muscle weakness, n (%) | | | | |
| Yes | 12 (9.3) | 4 (6.5) | 8 (11.9) | 0.284 |
| No | 117 (90.7) | 58 (93.5) | 59 (88.1) | |
| Popeye sign, n (%) | | | | |
| Yes | 13 (10.1) | 0 (0) | 13 (19.4) | <0.001 |
| No | 116 (89.9) | 62 (100) | 54 (80.6) | |
| Anterior shoulder pain, n (%) | | | | |
| Yes | 19 (14.7) | 10 (16.1) | 9 (13.4) | 0.666 |
| No | 110 (85.3) | 52 (83.9) | 58 (86.6) | |
| Muscle cramp, n (%) | | | | |
| Yes | 13 (10.1) | 8 (12.9) | 5 (7.5) | 0.305 |
| No | 11 (89.9) | 54 (87.1) | 62 (92.5) | |
| Re-rupture, n (%) | | | | |
| Yes | 9 (7) | 4 (6.5) | 5 (7.5) | 0.822 |
| No | 120 (93) | 58 (93.5) | 62 (92.5) | |
| Frozen Shoulder, n (%) | | | | |
| Yes | 8 (6.2) | 2 (3.2) | 6 (9) | 0.178 |
| No | 121 (93.8) | 60 (96.8) | 61 (91) | |

Abbreviations: SD standard deviation, p<0.05 was defined as significant and defined bold

Table 3: Mean difference of preoperative and postoperative clinical scores compared between two groups

| Clinical Score | All patients | Group 1 | Group 2 | p |
|---|---|---|---|---|
| ASES | 36.79 ± 9.32 | 37.09±8.82 | 36.52±9.81 | 0.728 |
| CM | 37.20±10.27 | 38.48±9.27 | 36.01±11.06 | 0.174 |
| VAS | 4.12 ± 1.40 | 4.16±1.35 | 4.08±1.45 | 0.773 |
| SF-36 | | | | |
| Physical functioning | 25.07±9.78 | 24.03±9.18 | 26.04±10.28 | 0.245 |
| Role limitations due to physical health | 56.58±20.13 | 58.06±19.61 | 55.22±20.66 | 0.426 |
| Role limitations due to emotional problems | 44.81±29.44 | 44.22±31.41 | 45.35±27.72 | 0.828 |
| Energy/fatigue | 46.66±18.12 | 46.45±16.35 | 46.86±19.74 | 0.897 |
| Emotional well-being | 34.07±13.79 | 35.48±12.70 | 32.77±14.70 | 0.267 |
| Social functioning | 44.99±12.69 | 44.19±13.85 | 45.73±11.57 | 0.494 |
| Pain | 52.62±15.68 | 51.90±13.64 | 53.29±17.44 | 0.616 |
| General health | 49.06±16.01 | 49.67±17.31 | 48.50±14.82 | 0.680 |
| Health change | 62.69±23.20 | 62.09±22.96 | 63.43±23.56 | 0.745 |

ASES American Shoulder and Elbow Surgeons Score, CM Constant Murley score, VAS Visual analogue scale

between the two groups. Nevertheless, all post operational improvement scores were similar between both groups (p>0.05).

## DISCUSSION

The most important findings of this study were that clinical scores, quality of life scores and complication rates were not significantly different in patients undergoing tenodesis or tenotomy of the biceps tendon, concomitant to ASR. However, two significantly different results were found between the two techniques: return to work time was higher in the tenodesis group and the Popeye sign was present in 13 patients in the tenotomy group, while none of the patients in tenodesis group presented with it.

The debate between biceps tenodesis and tenotomy is challenging to surgeons and patients. Many studies have found no significant differences in pain, function or limitations between tenotomy and patients with tenodesis [8, 16, 17]. Elsewhere, some authors favoring tenodesis have compared it with tenotomy in terms of increased shoulder pain and loss of supination power with biceps tenotomy [18, 19]. For our part, we found no significant difference in terms of clinical scores, pain score

and quality of life scores between tenotomy and tenodesis. Physical examination findings were assessed and only the Popeye sign was seen, significantly high, in the tenotomy group, though subjective muscle weakness rates were similar.

In a previous prospective double-blinded randomized controlled trial, MacDonald et al. reported that there was no difference in subjective clinical outcomes, between patients who underwent biceps tenodesis and tenotomy at postoperative 24 months. In that study, the Popeye sign was 3.5 times higher after tenotomy, compared with tenodesis [7]. The significant improvements in ASES and CM scores for both groups that were found in our study is similar to that seen in previous studies [7, 8, 20]. However, Godeneche et al. [21], Meraner et al. [10] and some other previous studies have reported better results after tenodesis than tenotomy, based on the CM score.

The Popeye sign may be seen following biceps tenotomy because of the retracted LHBT. Literature has many varying reports regarding Popeye deformity. Aflatooni et al. reported that although a higher proportion of patients with tenotomy reported limitations and the Popeye sign (+3%) compared with patients with tenodesis, those disparities were larger for weakness (+6% in tenotomy) and even greater for spasms/cramping (+12%), biceps pain (+9%), and shoulder pain (+17%) [22]. Castricini et al. and Hassan et al. found little to no significant difference in downsides such as the Popeye sign between the two procedures, except that patients with tenotomy experienced more shoulder pain, as well as biceps spasms and cramping [8, 23]. Lee et al. also found no difference in outcomes of function or pain between tenodesis and patients with tenotomy [18]. Our findings showed only higher Popeye sign rates in the tenotomy group compared with tenodesis and in terms of clinical scores, no significant difference was found.

Studies that we have discussed so far, however, were not completely biceps procedures with concomitant ARCR. A previous meta-analysis and systematic review comparing tenotomy and tenodesis procedures performed concomitantly with RCR, found that patients undergoing RCR with tenotomy were significantly more likely to generate a lower Constant-Murley score and develop a Popeye deformity, however these differences were not clinically significant and there was no significant difference in patient satisfaction [3, 19]. Our results are also consistent with these findings. Delayed failure of tenodesis fixation may help to explain our finding that showed none of patients in the tenodesis group had the Popeye sign. Long-term follow-up results may show more reliable outcomes of tenodesis failure rates.

Aflatooni et al. [22] found a delayed rupture or failure rate of 11% in their tenodesis group, consistent with other studies reporting delayed failure or rupture rates up to 20% [24]. The senior author has found that delayed failures do occur with interference screw fixation devices, and is most common within 3–12 weeks post-op. This is consistent with other authors' findings [24]. We found no tenodesis failure with physical examination. Extra magnetic resonance imaging may give more reliable results for failure of tenodesis.

Limitations: In our study, mean age, LHBT pathology and gender distributions were statistically different between groups. In group 2, both mean age and degenerative SLAP lesion numbers were high, and these differences may affect the clinical outcomes. Additionally, patients were not randomized but rather received biceps tenodesis or tenotomy after consulting with the senior author, which may have influenced our results due to selection bias. Finally, follow-up time was relatively short: longer follow-up may give more reliable results.

**Conclusion:** The results show that arthroscopic biceps tenotomy and tenodesis are both viable treatments for proximal biceps tendon pathology, yielding similar clinical outcomes with concomitant supraspinatus tendon repair. Tenotomy can be chosen instead of tenodesis, which is technically more difficult and expensive.

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

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**Ethics Committee Approval:** Ethics Committee of the Faculty of Medicine of Uludağ University, (Approval date-number: 02/06/2021, 2021-7/24),

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CASE REPORT

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# A rare case of Munchausen syndrome: foreign body ingestion

## Nadir bir munchausen sendromu olgusu: yabancı cisim yutulması

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**ABSTRACT**

Munchausen Syndrome (MS) is characterized by mental or physical diseases intentionally induced by the patients. In this case report, we aimed to present MS and review current literature. A 36-year-old female patient was admitted to the emergency room with the complaint of abdominal pain that developed after swallowing stitch needles. Physical examination revealed minimal tenderness in the abdomen with palpation. The plain abdominal X-ray revealed more than ten needle-shaped opacities. During the anamnesis, the patient was incoherent and too determined to have a surgical intervention, which gave rise to a suspicion of MS. A psychiatrist consultation was conducted, she was diagnosed with MS and hospitalization of the patient was planned. Clinical suspicion and detailed anamnesis are the key for the diagnosis. Physicians should bring to mind the diagnosis of MS and seek psychiatric support in patients with frequent hospital admissions, incompatibility between their complaints and a history and examination findings.

Keywords: Munchausen syndrome, foreign bodies, diagnosis

**ÖZ**

Munchausen Sendromu (MS), hastalar tarafından kasıtlı olarak üretilen zihinsel veya fiziksel hastalıklarla karakterizedir. Bu olgu sunumunda MS'i sunmayı ve güncel literatürü gözden geçirmeyi amaçladık. 36 yaşındaki kadın hasta, dikiş iğneleri yutma sonrası gelişen karın ağrısı şikâyetiyle acil servise başvurdu. Fizik muayenede palpasyonla batında minimal hassasiyet vardı. Çekilen ayakta direkt batın grafisinde ondan fazla iğne şeklinde opasite mevcuttu. Hastanın anamnezinde tutarsızlıklar ve cerrahi girişim için aşırı istekli olması MS şüphesini uyandırdı. Psikiyatri konsültasyonu istendi, MS tanısıyla yatışı planlandı. Klinik şüphe ve ayrıntılı anamnez, tanı için anahtardır. Hekimler, hastaneye sık başvuran, şikâyetleri ile öykü ve muayene bulguları arasında uyumsuzluk olan hastalarda MS tanısını akla getirmeli ve psikiyatrik destek almalıdır.

Anahtar Kelimeler: Munchausen sendromu, yabancı cisim, tanı

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## INTRODUCTION

Munchausen Syndrome (MS) was first defined by Asher in 1951, for a group of persons who presented to various hospitals with fabricated medical history and were allowing unnecessary surgical interventions to be performed on them. This syndrome is named after Karl Friedrich von Munchausen (1720-1797), known for his exaggerated war hero stories who worked as mercenaries in the Russian Army. The main feature of the syndrome is the deliberate production of physical or psychiatric symptoms related to almost any system [1]. Patients with a wide range of signs and symptoms apply to the emergency departments as well as other clinics with the hope of hospitalization and surgery. In this case report we present a patient with the complaint of abdominal pain who was ultimately diagnosed with MS.

## CASE REPORT

A 36-year-old female patient was admitted to the emergency room with the complaint of abdominal pain developing after allegedly swallowing stitch needles. No history of drug use was reported and her medical history revealed abdominal surgery using stitch needles had been performed. Vital signs findings were normal and after physical examination, a number of old scars and freshly injured wounds were observed on the abdominal skin, though no abdominal guarding and rebound tenderness was induced. Radiopaque foreign bodies compatible with a large number of stitch needles within the abdomen, as well as staple stitching in two different areas on the left side of the abdominal skin were observed on the plain abdominal X-ray (Figure 1). The patient reported accidentally swallowing stitching needles and after an investigation of her earlier medical reports, we found multiple earlier presentations, with complaints such as skin incision, foreign body ingestion and skin cut wounds. During the anamnesis, the patient was incoherent and very determined to undergo a surgical intervention, which gave rise to a suspicion of MS. The psychiatrist's consultation resulted in a decision to admit, however she refused to be admitted and left the hospital against medical advice.

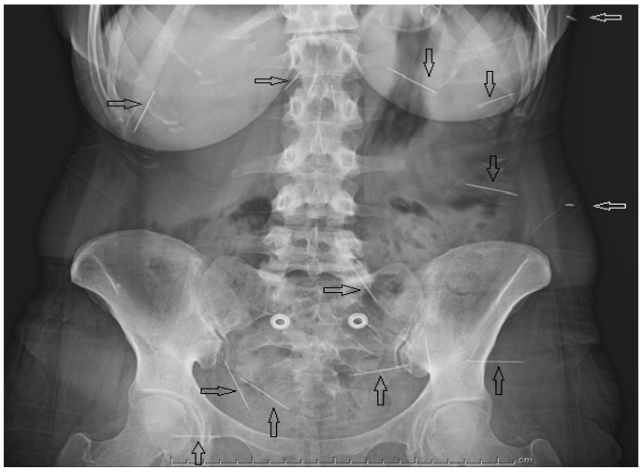

Figure 1. On the plain abdominal X-ray radio-opaque foreign bodies (black arrow) and metallic stitches (white arrow) on the left side of the abdomen is shown.

## DISCUSSION

Patients with MS have a long-term medical history of a wide range of medical and surgical applications in different hospitals, imitating the symptoms of actual diseases. The presence of psychiatric diseases in the family increases the risk for this syndrome [2]. The disease usually occurs in early adult life and mostly in males of low socioeconomic status. Childhood history usually reveals neglect, abandonment and harmful behavior by a parent. Hospital staff and doctors are perceived as a "tool of interest and love" by these patients. Those with complaints that are incompatible with the physical examination should be carefully evaluated. Patients with dramatic, aggressive, attention-grabbing behavior, atypical symptoms, consulting a large number of doctors, being themselves a health-care personnel and using literary language, are relevant behaviors for the diagnosis [3]. The inconsistency in the present medical history, medical records showing up to 100 applications on different occasions and the persistence of the same complaints, lead us to a diagnosis in this case. The patient had a number of old scars as well as newly sutured wounds. Typical MS patients apply to emergency departments and other clinics with complaints of non-healing wounds, recurrent infections, foreign bodies, necrotizing fasciitis; patients will allow many invasive procedures for diagnosis and treatment. It has been reported that the syndrome could coexist with borderline personality disorder [4]. One patient inserted a needle into her urethra for

4 days, to the point that she could not remove it by herself and applied to the emergency department with abdominal pain; the needle was removed by surgery in the abdomen [5]. Our patient also had a surgery after swallowing a sewing needle and had applied to another hospital two years prior, with complaints of abdominal pain.

MS should be distinguished from other medical and psychiatric diseases. In somatoform diseases such as somatization disorder and conversion, symptoms appear completely unconsciously and involuntarily: the patients believe they are ill and there is no simulation. In malingering (lying), the patient deliberately mimics the symptoms of the disease for secondary gains, such as money or for protection. MS is situated midway of these two conditions in this spectrum. Symptoms appear consciously with subconscious impulses [6]. Patients with MS are not mindful of any purpose and don't seek to gain any profit [7].

The pathophysiology of the disease is uncertain, as in the case of many psychiatric diseases, and its prognosis is quite poor. In addition, the constant changing of physicians or hospitals makes it difficult to follow up on those patients [4]. A multidisciplinary approach, especially psychiatric evaluation, plays an important role in the management of the MS [8]. In the treatment of the disease, psychotherapy, family therapy and behavioral therapy are recommended, along with pharmacotherapy.

## CONCLUSION

The most important and first step in the diagnosis of MS is to consider the prospect of the existence of the disease in our patients. Early diagnosis reduces unnecessary, repeated health costs and the risk of self-harm.

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CASE REPORT

OLGU SUNUMU

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# Can superficial veins be used as a drainage path in deep venous injuries?

## Derin venöz yaralanmalarda yüzeysel damarlar drenaj yolu olarak kullanılabilir mi?

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**ABSTRACT**

Peripheral vascular injuries are frequently encountered traumas that require emergency intervention. Rapid recognition of vascular injury, fluid replacement, blood transfusion, and early revascularization are very critical in the potential prevention of mortality, extremity loss, and functional defect. In the present report, we aimed to emphasize the importance of arterial and superficial venous and also deep venous revascularizations on the mortality and morbidity of a patient with burts vascular injuries.

Keywords: Superficial veins; deep venous injuries; drainage.

**ÖZ**

Periferik damar yaralanmaları sıklıkla karşılaşılan ve acil müdahale gerektiren travmalardır. Vasküler hasarın, erken revaskülarizasyonu, sıvı replasmanının veya kan transfüzyonunun hızlı bir şekilde yapılması, mortalitenin, ekstremite kaybının veya fonksiyonel kusurun potansiyel önlenmesinde çok kritiktir. Bu raporda, damar yaralanması olan bir hastanın mortalite, morbiditesi üzerinde arteriyel, yüzeyel venöz ve ayrıca derin venöz revaskülarizasyonların önemini vurgulamayı amaçladık.

Anahtar sözcükler: Yüzeysel damarlar; derin venöz yaralanmalar; drenaj

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## INTRODUCTION

Peripheral vascular injuries are frequently encountered traumas that require emergency intervention. Vascular injuries make up 2-3% of all traumas [1]. Treatment approaches for vascular injuries have been developed most commonly during wars. While, mortality rates were around 80%, in the era where ligation was accepted as the unique treatment option, developments in the field of vascular surgery, thanks to the successful anastomosis and revascularization techniques, nowadays mortality rate has decreased down to 1-1.5 percent [2]. Rapid recognition of vascular injury, fluid replacement or blood transfusion, and early revascularization are very critical in the potential prevention of mortality, extremity loss, or functional defect. Since injuries of neighboring venous, neural, bony structures, and other tissue traumas play an important role in morbidity and mortality, a comprehensive systemic evaluation and avoidance of focusing only on the arterial system carry vital importance. We present a case in a hypovolemic shock with major arterial and venous injury on his right lower extremity as a result of a mine explosion that occurred after a terrorist attack who was transported by a helicopter to the Emergency Service and operated rapidly.

In the present report, we aimed to emphasize the importance of arterial and superficial venous and also deep venous revascularizations on the mortality and morbidity of a patient with percutaneous penetrating vascular injuries.

## CASE REPORT

A 16-year-old male patient whose bleeding was partially controlled by an external tampon packing applied on the right femoral region 2 hours after penetrating vascular injury, was brought to our emergency service by airway transfer. The patient, who received 2 units of erythrocyte suspension, 2 units of whole blood transfusion, and fluid replacement during transfer, had a GCS (Glasgow Coma Scale) score of 6-8 points when he was brought to the hospital. He was unconscious and in hypovolemic shock. In the emergency service, a jugular catheter was inserted to provide a rapid fluid replacement. At the same time, hemogram, blood typing, and biochemical tests were performed and he was transferred urgently to the previously prepared Cardiovascular Surgery Operating Room.

Under general anesthesia, the compressed tampon dressing 5 cm above the femoral region of the right lower extremity was opened. It was found that the right superficial artery, superficial femoral vein, and deep femoral vein had lost their integrity. Vascular clamps were rapidly applied to the superficial femoral artery, superficial and deep femoral veins. The open wound area was cleaned using povidone iodine and 100 IU/kg heparin was administered to the patient after appropriate draping. A tissue defect (10 x 10 cm) was found approximately 5 cm below the femoral region. The exploration field was widened up to the femoral region with the aid of a horizontal skin incision extended proximally. Then main femoral artery, superficial femoral artery, and deep femoral artery, superficial and femoral veins were encircled from their proximal parts and clamped. Afterward, free ends of the lacerated vascular structures were found and clamped. After hemostatic control, defective vascular segments were found between proximal and distal ends of femoral veins and superficial femoral artery, and primary end-to-end repair was not considered to be applicable. To repair femoral veins and superficial femoral artery of the same extremity using autogenous vascular graft, vena saphena magna was explored through a cutaneous incision over the vein course, then prepared for anastomosis with sharp dissection and excision. In consideration of the corrosive effect of the mine explosion, 1-1.5 cm sections were excised from distal and proximal ends of the superficial femoral artery and superficial femoral vein together with the distal end of the deep femoral vein. Then proximal and distal ends of vascular segments were trimmed and prepared for anastomosis. Before the anastomosis, an arterial embolectomy was performed using a 5F Fogarty catheter inserted into the distal end of the superficial femoral artery. Any thrombus material was not found after embolectomy and improved retrograde flow was achieves. The saphenous vein harvested from the right leg was reversed, the saphenous vein was end-to-end anastomosed firstly between distal and proximal ends of the femoral artery, and then between distal and proximal ends of the superficial femoral vein in the

direction of normal blood flow (Figure 1). Finally, a deep femoral vein was explored. The distal end of the deep femoral vein was appropriate for vascular repair, whereas the proximal end was damaged due to corrosive effect and any intact segment couldn't be explored through the bundle of deep muscles. Thus, it was decided to drain the deep femoral vein into saphenofemoral junction. The saphenofemoral junction was explored. Saphenous vein was prepared from its insertion point into the femoral junction (2 cm below saphenofemoral junction) preserving proximal venous valve. First, end-to-end distal anastomosis of deep the femoral vein was completed using a saphenous vein graft. Then, this saphenous vein graft was brought to the saphenofemoral junction through the tunnel created between groups of extensor muscles of the lower extremity. The Saphenous vein graft was end-to-end anastomosed 2 cm below the saphenofemoral junction and the procedure was completed (Figure 2). Since the distal segment of the deep femoral artery was destructed, it was ligated by 2/0 silk suture from its proximal segment. Peripheral vascular pulses were evaluated following the completion of all vascular repairs. All distal pulses were palpable. The patient had no symptoms of venous congestion. Following hemostatic control, two hemovac drains were placed. Regional tissue defect was closed primarily by liberating cutaneous and subcutaneous tissues and the operation was completed by the Department of Plastic and Reconstructive Surgery.

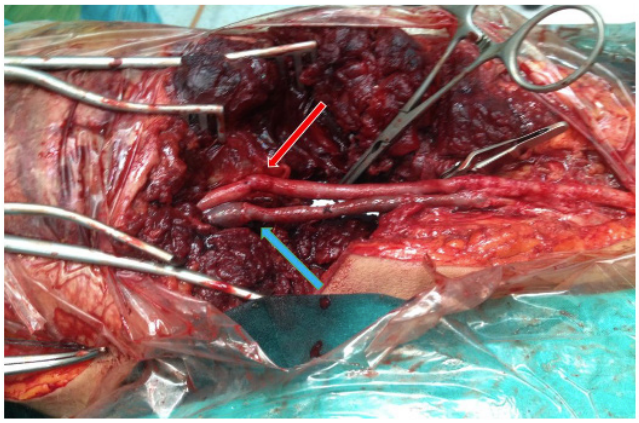

Figure 1. Femoral artery (red arrow) and femoral vein (blue arrow) following repair by saphenous vein graft

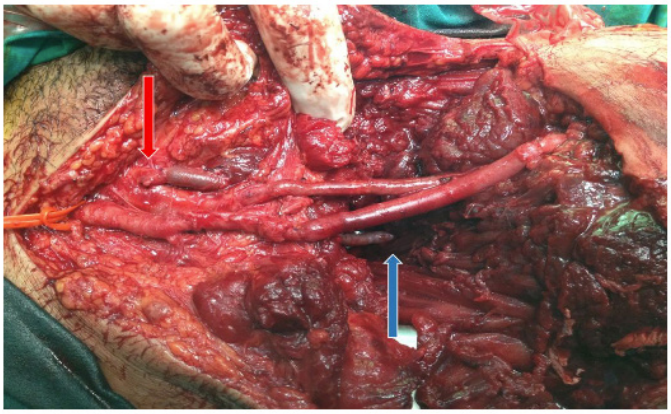

Figure 2. Drainage of the distal end of the deep femoral vein (blue arrow) through saphenous vein graft into saphenofemoral junction (red arrow)

The patient was administered to the postoperative intensive care unit and triple antibiotics, low weight heparin, acetylsalicylic acid, clopidogrel, and N-acetylcysteine. The patient was followed up for two days in the intensive care unit and seven days in the ward. There was no lower extremity edema or compartment syndrome during follow-up. The patient was discharged after anticoagulant, antiplatelet and venoactive drugs were administered. The 4th degree (strong) peripheral pulse was palpated in the postoperative 2nd month. Open superficial and deep femoral vessels were seen in control venography (Figure 3). During the lower extremity vascular examination in the follow-up of the patient, no sign of arterial or venous insufficiency was detected.

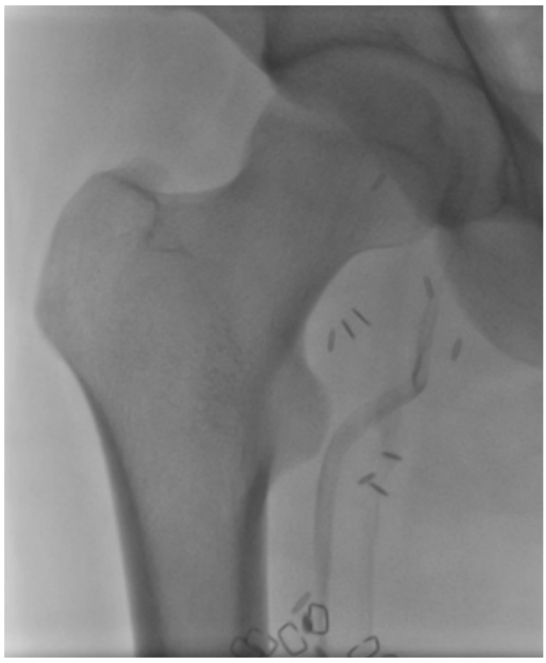

Figure 3. Control venogram obtained at the postoperative 2nd month

## DISCUSSION

The most frequent cause of peripheral vascular injuries is penetrating traumas. Blunt traumas and iatrogenic causes are more common in the developed North European countries with a lower incidence of violence. In the USA, firearm injuries are more frequently seen, whereas penetrating stab injuries take the first place in our country [1]. Penetrating peripheral vascular injuries are more commonly found in the younger age group and male individuals [2]. Early diagnosis and surgical intervention shorten ischemia times and reduce mortality and morbidity rates in cases with penetrating peripheral vascular injuries. Physical examination is generally sufficient for diagnosis [3]. However, advanced diagnostic methods such as Doppler ultrasonography and angiography are required in suspect cases. In major arterial injuries, patients apply to the emergency services with externally compressed wounds and in hypovolemic shock [4]. In arterial injuries, the first prerequisite for successful reconstruction is performing a revascularization procedure within the first 8 hours after the traumatic incident [5]. Vascular defects due to smooth-edged partial and total cuts not exceeding 2 cm can be primarily repaired, whereas large defective segments may be found in the vascular structures because of the corrosive effects of firearm injuries. To ensure a successful arterial and venous revascularization in such traumatic wounds not amenable to primary repair, autogenous vein grafts are generally preferred for the high patency rates they provide. However, synthetic grafts are also used for revascularization if suitable autogenous vein segments are not available [6-7].

A considerable part of the peripheral arterial injuries is accompanied by venous injuries. It has been stated that repair of venous injuries accompanying arterial injuries has a favorable impact on the prognosis [8]. In venous injuries, ligation can be performed in cases of larger defects, prolonged shock picture, and multiple organ injury. In their study involving 322 patients with venous injury, Timberlake et al. performed ligations in 170 of 239 patients with arteriovenous and 54 of 83 patients with isolated venous injuries. During an average follow-up period of 32 months, any permanent edema was not found in the group with isolated venous injuries, whereas they detected the development of permanent edema in 4 patients of the group with arteriovenous injuries [9]. Skin, soft tissue (muscle/tendon), and/or nerve damage, and bone fractures are often complex limb injuries and carry a high risk of amputation. In such cases, the primary goal is to prevent functional loss by correcting vascular events that disrupt hemodynamics [10].

In the study examining the effect of repair time on the results in arterial injuries, if the patient is operated on and revascularized within one hour in extremity injuries, this time is defined as the optimal time to save the limb. The mortality and morbidity rates become lower when the limb perfusion is performed more planned and faster in arterial injuries [11]. Peripheral vascular injuries are usually emergency cases. Often life-threatening and urgent repair (revascularization) is required. These injuries are often life-threatening and require immediate repair(revascularization). The approach to cases with vascular injury should be multidisciplinary. Rapid interdisciplinary communication and coordination decrease the mortality and morbidity rates as well as the serious loss of function and amputation rates [12].

In our case, large defective segments were found in all arterial and venous vascular structures, because of the development of a corrosive effect developed due to firearm injury in the right lower extremity. First, the superficial femoral artery and the following superficial femoral vein were repaired using a saphenous vein graft to prevent extremity loss. In the patient; Symptoms of venous congestion persisted following vascular anastomoses performed in the lower extremity. Deep femoral vein repair was planned. The destructed proximal segment and the intact segment of the deep femoral vein couldn't be explored through the group of deep extensor muscles. Consequently, the conversion of the drainage of the deep femoral vein into the saphenofemoral region was decided. The completion of revascularization by only end-to-end anastomosis to the deep femoral vein preserving the proximal segment of the saphenous vein could be appropriate. Since in our case proximal segment of the saphenous vein was defective due to corrosive effect, end-to-end anastomosis to either a saphenofemoral junction

or deep femoral vein had to be performed for the repair of the deep femoral vein. As we discerned intraoperatively in our case, signs of congestion in the leg disappeared after repair of the deep femoral vein rather than the superficial femoral vein. Therefore, we think that in combined injuries of superficial and deep femoral veins, primary repair of the especially deep femoral vein or if not possible, drainage of this vein through saphenous vein graft has critical importance in preventing the development of postoperative permanent edema or compartment syndrome.

## CONCLUSION

In peripheral vascular injuries, early vascularization, as well as rapid transport and fluid replacement is very critical with respect to the prevention of mortality and morbidity. We conclude that in cases where primary repair is not feasible, ensuring drainage of the deep venous system via superficial venous system would be a good alternative approach

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REVIEW

DERLEME

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# A point to evaluate in the COVID-19 pandemic process: Frailty

## COVID-19 Pandemi Sürecinde Değerlendirilmesi Gereken Bir Nokta: Kırılganlık

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**ABSTRACT**

Globally, as of September 16, 2020, COVID-19 had infected approximately 29 million people and caused the death of 930 thousand people around the world. While the whole world is at risk of COVID-19 infection, the elderly in particular represent the highest risk group. Those who are very old and frail and have cognitive impairment and multi-comorbidity, are the most vulnerable to the severe consequences of this infection. Frailty is a less well-known and often overlooked issue compared to other factors. In the world, the prevalence of frailty is seen to rise with the increase of the aging population, and the prevalence of frailty among the elderly people living in society is thought to be 4 to 59%. The COVID-19 pandemic, which has affected the entire world, leads to the necessity of taking care of elderly individuals in nursing homes and care centers. The fact that most persons in such facilities are advanced in age, frail and have multi-comorbidity, makes the management of the care even more difficult. It is thought that there may be a potential relationship between frailty and the course of COVID-19 and deaths resulting from the infection. The need for a holistic assessment of not only the age of the patient but also the state of frailty, is emphasized to ensure the triage of elderly persons and resource allocation during COVID-19 pandemic process. This review was performed to raise awareness of the healthcare professionals on the importance of frailty among the elderly during the COVID-19 pandemic process and to provide general information about the instruments that can be used to measure the frailty status of the elderly, when managing the COVID-19 pandemic process.

Keywords: Coronavirus disease (COVID-19), frailty, old age

**ÖZ**

Küresel olarak, 16 Eylül 2020 tarihi itibariyle COVID-19 dünyada yaklaşık olarak 29 milyon insanı enfekte etmiş ve 930 bin insanın ise ölümüne neden olmuştur. Tüm dünya COVID-19 enfeksiyonu riski altındayken, özellikle yaşlılar en yüksek risk altında olan gruptur. Özellikle çok yaşlı, kırılgan, bilişsel bozukluk ve multikomorbiditeye sahip olanlar bu enfeksiyonun ciddi sonuçlarına karşı en savunmasız olanlardır. Diğer faktörlere kıyasla kırılganlık daha az bilinen ve göz ardı edilen bir konudur. Dünyada, yaşlanan nüfusun artmasıyla birlikte kırılganlık prevelansının da arttığı görülmekte ve toplumda yaşayan yaşlı bireylerdeki kırılganlık prevelansının %4-59 olduğu düşünülmektedir. Tüm dünyayı etkisi altına alan COVID-19 pandemisi yaşlı bireyleri huzurevlerinde ve bakım merkezlerinde bakılmaya zorlamaktadır. Bu tür tesislerde çoğu kişinin ileri yaşta olması, genellikle kırılgan olması ve sıklıkla multikomorbiditeye sahip olması bakımın yönetimini daha da zorlaştırmaktadır. Kırılganlık ile COVID-19 'un seyri ve COVID-19 nedeniyle ölüm arasında potansiyel bir ilişki olabileceği düşünülmektedir. COVID-19 pandemi sürecinde yaşlı bireylerin triyajını ve kaynak tahsisini sağlama da sadece yaşın değil kırılganlık durumunun bütüncül bir şekilde değerlendirilmesi gerekliliğinin önemi vurgulanmaktadır. Bu derleme sağlık profesyonellerine COVID-19 pandemi sürecinde yaşlılarda kırılganlığın önemi konusunda farkındalık oluşturmak, COVID-19 pandemi sürecini yönetirken yaşlı bireylerin kırılganlık durumlarının ölçülmesi için yararlanılabilecek araçlar hakkında genel bilgi vermek amacıyla oluşturulmuştur.

Anahtar kelimeler: Koronovirüs hastalığı (COVID-19), kırılganlık, yaşlılık

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## INTRODUCTION

The COVID-19 (coronavirus disease) cases caused by the newly-identified SARS-CoV-2 (Severe Acute Respiratory Syndrome causing Coronavirus 2) first appeared in Wuhan, China in December 2019 [1]. This rapidly spreading disease was recognized as a pandemic, in other words, a global epidemic, by the World Health Organization (WHO) in March 2020 [2]. As of September 16, 2020, COVID-19 has infected approximately 29 million people and caused the death of 930 thousand persons around the world [3]. It appears that the majority of COVID-19 cases are individuals aged 65 and over, and that the mortality rate due to COVID-19 increases exponentially with age (3.6% between 60-69 years of age, 8.0% between 70-79 years of age, and 14.8% between 80 years and over) [4]. In addition, it has been determined that the mortality rate increases even more among elderly individuals with chronic diseases, such as cardiovascular disease, diabetes, respiratory system diseases, hypertension, etc. [5,6]. Frailty is one of the conditions that increases mortality and causes serious clinical symptoms among elderly individuals with COVID-19 [7,8].

## FRAILTY

Frailty is a concept that has physical, cognitive and emotional dimensions and is difficult to define [9,10]. It is one of the most common geriatric syndromes, is defined as [11,12] "a clinical condition characterized by weakness, vulnerability, functional regression, increase of dependency and disability in daily life activities, and decreased resistance to stressors, which develops due to the decrease in physiological reserves with aging" [13,14]. Although frailty, which is a complex condition, increases with age, it may also be seen among young adult individuals [13,15].

The number of studies conducted to determine the prevalence of frailty has increased with the rise in numbers of the aging population [16]. A systematic review performed to determine the prevalence of frailty among elderly persons living in society found that 10.7% of these are frail, and the prevalence of frailty ranges from 4% to 59% [17]. In a study conducted with 906 elderly persons living in Kayseri, the prevalence of frailty was determined to be 27.8%, according to the Fried Frailty Index and 10% according to the FRAIL scale [18]. In the study by Özdemir et al. (2017), the prevalence of frailty among geriatric and hospitalized patients aged 65 years and over is 63.1% to 91.2% [19]. Although the incidence of frailty increases with age, frailty is more common among women, individuals with low socioeconomic levels and inactive persons [11,20]. Frailty, which increases mortality and morbidity rates [7,21–23], causes falls [24], delirium [25], disability [26] and, long-term hospitalizations [22] among elderly individuals. In addition, it adversely affects the quality of life of elderly persons and increases the burden and cost of care [27,28]. Genetics/epigenetics, acute-chronic diseases, chronic inflammation and environmental factors, such as stress, malnutrition, etc., play an important role in the etiology of frailty, which is a dynamic condition [7,12,28].

### Stages of Frailty

Frailty is generally classified into three stages: pre-frail period, frail period and severe frail period. The symptoms seen in each period differ from another. Despite the decrease in physiological reserves during the pre-frail period, which is a clinically quiet period, it is sufficient to cope with stressors and there is a potential for a full recovery. Individuals during this period have two or fewer risk factors. In the frail period, functional capacity is progressively decreasing. Due to the decrease in physiological reserves, the healing process takes a long time and full recovery is not achieved. Coping with stress is insufficient in the frail period when individuals have three or more risk factors. The severe frail period is the stage in which frailty complications increase, the capacity to withstand progressive stress decreases, and disability and death are seen [20,29].

## FRAILTY MEASURING INSTRUMENTS

It is emphasized that it is very important to evaluate the frailty of individuals in the management of COVID-19 cases [21,30]. Various instruments have been developed to determine frailty [10]. The most commonly used instruments include the Clinical Frailty Scale (CFS) [31], the Fried Frailty Scale (FFI) [32], the FRAIL scale [33], the Study of Osteoporotic Fractures (SOF) [34] and the

**Table 1. Most Frequently used Frailty Measuring Instruments**

| INSTRUMENTS | ITEMS | CLASSIFICATION | POPULATION | EXPLANATION |
|---|---|---|---|---|
| Clinical Frailty Scale-CFS | 70 items | 7 categories<br>*Very healthy<br>*Healthy<br>* Healthy with comorbid diseases treated<br>* Invulnerable view<br>* Slightly fragile<br>* Moderately fragile<br>* Severe / severely fragile | The elderly | Due to the high number of items, it is not practical to apply in the clinical setting and a comprehensive geriatric evaluation is required when using the scale. |
| Fried Frailty Scale -FFI | 5 criteria | 3 categories<br>*Normal<br>*Pre-frail<br>*Frail | The elderly | The scale is applied face to face and requires some physical examination techniques. |
| FRAIL scale | 5 criteria | 3 categories<br>*Healthy<br>*Pre-frail<br>*Frail | Individuals between the ages of 49-65 /<br>The Elderly | This scale can be applied by phone or by itself without face-to-face examination. |
| Study of Osteoporotic Fractures -SOF | 3 criteria | 3 categories<br>*Normal<br>*Pre-frail<br>*Frail | Elderly people living in the community | The scale is applied face to face and requires some physical examination techniques. |
| Edmonton Frailty Scale-EFS | 10 domain | 5 categories<br>* Not fragile<br>* Sensitive<br>* Slightly fragile<br>* Moderately fragile<br>* Extremely fragile | The elderly | The scale was developed for use by healthcare professionals who are not experts in geriatrics and gerontology in hospitalized elderly patients.<br>The scale is applied face to face and requires some physical examination techniques. |

Edmonton Frailty Scale (EFS) [35] (Table 1).

CFS developed by Rockwood et al. (2005) consists of 70 items. The frailty status of individuals is interpreted in seven categories [31]. It has been confirmed that CFS is an instrument that can provide very useful information in the clinical use for prioritization, such as triage in emergency services [36]. In the COVID-19 Guide presented by the National Institute for Health and Care Excellence (NICE), the use of CFS is recommended in the assessment of frailty during intensive care management [37]. However, although its application in the clinical setting is not practical [34], a comprehensive geriatric evaluation is required when using the scale [35].

Weight loss, exhaustion, physical activity, walking speed and handgrip strength are evaluated with the FFI developed by Fried et al. (2001). The individuals who meet three or more of these five criteria are considered to be frail, those who meet one or two criteria as pre-frail, and the individuals who do not meet any criteria are considered normal [32].

10 domains (cognitive status, general health status, functional independence, social support, drug use, nutrition, mood, continence and functional performance) are evaluated with the EFS which was developed by Rolfson et al. (2006) to determine the frailty of the elderly, for use by the healthcare professionals who are not experts in geriatrics and gerontology. Frailty levels are grouped into 5 categories with the scale, used to evaluate the frailty of the elderly hospitalized patients [35].

Morley et al. (2012) developed the FRAIL scale to assess the frailty states of individuals aged 49-65. With the scale, it is aimed to determine the frailty at an early stage and thus slow down the development rate of frailty. Each letter in the word FRAIL represents a frailty criterion (F: Fatigue, R: Resistance, A: Ambulation, I: Illness, L: Loss of weight) [33]. Although the scale was first created to determine the frailty of middle-aged individuals, subsequent studies showed that the FRAIL scale

was a reliable scale for determining the frailty of elderly individuals [38–40]. This scale can be applied by phone or by the individual, without face-to-face examination [33].

The SOF developed by Ensrud et al. (2008) is a scale that can be easily applied to elderly persons living in society. With this scale, unintentional weight loss, decrease in energy level and low mobility (using the chair test) are evaluated. However, since it is not appropriate to apply the chair test to hospitalized individuals, this scale is not recommended to be applied to the hospitalized patients. On the scale, 0 point are evaluated as normal, 1-2 points as pre-frail, and 3 or more points as frail [34].

In addition, it is recommended that the measuring instruments related to frailty should not be used solely when providing health care services to elderly individuals. Within the scope of a patient-centered approach, frailty- measuring instruments are recommended to be used together with factors such as the severity of the disease, the probability that the intervention will be successful and the state of frailty [41].

## COVID-19 AND FRAILTY

In reviewing the literature, it is thought that there may be a potential relationship between frailty and the course of COVID-19 and deaths resulting from the pandemic. A study conducted by Baker et al. (2020) found that the patients who died due to COVID-19 had a high level of frailty [42]. In a cohort study by Brill et al. (2020), it was determined that most of the patients who died as a result of COVID-19 were elderly individuals and their frailty levels were higher than the surviving patients [43]. Similarly, another cohort study found that the frailty scores of organ transplant recipients who died due to COVID-19 were higher than those of the surviving patients, and that frailty was associated with death from COVID-19 [44]. It is supported by the studies that patients who die after contracting COVID-19 are frail individuals, although the number of these studies at present time is low. In a study conducted by Bellelli et al. (2020) to determine the role of frailty among the COVID-19 patients, it was discovered that frailty was associated with hospital mortality and intensive care admission [45]. In a retrospective observational study conducted by Turner et al. (2020) with palliative care patients, it was revealed that the level of frailty was associated with the course of COVID-19 disease and the duration of death [46]. In a retrospective observational study conducted to determine the relationship between the frailty and mortality rate of the elderly people hospitalized for COVID-19, age and frailty were found to have a weak positive relationship with mortality [47]. For this reason, during the COVID-19 pandemic, it is one of the recommended practices to evaluate the frailty of the elderly in particular.

Early life, middle life and late-life predictors and risk factors (e.g. genetic, epigenetic, psychological, socio-economic, lifestyle characteristics, frailty, nutritional status, multiple morbidity and medication) should be taken into account to more accurately explain the diversity in the aging of the population [9]. With the evaluation of frailty, which is found among these, the functional status of the patients can be closely monitored and elderly individuals who require special interventions can be identified, in order to reduce the risk of negative consequences [8].

Moreover, in the event of a pandemic such as COVID-19, screening of frailty and monitoring of changes in the health and frailty status of the elderly can be very useful in assessing the disease severity and the likelihood of successful intervention and directing patients to appropriate points, as well as preventing the resulting hospital occupancy [8].

Determining the factors related to frailty, preventing frailty and developing the appropriate protocols for a treatment plan, are also very important for the economic situation of countries [16]. For ensuring proper triage and resource allocation of elderly individuals during the COVID-19 pandemic, the importance of a holistic evaluation of not only the age of the patient, but also the frailty situation and the balance of benefit-harm by taking into account the patient's comorbidities, are also emphasized [48].

Prevention of frailty results in the aging of individuals in a healthy and desirable way [49]. For this reason, especially during the epidemic progression in the world, pre-frail individuals should be identified at an early stage and

necessary measures should be taken to reduce or prevent the progress towards frailty [50]. Frailty screening should be performed as early as possible to evaluate the frailty of the elderly persons in the community by health care professionals who take part in the care of the frail elderly individuals [24].

Finally, as suggested in the conveyed studies, the frailty situation can be reversed by exercise (strength, cardiovascular exercise), adequate protein intake, vitamin D supplements, nutritional supplements, health education, counseling, special comprehensive geriatric assessment, home visits, treatment of anemia, depression and immunization [7,16,28].

## CONCLUSION AND RECOMMENDATION

It is known that COVID-19 infections, which negatively affect human health all over the world, pose a greater threat to the elderly. Frailty, which is one of the criteria used to evaluate the healthy aging status of elderly persons, is also seen as one of the important evaluation parameters in the COVID-19 pandemic process. Screening, early detection, stage determination, careful monitoring and prevention of frailty are very important in health management and referral of elderly persons who have experienced a COVID-19 infection to the necessary medical institutions. Further studies are needed to assess the impact of frailty on elderly patients with COVID-19, to provide evidence-level recommendations in the effective execution of the process.

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