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# Muğla Sıtkı Koçman Üniversitesi Tıp Dergisi

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Muğla Sıtkı Koçman Üniversitesi Tıp Dergisi

Medical Journal of Mugla Sitki Kocman University

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## AMAÇ VE KAPSAM

Muğla Sıtkı Koçman Üniversitesi Tıp Dergisi, Muğla Sıtkı Koçman Üniversitesi Tıp Fakültesinin süreli yayın organıdır ve Nisan, Ağustos ve Aralık aylarında olmak üzere yılda 3 sayı yayınlanmaktadır. Derginin yayın dili Türkçe ve İngilizcedir.

Dergide, tıbbın her dalı ile ilgili prospektif, retrospektif ve deneysel araştırmalar, olgu sunumu ve derlemeler yayınlanır. Yayınlanan makalelerde konu ile ilgili en yüksek etik ve bilimsel standartlarda olması ve ticari kaygılarda olmaması şartı gözetilir. Yayın için gönderilen çalışmalar; orijinal, başka bir dergide değerlendirme sürecinde olmayan ve daha önce basılmamış olması koşullarıyla kabul edilir.

Muğla Sıtkı Koçman Üniversitesi Tıp Dergisi; TR Dizin, Google Akademik, Crossref, Türkiye Atıf Dizini, Türk Medline, Academic Keys ve Research Bible Index tarafından indekslenmektedir.

Dergide yayınlanacak olan yazılar http://dergipark.gov.tr/muskutd web sayfası üzerinden gönderilmelidir. Yazım kuralları, teknik bilgiler ve diğer gerekli formlara bu sayfadan erişilebilir. Gönderilen yazılardaki tüm içerikler yazarların sorumluluğundadır.

Bu dergide yer alan yazı, makale, fotoğraf ve illüstrasyonların elektronik ortamlarda dahil olmak üzere kullanma ve çoğaltma hakları Muğla Sıtkı Koçman Üniversitesi Tıp Dergisi'ne aittir. Yazılı ön izin olmaksızın materyallerin tamamının ya da bir bölümünün çoğaltılması yasaktır. Her hakkı saklıdır. Muğla Sıtkı Koçman Üniversitesi Tıp Dergisi'nde yayınlanan içerikler yazar(lar)a aittir. Muğla Sıtkı Koçman Üniversitesi Tıp Fakültesi, dergi editörleri, çalışanları ve yayıncı bu yazılar için mali, hukuki ve diğer yönlerden sorumluluk kabul etmemektedir.

## AIMS AND SCOPE

Medical Journal of Mugla Sitki Kocman University is a periodical of Medical School of Mugla Sitki Kocman University and is published three times per year; in April, August, and December. The Medical Journal of Mugla Sitki Kocman University's publication languages are both Turkish and English.

The articles which could be prospective or retrospective on investigational studies, case reports and reviews of every aspect of medicine are published. The studies should have paramount ethical and scientific standards as well as no commercial concerns. Articles are accepted for publication on the condition that they are original, are not under consideration by another journal, or have not been previously published.

The Medical Journal of Mugla Sitki Kocman University is indexed by TR Dizin, Google Scholar, Crossref, Türkiye Atıf Dizini, Türk Medline, Academic Keys and Research Bible.

All manuscripts must be submitted electronically on the http://dergipark.gov.tr/muskutd. Instructions for authors, technical information, and other necessary forms can be accessed over this web page. Authors are responsible for all content of the manuscripts.

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## YAZARLARA BİLGİ

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**Genel Bilgiler**

Muğla Sıtkı Koçman Üniversitesi Tıp Dergisi Muğla Sıtkı Koçman Üniversitesi Tıp Fakültesinin süreli yayın organı olup özel sayılar hariç düzenli olarak yılda üç sayı olarak yayımlanır. Tıbbın her dalı ile ilgili prospektif, retrospektif ve deneysel araştırmalar, olgu sunumu ve derlemeler yayınlanmak için değerlendirilmektedir. Konu ile ilgili etik ve bilimsel standartlar olması ve ticari kaygıların olmaması şartı gözetilir. Yazarlar tarafından, başka bir dergide değerlendirme sürecinde olmayan ve daha önce basılmadığı beyan edilen yazılar incelemeye alınır. Editör onayı sonrasında en az yurt içi-yurt dışı iki hakem incelemesinden geçip gerek görüldüğü takdirde istenen değişiklikler yazarlar tarafından yapılıp hakemlerce kabul edildikten sonra yayımlanır.

**Bilimsel Sorumluluk**

Yazar olarak belirtilmiş tüm kişiler çalışmayı planlamalı veya gerçekleştirmeli, çalışmanın yazılmasında, gözden geçirilmesinde ve son halinin onaylanmasında rol almış olmalıdır. Kriterleri karşılayan bir metnin ortaya çıkması tüm yazarların sorumluluğudur.

**Etik Sorumluluk**

Muğla Sıtkı Koçman Üniversitesi Tıp Dergisi, etik ve bilimsel standartlara uygun yazıları yayımlayarak bilimin doğru bir şekilde ilerlemesine katkı sağlamayı hedeflemektedir. Bu kapsamda, çalışmaların bilimsel etiğe uygunluğu önemlidir. Etik ilkeler COPE (Committee on Publication Ethics) tarafından hazırlanan yönerge (https://publicationethics.org/resources/resources-and-further-reading/international-standards-editors-and-authors) temel alınarak Muğla Sıtkı Koçman Üniversitesi Tıp Dergisi tarafından benimsenmiş ve yazarlar, hakemler ve editörler tarafından da benimsenmesi önerilmektedir. Bu önerilerin bir kısmı aşağıda verilmiştir.

**Yazarların Etik Sorumlulukları:**

- Çalışmayla ilişkili veri kayıtlarını düzenli tutmak ve olası bir talep üzerine bu verilere erişim verebilmelidir.
- Gönderdiği makalenin başka bir yerde yayınlanmadığından veya kabul edilmediğinden emin olmalıdır.
- İnsan veya hayvan denek içeren tüm çalışmalar için ulusal ve uluslararası yasalara ve yönergelere uygun olmasını sağlamak, (örneğin, WMA Helsinki Bildirgesi, NIH Laboratuar Hayvanlarının Kullanımına İlişkin Politika, Hayvanların Kullanımına İlişkin AB Direktifi) gerekli onayların alındığını onaylamak, denek mahremiyete saygı göstermek. Çalışmasına dair ilgili etik kurul onaylarını ve araştırma detaylarını çalışmanın "Gereç ve Yöntem" kısmında belirtmek.
- Herhangi bir çıkar çatışması durumunda, makalesiyle ilgili etik bir ihlal tespit ettiğinde bunu editör ve yayıncı ile paylaşmak, hata beyanı, zeyilname, tazminat bildirimi yayınlamak veya gerekli görüldüğü durumlarda çalışmayı geri çekmelidir.

**Hakemlerin Etik Sorumlulukları:**

- Editörün karar verme sürecine katkıda bulunmak için makaleyi objektif olarak zamanında incelmeli ve sadece uzmanlık alanı ile ilgili çalışma değerlendirmeyi kabul etmelidir.
- Değerlendirmeyi nesnel bir şekilde sadece çalışmanın içeriği ile ilgili olarak yapmalıdır.
- Dini, siyasi ve ekonomik çıkarlar gözetmeden çalışmayı değerlendirmelidir.
- Yayınlanacak makalenin kalitesini yükseltmeye yardımcı olacak yönlendirmelerde bulunmalı ve çalışmayı titizlikle incelemelidir. Yorumlarını yapıcı ve nazik bir dille yazara iletmelidir.
- Editör ve yazar tarafından sağlanan bilgilerin gizliliğini korumalı, kör hakemliğe aykırı bir durum varsa editöre bildirmeli ve çalışmayı değerlendirmemelidir.
- Potansiyel çıkar çatışmalarının (mali, kurumsal, işbirlikçi ya da yazar ve yazar arasındaki diğer ilişkiler) farkında olmalı ve gerekirse bu yazı için yardımlarını geri çekmek konusunda editörü uyarmalıdır.

**Editörlerin Sorumlulukları:**

- Cinsiyet, dini veya politik inançlar, yazarların etnik veya coğrafi kökenleri üzerine ayrım yapılmaksızın görevlerini yerine getirirken dengeli, objektif ve adil bir şekilde hareket etmelidir.
- Dergiye gönderilen çalışmaları içeriğine göre değerlendirmeli, hiçbir yazara ayrıcalık göstermemelidir.
- Olası çıkar çatışmalarını önlemek adına gerekli önlemleri almalı ve varsa mevcut beyanları değerlendirmelidir.
- Etik ihlali niteliğinde bir şikayet olması durumunda, derginin politika ve prosedürlerine bağlı kalarak gerekli prosedürleri uygulamalıdır. Yazarlara, gelen şikâyete cevap vermek için bir fırsat vermeli, çalışma kime ait olursa olsun gerekli yaptırımları uygulamaktan kaçmamalıdır.
- Derginin amaç ve kapsamına uygun olmaması durumunda gelen çalışmayı reddetmelidir.

Tüm araştırma makalelerinde (retrospektif çalışmalarda dahil olmak üzere), çalışma için Etik Kurul Onayı alınmalı ve Etik Kurul Onayının alındığı yer, tarih (gün, ay ve yıl olarak) ve onay numarası Gereç ve Yöntem bölümünde belirtilmelidir. İnsan ile ilgili tüm çalışmalarda Helsinki Deklarasyonu'na (World Medical Association Declaration of Helsinki http://www.wma.net/en/30/publications/10policies/b3/index.html) göre çalışmanın yapıldığı mutlaka belirtilmelidir. Olgu sunumlarında, hastadan (ya da yasal vasisinden) tıbbi verilerinin yayınlanabileceğine ilişkin yazılı hasta onam belgesi alındı cümlesinin hasta onam tarihi ile birlikte belirtilmesi gereklidir. Hayvan deneyleri için laboratuvar hayvanlarının bakım ve kullanımı konusunda kurumsal veya ulusal yönergelerin takip edilmeli ve bildirmelidirler. Yazarların çalışmalarında kullandıkları cümlelerinden editör ve yayın kurulu sorumlu değildir. Bilimsel, hukuki ve etik sorumluluğu yazarlara aittir.

Sorumlu yazar, gönderilen çalışmanın başka bir yerde yayımlanmadığını ve aynı anda bir diğer dergide değerlendirilme sürecinde olmadığını belirtmelidirler. Çalışmanın bir kısmı kongrede sözlü veya poster bildiri olarak sunuldu ise başlık sayfasında kongre adı, yer ve tarih verilerek belirtilmesi gereklidir.

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Kabul edilen yazının tüm kullanım ve yayın hakkı derginin olur ve izinsiz olarak başka bir yerde yayınlanamaz.

**Değerlendirme:** Tüm makaleler çift-kör değerlendirme yöntemi kullanılarak en az iki yerli veya yabancı hakem tarafından değerlendirilir. Makalelerin değerlendirilmesi, bilimsel önemi, orijinalliği göz önüne alınarak yapılır. Yayına kabul edilen yazılar editörler kurulu tarafından içerik değiştirilmeden yazarlara haber verilerek yeniden düzenlenebilir.

**İntihal taraması:** Dergiye gönderilen makaleler format ve intihal açısından kontrol edilir. Formata uygun olmayan veya intihal benzerlik oranı yüksek (%20'den az olmalıdır) makaleler değerlendirilmeden sorumlu yazara geri gönderilir.

**Çıkar çatışması:** Çalışmaları ile ilgili taraf olabilecek tüm kişisel, ticari bağlantı veya çalışma için doğrudan veya dolaylı olarak maddi destek veren kurum var ise yazarlar; kullanılan ticari ürün, ilaç, firma ile ticari hiçbir ilişkisinin olmadığını veya varsa nasıl bir ilişkisinin olduğunu (konsültan, diğer anlaşmalar vs.), editöre sunum sayfasında bildirmek zorundadır. Herhangi bir çıkar çatışmasının olmadığı durumda metin içerisinde 'Yazarlar çıkar ilişkisi olmadığını beyan eder' şeklinde ifade edilmelidir.

**Lisan**

Derginin yayın dilleri Türkçe ve İngilizcedir. Türkçe metinlerde Türk Dil Kurumu'nca (www.tdk.gov.tr) yayınlanan Türkçe sözlük temel alınmalıdır. Gönderilmiş makalelerdeki tüm yazım ve imla hataları, anlam ve verileri değiştirmeksizin editör tarafından düzeltilebilir. Metnin kurallara uygun olarak düzenlenmesi yazarların sorumluluğundadır.

**Telif Hakkı Bildirimi**

Telif hakkı devrini bildirmek için kapak mektubunda 'Bu makalenin telif hakkı; çalışma, basım için kabul edilmesi koşuluyla Muğla Sıtkı Koçman Üniversitesi Tıp Dergisi'ne devredilir' şeklinde belirtilmelidir. Yazarlara ücret ödenmez.

**Yazı Tipleri**

***Derleme:*** Derlemeler yeni veya tartışmalı alanlara ışık tutmalıdır. Türkçe ve İngilizce başlık ve tek paragraflık özetler ve anahtar kelimeler içermelidir. Dergi editörü derleme yazımı için davette bulunur.

***Orijinal makaleler:*** Orijinal makaleler temel veya klinik çalışmalar veya klinik denemelerin sonuçlarını bildirir. Makale dili Türkçe veya İngilizce fark etmeksizin Türkçe özet, İngilizce özet, giriş, gereç ve yöntemler, bulgular/sonuçlar, tartışma, teşekkür (gerekliyse), kaynaklar ve şekiller ve tablolardan oluşmalıdır.

***Olgu Sunumu:*** Tıbbın her alanındaki önemi olan olgu sunumlarını yayınlanır. Türkçe özet, İngilizce özet, giriş, olgu, tartışma, kaynaklardan oluşmalıdır.

**Yazı Gönderimi**

Tüm yazılar elektronik ortamda http://dergipark.gov.tr/muskutd adresi üzerinden gönderilmelidir.

**Yazının Hazırlanması**

Yazı hazırlığı iki satır aralıklı, satır numaraları verilmiş ve Times New Roman 12 punto karakter büyüklüğünde yapılmalıdır. Sayfalar başlık sayfasından başlamak üzere, sağ alt köşesinden numaralandırılmalıdır. Makale sistemine yüklenen word (*.doc, *.docx) dosyasının başlık sayfasında yazarlara ait isim ve kurum bilgileri yer almamalıdır.

***Kapak Mektubu:*** Kapak mektubu gönderilen makalenin kategorisini, daha önce başka bir dergiye gönderilmemiş olduğunu, çıkar ilişkisi bildirimini, yayın hakkı devri bildirimini ve varsa çalışmayı maddi olarak destekleyen kişi ve kurumların adlarını mutlaka içermelidir.

***Başlık sayfası:*** Bu sayfada çalışmanın tam Türkçe ve İngilizce ismi ve kısa başlığı olmalıdır. Katkıda bulunanların tüm yazarların isimleri, çalıştıkları kurumları ve ORCID numaraları listelenmelidir. Ücretsiz olarak bireysel ORCID numaraları http://orcid.org adresinden alınabilmektedir. Basım sürecinde dergi editörü ile iletişimde bulunacak olan yazışma yazarı belirtilmelidir. Çalışmanın bir kısmı kongrede sözlü veya poster bildiri olarak sunuldu ise başlık sayfasında kongre adı, yer ve tarih verilerek belirtilmesi gereklidir.

***Özet ve Anahtar Kelimeler:*** Özet 250 kelimeyi geçmemelidir. Çalışmanın amacını, yöntemi, bulgu ve sonuçları özetlemelidir. En fazla 5 anahtar kelime verilmelidir. Kelimeler birbirlerinden virgül (,) ile ayrılmalıdır. İngilizce kelimeler Index Medicus'taki Medical Subjects Headings listesine uygun olmalıdır www.nlm.nih.gov/mesh/MBrowser.html. Türkçe anahtar kelimeler Türkiye Bilim Terimleri (TBT)'ne uygun olarak verilmelidir www.bilimterimleri.com

***Giriş:*** Kısa ve açık olarak çalışmanın amaçlarını tartışmalı, çalışmanın neden yapıldığına dair temel bilgileri içermeli ve hangi hipotezlerin sınandığını bildirmelidir.

***Gereç ve Yöntemler:*** Açık ve net olarak yöntem ve gereçleri açıklanmalıdır. İlk vurgulamada kullanılan araç ve cihazların model numaraları, firma ismi ve adresi (şehir, ülke) mutlaka belirtilmelidir. Tüm ölçümler metrik birim olarak verilmeli ve ilaçların jenerik adları kullanılmalıdır.

***İstatistiksel Değerlendirme:*** Tüm çalışma makaleleri istatistiksel olarak değerlendirilmeli ve uygun plan, analiz ve bildirimde bulunmalıdır. p değeri yazı içinde belirtilmelidir. Kullanılan istatistik yöntem açıkça belirtilmelidir.

***Sonuçlar:*** Sonuçlar metin, tablo ve şekiller kullanılarak sunulmalıdır. Tablo ve metinler tekrarlanmamalıdır. p değeri yazı içinde belirtilmelidir (p=0.014 gibi).

***Tartışma:*** Çalışmanın farklılıklarına ve sonuçlarına vurgu yapılmalıdır. En önemli bulgu kısa ve net bir şekilde belirtilmeli, gözlemlerin geçerliliği tartışılmalı, aynı veya benzer konulardaki yayınların ışığında bulgular yorumlanmalı ve yapılan çalışmanın olası önemi belirtilmelidir. Çalışmanın esas bulgularının kısa ve özlü bir paragrafla vurgulanması önerilir.

***Teşekkür:*** Yazarlar araştırmaya katkıda bulunan ancak yazar olarak yer almayan kişilere teşekkür etmelidir.

***Tablo, Resim, Şekil ve Grafikler:*** Tüm tablo, resim, şekil, grafik ve diğer görseller ana metnin içinde geçiş sıralarına uygun şekilde, ardışık olarak numaralandırılmalıdır. Kullanılan görsellerde hasta ve doktor kimlikleri içeren bilgiler ve kurum adları görülmeyecek şekilde hazırlanmalıdır. Tablolar ana metin içinde kaynak listesinin sonrasında sunulmalıdır. Tablolar JPEG, TIFF veya diğer görsel formatlarda gönderilmemelidir. Mikroskopik şekillerde açıklayıcı

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bilgilere ek olarak, büyütme oranı ve kullanılan boyama tekniği de belirtilmelidir. Görseller sisteme minimum 300 DPI çözünürlükte yüklenmelidir. Şekil, resim, grafik ve fotoğrafların her biri ayrı *.jpg* veya *.gif* dosyası olarak sisteme eklenmelidir. Şekiller metin içinde kullanım sıralarına göre Arabik (1, 2, 3, v.b.) rakamla numaralandırılmalı ve metinde parantez içinde gösterilmelidir. Grafiklerde kullanılan çizgiler yayın hazırlığı aşamasında yeniden boyutlandırma sırasında meydana gelecek bozulmaları engellemek amacıyla yeterli kalınlıkta olmalıdır. Tablolarda kullanılan kısaltmalar tablo altlarında tanımlanmalıdır. Tablo ve şekil başlıklarında ve tablonun yazı içinde anılmasında Roma (I, II, II, v.b.) rakamları kullanılmamalıdır.

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The Standard Task Force, American Society of Colon and Rectal Surgeons: Practice parameters for the treatment of haemorrhoids. Dis Colon Rectum 1993;36:1118-20.

***Kitaptan konu için örnek***

Milson JW. Haemorrhoidal disease. In: Beck DE, Wexner S, eds. Fundamentals of Anorectal Surgery. 1 1992; 192-214. 1a ed. New York: McGraw-Hill

***Kitap için örnek***

Bateson M, Bouchier I. Clinical Investigation and Function, 2nd edn. Oxford: Blackwell Scientific Publications Ltd, 1981.

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***ORİJİNAL MAKALE / ORIGINAL ARTICLE***



***OLGU SUNUMU / CASE REPORT***

*Doi:10.47572/muskutd.1292120*

# A Retrospective Analysis of Cases Evaluated by Endoscopy in a Newly-Established Endoscopy Unit at A Second-Level Hospital

# 2. Basamak Bir Hastanede Yeni Kurulan Endoskopi Ünitesinde Endoskopik Değerlendirme Yapılan Olguların Retrospektif Analizi

**Cenk YAZKAN, Okay NAZLI**

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**Öz**

Bu çalışmanın amacı 2. basamak bir hastanede ilk kez kurulmuş olan endoskopi ünitesinde 2021-2022 yılları arasında üst ve alt gastrointestinal sistem endoskopik değerlendirme yapılan olguların retrospektif olarak analizi ve merkez hastanelerden uzakta bulunan ilçe hastanelerinde de endoskopi ünitelerinin önemini vurgulamaktır. Çalışma ilk kez endoskopi ünitesi kurulmuş bir ilçe hastanesinin verilerini ele alması açısından ön plana çıkmaktadır. Endoskopik değerlendirme yapılan toplam 440 hastanın verileri analiz edildi. Üst gastrointestinal sistem endoskopik değerlendirme yapılan toplam 320 hasta ve alt gastrointestinal sistem endoskopik değerlendirme yapılan 120 hasta çalışmaya dahil edildi. Elde edilen veriler üzerinden endoskopik ve patolojik olarak hastalara konulan tanılar değerlendirilerek tanımlayıcı istatistik çalışmaları yapıldı. Üst gastrointestinal sistem endoskopik değerlendirme yapılan olguların yaşları ortalama 53.93±16.14 yıl, alt gastrointestinal sistem endoskopik değerlendirme yapılan olguların ise yaşları ortalama 53.71±15.86 yıl olarak bulundu. Üst gastrointestinal sistem endoskopik değerlendirme yapılan olguların %70.3'ünde antral gastrit (n=225), %34.8'inde ülser (n=111), %10.3'ünde hiatus hernisi (n=28), %9.1'inde özofajit (n=29), %9.1'inde duodenit (n=29) saptandı. Endoskopik olarak kanser tanısı alan olguların %0.9'u distal özofagusta (n=3), %0.6 antrumda (n=3) lokalizeydi. Alt gastrointestinal sistem endoskopik değerlendirme yapılan olguların %20'sinde polip (n=24), %10'unda divertikül (n=12), %3.3 internal hemoroidal hastalık (n=4), %5'inde kanser (n=6) tanısı endoskopik olarak konuldu. Sonuç olarak çalışmadan elde edilen veriler merkez hastanelerden uzak ilçe hastanelerinde de endoskopi ünitelerinin önemini vurgulamaktadır.

**Anahtar Kelimeler:** Endoskopi, Helicobakter Pylori, Malignite, Polip

**Abstract**

The aim of this study is to retrospectively analyze the cases who underwent upper and lower gastrointestinal system endoscopic evaluation between 2021-2022 in the endoscopy unit, which was established for the first time in a second level hospital, and to emphasize the importance of endoscopy units in district hospitals located far from central hospitals. The study comes to the fore in terms of dealing with the data of a district hospital where an endoscopy unit was established for the first time. The data of 440 patients who underwent endoscopic evaluation were analyzed. The study included 320 patients who underwent upper gastrointestinal tract and 120 who underwent lower gastrointestinal tract endoscopic examination. The endoscopic and pathological diagnoses given to patients were examined based on the data obtained, and descriptive statistics were performed. The cases undergoing upper gastrointestinal endoscopic examination had a mean age of 53.93±16.14 years, and those undergoing lower gastrointestinal tract endoscopic examination had a mean age of 53.71±15.86. Of cases undergoing upper gastrointestinal tract endoscopic examination, 70.3% had antral gastritis (n=225), 34.8% had ulcers (n=111), 10.3% had hiatus hernia (n=28), 9.1% had esophagitis (n=29), and 9.1% had duodenitis (n=29). Among the cases endoscopically diagnosed with cancer, 0.9% were localized in the distal esophagus (n=3) and 0.6% in the antrum (n=3). Of cases undergoing lower gastrointestinal tract endoscopic examination, 20% were diagnosed with polyp (n=24), 10% with diverticulum (n=12), 3.3% with hemorrhoidal disease (n=4), and 5% with cancer (n=6). As a result, the data obtained from the study emphasize the importance of endoscopy units in district hospitals located far from central hospitals.

**Keywords:** Endoscopy, Helicobacter Pylori, Malignancy, Polyp

## Introduction

The advent of fiber optic technology and the transmission of cold light from an outside source in the mid-20th century enabled the development of flexible endoscopes. Fiber optic endoscopy was first used in 1957 by Basil Hirschowitz (1). In time, modern endoscopes have been developed. Using these endoscopes to examine mucosal and luminal pathologies of the upper and lower gastrointestinal system (GIS) has become the golden standard (2).

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Endoscopy stands out as it offers the possibility to make an objective diagnosis as well as the opportunity to perform a biopsy and endoscopic treatment. It can be used for treatment in numerous procedures, such as controlling gastrointestinal system bleeding, removing foreign bodies, placing percutaneous gastrostomy tubes, polypectomy, and endoscopic mucosal resection (3-5).

The aim of the study is to analyze the endoscopic evaluation results of a district hospital where an endoscopy unit was established for the first time, and to emphasize the importance of endoscopic evaluation and endoscopy units in secondary level district hospitals, which are far from central hospitals and where it is very difficult for patients to reach central hospitals. This study differs from similar studies in that it presents the data of the endoscopy unit, which was established for the first time in a region where access to the central hospital is difficult.

## Material and Method

The research is a descriptive, correlational and retrospective study. The study population consisted of all patients who underwent GIS endoscopy between the years 2021-2022. In the study, the data of 440 patients who underwent upper and lower GIS endoscopic examinations between 2021 and 2022 in the newly-established endoscopy unit of a second-level hospital were retrospectively analyzed.

The study involved 320 patients who presented to the general surgery polyclinic with dyspeptic problems and underwent upper GIS examination and 120 patients who presented with various symptoms such as rectal bleeding, constipation, anemia, anal fissure, and tenesmus and underwent lower GIS examination.

The file data of 500 patients who underwent endoscopic evaluation were analyzed. In accordance with the inclusion and exclusion criteria, 440 patients were included in the study. The cases who underwent endoscopic evaluation of the upper and lower GIS were evaluated within themselves as two separate groups. The flow chart of the study is given in Figure 1.

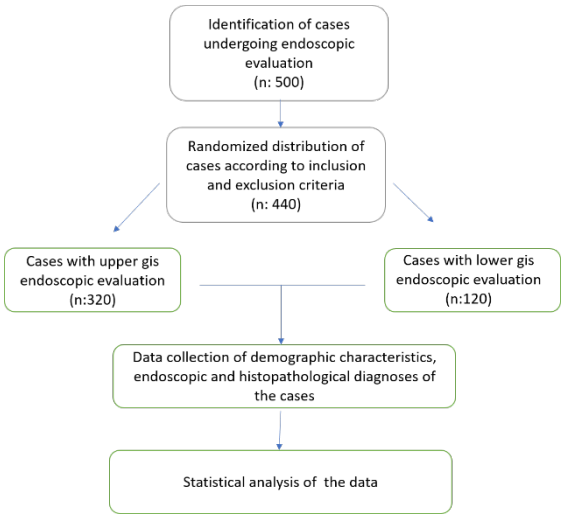


**Figure 1.** The chart showing the association between growing age and the percentage of hiatus hernia diagnosis.

In the study, patients with inadequate lower gastrointestinal system endoscopic evaluation due to suboptimal bowel cleansing, and upper gastrointestinal system patients who could not undergo the procedure under anesthesia because it was not suitable for anesthesia and who could not be optimally evaluated because they could not tolerate the procedure were not included in the study. All endoscopy patients with optimal evaluation and complete file information were included in the study.

Oral food intake was stopped 8h before the procedure. Xylocaine 10% spray was used locally for oropharyngeal anesthesia. Before the procedure, iv 2-milligram midazolam + iv 1-microgram/kilogram fentanyl + iv 1-milligram/kilogram propofol was given to the patients for sedation. Procedures were implemented after adequate sedation was achieved.

The examination was performed with a Fujinon EG-600 WR gastroscope. All the procedures were performed by the same surgeon.

The staging was performed in cases diagnosed with esophagitis based on the Los Angeles classification (**Grade A:** one or several erosions limited to the mucosal fold(s) and no larger than 5 mm in extent. **Grade B:** one or several erosions limited to the mucosal fold(s) and larger than 5 mm in extent. **Grade C:** erosion(s) extending over mucosal folds, but over less than three-quarters of the circumference. **Grade D:** confluent erosions extending over more than three-quarters of the circumference.)

In addition to suspicious lesions in cases undergoing upper GIS endoscopic examination, biopsies were performed from prepyloric antrum and corpus in all patients.

The R software version 2.15.3 (R Core Team, 2013) was used for statistical analyses. Minimum, maximum, mean, standard deviation, median, frequency, and percentage were used to report the study data. The Pearson chi-square, Fisher's Exact, and Fisher-Freeman-Halton Exact tests were used to compare the qualitative data. The level of significance was accepted as $p<0.05$.

Prior to the study, an approval was obtained from the Ordu University Clinical Research Ethics Committee (approval no: 2022/59). Patient information was kept confidential during the study, paying attention to patient privacy.

## Results

The ages of cases who underwent upper GIS endoscopic examination varied between 18–90, with a mean of 53.93±16.14 years. Among the cases, 43.1% were males (n=138), while 56.9% were females (n=182). On the other hand, the ages of cases who underwent lower GIS endoscopic examination varied between 19–88, with a mean of 53.71±15.86 years. Of these cases, 58.3% were males (n=70), and 41.7% were females (n=50). Cases within the age range of 52–67 years had examinations for both upper GIS (35%) and lower GIS (38.3%) the most frequently. Age distribution and gender characteristics are summarized in Table 1.

**Table 1.** Information regarding age and sex in upper and lower GIS endoscopic examinations

| | Upper GIS | | Lower GIS | |
|---|---|---|---|---|
| | **Min – Max (Median)** | **Mean ± sd** | **Min – Max (Median)** | **Mean ± sd** |
| **Age** | 18–90 (54) | 53.93 ± 16.14 | 19–88 (55) | 53.71 ± 15.86 |
| | **n** | **%** | **n** | **%** |
| **Sex** | | | | |
| Male | 138 | 43.1 | 70 | 58.3 |
| Female | 182 | 56.9 | 50 | 41.7 |
| **Age** | | | | |
| 20–35 | 44 | 13.8 | 13 | 10.8 |
| 36–51 | 95 | 29.7 | 40 | 33.3 |
| 52–67 | 112 | 35.0 | 46 | 38.3 |
| 68–83 | 55 | 17.2 | 17 | 14.2 |
| 84–99 | 14 | 4.4 | 4 | 3.3 |

The clinical diagnoses of cases who underwent upper GIS endoscopic examination involve the following: antral gastritis (70.3%, n=225), pangastritis (22.2%, n=71), ulcer (34.8%, n=111), hiatus hernia (10.3%, n=28), polyp (5.3%, n=17), esophagitis (9.1%, n=29), and duodenitis (9.1%, n=29). The ulcer was not detected in 65.3% of the cases. According to the investigation of ulcer cases, 19.4% were localized in the antrum (n=62), 7.5% in the pylorus (n=24), 2.5% in the duodenitis (n=8), 2.2% in the corpus (n=7), 1.6% in the incisura angularis (n=5), and 1.6% in both the antrum and the duodenitis (n=5). The investigation of the cases with polyps showed that 2.2% of the polyp cases were localized in the fundus (n=7, in the form of numerous gland polyps), 1.3% in the antrum (n=4), 0.9% in the corpus (n=3), 0.3% in the fundus (n=1, single polyp), and 0.3% in the pylorus (n=1). The cases in which esophagitis was detected, on the other hand, were grade A in 6.6% of the cases (n=21), grade B in 1.3% of the cases (n=4), grade C in 0.3% of the cases (n=1), and candida esophagitis in 0.9% of the cases (n=3). Among the cases endoscopically diagnosed with cancer, the cancer was localized in the distal esophagus in 0.9% of the cases (n=3) and the antrum in 0.6% of the cases (n=3) (Figure 2). Intramucosal adenocarcinoma was diagnosed in one patient who had a biopsy for ulcer base in the antrum.

Data on the age variable and the incidence of hiatus hernia are given (Figure 3).

The histopathological diagnoses of cases undergoing upper GIS endoscopic examination include the following conditions: chronic gastritis (98.8%, n=316), *H. Pylori* (72.8%, n=233), intestinal metaplasia (15%, n=48), atrophy (2.2%, n=7), malignancy (1.9%, n=6), focal low-grade gastric epithelial dysplasia (0.9%, n=3), hyperplastic polyp (1.6%, n=5), candida esophagitis (0.6%, n=2), atypical reactive changes (3.8%, n=12), and inlet patch in upper esophagitis (0.3%, n=1). Endoscopic and histopathological diagnoses are summarized in Table 2.

Of lower GIS endoscopic examinations, 84.2% were total colonoscopy (n=101), 10% were rectosigmoidoscopy (n=12), and 5.8% were rectoscopy (n=7). The endoscopic diagnoses included polyps in 20% of the cases (n=24), diverticulum in 10% (n=12), internal hemorrhoidal disease in 3.3% (n=4), and cancer in 5% (n=6). Among cases diagnosed with polyps, 7.5% had polyps localized in the sigmoid colon (n=9), 5.8% in the rectum (n=7), 1.7% in the ascending colon (n=2), 1.7% in the sigmoid colon + descending colon (n=2), 1.7% in the rectum + sigmoid colon (n=2), 0.8% in the splenic flexure, and 0.8% in the descending colon. Diminutive polyps were the most frequently observed type in terms of polyp size and single polyps in terms of the number of polyps. Patients diagnosed with cancer had a diagnosis of rectum cancer (3.3%, n=4) and sigmoid colon cancer (1.7%, n=2) (Figure 4). Table 3 summarizes the histopathological and endoscopic diagnoses of cases undergoing lower GIS endoscopic examination.

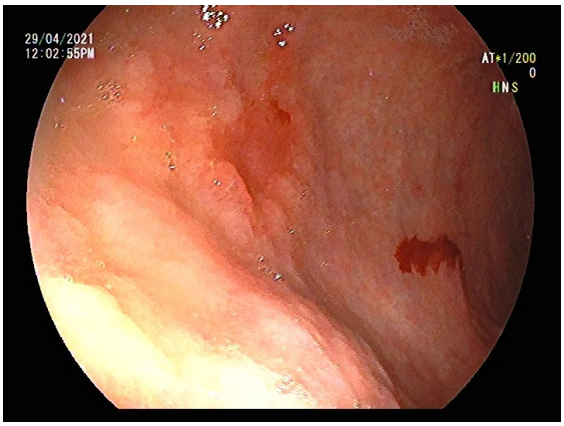


**Figure 2.** Endoscopic image of the case who had a biopsy of ulcer base in the antrum and was diagnosed with intramucosal adenocarcinoma.

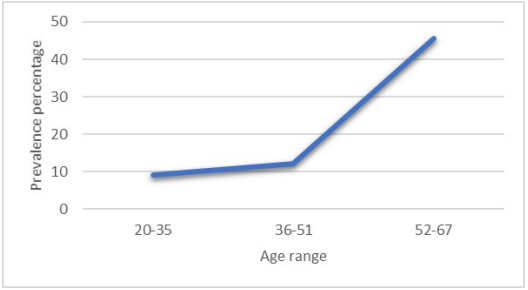


**Figure 3.** The chart showing the association between growing age and the percentage of hiatus hernia diagnosis

**Table 2.** Table showing the clinical and histopathological diagnoses of cases undergoing upper GIS endoscopic examination.

| | **Min – Max (Median)** | **Mean ± sd** |
|---|---|---|
| **Age** | 18–90 (54) | 53.93 ± 16.14 |
| | **n** | **%** |
| **Sex** | | |
| Male | 138 | 43.1 |
| Female | 182 | 56.9 |
| **Age** | | |
| 20–35 | 44 | 13.8 |
| 36–51 | 95 | 29.7 |
| 52–67 | 112 | 35.0 |
| 68–83 | 55 | 17.2 |
| 84–99 | 14 | 4.4 |
| **Endoscopic Diagnosis** | | |
| **Antral gastritis** | 225 | 70.3 |
| **Pangastritis** | 71 | 22.2 |
| **Erosion** | 66 | 20.6 |
| **Ulcer** | | |
| Ulcer in pylorus | 24 | 7.5 |
| Ulcer in antrum | 62 | 19.4 |
| Ulcer in corpus | 7 | 2.2 |
| Ulcer in duodenum | 8 | 2.5 |
| Ulcer incisura angularis | 5 | 1.6 |
| Ulcer in antrum + duodenum | 5 | 1.6 |
| **Hiatus hernia** | 33 | 10.3 |
| **Gastric polyp** | | |
| Polyp in antrum | 4 | 1.3 |
| Polyp in corpus | 3 | 0.9 |
| Polyp in fundus | 1 | 0.3 |
| Polyp in pylorus | 1 | 0.3 |
| Gland polyp in fundus | 7 | 2.2 |
| Polyp in duodenum | 1 | 0.3 |
| **Esophagitis** | | |
| Grade A | 21 | 6.6 |
| Grade B | 4 | 1.3 |
| Grade C | 1 | 0.3 |
| Candida | 3 | 0.9 |
| **Duodenitis** | 29 | 9.1 |
| **Histopathological Diagnosis** | | |
| *H. Pylori* | 233 | 72.8 |
| Intestinal metaplasia | 48 | 15.0 |
| Atrophy | 7 | 2.2 |
| Malignity | 6 | 1.9 |
| Focal low-grade gastric epithelial dysplasia | 3 | 0.9 |
| Hyperplastic polyp | 5 | 1.6 |
| Candida esophagitis | 2 | 0.6 |
| Antrum intramucosal adenocarcinoma | 1 | 0.3 |
| Reactive atypical changes | 12 | 3.8 |
| Antrum adenocarcinoma | 2 | 0.6 |
| Fundic gland polyp | 5 | 1.6 |
| Distal esophagus adenocarcinoma | 3 | 0.9 |
| Inlet patch in the upper esophagus | 1 | 0.3 |
| Autoimmune metaplastic atrophic gastritis | 1 | 0.3 |
| Hyperplastic polyp + autoimmune metaplastic atrophic gastritis | 1 | 0.3 |

It was observed that the number of endoscopically detected polyps decreased starting from the rectum and sigmoid colon to the cecum (Figure 5).

Among cases undergoing upper GIS endoscopic examination, no statistically significant differences were detected between sexes regarding endoscopic diagnoses ($p>0.05$). However, significant differences were found when histopathological diagnoses were considered ($p=0.008$, Fisher-Freeman-Halton exact test, test value: 18,678). It was determined that there was a statistically

significant difference in the percentage of metaplasia in terms of sex (p=0.003, Pearson ki-square test, test value: 8,643). The percentage of incidence was higher among males than females. Similarly, there was a statistically significant difference in the percentage of atrophy regarding sex (p=0.045, Fisher's exact test, test value: 5,292). The percentage of incidence was higher among males than females. It was found that males had higher incidences of focal low-grade gastric epithelial dysplasia and distal esophagus adenocarcinoma but lower incidences of fundic gland polyp than females (p=0.045, p=0.045, p=0.049, respectively).

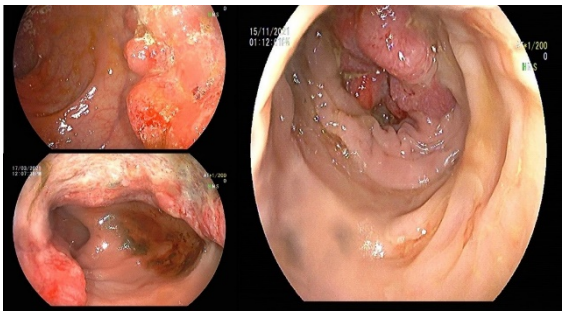

**Figure 4.** Endoscopic images of cases in whom malignancies were detected in lower GIS endoscopic examinations.

**Table 3.** Table showing the clinical and histopathological diagnoses of cases undergoing lower GIS endoscopic examination.

| | **Min – Max (Median)** | **Mean ± sd** |
|---|---|---|
| **Age** | 19–88 (55) | 53.71 ± 15.86 |
| | **n** | **%** |
| **Sex** | | |
| Male | 70 | 58.3 |
| Female | 50 | 41.7 |
| **Age** | | |
| 20–35 | 13 | 10.8 |
| 36–51 | 40 | 33.3 |
| 52–67 | 46 | 38.3 |
| 68–83 | 17 | 14.2 |
| 84–99 | 4 | 3.3 |
| **Procedure** | | |
| Total colonoscopy | 101 | 84.2 |
| Rectosigmoidoscopy | 12 | 10.0 |
| Rectoscopy | 7 | 5.8 |
| **Clinical Diagnosis** | | |
| **Polyp** | | |
| Polyp in the rectum | 7 | 5.8 |
| Polyp in the sigmoid colon | 9 | 7.5 |
| Polyp in the descending colon | 1 | 0.8 |
| Polyp in the sigmoid colon + descending colon | 2 | 1.7 |
| Polyp in the ascending colon | 2 | 1.7 |
| Polyp in the rectum + sigmoid colon | 2 | 1.7 |
| Polyp in the splenic flexure | 1 | 0.8 |
| **Diameter of polyps** | | |
| <1cm | 19 | 15.8 |
| >1cm | 2 | 1.7 |
| <1cm or >1cm | 3 | 2.5 |
| **Number of polyps** | | |
| 1 | 16 | 13.3 |
| 2 | 5 | 4.2 |
| 3 | 2 | 1.7 |
| ≥4 | 1 | 0.8 |
| **Diverticulum** | 12 | 10.0 |
| **Malignancy** | 6 | 5.0 |
| Malignancy in rectum | 4 | 3.3 |
| Malignancy in the sigmoid colon | 2 | 1.7 |
| **Hemorrhoidal disease** | 4 | 3.3 |
| **Histopathological Diagnosis** | | |
| Adenocarcinoma in rectum | 4 | 3.3 |
| Sigmoid colon adenocarcinoma | 2 | 1.7 |
| Tubular adenoma | 13 | 10.8 |
| Tubulovillous adenoma | 4 | 3.4 |
| Tubular adenoma + tubulovillous adenoma | 2 | 1.7 |
| Serrated adenoma | 1 | 0.8 |
| Hyperplastic polyp | 4 | 3.4 |
| Rectitis | 2 | 1.7 |

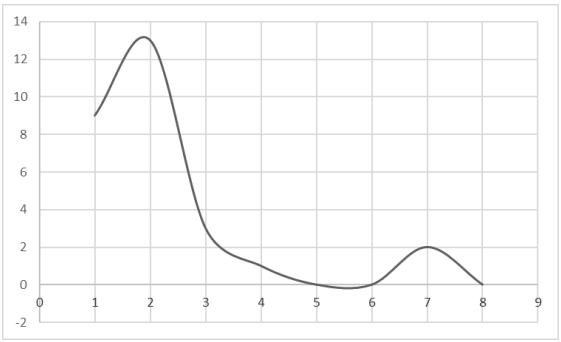


**Figure 5.** The chart showing the decrease in the number of detected polyps starting from the rectum and sigmoid colon to the cecum (1: Rectum, 2: Sigmoid colon, 3. Descending colon, 4. Splenic flexure, 5. Transverse colon, 6. Hepatic flexure, 7. Ascending colon, 8. Cecum).

A statistically significant difference was detected in terms of the percentage of pangastritis among cases with ulcers (p=0.037, Pearson ki-square test, test value: 4,342). The incidence rate of pangastritis was higher in cases with ulcers than those without ulcers. Likewise, it was determined that there was a statistically significant difference in the percentage of erosion (p=0.008, Pearson chi-square test, test value: 6.987). Its incidence rate was higher in cases with ulcers than those without ulcers.

A statistically significant difference was detected in duodenitis incidence in terms of the existence of ulcers (p=0.043, Pearson chi-square test, test value: 4.086). The incidence of duodenitis was higher in cases with ulcers. Similarly, histopathological examinations detected a significant difference in the percentage of *H. Pylori* in terms of ulcers (p=0.031, Pearson ki-square test, test value: 4.660). Cases with ulcers had higher incidence rates than those without ulcers. Similar results were obtained in terms of the existence of intestinal metaplasia (p=0.006, Pearson chi-square test, test value: 7,543).

No statistically significant differences were detected between age groups in terms of the incidence of hiatus hernia (p=0.047, Pearson chi-square test, test value: 9.060).

Statistically significant differences were detected between age groups regarding the incidence of malignancy (p=0.003, Fisher-Freeman-Halton exact test, test value: 12.309). It was observed that the incidence of hernia was higher among those aged between 84–99 (p<0.001).

Lower GIS endoscopic examinations determined a statistically significant difference in the percentage of diverticulum in terms of sex (p=0.002, Pearson chi-square test, test value: 9.524). The percentage of incidence was higher among males than females. A statistically significant difference was detected in histopathological diagnosis in terms of the existence of polyp (p<0.001). It was found that the incidence of tubular adenoma was higher among those with polyp (p=0.043).

In the post-hoc power analysis, the effect size for the gender difference was found to be 0.27, and the power of the test was calculated to be 0.98 over 320 patients.

The examination of other parameters did not reveal any statistically significant differences.

## Discussion

Dyspeptic complaints refer to symptoms such as pain in the epigastric region, retrosternal burning, swelling, gas, a sensation of early satiety, nausea, and vomiting (6). No underlying organic pathologies were observed among 75% of patients presenting with dyspeptic complaints. However, symptoms might indicate a severe problem in 25% of the cases (7). Malignancies are the most important of such problems. Upper GIS endoscopic examination is essential for early diagnosis and treatment, particularly in at-risk patient groups and patients with alarm symptoms (8). When the cases in our study are considered, dyspepsia was the primary complaint of cases undergoing upper GIS endoscopic examination. Furthermore, patient groups at risk and patients with alarm symptoms underwent endoscopic examination for further evaluation.

Rectal bleeding, tenesmus, constipation, iron deficiency anemia, fecal occult blood positivity, and weight loss may be severe symptoms of a serious colorectal disease. It is vital to make a differential diagnosis of malignancies initially in these patient groups and patients presenting with dyspeptic complaints. Diagnostic lower GIS endoscopic examination is the most effective method to detect these pathologies (9).

Endoscopy is regarded as a reliable method with low rates of complications. The rate of procedure-related complications is 0.13% in upper GIS endoscopic examinations (10). This rate has been reported to be 0.4% in lower GIS endoscopic examinations (11).

The number of patients requiring endoscopic examination has gradually increased in recent years. The main reasons are rising outpatient admissions due to symptoms such as the dyspeptic complaints mentioned above, rectal bleeding, constipation, and anemia. Similar rises in GIS cancers are observed in our country and worldwide (12). Therefore, endoscopic examination can be used for diagnostic purposes in symptomatic patients, and it has become a part of cancer screening programs (13).

In a study that investigated cases undergoing upper GIS endoscopic examination in 2017, the data of 396 patients were retrospectively analyzed, and gastric cancer was detected in one case (0.02%) (14). In a study carried out on 5551 patients, the malignancy rate was detected as 2.3%. A similar

study that investigated cases who underwent an endoscopic examination at a second-level state hospital reported a rate of 1.8%. Also, another study reported a malignancy rate of 1.1% (15-17). On the other hand, our study found a rate similar to previous studies (1.9%).

The present study detected the rate of antral gastritis as 70.3%, and the rate of *H. Pylori* was 72.8%, according to endoscopic biopsy results. The literature data shows that this rate varies between 43.66% and 93.7% (18-21). Two contemporary studies reported this rate as 60% and 45%, respectively (22,23). Although the prevalence of *H. Pylori* varied between 76.8% and 85.9% in our country, in recent years, this rate has dropped to 23–65% (24-27). This change is associated with improvements in hygiene practices in our country, non-invasive diagnostic methods for *H. Pylori*, and empirical treatments that have become widespread. The literature data are similar to our findings.

The lifelong prevalence of peptic ulcer disease changes between 5% and 10% (28). The ulcer rate was found to be 34.7% in our study. The most frequent localization of ulcers was the antrum (19.4%). Also, the prevalence of duodenal ulcers was 2.5%. The prevalence of ulcers varies between 4.6% and 9.4% in previous studies (14-17). The ulcer rate was higher in our study when gastric and duodenal ulcers were considered together. However, the rate of duodenal ulcers was lower in our study than that in previous similar studies.

The rate of hiatus hernia was found to be 10.3% in our study. It was observed that the prevalence of hiatus hernia increased with growing age. The literature data also show that its prevalence rises with age in society, which is 10% among young adults, while it exists in 70% of those over 70 years (29,30). The results of a current study conducted in our country do not agree with the results of our study. The small number of cases in our study may explain this situation. We think that there is a need for comparison with the larger series.

Gastric polyps are abnormal tissue growths protruding from stomach mucosa into the lumen. Their prevalence varies between 2% and 6%, and they are often diagnosed incidentally (31). The rate of polyps was found to be 5.3% in our study, which was consistent with the literature. They were predominantly localized in the fundus in the form of multiple fundic gland polyps. The second most frequent incidence was in the antrum (1.3%) and the corpus (0.9%). At the end of the histopathological examination, the polyps were reported to be hyperplastic polyps and fundic gland polyps. The subtypes of gastric polyps were investigated in a large series of cases, and they were reported to be hyperplastic polyps (71.2%) and fundic gland polyps (16.3%) (32). In our study, long-term proton-pump inhibitor (PPI) use was held responsible for the high incidence rate of gland polyps. The rate of polyp development following long-term PPI use varies between 1% and 36% (33).

Various studies about the rates of colorectal cancer cases detected in lower GIS endoscopic examinations performed in our country have obtained different results. These rates range between 1% and 14% (34-37). In a large series in which 4001 patients were evaluated in 2022, this rate was presented as 2.92% (38). This rate was found to be 5% in our study. Out of six cases in whom malignancies were detected, cancer was observed in the rectum in four cases while in the sigmoid colon in two cases. All the cases were reported as adenocarcinoma at the end of the histopathological examination. The data we obtained as to the localization and type of cancer were in line with previous studies (35-38).

Colorectal polyps are precancerous lesions that are extremely important for colon cancer development. They are often detected in the colon and rectum. According to a recent study including 7503 patients conducted in Turkey, the most frequent localization of cases who underwent polypectomy was the rectum (29.5%) and sigmoid colon (25.3%) (39). Similar results were obtained in our study. Our results show that the most frequent localization was in the colon in cases with polyps (7.5%). According to the size and the number of polyps, diminutive polyps, and single polyps were the most frequently detected, respectively.

Endoscopy has been extensively used due to the rising number of patients requiring endoscopic examination, the possibility of early diagnosis for GIS cancers, endoscopic treatment of precancerous lesions, its therapeutic applications in conditions such as bleeding or stricture, the potential of mucosal resections with the introduction of advanced endoscopic methods in recent years, low rates of complications, and the opportunity of making objective diagnoses. In conclusion, the need for endoscopy units and endoscopists has risen. Therefore, this study mainly emphasized the importance of both endoscopy units and surgical endoscopy.

## Conclusion

Endoscopy has become the golden standard in the diagnosis of GIS cancers. It is crucial that patients can access endoscopy units easily and that their examinations be performed in a timely manner for early diagnosis and treatment of these cancers. The opportunity to carry out these procedures at second-level hospitals in the periphery may contribute to the early diagnosis and treatment of patients. This is especially important in regions where it is difficult for patients to reach central hospitals. Delays in endoscopic evaluations also lead to delays in diagnosis.

## Limitations of the Study

The major limitations of the study are its retrospective design and the limited number of cases due to the data being obtained within only one year

## Conflict of interest statement

In our study, there is no financial conflict of interest with any institution, organization, person and there is no conflict of interest between the authors.

**Ethics Committee Approval:** It was approved by Ordu University Clinical Research Ethics Committee on 11.03.2022 with protocol code 2022/59.

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# Mid-term Results of Double Chamber Right Ventricle in Association with Genetic Syndromes

# Genetik Sendromların Eşlik Ettiği Çift Odacıklı Sağ Ventrikülde Orta Dönem Sonuçlarımız

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**Öz**

Çift odacıklı sağ ventrikül (DCRV), hipertrofik kas bantlarının sağ ventrikülü ikiye böldüğü konjenital bir hastalıktır. Genetik sendromun da eşlik ettiği 6 hastamızın erken-orta dönem cerrahi sonuçlarını sunmaktayız. DCRV tanılı, genetik sendromun eşlik ettiği 6 hasta ile ilgili bulguları derlendi. Ortalama yaş 3.9±1.4 yıldı. Eşlik eden ek kalp anomalileri perimembranöz ventriküler septal defekt (n=3), atrial septal defekt (n=1), orta aort yetmezliği (n=1), diskret subaortik membran (n=1) idi. Eşlik eden genetik sendromlar Costello (n=1), Seckel (n=1), Down sendromuydu (n=4). Ortalama takip süresi 4.86±4.6 yıldı. Sağ ventriküldeki ortalama sistolik basınç gradienti 18.5 ± 11.5 mmHg idi. Takipte mortalite ya da tekrar operasyon gerekliliği olmadı. Çift odacıklı sağ ventrikül, Costello ve Seckel Sendrom birlikteliğinin literatürde ilk kez yayımlandığı düşüncesindeyiz.

**Anahtar Kelimeler:** Costello Sendromu, Double Chambered Right Ventricle, Down Sendromu, Seckel Sendromu

**Abstract**

Double chamber right ventricle (DCRV) is a congenital disease in which a hypertrophied muscle band divides the right ventricle chamber into two. The early-mid-term follow-up of 6 patients with DCRV and distant genetic syndromes is reported in this paper. A retrospective analysis was performed of 6 DRCV patients with a mean age of 3.9±1.4 years. Concomitant cardiac anomalies were perimembranous ventricular septal defect (n=3), atrial septal defect (n=1), mild aortic regurgitation (n=1), discrete subaortic membrane (n=1). Associated genetic syndromes were Costello (n=1), Seckel (n=1) and Down syndromes (n=4). The mean follow-up period was 4.86±4.6 years. Mean systolic pressure gradient in the right ventricle in the postoperative was 18.5±11.5 mmHg. No mortality occurred and there was no requirement for reintervention. To the best of our knowledge, this is the first report in literature of concomitant DCRV with Costello and Seckel syndromes.

**Keywords:** Costello Syndrome, Double Chambered Right Ventricle, Down Syndrome, Seckel Syndrome

## Introduction

Double chamber right ventricle (DCRV) is a congenital disease in which a hypertrophied muscle band divides the right ventricle chamber into two, and constitutes 0.5-2% of all congenital heart diseases (1). An abnormal hypertrophic muscle band divides the right ventricle (RV) into two chambers (1). The defect between two chambers has a pressure gradient relative to the diameter (2). Although DCRV is usually asymptomatic, a progressive course (chest pain, heart failure symptoms) may occur. Ventricular septal defect (VSD) is the most associated congenital heart defect at the rate of 80-90% (2). In the absence of a co-existing defect, surgery is not indicated unless the intracavitary systolic pressure gradient is higher than 40 mmHg or the obstruction is progressive to maintain normal RV function (1). The early and mid-term follow-up is here presented of 6 cases with DCRV associated with different genetic syndromes, which were successfully treated with surgical correction.

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## Material and Method

*Patient Selection*

A retrospective analysis was made of 6 patients diagnosed with DCRV who were operated on by the same surgeon in two separate centers, using data collected from the hospital database between 2016-2022. Demographic data of gender, age, weight, RV pressures, gradients measured with transthoracic echocardiography (Figure 1A), co-existing cardiac anomalies, and associated genetic syndromes were recorded. Transthoracic echocardiography was performed preoperatively, 10 days postoperatively, and during early and mid-term follow up. The diagnostic criteria for DCRV were: a. Echocardiographic or angiographic evidence of a mid-ventricular obstruction (a systolic pressure gradient between the RV proximal chamber (inflow) and distal chamber (outflow), and b. Absence of infundibular hypoplasia.

The indication for surgery was that the right ventricle intracavitary systolic pressure gradient did exceed 40 mmHg on the echocardiogram.

Approval for the study was obtained from the Institutional Ethics Review Board (16.03.2022 6/VII). All the study procedures were in compliance with the Helsinki Declaration. Informed consent was obtained from the parents or legal guardian of all the patients. All the patients had additional syndromes: Costello Syndrome (n=1) (Figure 1B), Seckel Syndrome (Figure 1C) (n=1), and Down Syndrome (n=4). The patients with Costello and Seckel

Syndromes did not have any other intracardiac defect.

*Operation Technique*

Corrective surgery was indicated according to the pressure values of the preoperative echocardiograms. All patients underwent cardiac surgery under CPB. Custodiol was used to establish diastolic cardiac arrest. Following right atriotomy, the membrane on the hypertrophic muscle bands dividing the RV, were resected (Figure 2A, 2B, 2C). Ventriculotomy of RV outflow was not needed for any further exploration or for resection of muscle bands. Three patients had perimembranous VSD, which was repaired via right atriotomy using a PTFE patch (Figure 2D). ASD repair was performed using an autologous pericardial patch in one patient. A subaortic ridge in one patient was considered to be non-significant, and did not require any ridge resection.

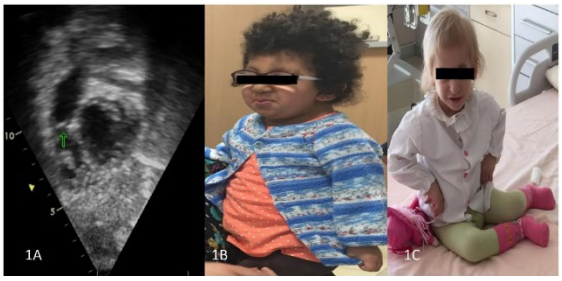


**Figure 1A.** Preoperative echocardiography of a patient with DCRV. B: Characteristic facial features of Costello Syndrome. C: Characteristics of 'bird head dwarfism' in Seckel Syndrome.

## Results

Postoperative evaluation was made of 6 patients diagnosed with DCRV. The mean age at the time of diagnosis was 3.9±1.4 years, and mean weight was 12.1±3.4 kg at the time of the operation.

The mean value of the pressure gradient between the proximal and distal chambers in RV was 72±25 mmHg on the preoperative echocardiograms.

The mean postoperative length of stay in the intensive care unit was 2.1±1.4 days, and the mean length of hospital stay was 6.5±3.2 days.

The patient diagnosed with Costello Syndrome, had atrial fibrillation on the 5th postoperative day and was treated with amiodarone. No patient required a temporary or permanent pacemaker.

Echocardiography in the postoperative early follow-up revealed that the residual mean systolic pressure gradient in the RV was 18.5±11.5 mmHg. No residual VSD was detected.

Echocardiography showed residual discrete aortic membrane in one patient with a 10-mmHg pressure gradient in the early follow-up period.

The mean follow-up period was 4.86±0.6 years. According to the clinical and laboratory findings of the patients during the follow-up period, echocardiography and electrocardiography were performed in the first, third, and sixth months following the operation, then once a year thereafter. The mean systolic pressure gradient in the RV (on echocardiography) at mid-term after surgical intervention was 10.4±6.9 mmHg.

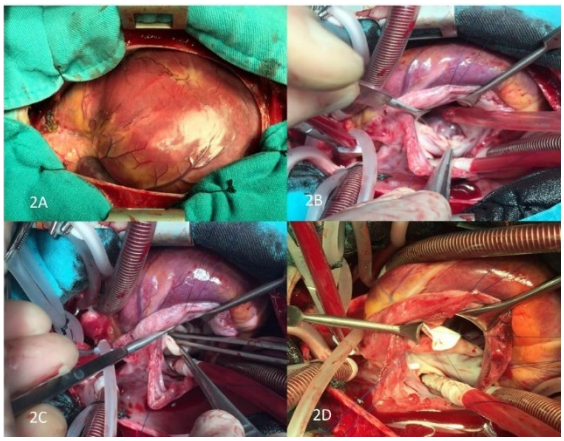


**Figure 2A.** Intraoperative view of RV in DCVR. B and C: Intraoperative view of membrane in the RV cavity. D: VSD closure through right atriotomy with PTFE patch.

The mid-term follow-up examinations showed that the patient with preoperative mild aortic regurgitation had the same degree of regurgitation, and 2 patients who underwent pulmonary valvulotomy also had the same degree of regurgitation. The patient with discrete aortic membrane had no increase of pressure gradient in the mid-term follow up, and no additional surgery was required.

No mortality occurred in any patient, and there was no requirement for surgical re-intervention during the mid-term follow-up period due to residual VSD.

## Discussion

Double chamber right ventricle is considered an acquired congenital heart defect caused by an abnormal membrane on the hypertrophic muscle band formation inside the RV (3). Various mechanisms of development of DCRV have been suggested. Superior displacement of the moderator band especially in association with a VSD and flow turbulence in the RVOT may lead to DCRV, or DCRV may occur due to anomalous muscular bands causing obstruction (3). The frequent associations are tetralogy of Fallot, perimembranous VSD or transposition of great arteries (3). Pulmonary valvular stenosis, ASD, aortic or tricuspid valve regurgitation, persistent left superior vena cava, ruptured sinus of valsalva aneurysm, and Ebstein anomaly are less common associations (4). Pulmonary valve agenesia, main pulmonary artery stenosis have also been reported (1,2).

In the absence of a co-existing defect, surgery is not indicated if the intracavitary systolic pressure gradient is not higher than 40 mmHg or the obstruction is not progressive to maintain normal RV function (1). Different surgical techniques have been described for removing the membrane on the hypertrophic muscle band in the RV, including transventricular, transatrial, and combined approaches (5). The transatrial approach is preferable to the transventricular approach depending on the RV dysfunction and risk of arrhythmia in long-term follow-up. However, in the presence of severe obstructing RV muscle bundles, right ventriculotomy permits adequate relief of the RV cavitary obstruction, allowing better exposure than the transatrial approach

The incidence of additional aortic valve insufficiency has been reported at the rate of 40% in adult DCRV patients and 5% to 20% in pediatric patients (6). In the current case series, aortic valve prolapse and/or mild aortic regurgitation were diagnosed in only 1 patient and no surgical intervention was required.

Williams Syndrome, VACTER-L Syndrome, and Noonan Syndrome may be seen in association with DCRV (7,8). Eltohami reported the high rate of 25% of associated Down's syndrome in a series of DCRV (9).

Costello Syndrome was first described in 1971, with findings of relative macrocephaly, curly-sparsely implanted hair, strabismus, downward slanted palpebral fissures, bulbous nose, large mouth, thick lips, low-set pinnae with large lobes with PS, VSD, ASD, bicuspid aortic valve, aortic stenosis, mitral stenosis, thickening of the intraventricular septum, and hypertrophic cardiomyopathy associations (10). Supraventricular tachycardia and atrial fibrillation have been reported. In the current series, DCRV was detected in routine echocardiographic examinations. Preoperative electrocardiogram showed sinus tachycardia, and the perioperative course was uneventful except for atrial fibrilation on the postoperative 5th day.

Seckel Syndrome was first determined in 1959 as a type of microcephalic primordial dwarfism-bird head dwarfism, neurodevelopmental defects and retinopathy (11). Concomitant congenital heart defects have been recorded as atrioventricular septal defect, ASD, VSD, PDA, tetralogy of Fallot, pulmonary atresia and overriding aorta, and tricuspid atresia (12-14).

In the current study, patient with Seckel Syndrome, the operation and postoperative follow up were uneventful.

Complete relief of the right ventricular obstruction demonstrated excellent functional and hemodynamic mid and long-term results. There was no death or surgical reintervention. In the current study, only 2 patients were followed up for 25 mmHg residual pressure gradient across the RV and for a discrete subaortic membrane with 10 mmHg pressure gradient postoperatively. No arrhythmia was detected.

This study had some limitations, primarily the retrospective design and that only 6 patients were evaluated. There is a need for further studies of larger populations to be able to obtain more information.

## Conclusion

The surgical outcomes in this series were excellent due to the right atriotomy approach. Right atriotomy provides adequate exposure and less risk of postoperative arrhythmia. To the best of our knowledge, this is the first report in literature of concomitant DCRV with Costello and Seckel syndromes.

**Ethics Committee Approval:** Ethical approval for this study was obtained from the Mugla University Institutional Review Board (16.03.2022 6/VII).

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# Hyperhomocysteinemia Transcriptionally Regulates Expression of a Set of Ion Channels in Brain and Heart Tissues in Mice

# Hiperhomosisteinemi, Farelerde Beyin ve Kalp Dokularında Bir İyon Kanalları Kümesinin Ekspresyonunu Transkripsiyonel Olarak Düzenler

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**Öz**

Hiperhomosisteinemide (HHcy) beyin ve kalp dokularındaki iyon kanalı gen ifadelerindeki değişiklikler daha önce bildirilmemiştir. Araştırmamızda HHcy farelerinin beyin ve kalp dokularındaki 36 iyon kanalının ekspresyonunu karakterize etmek için kontrol fareleri ile kıyasladık. C57BL/6 J. fareleri, her biri 15 hayvandan oluşan iki gruba ayrıldı: (1) kontrol ve (2) HHcy grubu. HHcy, metiyonin uygulamasıyla indüklendi. İyon kanallarının mRNA seviyeleri, qRT-PCR kullanılarak analiz edildi. HHcy'nin kalp ve beyin dokularındaki olumsuz yan etkilerini doğrulamak için TUNEL boyama ve MDA testi kullanıldı. RT-PCR sonuçlarına göre kontrol ile karşılaştırıldığında HHcy grubunun beyin dokularında Hcn4, Trpc3, Trpm2'nin ifadesinin arttığı ve Abbc8, Cacna1b, Cacna1c, Cacna1e, Cacna1h, Hcn1, Kcnc3, Kcnh7, Kcnj8, Trpc4, Trpc5, Trpc6, Trpm3, Trpm4, Trpv4, Trpv6'nın ifadesinin azaldığı belirlendi. Kalp dokularında iyon kanalı ifadelerinde artış tespit edilmedi ancak Accn1, Accn2, Accn3, Hcn1, Kcnc4 ve Trpv6 iyon kanallarının ifadesinin azaldığı bulundu. HHcy grubunun beyin ve kalp dokularında apoptozis ve MDA düzeyinin kontrole göre anlamlı olarak yüksek olduğu belirlendi. Kalp dokularıyla karşılaştırıldığında beyin dokuları, HHcy'li farelerde kontrole göre çok önemli ve çeşitli bir iyon kanalı gen ekspresyon paterni sergiler. İyon kanallarının HHcy'deki rollerinin açıklığa kavuşturulması, yeni terapötik stratejilerin geliştirilmesine ışık tutabilir ve sonuçta HHcy yan etkilerini iyileştirebilir.

**Anahtar Kelimeler:** Asit Duyarlı İyon Kanalları, Geçici Reseptör Potansiyel Kanalları, Hiperhomosisteinemi, Kalsiyum Kanalları, Potasyum Kanalları

**Abstract**

The alterations of ion channel gene expressions in brain and heart tissues in HHcy have not been previously reported. We investigated the mRNA expression levels in brain and heart tissues of the HHcy mice compared to the control mice to characterize distinct expression of 36 ion channels. C57BL/6 J. mice were divided into two groups of 15 animals each: (1) control group and (2) HHcy group. The HHcy was induced by methionine administiration. The mRNA levels of ion channels were analyzed using qRT-PCR. TUNEL staining and MDA assay were used for verification of the negative side effects of HHcy in heart and brain tissues. RT-PCR revealed the upregulation of Hcn4, Trpc3, Trpm2 and the downregulation of Abbc8, Cacna1b, Cacna1c, Cacna1e, Cacna1h, Hcn1, Kcnc3, Kcnh7, Kcnj8, Trpc4, Trpc5, Trpc6, Trpm3, Trpm4, Trpv4, Trpv6 in brain tissues of the HHcy group compared to the control. The upregulation of ion channel expressions in heart tissues were not detected, but we found only the downregulation of Accn1, Accn2, Accn3, Hcn1, Kcnc4 and Trpv6 ion channels. Apoptozis and MDA level were significantly increased in brain and heart tissues of the HHcy group compared to the control. Brain tissues compared to heart tissues exhibit a very considerable and diverse ion channel gene expression pattern in mice with HHcy than control. Clarifying the roles of ion channels in HHcy could shed light on the development of novel therapeutic strategies and ultimately improve HHcy side effects.

**Keywords:** Acid-Sensing Ion Channels, Transient Receptor Potential Channels, Hyperhomocysteinemia, Calcium Channels, Potassium Channels

## Introduction

Homocysteine (Hcy) is a sulfur-containing amino acid formed during the intracellular conversion of methionine to cysteine. Hcy levels are affected by genetic defects (such as enzyme deficiencies), chronic diseases, vitamin and nutritional deficiencies, individual features (sex, age, etc.) and certain drugs. Elevated Hcy levels cause hyperhomocysteinemia (HHcy) and can be normalized by the administration of folic acid. Homocysteine is a well known toxic substance, and is associated with cardiovascular and neurodegenerative diseases; its mechanisms are only poorly understood (1). Toxic effects of homocysteine and the product of its spontaneous oxidation, homocysteic acid, are based on their ability to activate NMDA receptors, increasing intracellular levels of ionized calcium, reactive oxygen species and activating of MAP kinase. Even a short-term exposure of cells to high homocysteic acid concentration induces their apoptotic transformation (2). NMDA receptors are found in neutrophils, red blood cells, cardiomyocytes, osteoblasts and especially neurons (1).

Ion channels are essential components for neuronal and cardiac excitability. Numerous cellular

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processes including cell size regulation, apoptosis, cell proliferation, muscle contractions, immune system activation, or hormone release depend on ion channels activities (3). Multiple ion channels expressed by a specific neuron contribute to determine cellular responses to humoral or synaptic inputs in the nervous system. Distinct ion channels are recognized to be of high importance for excitable cells of the heart: cardiomyocytes of the working myocardium as well as cells of the cardiac conduction system (CDS). In the heart, specific ion channels are responsible for the regulated generation of action potentials and for cardiac muscle contraction strength and time. Due to complex interaction with other signaling pathways in HHcy, ion channels may play a role in neuronal and cardiomycte apoptosis (3). But, it remains unclear how interactions of homocysteine with ion channels or ions occur.

In the current study, ion channels, which are especially highly expressed in the brain and capillary and are responsible for the pathophysiology of common heart and brain diseases, were selected and the expressions of these ion channels were evaluated at the mRNA level in the HHcy mouse model, and ion channels that could mediate the toxic effects of HHCy were identified. Selected ion channel families are ATP-binding cassette channels (ABCC) or Adenosine-triphosphate-sensitive K+ channels (KATP) (4). Acid-sensing ion channels (ASICs), Voltage-gated calcium channels (5), Hyperpolarization-activated and cyclic nucleotide-gated channels (HCN) (6), Potassium Channels (PC) (7), Transient Receptor Potential channels (TRP) (8) and the selection was made according to www.proteinatlas.org data. Although the expression of ion channels is rather stable, being conductivity regulated by gating mechanisms is linked to signaling cascades (3). The present study aims to compare ion channels expression in brain and heart tissues of the HHcy and the control mice to give a starting point for further analyses of their distinct roles at neurons and cardiomyocytes. These comparisons could be a starting point to evaluate the contribution and function of different ion channels on the apoptotic effects at neurons and cardiomyocytes in HHcy condition.

## Material and Method

*Study design and HHcy model*

The study was approved by Fırat University Animal Experiments Ethical Committee Directorate (2014/08-21). Thirty C57BL/6J mice at 8 wk of age were obtained from FUDAM (Turkiye, Elazig). All mice were housed in a temperature-controlled room (23°C) with a 12:12 hour light/dark cycle with food and water ad libitum during the course of the study. Thirty mice were divided into two groups (15 animals per group): Group I (control mice); Group II (HHcy mice). The control group were fed a normal chow diet and the hyperhomocysteinemic group were fed a 2% (w/v) L-methionine (Sigma-Aldrich, St. Louis, MO, USA) supplementation with drinking water for induced hyperhomocysteinemia (9,10). L-methionine was obtained from Sigma-Aldrich (St. Louis, MO, USA). Mice were sacrificed after 2 months on the diets. After collecting blood, the brain and heart tissues were quickly removed, then cut into three portions, rapidly frozen in liquid nitrogen and stored at -80 until laboratory analysis.

*Measurement of homocysteine*

Blood samples were drawn from the decapitation and centrifuged to obtain plasma, which was frozen at –80°C for subsequent analysis. Homocysteine levels were measured using Fluorescent Polarization Immunoassay (FPIA) procedures (Axsym Plus, Abbott, USA).

*Terminal deoxynucleotidyl transferase mediated dUTP nick-end labeling (TUNEL) assay*

One portion of the brains and hearts were fixed with 10% neutral buffered formalin and embedded in paraffin. Sections obtained from paraffin blocks with a thickness of 5 μm were taken on polylysine slides. Cells undergoing apoptosis were determined using the ApopTag Plus Peroxidase In Situ Apoptosis Detection Kit (Chemicon, cat no: S7101, USA) in line with the manufacturer's recommendations. The obtained preparations were examined under the research microscope (Olympus BX50), evaluated and photographed (Olympus Corp., Tokyo, Japan). In the evaluation of the tunnel staining process, nuclei stained blue with Harris hematoxylin were considered normal, cells stained as brown nuclear were considered apoptotic. In the evaluation of tunnel staining, the extent of staining was taken as a basis. The extent of tunnel staining was scored semi-quantitatively with numbers from 0 to +4 (0: No, +1: very little, +2: less, +3: medium, +4: severe).

*Measurement of lipid peroxidation*

Malondialdehyde (MDA) level used as a marker of lipid peroxidation index in one portion of the brain and heart was detected with TBARS reaction according to Yagi (11). Samples were homogenized by using Next advance homogenizer and supernatants were removed. TCA and TBARS reagent were added to the supernatant aliquots and mixed and incubated at 100°C for 120 minutes. Samples were centrifuged 10 min at 1000g and the absorbance was read at 532nm. Tetraethoxypropane was used as standard. The results of TBARS measurements were expressed as nmol/gr tissue MDA equivalents.

*Quantitative Real Time Polymerase Chain Reaction (qRT-PCR) Analysis*

We characterized the expression of 36 ion channels in mice with a focus on the brain and heart tissues. These ion channels were given in Table1. Total RNA from one portion of the brain and heart was isolated using TRIzol reagent and High Capacity RNA to cDNA Synthesis kit used for cDNAs synthesis (Invitrogen, Carlsbad, USA). GAPDH was used as a reference gene (housekeeping). Gene expression levels were measured with the Applied Biosystems 7500 Real-Time PCR system using Tag Man Master Mix. The 2-ΔΔCT method was used to calculate the differences between the gene expressions of the groups (Applied Biosystems, Foster City, CA).

**Table 1.** Body weight gain and plasma Hcy levels of mice fed on the experimental diets

| | Diet type | | |
|---|---|---|---|
| | **Chow diet (n=15)** | **2% (w/v) L-methionine (n=15)** | **p value** |
| **Body weight gain (gr)** | 30.13±1,90 | 28.81±2.17 | 0.167 |
| **Plasma Hcy level (µM/L)** | 10.2±0.28 | 38.1±0.97 | <0.001 |

*Each value is given the mean±SE.*

*Statistical analysis*

Statistical evaluations of this study were made using IBM SPSS 22.0 package program, licensed by Fırat University (193.255.124.131). The data were expressed as mean±SD Shapiro-Wilk test was used in the normality test of numerical variables. T test was used to determine the difference between the means of two independent samples in the comparison of the groups for the numerical variables with normal distribution (parametric) and Mann-Whitney U test was used in the comparison of these two groups in terms of numerical variables that do not show normal distribution (non-parametric). The ΔΔCt method was used to determine fold increase and statistical differences according to Ct values in qPCR data, and the Qiagen GeneGlobe program, which is open to all users, was used for the analysis (http://www.qiagen.com/us/shop/genesand-pathways/data-analysis-center-overview-page/).
The $p<0.05$ value was considered statistically significant in the interpretation of the results obtained.

## Results

All mice with diet-induced hyperhomocysteinemia appeared normal and their body weights were similar to those of mice fed on control diets ($p>0.05$). 2% (w/v) L-methionine was induced HHcy. Mean plasma Hcy concentration of the Hcy group was significantly higher than that of the control mice ($P<0.000$) (Table1).

*Neuronal and Myocardial Apoptosis*

We show TUNEL staining positive in most apoptotic cardiac and neuronal cells. TUNEL positivity, assessed under light microscopy, was observed as +1 in the brain and heart tissues of the control group (Fig 1A and Fig 1C). The spreading of TUNEL positivity was significantly increased in the HHcy group compared to the control group and the extent was determined to be +4 (Fig 1B and Fig 1D). Breast tissue was used as the positive control. TUNEL positivity was not determined in negative control.

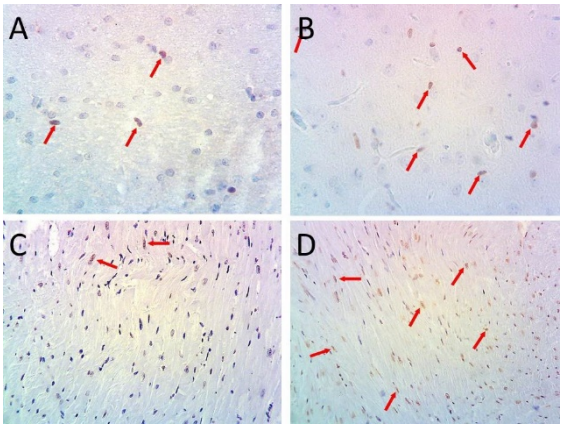


**Figure 1.** Representative sections of TUNEL-positive cells in HHcy and control. TUNEL staining to identify apoptotic cells; apoptotic cells (arrow) in brain and heart control group spreading were +1 (A, C), apoptotic cells (arrow) in Hcy group spreading were +4 (B, D).

*MDA levels*

MDA level is an indicator of oxidative stress. This indicator was determined in myocardial and brain tissues identifying the possible oxidative effect induced by HHcy. As illustrated in Figure 2, the brain and heart MDA levels in the HHcy groups were significantly increased compared to the control group (P=0.000 and p=0.000; Fig. 2A and Fig. 2B).

*Real Time PCR results*

Hcn4, Trpc3 and Trpm2 expressions significantlly increased in fold change > 1.5-2 (p=0.040, p=0.033 and p=0.047; respectively) and Abbc8, Cacna1b, Cacna1c, Cacna1e, Cacna1h, Hcn1, Kcnc3, Kcnh7, Kcnj8, Trpc4, Trpc5, Trpc6, Trpm3, Trpm4, Trpv4 and Trpv6 gene mRNA levels showed a downregulation in fold change <0.5 (p=0.041, p=0.028, p=0.021, p=0.023, p=0.017, p=0.037, p=0.012, p=0.046, p=0.047, p=0.026, p=0.046, p=0.048, p=0.015, p=0.036, p=0.011, p=0.011; respectively) in the brain tissue of HHcy treated mice than nontreated mice.

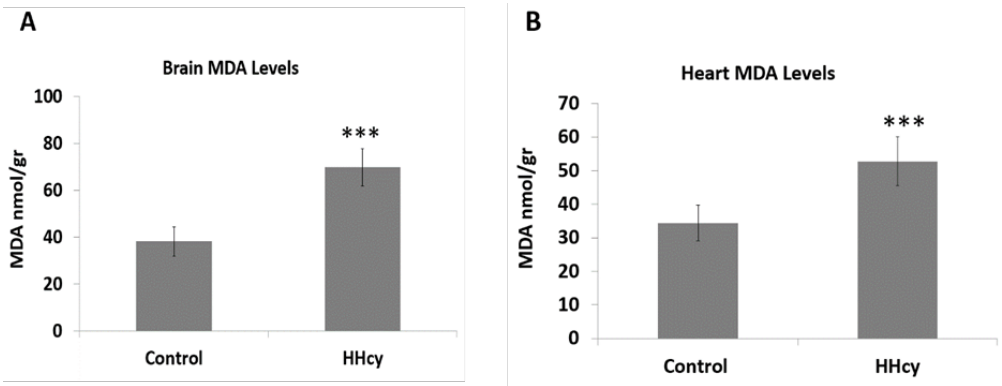


**Figure 2.** Brain and heart tissues MDA levels in mice induced by HHcy. Data represent the means ± SD in each group (n = 15/each); ***p<0.01 compared to the control group.

For the heart tissues, mRNA levels of Asic1, Asic2, Asic3, Hcn1, Kcnc4 and Trpv6 ion channel members were downregulated in fold change <0.5 (p=0.008, p=0.011, p=0.012, p=0.018, p=0.042, p=0.038; respectively) and other ion channel mRNA levels did not show a significant change in the brain tissue of HHcy treated mice than nontreated mice. Table 2 shows the fold changes and p value in the ion channel expression in the brain and heart tissues in the HHcy group compared to the control group.

## Discussion

We investigated neuronal and cardiac ion channel remodeling associated with elevated high homocysteine levels in mice brain and heart tissues. The principal findings of this study are as follows (1). In the brain; the upregulation levels of Hcn4, Trpm2 and Trpc3; the downregulation of Abcc8, Cacna1b, Cacna1c, Cacna1e, Cacna1h, Kcnc3, Kcnh7, Kjnj8 Trpc4, Trpc5, Trpc6, Trpm3, Trpm4, Trpv4 and Trpv6 were detected in the mice with HHcy compared to the control mice (2). The downregulation of Accn1, Accn2, Accn3, Hcn1, Kcnc4, and Trpv6 mRNA expressions were found in the heart tissues of HHcy mice compared to the control. Apoptosis and oxidative stress in the brain and heart tissues of HHcy mice significantly increased compared to the control.

Recent studies have shown in cultured neurons that the toxicity mechanism of homocysteine involves the activation of NMDA receptors (12) or the apoptosis trigger by DNA damage (13**).** The apoptosis apparently depends on the magnitude and temporal organization of $Ca^{2+}$ entry and on the functional state of cell (3). The increased Ca uptake in neurons is mediated mainly by the NMDA receptors and group I mGluRs, known to be important mechanisms of calcium influx in HHcy (1). In addition, $Ca^{2+}$-channels including a voltage-gated $Ca^{2+}$ channel (VGCC) and store-operated channels (SOC) are essential for apoptosis (14,15).

Adenosine-triphosphate-sensitive K+ channels (KATP) are responsible for metabolic control of membrane potential. The inwardly rectifying potassium channel (Kir) subunits 6.1 (KJNJ8 gene product) and 6.2 (KJNJ11 gene product) form the ion-conducting pore with regulatory sulfonylurea receptor (SUR2; ABCC9 gene product)) or SUR1 (ABCC8 gene product) respectively. SUR1/KIR6.2 channels are broadly distributed in the neuroendocrine system. SUR2 assembles with Kir6.1 in vascular smooth muscle or Kir6.2 in ventricular and skeletal muscle (4). The expression of ABCC8, ABCC9, KCNJ8, and KCNJ11 upregulate in the central nervous system (CNS) in some pathological situations (16). Both SUR1 (Abcc8) and Kir6.2 (KJNJ11) expression in the current study significantly decreased in the brain tissues of the HHcy group compared to the control. Weekman et al. reported that treating with moderate levels of homocysteine the astrocyte cell significantly upregulated at 48 hr KCNJ10 mRNA levels compared to the controls and the levels significantly decreased at 72 hr compared to the 48 hr homocysteine-treated cells (16). When clonal BRIN-BD11 beta-cells were exposed to homocysteine (250-1000 micromol/L) for 18h, SUR1 and Kir6.2 gene expressions did not noticeably change (17). Hcy significantly decreased nucleotide hydrolysis and increased ATP levels in the cerebral cortex of Hcy-treated rats for the 30th to the 60th day of life. These findings propose that the unbalance in ATP may lead to the cerebral toxicity of mild hyperhomocysteinemia (18). SUR1/Kir6.2 channels were activated by the increased ATP/ADP ratio (19). The downregulated SUR1/Kir 6.2 expression in response to the enhanced channel activation because of the increased ATP/ADP ratio caused by HHcy may be a protective mechanism for the brain from the toxic effects of HHcy in mice brain.

**Table 2.** The mRNA expression levels for ion channel genes in brain and heart tissue of HHcy mice

| Gene Symbol | Gene Name | Brain Tissue | | Heart Tissue | |
|---|---|---|---|---|---|
| | | Fold Change | p value | Fold Change | p |
| **Gapdh** | Glyceraldehyde-3-phosphate dehydrogenase | 1 | 0 | 1 | 0 |
| **Abcc8** | ATP-binding cassette, sub-family C (CFTR/MRP), member 8 | **0.450** | **0.041** | 0.908 | 0.676 |
| **Abcc9** | ATP-binding cassette, sub-family C (CFTR/MRP), member 9 | 0.920 | 0.715 | 0.993 | 0.952 |
| **Accn1** | Amiloride-sensitive cation channel 1, neuronal (degenerin) | 1.086 | 0.758 | **0.137** | **0.008** |
| **Accn2** | Amiloride-sensitive cation channel 2, neuronal | 1.602 | 0.117 | **0.207** | **0.011** |
| **Accn3** | Amiloride-sensitive cation channel 3 | 0.669 | 0.169 | **0.222** | **0.012** |
| **Cacna1a** | Calcium channel, voltage-dependent, P/Q type, alpha 1A subunit | 0.659 | 0.159 | 0.859 | 0.531 |
| **Cacna1b** | Calcium channel, voltage-dependent, N type, alpha 1B subunit | **0.389** | **0.028** | 0.732 | 0.254 |
| **Cacna1c** | Calcium channel, voltage-dependent, L type, alpha 1C subunit | **0.341** | **0.021** | 1.248 | 0.403 |
| **Cacna1e** | Calcium channel, voltage-dependent, R type, alpha 1E subunit | **0.356** | **0.023** | 0.504 | 0.058 |
| **Cacna1h** | Calcium channel, voltage-dependent, T type, alpha 1H subunit | **0.301** | **0.017** | **0.518** | **0.063** |
| **Hcn1** | Hyperpolarization-activated, cyclic nucleotide-gated K+ 1 | **0.432** | **0.037** | **0.302** | **0.018** |
| **Hcn2** | Hyperpolarization-activated, cyclic nucleotide-gated K+ 2 | 0.717 | 0.230 | 0.889 | 0.619 |
| **Hcn3** | Hyperpolarization-activated, cyclic nucleotide-gated K+ 3 | 0.717 | 0.230 | 1 | 0.996 |
| **Hcn4** | Hyperpolarization-activated, cyclic nucleotide-gated K+ 4 | 2.084 | 0.040 | 0.559 | 0.082 |
| **Kcna4** | Potassium voltage-gated channel, shaker-related subfamily, member 4 | 1.064 | 0.824 | 1.072 | 0.802 |
| **Kcnc3** | Potassium voltage gated channel, Shaw-related subfamily, member 3 | **0.230** | **0.012** | 0.824 | 0.440 |
| **Kcnc4** | Potassium voltage gated channel, Shaw-related subfamily, member 4 | 0.673 | 0.175 | **0.454** | **0.042** |
| **Kcnd1** | Potassium voltage-gated channel, Shal-related family, member 1 | 0.598 | 0.106 | 0.697 | 0.207 |
| **Kcnd2** | Potassium voltage-gated channel, Shal-related family, member 2 | 0.817 | 0.426 | 0.790 | 0.362 |
| **Kcnd3** | Potassium voltage-gated channel, Shal-related family, member 3 | 0.687 | 0.191 | 1.149 | 0.598 |
| **Kcnh2** | Potassium voltage-gated channel, subfamily H (eag-related), member 2 | 1.505 | 0.158 | 0.586 | 0.099 |
| **Kcnh7** | Potassium voltage-gated channel, subfamily H (eag-related), member 7 | **0.466** | **0.046** | 0.578 | 0.093 |
| **Kcnj11** | Potassium inwardly rectifying channel, subfamily J, member 11 | 0.913 | 0.6952 | 0.637 | 0.138 |
| **Kcnj8** | Potassium inwardly-rectifying channel, subfamily J, member 8 | **0.469** | **0.047** | 0.790 | 0.362 |
| **Trpc1** | Transient receptor potential cation channel, subfamily C, member 1 | 0.683 | 0.186 | 0.727 | 0.246 |
| **Trpc3** | Transient receptor potential cation channel, subfamily C, member 3 | **2.219** | **0.033** | 1.2142 | 0.461 |
| **Trpc4** | Transient receptor potential cation channel, subfamily C, member 4 | **0.371** | **0.026** | 1.173 | 0.563 |
| **Trpc5** | Transient receptor potential cation channel, subfamily C, member 5 | **0.466** | **0.046** | 1.173 | 0.563 |
| **Trpc6** | Transient receptor potential cation channel, subfamily C, member 6 | **0.277** | **0.015** | 0.595 | 0.104 |
| **Trpm2** | Transient receptor potential cation channel, subfamily M, member 2 | **1.986** | **0.048** | 0.863 | 0.549 |
| **Trpm3** | Transient receptor potential cation channel, subfamily M, member 3 | **0.275** | **0.015** | 0.801 | 0.387 |
| **Trpm4** | Transient receptor potential cation channel, subfamily M, member 4 | **0.429** | **0.036** | 1.087 | 0.759 |
| **Trpm7** | Transient receptor potential cation channel, subfamily M, member 7 | 0.959 | 0.841 | 0.599 | 0.107 |
| **Trpv2** | Transient receptor potential cation channel, subfamily V, member 2 | 0.582 | 0.096 | 0.722 | 0.238 |
| **Trpv4** | Transient receptor potential cation channel, subfamily V, member 4 | **0.205** | **0.011** | 0.908 | 0.676 |
| **Trpv6** | Transient receptor potential cation channel, subfamily V, member 6 | **0.205** | **0.011** | **0.435** | **0.0379** |

*Significant changes are labeled in bold for upregulation and downregulation in HHcy groups than control.*

Voltage-gated calcium channels are involved in the $Ca^{2+}$-influx, thereby playing an important role in calcium signaling of actually all cells (8). They have four to five different subunits, $\alpha_1$, $\beta$, $\alpha_2$, $\delta$ and $\gamma$. This study is focused on the calcium channel subunit $\alpha_1$ (CACNA1) which is the largest subunit that forms the actual channel. In mice, there are ten different CACNA1 genes, divided into three families, L-, P/Q/N/R- and T-type. The L-type family of the CACNA1 subunits includes four different proteins in humans: CACNA1S, 1C, 1D and 1F. The P/Q-type (CACNA1A), N-type (CACNA1B) and the R-type (CACNA1E) form one distinct family and are activated by strong depolarization. CACNA1 gene family members are expressed in brain and heart tissues. CACNA1B primary are expressed in brain tissues (5). CACNA1B, C, E and H mRNA levels were downregulated in the brain tissues of HHcy mice compared to the control in current study. Phelan et al. (2013) reported a role of L-type $Ca^{2+}$ channel-dependent, NMDAR-independent hippocampal L-LTP in the formation of spatial memory in behaving animal and for a function of the MAPK/CREB (CRE-binding protein) signaling cascade in linking CACNA1C channel-mediated $Ca^{2+}$ influx to either process (20). Homocysteine-induced neuronal cell death played a role in the activation of extracellular signal-regulated kinase-mitogen activated protein kinase (ERK-MAPK) by NMDA receptor (2). Downregulated VGCC genes expression and altered MAPK/CREB signaling cascade may contribute the neuronal cell death.

Potassium channels ($K^+$) are membrane-spanning proteins and the most abundant ion channels in different tissues. Their activity may be regulated by voltage, calcium and neurotransmitters. These channels have an important role in maintaining the normal physiology of cellular repolarization, cardiac action potential repolarization, smooth muscle relaxation, neurotransmitter release, immune function and insulin secretion (6). $K^+$ channels have been suggested as an important physiological target of NO in the brain. Recent research has shown that NO *release* or via cGMP production in various tissues can activate different $K^+$ channels (22). NO release from sinusoidal endothelial cells was reduced by homocysteine (21). The decrease in NO production in brain of HHcy mice might cause the diminished $K^+$ channels activity and expression.

Hyperpolarization-activated and cyclic nucleotide-gated channels (HCN) are widely expressed throughout the heart and the central nervous system. They contribute to the control of cardiac and neuronal rhythmicity (pacemaker currents). In neurons the HCN channels play a role in several neuronal functions including several other neuronal processes, including determination of resting membrane potential, dendritic integration and synaptic transmission (6). Of four HCN channels, HCN4 is the most sensitive to cAMP and the open probability of HCN4 is increased by cyclic adenosine monophosphate (cAMP) (23). The increased expression of HCN4 in HHcy mice may contribute to the Hcy induced cAMP inhibition.

TRPs defects in the genes encoding TRP channels (so-called "TRP channelopathies") underlie certain neurodegenerative disorders due to their abnormal $Ca^{2+}$ signaling properties, and changes in TRP channel expression and functionality are related to diabetic thermal hyperalgesia, painful neuropathies and headache (8,24,25). TRPC proteins might play a critical role in neuronal survival, proliferation, and differentiation. TRPC3, TRPC4, TRPM2 and TRPM7 are influenced by oxidative stress (14,15). Our study found increased TRPC3 and decreased TRPC4, 5, 6 mRNA expression. Recent studies stated that comparisons of transcript abundance of TRP ion channels showed a consistent dominance TRPC3 in most tissues where TRPC3 channels are directly activated in response to oxidative stress (25). TRPC1/4 double-knockout (DKO) mice lack epileptiform bursting in lateral septal neurons and exhibit reduced seizure-induced neuronal cell death (20). TRPC5 was activated by nitric oxide (NO). NO release from sinusoidal endothelial cells was reduced by homocysteine (21). TRPC6 inhibited NMDA receptor-triggered neurotoxicity and protected neurons from ischemic brain damage (26). Decreased NO levels in HHCY may act to reduce TRPC4, 5 and 6 activity and expression. Investigation of the effects of NO levels on TRP ion channel expression is a new field of study.

TRPM2, TRPM4, and TRPM7 are the oxidative stress-modulated TRPM ion channels. Especially TRPM2 channels integrate calcium signaling and oxidative stress in the brain (27). NMDA-induced burst firing in substantia nigra pars reticulata (SNr) GABAergic neurons require TRPM2 channel (28). A cell culture study examining the TRPM2 expression in rat cortical neurons after oxidative stressor rotenone and paraquat treatment showed the increase of TRPM2 mRNA levels but not protein levels after acute and chronic rotenone treatment (27). We suggest that TRPM2, acting in concert with NMDARs, may provide the basis for a positive feedback loop in which $Ca^{2+}$ influx is facilitated through a pathway involving aberrant NMDAR activation, the formation of ROS, all of which lead to the activation of TRPM2. We speculate that TRPM2 and NMDA signaling mechanisms can the one of the main pathways in the deterious effects of HHcy. This pathway may mediate Hcy-induced neuronal cell death by contributing to the sensitivity to $Ca^{2+}$ overload stress and through ROS increase in HHcy mice. Exposure to $H_2O_2$ of cells abolishes TRPM4 channel deactivation, leading to permanent TRPM4 activity without alterations in the $[Ca^{2+}]_i$ dependence (15). Additionally, ROS occurring in the injured region during trauma are also effective in regulating TRPM4 activity via upregulating TRPM4 expression (29). We determined that TRPM4 diminished in the HHcy mice compared to the control. Molecules that cause cell death by inducing oxidative stresses such as Hcy, $H_2O_2$, etc. may induce different signaling mechanisms within the cell, causing TRPM3 and TRPM4 gene expression to be activated in different intracellular signaling mechanisms. The upregulated TRPM2 and TRPC3 mRNA expression depend on increased oxidative stress in HHCY mice may cause neuronal apoptosis via TRPC3 and TRPM2-mediated ($Ca^{2+}$) overload. The remarkable feature in terms of calcium ion channel expression changes is that there is an increase in the expression of channels that are active with oxidative stress, while calcium channels that are active with other mechanisms show a decrease. This data puts ion channel inhibitions activated by oxidative stress into therapeutic targets for HHCy.

Acid-sensing ion channels (ASICs) are voltage-independent proton-gated cation channels that are largely expressed in the nervous, cardiac and muscle tissues as well as in some non-neuronal tissues (30). Each ASIC channel is activated in different extracellular pH (pH 7.2–6.8) that are released from muscle during ischemia (31). The elevated levels of homocysteine are associated with decline in cardiac performance. Hcy impairs the endocardial endothelial-myocyte (EM) uncoupling functions associated with the induction of ventricular hypertrophy leading to cardiac stiffness and diastolic heart failure. NMDA-R is expressed in the heart. Hcy increases calcium overload and oxidative stress in the mitochondria and causes the opening of mitochondrial permeability transition pore leading to mechano-electrical dysfunction in the heart (32). An interesting finding of the present study was a tendency towards decreased expression of DEG/ENAC gene family members including *ASIC1, ASIC2* and ASIC3 in heart tissue. In mammalian cells, caspase-8-mediated apoptosis is induced by intracellular calcium overload that is dependent on the hyperactivation of DEG/ENaC channels family including ASICs (2). An isoproterenol-induced cardiac ischemia model mimicking clinical conditions of early cardiac angina was used to demonstrate that ASIC3 plays a protective role in sensing cardiac ischemia (33). Asic2 knockout mice

in $Ca^{2+}$ imaging experiments exhibited normal physiological responses (increases in intracellular $Ca^{2+}$ concentrations) to acid taste stimuli (34). Multiple measures of baroreceptor activity suggest that mechanosensitivity is diminished in ASIC2 null mice. The results define ASIC2 as an important determinant of autonomic circulatory control and of baroreceptor sensitivity. The genetic disruption of ASIC2 recapitulates the pathological dysautonomia seen in heart failure and hypertension, and defines a molecular defect that may be relevant to its development (15).

HCN channels are essential for cardiac pacemaker and electric conduction (35). HCN1-knockout mice show the Congenital Sinus node dysfunction (SND) associated with a severely reduced cardiac output (36). K channels are the essential for the change in action potential in response to variation in heart rate. KCNC4 responsible slow transient outward current and voltage depolarization (37). The downregulated ASICs, HCN1 and KCNC4 (Kv3.4) mRNA levels expressions may contribute to the change action potential in heart tissue and to the decline in cardiac performance as seen in the hearts of HHcy mice.

Concerning the current study, it is clear that gene regulations on transcript level do not explicitly mimic either protein levels and posttranslational modifications or protein activity. This set of data is thought to describe a global overview on transcript regulation of ion channels in brain and heart tissues of HHcy mice. More detailed studies of ion channel splice variants could give insights into their function and broaden the still scarce knowledge.

## Conclusion

This is the first study to investigate the homocysteine-induced brain and heart ion channel expressions in mice with HHcy. The ion channel expression changes, combined with oxidative stress shown in HHcy, appear to play an important role in Hcy-induced neurol and cardiomyocytes cell death pathways, and are probably key mediators of the long-term neuronal cell adaptation to raised homocysteine concentrations. Hcy indirectly increases calcium influx by binding to NMDA receptor. However, it is clear that HCY can also increase calcium influx through other calcium ion channels. Patch clamp studies to reveal the administration time and dose dependent effects of homocysteine on ion channels are likely to determine whether there are any differences in electrophysiological properties of ion channels in HHcy condition.

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## Conflict of interest statement

The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

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# Prolidase Enzyme Activity in Endometrial Polyps and Its Relationship with Oxidative Stress

# Endometriyal Poliplerde Prolidaz Enzim Aktivitesi ve Oksidatif Stres ile İlişkisi

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**Öz**

Endometrial polip (EP) kadın hastalıkları ve doğum kliniği pratiğinde sıklıkla karşılaşılan bir durumdur. Biz çalışmamızda endometrial polip tanısı olan hastaların prolidaz enzim aktivitesi ve oksidatif stres (OS) ile ilişkisini ortaya çıkarmayı amaçladık. Araştırmaya dahil edilme kriterlerini karşılayan histopatolojik olarak EP tanısı konan 35 hasta ile endometrial örnekleme sonucunda patoloji tespit edilmeyen 35 anormal uterin kanaması (AUK) olan kontrol grubu hastası dahil edilmiştir. EP ve kontrol grupları arasında TAS, TOS, OSI, prolidaz ve endometrium kalınlığı değerleri karşılaştırıldığında EP grubunda, kontrol grubuna göre TOS, OSI, prolidaz ve endometrial kalınlık değerleri istatistiksel olarak anlamlı düzeyde yüksek bulunmuştur. Çalışmamız, daha geniş hasta grupları ile yapılan çalışmalarla desteklendiğinde patogenezin daha ayrıntılı anlaşılması ve klinik olarak hasta takibinde yararlı bir belirteç olabileceği görüşündeyiz.

**Anahtar Kelimeler:** Endometrial Polip, Oksidatif Stres, Prolidaz Enzim Aktivitesi

**Abstract**

Endometrial polyp (EP) is a condition that is often encountered in obstetrics and gynecology clinic practices. In our study, we aimed to reveal the relationship between prolidase enzyme activity (PEA) and Oxidative Stress (OS) in patients with endometrial polyps. Thirty-five patients who were histopathologically diagnosed with EP and 35 patients with abnormal uterine bleeding (AUB) without pathology as a result of endometrial sampling were included in the control group. Serum TOS, OSI, tissue PEA, and endometrial thickness values were found to be statistically significantly higher in the EP group compared to the control group. We believe that our study, when supported by studies with larger patient groups, may be a useful marker for a more detailed understanding of the pathogenesis and clinical follow-up of patients.

**Keywords:** Endometrial Polyp, Oxidative Stress, Prolidase Enzyme Activity

## Introduction

Endometrial polyps (EPs) are benign growths that extend from the endometrial surface into the cavity. They arise due to hyperplasia of the endometrial gland and stroma surrounding a vascular structure (1). Its incidence is estimated to be about 8% in the general population, and between 10% and 30% in females with abnormal uterine bleeding (AUB) (2,3). Advanced age and tamoxifen use are the most important known risk factors (4). From this background, endometrial cancer may develop (5). In addition to malignancy potential, in various sources, it has been reported that it can regress spontaneously at different rates (27.0-57.1%) (6). The EP diagnosis is made after histopathological evaluation with endometrial sampling.

The pathophysiology of EP is indeed multifactorial and complex. Increased expression of Bcl-2, a protein that regulates cell death, and estrogen receptors, which mediate the effects of estrogen, have been implicated in the development of EP. Estrogen-dependent causes relate to the actions of estrogen, a hormone associated with the female reproductive system. These conditions can fall under hypoestrogenism or hyperestrogenism, or any sensitivity to the presence of estrogen in the body. Estrogen-dependent cancers, like breast cancer, ovarian cancer and endometrial cancer, rely on estrogen to develop and grow. On the other hand, estrogen-independent causes do not rely on the presence of estrogen. These could be due to mutations that confer estrogen-independent activity to estrogen receptors, causing changes in gene expression. Some cancers can develop resistance to endocrine therapy, which is used to block the action of estrogen in estrogen receptor-positive cancers. However, the exact mechanisms and causes behind EP, particularly in different periods such as premenopausal and postmenopausal stages, are not yet fully understood. Research is ongoing to further understand these mechanisms and improve treatment strategies for EP (6).

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Oxidative stress (OS) is indeed a state of imbalance between the production and elimination of reactive oxygen species (ROS) in the body. When the production of ROS increases or the body's ability to eliminate them decreases, it leads to an accumulation of these reactive species, disrupting the balance and causing OS.

OS is a key factor in causing tissue or molecular damage in cells. It's been implicated in a variety of health conditions, including neurodegenerative diseases, cancer, and cardiovascular diseases (7).

In the context of endometriosis, recent studies have highlighted the role of OS. It's defined as an imbalance between ROS and antioxidants, which may be implicated in the pathophysiology of EP, causing a general inflammatory response in the peritoneal cavity. Local inflammation and increased levels of ROS contribute to the acquisition of a proliferative phenotype and proangiogenic features that are crucial to endometriotic lesion development (8).

However, more research is needed to fully understand the complex mechanisms by which OS contributes to diseases like EP and to develop effective therapeutic strategies (7). In the literature, OS status in EPs was evaluated using serum catalase, xanthine oxidase, and malondialdehyde, and as a result, data showing that OS may have a role in the pathophysiology were obtained (9).

Prolidase is a type of metalloproteinase that is found in abundance throughout the body. It plays crucial roles in various biological processes, including cell proliferation, collagen metabolism, and matrix remodeling. There is a significant correlation between prolidase activity and increased collagen turnover (10). It's hypothesized that an increase in collagen turnover may play a role in the pathophysiology of polyps, and this could potentially be determined by prolidase activity. However, to date, there have been no studies that have explored the role of the enzyme prolidase in the pathophysiology of EPs (11).

In our study, we aimed to compare the group of patients diagnosed with EP and the control group and to reveal the relationship of EPs with prolidase enzyme activity (PEA) and OS.

## Material and Method

This study is a prospective case-control study that was conducted between December 1, 2021, and March 1, 2022, at the Department of Obstetrics and Gynecology, Muğla Sıtkı Koçman University Faculty of Medicine (MSKU). The study received approval from the MSKU Faculty of Medicine Clinical Research Ethics Committee on January 6, 2021 (Decision no: 1/II) and was supported by the MSKU Scientific Research Projects Coordination Unit (Project No: 22/136/02/3/4). All patients were informed about the study and their written informed consent was obtained.

The study involved patients who met the inclusion criteria and were histopathologically diagnosed with EPs. The control group was composed of patients with abnormal uterine bleeding (AUB) who, following endometrial sampling, were found to have no pathological conditions.

*Exclusion Criteria*

The study did not include patients who had abnormal pap smear results, an adnexal mass, or a diagnosis of malignancy. Additionally, individuals with other pathological conditions that could lead to oxidative stress, such as pulmonary disease, pulmonary hypertension, inadequate cardiac function, renal and hepatic dysfunction, chronic ischemic disease, and systemic inflammatory disease were also excluded. Smokers, alcohol users, substance abusers, and those who take antioxidant vitamins and lipid-lowering drugs were not part of the study either.

*Data Collection Tools*

Within the scope of the study, data collection forms and biochemical analysis methods (laboratory analysis of blood and tissue samples) were used as data collection tools.

*Laboratory Analysis of Blood and Tissue Samples*

The polyp tissue that was surgically removed was first rinsed with 0.09% NaCl before being placed into Eppendorf tubes. These tubes were then stored in a deep freezer (Thermo Scientific, -80°C) located in the Biochemistry Department Research Laboratory at Muğla Training and Research Hospital. The samples remained there until the day of the study.

On the day of the study, each tissue sample was first weighed to ensure it was approximately 100±10 mg. The samples were then homogenized in a cold phosphate buffer (PBS; pH: 7.4; 50 mM) at a ratio of 1/10 using a homogenizer (IKA T10 Ultra-Turrax 10) operating at 20,000 rpm. Following this, the homogenate of the polyp tissue was centrifuged at 10000 xg for 5 minutes with Hettich Mikro 200 centrifuge (Andreas Hettich Co., Tuttlingen, Germany) and a temperature of +4°C for a duration of 5 minutes.

Approximately 6 cc of venous blood was taken from all females in the study and control groups into tubes containing separators. The blood was centrifuged at 2000 xg for 10 minutes at +4 and serum samples were taken. The samples were stored at -80°C. Prolidase with the supernatant obtained from the tissue homogenate, total antioxidant status (TAS) obtained from the serum, and total oxidant status (TOS) tests were studied from blood.

*Prolidase Activity*

PEA was quantified using the modified Chinard method and a photometric method with a commercial kit (Rel Assay Diagnostics, Türkiye, Catalog no: RL0025). The underlying principle of the method is that proline, a component of the glycine-proline substrate produced by the prolidase enzyme, forms a colored compound with ninhydrin under the influence of heat in an acidic environment. The color intensity is proportional to the proline concentration. The absorbance of the resulting proline was measured at 515 nm, and the enzyme activity was expressed as U/L creation (10).

*Total Antioxidant Status (TAS)*

TAS levels were studied colorimetrically (Rel Assay Diagnostics, Türkiye, Catalog no: RL0017) by taking 18 µL of supernatant (12). The assay was conducted using an ELISA plate, and the results were determined by performing two readings at a wavelength of 660 nm with an ELISA reader, with 5-minute intervals between each reading. The standard curve was established using the company's calibrator. This test demonstrated a repeatability value of %CV<10% and the reading range was between 0.1-3.5 mmol TroloxEq/L.

*Total Oxidant Status (TOS)*

TOS levels were studied colorimetrically (Rel Assay Diagnostics, Türkiye, Catalog no: RL0024) by taking 45 µL of supernatant (13). The assay was carried out using an ELISA plate, and the results were determined by taking two readings with an ELISA reader at a wavelength of 530 nm, with a 5-minute interval between the readings. The standard curve was created using the company's calibrator (Catalog number RL0024). This test had a repeatability value of %CV<10% and the reading range was between 0.2-80 µmol $H_2O_2$Eq/L.

*Calculation of Oxidative Stress Index (OSI)*

The OSI is the ratio of the TOS value in µmol $H_2O_2$Eq/L to the TAS value in mmol TroloxEq/L (14).

*Data Analysis*

The research data was analyzed using the SPSS 21.0 statistical software. The normal distribution of continuous variables was assessed using both visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). Descriptive statistics for the study included mean and standard deviation for data that followed a normal distribution, and median, minimum, and maximum for data that did not follow a normal distribution. The Student t-test was employed to compare continuous variables with parametric properties between independent groups, while the Mann-Whitney U-Test was used for comparing continuous variables without parametric properties between independent groups. A p-value of less than 0.05 was considered statistically significant.

## Results

The demographic characteristics of the cases in the patient and control groups are compared in Table 1. When the table is examined, no statistically significant relationship was found between the patient and control groups in terms of age, weight, BMI, gravidity, parity, and menopausal status (p<0.005).

**Table 1.** Comparison of demographic characteristics of cases in the control and patient groups.

| | | Group | | | | |
|---|---|---|---|---|---|---|
| | | Control (n:67) | | Patient (n=35) | | p |
| | | n | Median (min-max) | n | Median (min-max) | |
| **Age (years)** | | 35 | 44.0 (27.0-72.0) | 35 | 45.0 (31.0-66.0) | 0.986 |
| **Weight (kg)** | | 35 | 72.0 (58.0-110.0) | 35 | 74.0 (59.0-97.0) | 0.293 |
| **BMI (kg/m$^2$)** | | 35 | 28.0 (22.0-39.0) | 35 | 29.0 (24.0-82.0) | 0.512 |
| **Gravidity (n)** | | 35 | 2.0 (0.0-5.0) | 35 | 2.0 (0.0-5.0) | 0.333 |
| **Parity (n)** | | 35 | 2.0 (0.0-3.0) | 35 | 2.0 (0.0-4.0) | 0.240 |
| **Menopause** | **No** | 27 | 77.1 | 27 | 77.1 | 1.000 |
| | **Yes** | 8 | 22.9 | 8 | 22.9 | |

TAS, TOS, OSI measurement values in serum samples of the cases, PEA measurement values in tissue samples and endometrial thickness measurement results were evaluated. In this assessment mean TAS, mean TOS, mean OSI, mean tissue PEA, and mean endometrial thickness for all study group was found to be 1.4±0.2 mmol/L, 8.4±8.5 µmol/L, 0.6±0.7 AU, 24.8±13.8 U/L and 6.4±11.3 mm, respectively.

The TAS, TOS, OSI values measured in serum samples, PEA values measured in tissue samples, and endometrial thickness measurements of both the patient and control groups were compared (Table 2). Upon examining the table, it was found that the serum TOS, OSI, tissue PEA, and endometrial thickness values were statistically significantly higher in the patient group compared to the control group (p:0.027, p:0.043, p:0.046, p:0.000, respectively).

**Table 2.** The comparison of serum TAS, TOS, OSI, tissue prolidase enzyme activity and endometrial thickness values between the patient and control groups.

| | Group | | | | p |
|---|---|---|---|---|---|
| | Control (n:67) | | Patient (n=35) | | |
| | Mean±SD | Median (min-max) | Mean±SD | Median (min-max) | |
| **TAS (mmol/L)** | 1.4±0.2 | 1.4 (1.0-1.8) | 1.4±0.2 | 1.4 (0.4-1.9) | 0.747* |
| **TOS (µmol/L)** | 7.2±9.1 | 3.5 (1.2-39.1) | 9.6±7.8 | 8.0 (2.2-29.4) | **0.027*** |
| **OSI (AU)** | 0.5±0.6 | 0.3 (0.1-2.5) | 0.8±0.8 | 0.5 (0.2-4.2) | **0.043*** |
| **Prolidase (U/L)** | 21.7±13.5 | 23.6 (1.7-64.1) | 27.9±13.5 | 26.6 (5.8-62.3) | **0.046*** |
| **Endometrial thickness** | 2.7±11.4 | 0.0 (0.0-67.0) | 10.2±10.0 | 8.0 (0.0-60.0) | **0.000*** |

## Discussion

EPs are tumor-like formations covered by epithelial cells, primarily composed of endometrial glands and stroma, with a robust vascular network. These are primarily a result of excessive cellular proliferation. Several molecular mechanisms have been proposed to play a role in the development of EPs. These include the overexpression of endometrial aromatase, monoclonal endometrial hyperplasia, and gene mutations (14).

Another theory is that it was formed as a result of an irregularity in the mechanism of apoptosis (15). In a study conducted by Erdemoglu et al., EP formation was found to be associated with inflammation (16). In the literature, the OS status in EPs was assessed using serum catalase, xanthine oxidase, and malondialdehyde. The results obtained provided data suggesting that OS may play a role in the pathophysiology of EPs. In addition to OS markers, there is no information in the literature about the relationship between endometrial activity and the prolidase enzyme, which plays crucial roles in cell proliferation, collagen metabolism, and matrix remodeling. Our study elucidates the association of EPs with OS and PEA.

In our research, the average age of patients with endometrial polyps was determined to be 45.4±8.8 years (range: 27-72 years). When compared with the control group, there was no statistically significant difference in the average age values. These findings are consistent with the literature. In a study conducted by Demirtaş et al., no significant difference was observed in terms of menopause status when comparing patient groups with and without endometrial polyps (17). Özgen et al. also reported that no significant difference was found in menopause status when the EP cases and the control group in their study were compared (17). In our study, when the cases and control groups were compared in terms of menopause status in accordance with the literature, no statistically significant difference was found.

In the study of Nappi et al., multivariate analyses were used and no relationship was demonstrated between BMI, obesity, menopausal status and EP (4). Similarly, in the study of Çınar et al., no significant difference was found between the EP and control groups in terms of BMI (9). In our study, the groups were compared in terms of BMI in accordance with the literature; the mean BMI was found to be 28.9±4.4 kg/m$^2$ in cases with EP and 30.5±9.4 kg/m$^2$ in the control group, and it was found that this difference was not statistically significant.

Inflammation and OS pathways are indeed closely related. In the study by Çınar et al., they examined the relationship between some markers that play a role in OS and EPs. However, we couldn't find the specific details of Çınar et al.'s the study in current resources (8). In patients suspected of having EPs based on transvaginal ultrasound (TV-USG), blood samples were collected and levels of catalase, xanthine oxidase, and malondialdehyde were assessed. The results revealed higher levels of catalase, xanthine oxidase, and malondialdehyde in patients with EPs compared to the control group. These findings suggest a relationship between OS and EPs.

However, a study by Özgen et al. that compared TAS, TOS, and OSI levels between EP cases and a control group found no statistically significant difference between the groups (17). This indicates that the relationship between OS and EPs might be complex further investigation (18).

In a study by Nayki et al., the levels of superoxide dismutase, catalase, glutathione reductase, TAS and TOS were evaluated in all cases with endometrial biopsy due to AUB. No significant difference was found in terms of TOS and TAS levels between the patients divided into groups according to histopathologies (18). In both studies, cases with AUB that had histopathological differences were evaluated. It was suggested that the existing uterine bleeding could lead to an increase in oxidative products due to the inflammation it causes. In our study, we compared the OSI, TOS, and TAS levels of patients with EP and the control group. We found that the TOS and OSI levels were statistically significantly higher in patients with EP. Our study supports the data from Çınar et al., demonstrating a correlation between the presence of EP and OS (19).

In addition to OS markers, literature information on the relationship between endometrial activity and the prolidase enzyme, which has important functions such as cell proliferation, collagen metabolism and

matrix remodeling, could not be reached. However, studies are showing the relationship between PEA and other gynecological diseases. It has been shown that prolidase activity is increased in epithelial ovarian cancer (20). In a study, it was found that serum prolidase activity was high in patients with early pregnancy loss, and placental prolidase activity was found to be low due to possible placental use. With this study, it was concluded that low prolidase activity in the placenta may be an etiopathological factor in women with early pregnancy loss (21). Hilali et al. found that serum prolidase activity, OSI and TOS were significantly higher in patients with polycystic ovary syndrome (PCOS) compared to the control group. It was found that prolidase activity was positively correlated with TOS, follicle count and prolactin levels in patients (22). In our study, it was found that the PEA was significantly increased in patients with EP compared to the level of the prolidase enzyme in the control group.

## Conclusion

This is the first study in the literature to examine EP and prolidase activity, and if these results are supported by studies with larger patient groups, it can be a useful marker for a more detailed understanding of EP pathogenesis.

## Acknowledgements

This study was originally conducted as a graduation thesis in obstetrics and gynecology (FH).

## Conflict of interest statement

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Ethics Committee Approval:** Ethical approval was obtained from the MSKU Faculty of Medicine Clinical Research Ethics Committee, Türkiye (Date: 06/01/2021, Decision no: 1/II).

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# The Use of Gore-Tex Surgical Membrane in Preventing Spinal Epidural Fibrosis (Experimental Study)

# Spinal Epidural Fibrozisin Önlenmesinde Gore-Tex Cerrahi Membranın Yeri (Deneysel Çalışma)

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**Öz**

Spinal dural defektlerin onarılmasında zaman zaman dura greftlerine ihtiyaç duyulmaktadır. Duraplasti için ideal bir materyal arayışı halen tartışmalı bir konudur. Bu çalışmada Wistar-Albino cinsi, 260-380 gr ağırlığında, 26 adet erişkin erkek sıçanlar kullanılmıştır. Sıçanlar, Kontrol (n=10), Fasya (n=6) ve Gore-Tex (politetrafloroetilen) Cerrahi Membran (n=10) grupları olmak üzere üç gruba ayrılmışlardır. Sıçanlara laminektomi uygulanıp, dura defekti yaratılmış ve daha sonra sırasıyla 'defekt açık bırakılmış', 'fasya ile duraplastı' ve 'Gore-Tex cerrahi membran ile duraplasti' uygulanmıştır. Cerrahi operasyon sonrası 60. günde, sıçanlara ötenazi uygulanmış ve operasyon bölgesinde granülasyon dokusu ve yapışıklıkların değerlendirilmesi amacıyla histopatolojik incelemeler yapılmıştır. Her üç grupta da defekt bölgesini granülasyon dokusunun doldurduğu görülmüştür. Fasya grubunda, fasyanın dura mater ve nöral doku ile granülasyon dokusu arasında bir bariyer teşkil ettiği ancak yoğun granülasyon dokusuyla dolduğu ve yapışıklık olduğu gözlenmiştir. Gore-Tex cerrahi membran grubunda ise Gore-Tex cerrahi membranın dura mater ve nöral doku ile granülasyon dokusu arasında yapışıklığı önleyen iyi bir fiziksel bariyer oluşturduğu, granülasyon dokusunun Gore-Tex cerrahi membrana yapışmadığı bulunmuştur.

**Anahtar Kelimeler:** Dura Defekti, Duraplasti, Epidural Fibrozis, Gore-Tex

**Abstract**

Dural grafts are sometimes necessary for repairing spinal dural defects. The search for an ideal material for duraplasty remains a controversial topic. Twenty-six adult male Wistar-Albino rats, weighing 260-380 g, were used. The rats were divided into three groups: Control (n=10), Fascia (n=6), and Gore-Tex (polytetrafluoroethylene) Surgical Membrane group (n=10). After laminectomy was performed on the rats, a dural defect was created. Then, one of the procedures 'leaving the defect open,' 'duraplasty with fascia,' and 'duraplasty with Gore-Tex surgical membrane' was performed. On the 60$^{th}$ day after surgery, the rats were euthanized, and histological investigations were conducted to examine the granulation tissue and adhesions in the operative area. It was observed that granulation tissue filled the defect area in all three groups. In the fascia group, it was observed that the fascia formed a barrier between the dura mater and neural tissue and the granulation tissue, but it was filled with dense granulation tissue and there was adhesion. It was found that the granulation tissue did not adhere to the Gore-Tex surgical membrane in the Gore-Tex surgical membrane group, and the Gore-Tex surgical membrane created an excellent physical barrier preventing adhesion between the dura mater and neural and the granulation tissues.

**Keywords:** Dural Defect, Duraplasty, Epidural Fibrosis, Gore-Tex

## Introduction

Spinal dural defects often occur as a result of spinal dysraphism, Chiari malformation, vascular malformations, and tumor operations. After such operations, complications such as CSF leaks, pseudo meningocele, infection, arachnoiditis, and strain due to adhesions may occur (1). Dural grafts are often needed to repair spinal dural defects after these surgical procedures. The search for an ideal material for duraplasty remains current in clinical and experimental studies.

Autografts obtained from the temporal fascia, cranial periosteum, or fascia lata are popular in the repair of cranial dural defects (2,3). Gore-Tex (polytetrafluoroethylene) materials are used in various products, such as high-performance fabrics and sealants. Although the medical uses of Gore-Tex are still being actively researched, it is a material that has entered active use in general surgery, plastic surgery, and cardiovascular surgery (4). In neurosurgery, most experimental studies have focused on its beneficial effects in preventing postoperative peridural fibrosis. However, most of these studies were conducted for defects in the cranial dura, and less importance was given to the spinal dura (5,6). To assess the role of Gore-Tex surgical membrane in the repair of spinal dural defects and compare it to autologous fascia graft, a spinal dural defect was surgically generated in rats, and the effects of this material were studied histopathologically in this study.

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## Material and Method

26 Wistar-Albino adult male rats (260-380 g) were utilized in this study. The Animal Experiments Local Ethics Committee obtained the necessary approval for the study. The rats were allocated into

three distinct groups: the control group, the Gore-Tex Surgical Membrane group, and the fascia group. The study started with ten animals each for the control, fascia and Gore-Tex groups, with the idea that this would be sufficient for statistical evaluation. However, four animals in the fascia group died and the study continued without replacement. Therefore, the study was completed with six animals in the fascia group, while there were ten animals in the other groups.

Anesthesia was induced by administering a mixture of ketamine hydrochloride and xylazine (5/10 mg/kg, intramuscularly) to the rats. The rats were allowed to breathe spontaneously throughout the operation.

*Operation technique*

Rats were fixed on the dissection table in the prone position. Then, the area from the mid-lower thoracic region to the sacrum was shaved. The operation area was cleaned with 10% povidone-iodine solution, and a midline 4 cm skin incision was made. Fascia was cut bilaterally, and paravertebral muscles were stripped subperiosteally. A single-level lumbar total laminectomy was performed, and a dural defect was created at that distance (using loop glasses).

In the control group, the dural defect was left open. In the fascia group, a 1 cm autologous fascia graft was taken from the lumbar region and sutured to the defective area with 5/0 silk. In the Gore-Tex surgical membrane group, a 1 cm Gore-Tex Surgical Membrane was fixed with 5/0 silk, and dural repair was performed. Then, the layers were closed following the anatomy, and the operation was terminated. After the intervention, 400000 IU procaine penicillin was given to all rats for prophylactic purposes for five days.

Until the experiment's two-month duration, the rats were monitored in groups of 2 in standard cages, with ad-libitum feeding and daily wound care. At the end of the experiment, the rats were sacrificed by giving carbon dioxide euthanasia within a chamber.

*Histopathological examinations*

The thoracolumbar spine region was taken en bloc and fixed in 10% buffered formalin. It was then decalcified in 20% formic acid for three days. After decalcification, 2 mm thick samples were taken, and paraffin blocks were prepared. Sections of paraffin blocks were stained with hematoxylin-Eosin and histopathologically examined. To microscopically evaluate the adhesion to the dura mater, three levels of scoring were made, similar to the scoring of He et al. (7),

Grade 0 = when the dura mater was free of the scar tissue.

Grade 1 = when only thin fibrous band(s) between the scar tissue and dura mater were observed.

Grade 2 = when continuous adherence was observed but was less than two thirds of the laminectomy defect.

Grade 3 = when scar tissue adherence was large, more than two thirds of the laminectomy defect, and/or extended to the nerve roots.

*Statistical analysis*

The Shapiro-Wilk test was used to determine whether the data showed normal distribution. A comparative analysis among three groups was conducted utilizing the Kruskal-Wallis test. The Dunn-Bonferroni test was used as a post hoc test. According to the post-power analysis, the effect size for the adhesion difference between the groups was obtained as partial $\eta 2 = 0.291$ and statistical power = 0.732, with a type 1 error of 0.05. IBM SPSS Version 22 program was used for statistical tests.

## Results

After laminectomy, dense connective tissue with fibroblasts was determined to fill the operation region in the control group. It was observed that the dura was thickened and completely adhered to the granulation tissue in the posterior (Figure 1). In the fascia group, in the histopathological examination of the levels where the fascia autograft was placed, it is seen that the dura is thickened, the operation area is again filled with dense granulation tissue, the fascia forms a barrier between the dura mater and the granulation tissue, but the fascia shows adhesion with the dura mater (Figure 2). In the Gore-Tex group, granulation tissue covered the laminectomy defect area with the Gore-Tex surgical membrane. Still, there was no adhesion to the Gore-Tex surgical membrane. In this region, it is observed that the Gore-Tex surgical membrane creates a mechanical barrier between the dura mater, neural tissue, and granulation tissue (Figure 3). Descriptive statistics of the groups are shown in Table-1.

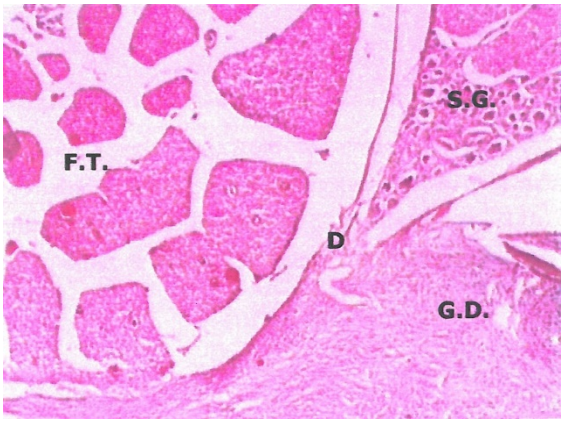


**Figure 1.** Findings of adhesion of granulation tissue with the dura mater and neural tissue in the laminectomy area. G.D., granulation tissue; D, dura mater; F.T., filum terminale; S.G., sympathetic ganglion. (HE x 44).

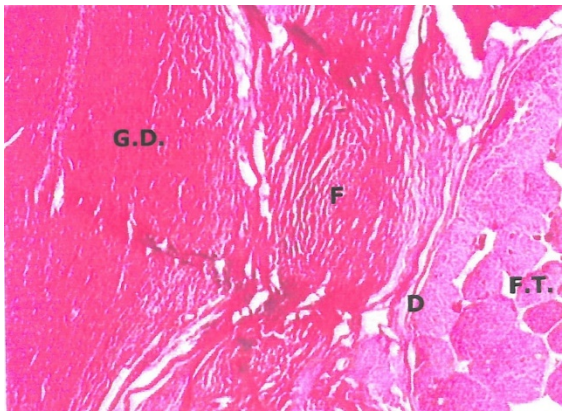


**Figure 2.** It is seen that fascia forms a barrier between the dura mater and neural tissue and granulation tissue but cannot prevent adhesion. G.D., granulation tissue; D,dura mater; F.T., filum terminale; F, Fascia. (HE x 27.5).

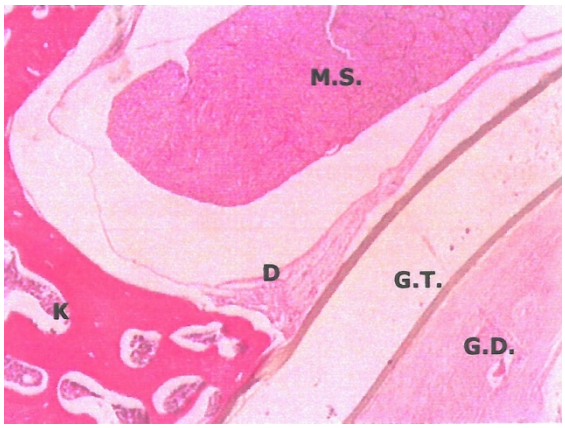


**Figure 3.** Although the dura mater thickens where the Gore-Tex Surgical Membrane is applied, no adhesion is observed. K, bone; G.D., granulation tissue; D, dura mater; M.S., medulla spinalis; G. T., Gore-Tex Surgical Membrane. (HE x 27.5).

The data did not show a normal distribution (p=0.022, the Shapiro-Wilk test). Then, it was understood that the three groups were different (p=0.028, the Kruskal Wallis test). Gore-Tex group was found better than both the control and the fascia group. The details of pairwise comparison results between the groups are shown in Table-2.

## Discussion

After laminectomy, fibroblasts originating from the deep surfaces of the paravertebral muscles migrate to the laminectomy area and replace the epidural hematoma found there, causing dense scar tissue to form (8,9). Ligamentum flavum, posterior longitudinal ligament, bone, and annulus fibrosus are known as the sources of fibroblasts. Factors that stimulate the chemotaxis and migration of fibroblasts include blood products resulting from the breakdown of red blood cells (10,11).

Dural grafts are needed for dural defects occurring after spinal dysraphism, Chiari malformation, vascular malformations, tumor operations, and other surgical procedures (3,5,6,12). Among the many materials used to prevent the formation of epidural granulation tissue and dural adhesions, the most accepted ones are those that create physical barriers (6,8,10,13,14). Researchers LaRocca and Macnab placed Gelfoam and a silastic sheath between the dura and muscles to prevent the formation of peridural granulation tissue after laminectomy (8). Yong-Hing et al. (13) and Chen et al. (14) investigated the effectiveness of applying Gelfoam and free fat grafts to the laminectomy area. These studies found that Gelfoam breaks down by creating a foreign body reaction and has no effect on epidural granulation tissue and adhesions to the dura.

**Table 1.** Summary of parameters in each group.

| | Groups | | | Test Statistics | | | |
|---|---|---|---|---|---|---|---|
| | Control Group | Fascia Group | Gore-Tex Group | Test value | *p* | partial $\eta^2$ | Power |
| Number of Animals | 10 | 6 | 10 | | | | |
| Adhesion scores to the dura mater | | | | | | | |
| Mean ± SD | 2.0±0.7$^a$ | 2.0±0.0$^a$ | 1.2±0.8$^b$ | 7.138$^\dagger$ | 0.028 | 0.291 | 0.732 |
| 95.0% Confidence interval | (1.5-2.5) | (2.0-2.0) | (0.6-1.7) | | | | |
| Median | 2 | 2 | 1 | | | | |
| Min; Max | 1; 3 | 2; 2 | 0; 2 | | | | |

*Data are given as mean±standard deviation (95.0% Confidence interval) and median (min; max). $^\dagger$: Kruskal-Wallis H test, a and b superscripts indicate differences between groups. There are no statistically differences between groups with the same superscripts. The p values of the Dunn-Bonferroni post hoc test comparing adhesion scores between groups: Control Group vs Fascia Group p= 1.000, Control Group vs Gore-Tex Group p= 0.031, Fascia Group vs Gore-Tex Group p= 0.019.*

**Table 2.** The p values of the Dunn-Bonferroni post hoc test comparing adhesion scores between groups.

| | Control Group | Fascia Group | Gore-Tex Group |
|---|---|---|---|
| **Control Group** | NA | 1.000 | 0.031 |
| **Fascia Group** | 1.000 | NA | 0.019 |
| **Gore-Tex Group** | 0.031 | 0.019 | NA |

**Purple: NA; Green: All p values <0.05; Blue: All p values >0.05*

Another dural graft material is lyophilized dura taken from human cadavers. It is used in the repair of spinal dural defects and meningomyeloceles. However, it has the disadvantage of carrying infectious diseases. Jakob-Creutzfeld (JCD) cases transmitted via lyophilized human dura mater have been reported (15-19).

It has been suggested that steroids inhibit fibroblast proliferation and mucopolysaccharide

protein synthesis. Based on this idea, steroidal materials and direct depot steroids have been applied to the laminectomy area experimentally and clinically, and it has been reported that adhesion is less in the areas where steroids are used (13,14).

Among the dural graft materials, autogenic fat grafts have the best results in repairing spinal dural defects and are very useful in preventing granulation tissue formation after lumbar laminectomy (3). Nevertheless, alternative graft materials become necessary when the donor areas are afflicted or compromised by disease, the person refuses graft removal for cosmetic reasons, or in traumatic and emergency cases where additional time cannot be spent for autogenous graft harvesting (12,20).

Another material is the Gore-Tex surgical membrane. Developed in 1969 and trademarked by W.L. Gore & Associates, Gore-Tex is a fabric membrane renowned for its exceptional waterproofing capabilities. It consists of expanded polytetrafluoroethylene (PTFE). It is a soft, synthetic, biologically inert, non-absorbable material that can be easily shaped and sutured (10). Its less porous and densely intertwined fibrous structure suppresses cell proliferation and creates minimal adhesion. It is used for various purposes, such as preventing tissue adhesion in cardiovascular surgery (21,22). Vakis et al., in their case series in 2006, stated that the Gore-Tex surgical membrane helps prevent adhesions after decompressive craniectomies (5). In their study on pediatric Chiari malformation patients 2008, Attenello et al. found the Gore-Tex surgical membrane superior to the pericranial autograft (6). In a study by Park et al., the Gore-Tex surgical membrane was compared with collagen-coated vicryl mesh and lyophilized spinal dural allograft material. It was more beneficial than these two materials in preventing arachnoidal adhesions and inflammation after spinal dural repair (1). Again, it was recommended in this study to do experimental trials comparing the Gore-Tex surgical membrane to autologous fascia.

Many studies have used dural materials for cranial dural repair (3,5,6,12,23), and there has been very little experimental research on spinal duraplasty. (4,10,24,25). In this study, we aimed to prevent the granulation tissue from coming to the epidural space and the resulting granulation tissue from adhering to the dura mater and nerve roots by placing a physical barrier between the granulation tissue and the dura mater, and we compared the effects of autologous fascia taken from the lumbar region and Gore-Tex surgical membrane. Based on the results obtained, it was observed that the fascia formed a physical barrier between the dura mater and neural tissue and the granulation tissue, whereas the granulation tissue adhered to the fascia. These findings make us think it will create difficulties in cases requiring reoperation. However, the Gore-Tex surgical membrane could interrupt the contiguity between the granulation tissue, the dura mater, and the neural tissue. It has been observed that even if epidural granulation tissue forms, no adhesion to the Gore-Tex surgical membrane occurs, and therefore, it is superior to autologous fascia. These findings are consistent with Kurt et al. in 2009 and Topsakal et al. in 2004 (4,25).

## Conclusion

This study showed that the granulation tissue did not adhere to the Gore-Tex surgical membrane, and the Gore-Tex surgical membrane created an excellent physical barrier preventing adhesion between the dura mater and neural and granulation tissues. The biologically inert nature of the Gore-Tex surgical membrane, its compatibility with the body, appropriate placement on the dural defect, and an ease of suturing can be considered surgical advantages.

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## Conflict of interest statement

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Ethics Committee Approval:** The Ege University Animal Experiments Local Ethics Committee granted the necessary approval for the study (dated 12.03.2002 and numbered 2002.08).

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# Rat PKU Model Display Gender-Based Neuroinflammatory Changes: Proinflamatuary Cytokines and Lipid Peroxidation

# Sıçan PKU Modeli Cinsiyete Dayalı Nöroinflamatuar Değişiklikler Gösterir: Proinflamatuar Sitokinler ve Lipid Peroksidasyonu

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**Öz**

Fenilketonüri (PKU), amino asit metabolizmasının konjenital kusurlarından kaynaklanır. Birikmiş fenilalanin kan-beyin bariyerini geçer ve kalıcı beyin hasarına neden olur, ancak fenilketonürinin altında yatan nöro-patofizyoloji tam olarak anlaşılamamıştır. Prefrontal kortekste inflamatuar yanıtın, lipit peroksidasyonunun ve oksidatif stresin rolünü incelemek için her iki cinsiyete ait kimyasal olarak indüklenmiş sıçan Fenilketonüri modeli oluşturuldu. Sonuçlarımız Fenilketonüride kontrollere kıyasla lipit peroksidasyonunda artış olduğunu gösterdi; bu artış sadece erkeklerde anlamlı derecede farklıydı ($p<0.001$). Erkek sıçan PKU gruplarında serum triptofan ($p<0.001$) ve interlökin-1β düzeylerinde ($p=0.014$) erkek kontrollere göre anlamlı farklılıklar gözlendi. Bu çalışma ile cinsiyete ilk kez bir PKU modelinde cinsiyete bağlı nöroinflamasyon ve lipid peroksidasyonunda değişiklikler rapor edilmiştir.

**Anahtar Kelimeler:** Cinsiyete Temelli, Fenilketonüri, Nöroinflamasyon, Oksidatif Stres, Proinflamatuar Sitokinler

**Abstract**

Phenylketonuria (PKU) results from congenital defects of amino acid metabolism. Accumulated phenylalanine crosses the blood-brain barrier and causes permanent brain damage, but the neuro-pathophysiology underlying phenylketonuria is not fully understood. Chemically-induced rat phenylketonuria model of both genders was generated to examine the role of inflammatory response, lipid peroxidation and oxidative stress in the prefrontal cortex. Our results showed that in phenylketonuria there was an increase in lipid peroxidation compared to controls, which was significantly different only in males ($p<0.001$). In male rat PKU groups, statistically significant differences were also observed in serum tryptophan ($p<0.001$) and interleukin-1β levels ($p=0.014$) as compared to male controls. In this study, gender-based changes in neuroinflammation and lipid peroxidation were reported for the first time in a PKU model.

**Keywords:** Gender Based, Phenylketonuria, Neuro-inflammation, Oxidative Stress, Proinflammatory Cytokines

## Introduction

Phenylketonuria (PKU, MIM 261600) is characterized by toxic levels of phenylalanine (Phe) accumulation due to the mutation of Phe hydroxylase and dihydropteridine reductase deficiency (PAH, EC 1.14.16.1) (1,2). In patients with PKU, phenylalanine that cannot be converted to tyrosine accumulates in blood and other tissues. Crossing the blood-brain barrier, the accumulated phenylalanine causes irreversible progressive brain damage, mental retardation, behavioral problems and neuro-inflammation (3). Many researches have been published to elucidate the mechanisms underlying brain damage caused by PKU; however, no single factor has been identified as being directly responsible for it.

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In recent years, many studies have reported alterations in oxidative stress parameters in many congenital disorders of mediator metabolism, including PKU, both in animal models and in patients (4,5). It has been suggested that biomolecules such as lipids, proteins and DNA are affected by oxidative damage (6,7). In this context, data from in vitro and in vivo studies have shown that accumulation of Phe and its metabolites leads to decreased antioxidant defenses and increased oxidative damage in the rat brain (8,9). All these findings on oxidative damage suggest that it might contribute to the neurological symptoms observed in PKU patients (10). Oxidative stress is commonly observed in some inborn errors of intermediary metabolism. Although the reason for this oxidative stress is not completely understood, it may be caused by the accumulation of toxic metabolites that lead to the excessive production of free radicals. It may also be that an unusual increase in metabolic by-products will directly, or indirectly, depletes a cell's antioxidant capacity (4).

Cytokines are signaling molecules released from peripheral immune cells (monocytes, macrophages and lymphocytes). They act as intercellular messengers and are associated with activation of the immune system, cell differentiation and cell death

(11). Cytokines can be divided into pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α, which activate inflammation (12). Inflammatory cytokines are released from glial cells in the brain. Pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α mediate microglial activation. By increasing inflammation, it contributes to the inflammation-mediated progression of neurodegenerative diseases (13).

Monoamine oxidase (MAO) (14) catalyzes the oxidation of monoamines which inactivates monoamine neurotransmitters such as serotonin, dopamine, adrenaline, and noradrenaline. A number of psychiatric and neurological disorders are thought to be caused by abnormal monoamine oxidase expression. Many studies have also linked increased MAO activity to increased oxidative stress and inflammation as potential deleterious by-products of oxidative deamination, aldehydes, hydrogen peroxide, and ammonia are constantly produced (15). Hence, MAO enzyme has been implicated with neuro-inflammation in several studies. Furthermore, MAO activation promotes cognitive impairment (16), cholinergic neuron destruction, and cholinergic system abnormalities (17).

In this study, an animal model of chemically-induced PKU was generated and proinflammatory cytokines, oxidative stress related parameters, and lipid peroxidation in the prefrontal cortex of rat brain were analyzed. Since the prefrontal cortex (PFC) has been reported to contribute to cognitive deficits (18), this study focused on the PFC. Our results display that while PKU rats had higher lipid peroxidation than controls, the significance was more pronounced in males. All observed parameters displayed a difference between control and PKU groups with a bias to gender. These findings suggest that IL-1β-proinflammatory cytokine-, and, MDA-lipid peroxidation-, in phenylketonuria differ by gender.

## Material and Method

*Reagents*

Unless otherwise stated in the text, all chemicals were purchased from Millipore Sigma (St. Louis, MO, USA). Both of the stock solutions containing 152 µmol/ml phenylalanine and 26 µmol/ml p-chloro-phenylalanine (p-Cl-Phe - a PAH inhibitor), were prepared in 0.9% sodium chloride solution (saline) by heating at 37 °C for 1 h on the day of the experiments and had its pH adjusted to 7.4.

*Animals*

20 female and 20 male Sprague-Dawley rat pups (6 days old, weight 5 ± 2 grams) were used. In total, the pups of three mothers were randomly divided into male and female groups before sacrification. Animals were maintained under 12 h light-dark-cycle (lights on at 7:00 AM), at constant temperature (22 ± 1 °C) and with free access to food and water. All experimental procedures were designed to minimize the number of animals used and their suffering. The procedures were approved by Hacettepe University Committee on Animal Ethics (Protocol No 2022/07-16).

*Generation of rat PKU model*

Rat PKU model was generated as previously described (19). A total of 20 female and 20 male Sprague-Dawley rat pups (6 days old, weight 5 ± 2 g) were used in the study. Briefly, animals were divided into two groups: control animals received subcutaneous administration of 0.9% sodium chloride solution (saline) daily. PKU animals received phenylalanine (5.2 mmol/g of body weight) injection daily while the phenylalanine hydroxylase inhibitor and tyrosine hydroxylase inhibitor (20), p-Cl-Phe (0.9 mmol/g of body weight) injection was administered every other day. For all groups, injections started on the postnatal 6$^{th}$ day and continued until the postnatal 20$^{th}$ day (19,21,22). Animals were euthanized by decapitation without anesthesia and the brain was rapidly excised on a Petri dish placed on ice. The prefrontal cortex was dissected and stored at -80°C until used for the experiments. All injections were performed between 7:00-9:00 AM for the duration of the experiment.

*Prefrontal cortex and hippocampus homogenate preparation*

The prefrontal cortex was removed for biochemical analysis. Prefrontal cortex was homogenized %10 (w/v) by Ultra-Turrax ® (S8N-5 g, IKA-Werke GmbH) on ice for 3 x 10 seconds in 50 mM Tris pH 7.4 buffer containing 2 mM EDTA, 0.5% Triton X-100, and protease inhibitor cocktail. All treatments were done on ice to prevent protein denaturation. The solutions used were also kept on ice. Homogenates were centrifuged for 15 minutes at 13,000 g at +4°C. The supernatant was removed and used in the determination of selected parameters. Total protein was measured by the method of Lowry et al. (23) using bovine serum albumin as a standard.

*Serum tryptophan determination*

Blood samples were collected in EDTA tubes in order to obtain serum by centrifugation at 10,000 g for 10 min at +4°C and then tryptophan level was measured by HPLC (Shimadzu DGU-20A3). All results were calculated as mg/dl.

*Determination of Lipid Peroxidation*

Malondialdehyde levels were used to determine lipid peroxidation using a Cayman Chemical TBARS assay kit (Catalog No. 10009055, Ann Arbor, MI, USA). In a summary, 100 µL of the supernatant were prepared by centrifuging the homogenate at 1600 g for 10 minutes at 4°C, then mixing it with 100 µL of sodium lauryl sulfate lysis solution in glass tubes. The mixture was boiled for 45 minutes at 95°C after being incubated with thiobarbituric acid (TBA). After cooling the tubes on ice for 5 minutes, they were centrifuged at 10,000 g for 15 minutes, collecting 200 µL of supernatant fluid and measuring absorbance at 532 nm with a Molecular Devices SpectraMax M2 microplate reader (San Jose, CA, USA). The results were calculated using standard graphics. MDA concentration was reported in µM/mg protein.

*Monoamine Oxidase Activity*

BioVision Total Monoamine oxidase activity kit (catalog number: K795-100) was used to measure total MAO activity. The test is based on the fluorometric detection of $H_2O_2$, one of the by-products produced during oxidative deamination of the MAO substrate. Total MAO activity was measured kinetically through fluorescence (Ex/Em = 535/587 nm) at 25°C on a SpectraMax M2 microplate reader (Molecular Devices, CA, USA) at 25°C. The results were determined by considering the amount of peroxide formed. All activity results were calculated as µU/mg protein.

*Oxidative Stress Related Enzyme Activity*

*Glutathione Peroxidase Activity*

Glutathione peroxidase (GPx) activity was measured using the method set forth by Flohe and Günzler (24). The final activity medium contained 100 mM potassium phosphate buffer, 0.2 mM NADPH, 1 mM reduced glutathione (GSH), 1 mM EDTA, 4 mM sodium azide, 100 U/ml glutathione reductase enzyme, 0.1 mM hydrogen peroxide. Enzyme activity was determined by monitoring the decrease in absorbance at 340 nm for 10 minutes on the SpectraMax M2 microplate reader. The GPx activity unit was defined as the amount of enzyme that catalyzed the oxidation of 1 µmol of NADPH in 1 minute under these conditions, and the results of the GPx activity were given as µU/mg protein.

*Glutathione Reductase Activity*

Glutathione reductase (GR) activity was determined according to the Stall method (25) with a slight modification. The activity medium contained 100 mM Sodium phosphate buffer pH = 7.4, 0.2 mM NADPH, 1 mM oxidized glutathione (GSSG) and sample in the final. The results were immediately read kinetically for 10 minutes at a wavelength of 340 nm on a Spectramax M2 microplate reader from Molecular Devices. The results were calculated on the absorbance decrease of NADPH at 340 nm. A unit of activity (U) was defined as the amount of enzyme that catalyzes the oxidation of 1 µmol of NADPH in 1 min under these conditions. GR activity was given as µU/mg protein.

*Superoxide dismutase Activity*

Superoxide dismutase (SOD) activity was measured using the BioVision SOD activity kit (Catalog no: K335-100). The principle of method is based on WST-1, which forms a water-soluble formazan dye when reduced with the superoxide anion, is used in the sensitive SOD assay kit. The reduction rate with a superoxide anion is proportional to the activity of Xanthine Oxidase (XO) suppressed by SOD. All results were given as µU/mg protein.

*Determination of proinflammatory cytokines: IL-1β, IL-6, TNF-α*

Bioassay Technology Laboratory IL-1β Elisa kit (catalog number: E0119Ra), Bioassay Technology Laboratory TNF-α ELISA kit (catalog number: E0764Ra) and Bioassay Technology Laboratory IL-6 ELISA kit (catalog number: E0135Ra) were used to measure IL-1β, TNF-α and IL-6, respectively. 50 µl of standard and 40 µl of sample was added to the antibody-coated wells. 10 µL of biotinylated TNF-α, IL-1β and IL-6 was added to the sample wells. Then, 50 µl of streptavidin-HRP was added to all wells and incubated at 37°C for 1 hour. Following incubation, the wells were washed four times with 200 µL of wash buffer solution. After removal of the liquid, 100 µL of chromogenic substrate was added to each well and incubated for 10 minutes at 37°C in the dark. 50 µL of stop solution was added per well to prevent color formation reaction and the results were quickly measured at 450 nm with a Molecular Devices SpectraMax M2 microplate reader. The results were obtained from the standard chart and given as pg/mg protein.

*Statistical analysis*

Since the Shapiro-Wilk test is a more appropriate method for small sample sizes (<50 samples), the Shapiro-Wilk test was used to test whether the samples showed normal distribution (26). Data with normal distribution were analyzed by the Student's *t*-test for unpaired samples and expressed as mean ± standard deviation (SD) (n=7). Values of $p<0.05$ were considered significant. All analyses were performed in GraphPad Prism Software Version 9.0 (San Diego, CA, USA).

## Results

To assess the involvement of neuro-inflammation and oxidative stress in PKU, a chemically-induced PKU model in male and female rats was established as performed previously (22)

and the selected parameters were analyzed in PFC homogenates. Neurotransmitter systems were assessed through monoaminergic neurotransmitter activities, while the lipid peroxidation products, anti-oxidant enzyme activities, and cytokines were analyzed to evaluate oxidative stress response and neuro-inflammation.

*Neuro-inflammation-related cytokine levels*

To evaluate neuro-inflammation, IL-1β, IL-6, and TNF-α from the prefrontal cortex were analyzed by ELISA. There was no significant difference in, IL-6 and TNF-α levels in neither group. The cytokine levels displayed a steady state. Briefly, median of IL-1β was found as 112.30 pg/mg vs 117.9 pg/mg in control males and PKU males, respectively (Fig 1B, Control male: 112.30±4.19, PKU male: 117.9±4.18 (p=0.014). IL-6 levels in control females were 1.54±0.35 pg/mg, and 1.41±0.29 pg/mg in PKU females (p=0.962) (Table 1).

*Serum Trp levels*

The serum Trp levels stayed at a constant level in female rat groups. Meanwhile, in PKU male rats statistically significant difference (p<0.001; Fig. 2C). In serum tryptophan levels were observed. Median of Trp in PKU male group was found 1.88 mg/dl while median of Trp in control male group was 2.56 mg/dl. These results display that Trp level differ with bias to gender in PKU (Table 1, Control male: 2.56±0.49 PKU male: 1.88±0.37).

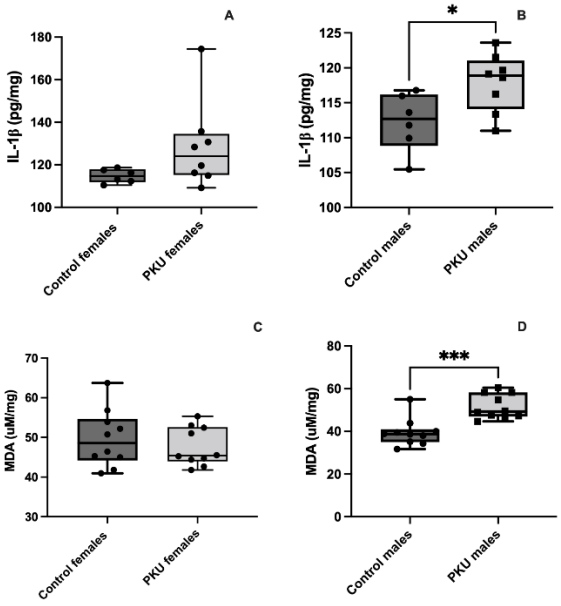


**Figure 1.** IL-1β (pg/mg) and MDA (uM/mg) levels in the PFC of phenylketonuria rat pups. P-Cl-Phe injection started on the 6th postnatal day and continued until the 20th postnatal day. **A)** IL-1β (pg/mg); Control Female: 114.7±3.20; PKU female: 128.7±20.50 **B)** IL-1β (pg/mg); Control male: 112.30±4.19. PKU male: 117.90±4.18 **C)** MDA (uM/mg); Control Female: 49.65±7.17; PKU female: 47.61±4.85 **D)** MDA (uM/mg); Control male: 39.51±6.42. PKU male: 51.56±5.82. Statistical significance was determined by One-tailed unpaired t test. The data were expressed as the as mean±standard deviation. *p≤0.05. **p≤0.01. ***p<0.001.

**Table 1.** Descriptive Statistics Table

| | PKU | | Control | | p* |
|---|---|---|---|---|---|
| | Mean | Std. Deviation | Mean | Std. Deviation | |
| **IL-1 β female** | 128.70 | 20.50 | 114.70 | 3.20 | 0.064 |
| **IL-1 β male** | 117.90 | 4.18 | 112.30 | 4.19 | 0.014 |
| **IL-6 female** | 1.41 | 0.29 | 1.54 | 0.35 | 0.962 |
| **IL-6 male** | 1.46 | 0.29 | 1.53 | 0.38 | 0.982 |
| **TNF-α female** | 27.21 | 14.97 | 22.81 | 7.39 | 0.234 |
| **TNF-α male** | 22.79 | 11.94 | 18.98 | 3.58 | 0.216 |
| **SOD female** | 94.03 | 0.94 | 96.86 | 1.52 | <0.001 |
| **SOD male** | 93.80 | 5.93 | 95.40 | 2.46 | 0.248 |
| **MDA female** | 47.61 | 4.85 | 49.65 | 7.17 | 0.232 |
| **MDA male** | 51.56 | 5.82 | 39.51 | 6.42 | <0.001 |
| **GR female** | 24.05 | 20.96 | 37.86 | 22.03 | 0.191 |
| **GR male** | 18.94 | 15.30 | 20.96 | 9.19 | 0.900 |
| **GPx female** | 24.51 | 21.86 | 17.43 | 6.16 | 0.169 |
| **GPx male** | 13.95 | 9.91 | 11.54 | 6.34 | 0.270 |
| **MAO female** | 3.02 | 2.81 | 10.97 | 13.61 | 0.077 |
| **MAO male** | 3.63 | 3.98 | 2.29 | 2.90 | 0.269 |
| **Trp female** | 2.18 | 0.40 | 2.15 | 0.63 | 0.456 |
| **Trp male** | 1.88 | 0.37 | 2.56 | 0.40 | <0.001 |

*One-tailed unpaired t test result. The mean difference is significant at the 0.05.

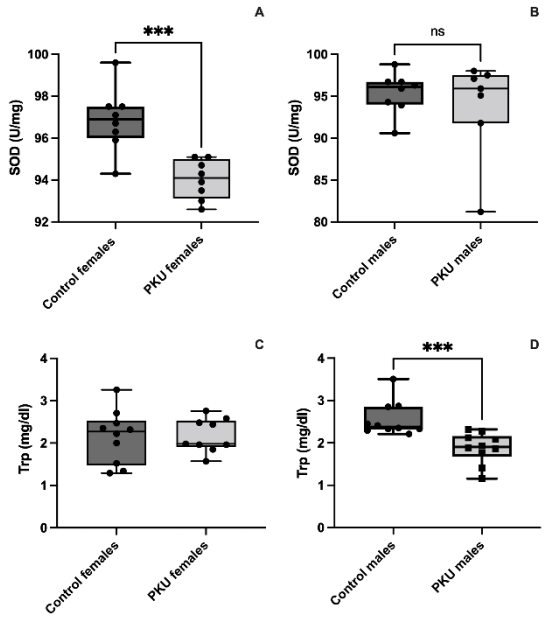


**Figure 2.** SOD (U/mg) and Trp (mg/dl). in the PFC of phenylketonuria rat pups. P-Cl-Phe injection started on the 6th postnatal day and continued until the 20th postnatal day. **A)** SOD (U/mg); Control Female: 96.86±1.52; PKU female: 94.03±0.94 **B)** SOD (U/mg); Control male: 95.4±2.46. PKU male: 93.80±5.93 **C)** Trp (mg/dl); Control Female: 2.15±0.63; PKU female: 2.18±0.40 **D)** Trp (mg/dl); Control male: 2.56±0.40. PKU male: 1.88±0.37. Statistical significance was determined by One-tailed unpaired t test. The data were expressed as the as mean±standard deviation. *p≤0.05. **p≤0.01. ***p <0.001.

*Measurements for oxidative stress and lipid peroxidation*

Superoxide dismutase (SOD) enzyme activity reduced in female PKU rats compared to female rats in control group (p<0.001; Fig 2A; 96.86±1.52 µU/mg protein vs 94.03±0.94 µU/mg protein in control and PKU groups, respectively). SOD activity did not display significant difference in male PKU rats compared to control group male rats (Table 1). The lipid peroxidation in the PFC revealed differences between genders in this rat PKU model. In the PFC mean of MDA levels in female groups, 49.65±7.17 µM/mg vs 47.61±4.85 µM/mg MDA in control and PKU females respectively, it was not statistically significant. However, in PKU male groups (51.56±5.81 µM/mg), MDA levels were significantly increased compare to their control (39.51±6.42 µM/mg) (p<0.001) (Figure 1D). These results showed that in this PKU model, males were more susceptible to the effects of lipid peroxidation as opposed to females.

A large variance in individual MAO activities it was not reflected in statistical results (Table 1). Mean of PKU female group was 10.97 µU/mg while mean of control female group was found as 3.92 µU/mg (Control Female: 10.97±13.61, PKU female: 3.02±2.81. Mean of PKU male group was 3.63 µU/mg while mean of control male group was 2.29 µU/mg (Control male: 2.94±2.90, PKU male: 3.63±3.98).

Oxidative stress was assessed through several parameters. The actual damage caused was measured through TBARs assay, which measured the major lipid oxidation product malondialdehyde, is a good index of the level of oxidative stress whereas the response was evaluated through the antioxidant enzymes glutathione peroxidase (GPx), and Glutathione reductase (GR) (Table 1).

Glutathione peroxidase (GPx) activity was not found significant as opposed to control group (Table 1). In males, mean of PKU group GPx activity was 13.95 µU/mg protein in their controls versus 11.54 µU/mg PKU group (Control male: 11.54±6.34, PKU male: 13.95±9.91). In female mean of PKU group GPx activity was 24.51 µU/mg protein while mean of their control was 17.43 µU/mg protein in PKU (Control Female: 17.43±6.16; PKU female: 24.51±21.86. The activity of GR, the anti-oxidant enzyme, not changed according to groups (Table 1).

Post hoc power analysis was performed according to the 5% type 1 error (p<0.05) threshold based on the IL-1β values of the male PKU and male control groups. In G*power 3.1.9.6 software, two independent means comparison was selected for t tests and by entering the mean values of the groups (male pku vs male control), the effect size was found to be 1.33. With this data and the number of cases per group, the power (1-beta) was found to be 0.75 (75%). Likewise, post hoc power analysis was performed according to the 5% type 1 error (p<0.05) threshold, based on the MDA values of the male PKU and male control groups. In G*power 3.1.9.6 software, two independent means comparison was selected for t tests and by entering the mean values of the groups (male pku vs male control), the effect size was found to be 1.96. With this data and the number of cases per group, the power (1-beta) was found to be 0.99 (99%).

## Discussion

MDA is a marker of lipid peroxidation associated with increases in patients with traumatic and non-traumatic brain injury and PKU. However, molecular studies identifying individual lipids and their oxidatively altered molecules are needed to understand how these relate to the development of chronic complications in PKU. Studies have reported that PKU causes an increase in oxidative stress, lipid peroxidation and inflammation (27). In our study, while there was no change in the PKU female group, lipid peroxidation increased along with inflammation in the PKU male group. Our study is the first to reveal the relationship between inflammation and lipid peroxidation in PKU on a

gender basis. In a study comparing the MDA levels of male PKU patients and the male control group, it was reported that the MDA level was statistically higher in the PKU group (28). In our study, lipid peroxidation in the PFC revealed gender differences in the rat PKU model. Oxidative stress has been identified as an important pathophysiological feature of various inborn errors of metabolism, including phenylketonuria. In the reported study, proinflammatory cytokines IL-1β and IL-6 were significantly increased in PKU. This indicates that inflammation has occurred and provides evidence that it has occurred (29). These results are consistent with our results.

Currently, the relationship between MAO and inflammation has not been fully explained. For decades, researchers have systematically supported the role of MAO-related oxidative stress in various metabolic pathologies and cardiovascular diseases. The induced inflammatory load has been associated with the contribution of the MAO enzyme to hypertension, metabolic disorders, chronic kidney disease, and vascular oxidative stress (30). It was recently reported that mitochondrial MAO enzymes contribute to inflammation associated with endothelial dysfunction in mice (31). Type A and B monoamine oxidases (MAO-A, MAO-B) mediate and modulate intracellular signaling pathways for neuronal cell survival or death. Although the effect of MAO activity on BDNF expression has not yet been reported, the use of MAO-B inhibitors has been reported to increase BDNF expression (32). In our study, we predicted that MAO activity would increase in the PKU model, but MAO activity did not change. MAO enzymes have been linked to neuro-inflammation and oxidative stress as a result of their activity which generates hydrogen peroxide and ammonia as byproducts. It has been reported that newborns have low MAO-B activity and act as a modifying gene in phenylketonuria (33). Discussion has been limited because measurement of MAO activity in the PKU model has not been previously reported. In our study, no difference between control and PKU groups in total MAO activity is found. A recent paper reported that tryptophan levels decreased in PKU patients compared to the control group (34) but the authors did not define the findings with regards to gender. In comparison, we reported a decrease in tryptophan level only in the male PKU group as compared to the male controls. There was no change in tryptophan level between PKU and control groups in the females. According to the results, SOD enzyme activity did not make a significant difference between the groups.

Our findings did not show a significant difference in PFC GPx activity when the control and PKU rat groups were compared according to gender. There is already a debate about whether GPx is impaired in PKU. GR activity in the PFC was not change PKU groups of both genders as compared to their controls. In the literature, it has been shown in previous study that GR activity decreased in the PKU group (35). However, in this study, the results were not published according to gender.

Superoxide free radicals are converted by SOD into hydrogen peroxide, a less reactive molecule. In the hippocampus, it has been reported that the PKU group showed lower SOD activity than the control group (36). Although no change was observed in the male PKU group. TNF-α level was unchanged in both groups. When the literature was searched, it was determined that neither brain nor serum levels of TNF-α were measured in PKU. There is no scientific study related to IL -1β and PKU. However, we report for the first time statistically increased IL-1β in the male PKU group compared to control males. In literature blood IL-6 levels in PKU state no difference between controls and PKU. However, there was no statistically significant difference between the blood levels of PKU and IL-6 (37). According to our results, no change was observed in IL-6 levels.

There is substantial evidence that the dorsolateral prefrontal cortex serves critical cognitive abilities even in early infancy. It has been shown that in adult monkeys these cognitive abilities are critically dependent on dopaminergic projection to the prefrontal cortex, and there is a change in the distribution of dopamine axons in the dorsolateral prefrontal cortex (38). In a four-year longitudinal study, it was shown that these deficits are in working memory and inhibitory control functions related to the dorsolateral prefrontal cortex in children with PKU whose plasma Phe levels are 3-5 times normal. In another study involving PKU and prefrontal cortex reports that the behavioral disorder in PKU is caused by changes in the dopamine system in the frontal cortex (39). Although the relationship between the prefrontal cortex and cognitive competencies was revealed in the PKU animal model created, the relationship between oxidative stress and prefrontal cortex in PKU was not revealed (40). Considering that the prefrontal cortex has an effect on cognitive functions, it can be thought that neuro-inflammation in the prefrontal cortex also has an effect on the pathophysiology of PKU.

It is known that cognitive abilities are weakened in phenylketonuria. There is substantial evidence that the prefrontal cortex serves critical cognitive abilities even in early infancy. In our study, high dose of Phe on the prefrontal increased lipid peroxidation and IL-1β level in male gender. Significant gender differences were found in phenylketonuria. Adjuvant agents/treatments affecting the prognosis of the disease on a gender basis can be considered. The increase in Phe in the brain increase the possible inflammation, but in the results, we found, it is clear that inflammation markers differ according to gender.

## Conclusion

Lipid peroxidation parameters, proinflammatory cytokines and, oxidative stress parameters were analyzed in the induced PKU rat model. In the study conducted with male and female groups, the male PKU group was found to have more abnormalities than the female PKU group.

## Conflict of interest statement

The authors declare that they have no conflicts of interest.

**Ethics Committee Approval:** The procedures were approved by Hacettepe University Committee on Animal Ethics (Protocol No 2022/07-16).

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# Traumatic Papillary Muscle Rupture Mimicking Infective Endocarditis

## Enfektif Endokarditi Taklit Eden Travmatik Papiller Kas Rüptürü

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**Öz**

Motorlu taşıt kazalarında künt travma, kalp kapakçıklarının ciddi bir şekilde yaralanmasına sebep olabilir. Sıklıkla mitral ve aort kapak etkilenir. Akut kalp kapakçığı hasarı, ciddi dispne ve kalp yetmezliğine yol açabilir. Triküspit kapağın etkilenmesi oldukça nadirdir. Klinik tablo çoğu vakada sessiz ilerleyebilir. Ekokardiyografi ile hastalar kolayca tanı alabilir. Olgu sunumumuzda, 46 yaşında erkek hastada triküspit kapak papiller kas rüptürü ve başarılı cerrahi tedavisi sunulmaktadır. Ateş ve kalp kapakçığı üzerindeki kitle görünümü efektif endokarditi taklit edebilir.

**Anahtar Kelimeler:** Kalp Yaralanması, Rüptür, Triküspit Kapak

**Abstract**

Blunt trauma due to motor vehicle accident may result in severe cardiac valve injury. The most part of lesions are about aortic and mitral valves. Acute on-set cardiac valve insufficiency may cause severe dyspnea or cardiac failure. Tricuspid valve injury due to blunt trauma is extremely rare and clinical manifestation is silent in majority of all cases. In case of echocardiographic evaluation, patient can be diagnosed easily with cardiac valve injury. Here we introduce a 46 year-old male patient suffering tricupid papillary muscle rupture and its successful surgical repair. Fever and a mass on the valve caused by multitrauma may mimic infective endocarditis.

**Keywords:** Heart Injury, Rupture, Tricuspid Valve

### Introduction

Isolated tricuspid valve insufficiency due to the valve apparatus pathology is extremely rare and accounted for 0.13% in all injury-related patients in the United States (1,2). One of the causes is the blunt chest trauma due to the motor vehicle accidents. This leads aortic isthmus injury, aortic dissection or transection, mitral valve chordal rupture and tricuspid valve (TV) chordal or papillary muscle rupture. We introduce a 46 year-old male who was hospitalized for the femur fracture after motor vehicle accident and his unexpected tricuspid valve insufficiency with symptoms and signs of infective endocarditis.

### Case

A 46 year-old male was admitted to the emergency service due to a motor vehicle accident. On arrival, he was asymptomatic and vital signs were in normal limits. Right tube thoracostomy was made due to pneumothorax and fracture left femur was detected. 3/6 pansystolic murmur was present at the left parasternal border. Clinical features of heart failure were not present. Blood tests parameters were in normal limits. Transthoracic echocardiogram revealed a moderate enlargement of right atrium and right ventricle (RV) with a normal ejection fraction. The flail TV leaflet was detected. Additional vegetation was seen on TV. Avulsion of the anterior papillary muscle was suspected. Colored Doppler determined a severe TV regurgitation with a velocity of 2.16 m/sec. However, intermittent fever as 38 °C was noted during the follow up. Leucocyte value was 25.97x103 / $mm^3$, C reactive protein value was 140, procalcitonin was 0.164, and blood culture showed a staphylococcus epidermidis presence in preoperative period. Infective endocarditis was suspected and antibiotic treatment was given. After 2 weeks of antibiotic treatment and feverness period, mass did not regress on transthoracic echocardiography and we decided to operate. We thought that visualization of the mass was adequate to diagnose of infective endocarditis and we did not prefer to perform a transesophageal echocardiography. Informed consent was taken from the patient.

Cardiopulmonary bypass (CPB) with bicaval cannulation was established in moderate hypothermia. Careful dissection to avoid any embolic event to the pulmonary arteries was performed. Via right atriotomy, anterior papillary muscle rupture was identified with a residual stump on the inner wall (Figure 1A). The anterior papillary muscle was reattached to its stump using 2 pledgeted 4-0 polypropylene sutures (Figure 1B). Modified De Vega annuloplasty was performed (Figure 1C, 1D). Saline test confirmed a good competency of TV (Figure 1C). Postoperative follow up was uneventful.

### Discussion

Motor vehicle accidents are important causes of isolated TV damage (3). The probable mechanism is a rapid deceleration force with an increased

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pressure in intracardiac right chambers (4). TV injury can occur by rupture of papillary muscle of any cusp or any of its chorda, and TV annular tear (5). Clinical presentation remains silent in some of cases (6). In the literature it is strongly advised that routine echocardiography should be performed in blunt chest trauma cases to be aware of silent TV damage and 3-dimensional echocardiography has a superiority (4,7).

Literature brings limited information about longterm follow up of repair of traumatic TV rupture. Serial echocardiographic examination is strongly advised to be aware of impairment of TV function (3). In case of RV dysfunction, early TV repair should be performed to recover RV function. Moreover, in urgent service physicians should be attentive of cardiac complications in case of blunt chest trauma and echocardiography is an initial and essential evaluation.

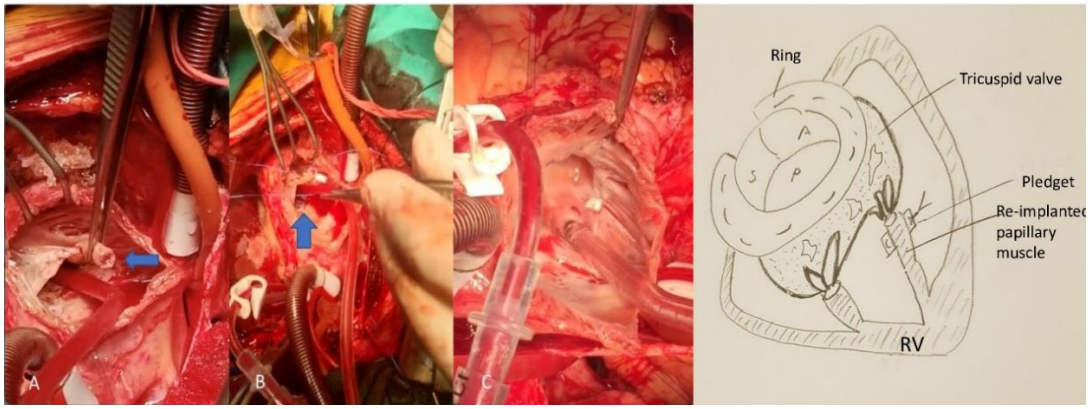


**Figure 1A.** The injured anterior papillary muscle, B: Re-implantation of ruptured anterior papillary muscle, C: Saline test after De-Vega annuloplasty, D: An illustration of complete tricuspid valve repair (illustrated by Hande İştar)

## Conclusion

Our patient was diagnosed with infective endocarditis due to the fever, mass on tricuspid valve, multiple positive blood culture that indicated staphylococcus epidermidis infection, consecutive leucocyte value increases, C reactive protein and procalcitonin positivity previously. This issue should be examined also with transesophageal echocardiography, this approach might give more information about nature of the mass on TV even if it would not change the decision for surgery. Moreover, computed tomography can be also used for determination of the mass on tricuspid in emergency service conditions. In conclusion, a mass on the valve and fever caused by multitrauma may mimic infective endocarditis and even though TV regurgitation can be silent in times, the earlier diagnosis and surgical repair provide prevention of right ventricular deterioration.

**Written consent:** Written consents of the patients were obtained on 13.05.2022.

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# A Case with Angelman Syndrome Carried *de novo* der(15q;15q) By *de novo* Paternal Uniparental Disomy

# *De novo* Paternal Uniparental Dizomiyle Ortaya Çıkan *de novo* der(15q;15q) taşıyan Angelman Sendromlu Olgu

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**Öz**

Angelman sendromu (AS; OMIM 105830), tipik olarak maternal kromozom 15q11.2-q13 delesyonu, Ubiquitin-protein ligaz E3A (UBE3A) gen mutasyonları, paternal uniparental disomi (UPD), imprinting merkez mutasyonlarının neden olduğu konjenital bir nörogelişimsel bozukluktur. UPD taşıyan sporadik AS oranı %2-3 olarak bilinmektedir. AS hastalarının yaklaşık %2-3'ünde paternal UPD saptanmıştır. Birçok rapor, UPD ile ilişkili AS olgularının heterodizomik olduğunu ileri sürmüştür. Bu yazıda AS tanısı konulan dört yaşında bir hasta sunulmaktadır. Hastanın dilini dışarı çıkaran geniş bir ağzı, her iki parmağını esnetmesi, zeka geriliğiyle birlikte salyası akması, konuşamaması, uyku bölünmesi, kendine zarar verme davranışı gibi dismorfik özellikleri gözlenmiştir. Angelman sendromu olgularının tanısında elektroensefalogram (EEG) bulguları önemli olmakla birlikte olgumuzda spesifik EEG ve ayrıca manyetik rezonans görüntüleme bulguları saptanmamıştır. Olgumuzda konvansiyonel sitogenetik yöntemle başlayan tanı sürecinde yeni nesil dizileme yöntemi kullanılarak genetik analiz tamamlanmıştır. 15. kromozomda iki uzun kolun Robertson tipi translokasyonu saptanan hastanın karyotipi 45,XX,der(15;15)(q10;q10)dn olarak tanımlanmıştır. Haplotip analizi, vakanın taşıdığı *de novo* rob(15q;15q) translokasyonun paternal kökenli 15 numaralı kromozom olduğunu göstermiştir. Literatürde UPD'li AS olgularının klinik bulgularının mikrodelesyonlulara göre daha hafif olması nedeniyle 15. kromozomun UPD'sini taşıyan AS olgularının gözden kaçabileceğini düşündürmektedir. Bu nedenle, burada sunulan vaka, geleneksel sitogenetik tarafından belirlenen der(15;15) translokasyonlarında, bireyin UPD için değerlendirilmesi gerektiğini gösteren iyi bir örnektir.

**Anahtar Kelimeler:** Angelman Sendromu, Sitogenetik, Translokasyonlar, Uniparental Dizomi, Yeni Nesil Dizileme

**Abstract**

Angelman syndrome (AS; OMIM 105830) is a congenital neurodevelopmental disorder typically caused by maternal chromosome 15q11.2-q13 deletion, Ubiquitin-protein ligase E3A (UBE3A) gene mutations, paternal uniparental disomy (UPD), or imprinting center mutations. The rate of sporadic Angelman syndrome carrying UPD is known to be 2-3%. Paternal UPD has been detected in approximately 2-3% of AS patients. Many reports have suggested that patients with UPD-associated AS cases are heterodisomic. We reported a case of a 4-year-old patient diagnosed with AS. She presented with dysmorphic features, including a wide mouth with protruding tongue, flexion of both fingers, drooling with mental retardation, absence of speech, disrupted sleep, without self-injuring behavior. Although electroencephalogram (EEG) findings are important to diagnosing AS, specific EEG and also magnetic resonance imaging (MRI) findings were not detected in our case. In the diagnostic process, which began with conventional cytogenetics, genetic analysis was completed using the next-generation sequencing method. A Robertsonian-type translocation of two long arms in derivative chromosome 15 was detected, defining the patient's karyotype as 45,XX,der(15;15)(q10;q10)dn. Haplotype analysis confirmed the presence of paternal uniparental disomy, indicating that the case carried a *de novo* rob(15q;15q) translocation. The literature, suggests that AS cases with UPD may exhibit milder clinical features compared to those with microdeletion. Consequently, AS cases involving UPD of chromosome 15 can sometimes be overlooked. Therefore, the case presented here serves as an example highlighting the need to evaluate individuals with translocations involving der(15;15) identified through conventional cytogenetics for potential UPD.

**Keywords:** Angelman Syndrome, Cytogenetics, Next-Generation Sequencing, Translocations, Uniparental Disomy

## Introduction

The frequency of Angelman syndrome is reported to be 1 in 15,000 and 1 in 20,000 individuals. Diagnosing Angelman syndrome involves considering clinical, behavioral, and developmental phenotypic features, along with electroencephalogram (EEG) findings. This diagnostic process is complemented by cytogenetic and molecular genetic analyses. Due to the gradual onset of signs and symptoms, which can overlap with other conditions, diagnosing the disease can be challenging (1). Genetic mechanisms associated with Angelman syndrome include the deletion of the 5-7 Mb 15q11.2-q13 region where the UBE3A gene resides, pathogenic intragenic deletions/insertions within the UBE3A gene, loss-of-function mutations involving missense, nonsense, or splice site mutations, and instances where both gene copies are inherited from the father, referred to as uniparental disomy (UPD). DNA methylation imprinting

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analyses aid in identifying these genetic anomalies in Angelman syndrome cases. Furthermore, cytogenetic methods have revealed chromosomal rearrangements, such as translocations and inversions, in some cases of Angelman syndrome (2).

In this report, cytogenetic testing was initially performed on a patient diagnosed with Angelman syndrome, followed by testing her family members to confirm the presence of a Robertsonian-type translocation involving two long arms of chromosome 15. The specific mild phenotype observed in the patient and the carrier of a *de novo* Robertsonian-type translocation involving two long arms of the derivative chromosome 15 suggested the need to confirm the presence of UPD in this case. Consequently, haplotype analysis was conducted on both the patient and her family to evaluate UPD.

## Case

A 4-year-old girl was referred to a pediatric clinic due to the absence of speech. She had non-consanguineous parents and a healthy one-year-old sister. Her mother was 40 years old, and her father was 39. She was born via caesarian section after an uneventful first pregnancy, weighing 2800 g at birth. During her physical examination at the age of 4, she weighed 19.8 kg (90th percentile) and measured 106 cm in length (50-75th percentile), with a head circumference of 51 cm. She exhibited dysmorphic features such as a wide mouth with a protruding tongue, finger flexion, drooling, mental retardation, absence of speech, disrupted sleep, and the inability to run by the age of 4. She started walking at 22 months without a history of seizures. Notably, both brain MRI and EEG results were normal. At the age of 7, she underwent another physical examination, showing a weight of 29.4 kg (94th percentile), a length of 118.5 cm (40th percentile), and a head circumference of 50 cm.

Peripheral blood samples were obtained from the patient and her parents. GTG banding chromosome analysis was performed on peripheral blood lymphocytes using standard procedures, at a resolution of 550 bands. Subsequently, their karyotypes were determined following the guidelines of the International System for Human Cytogenetic Nomenclature 2020 (3). The patient's karyotype was identified as 45,XX,der(15;15)(q10;q10), while her parents' karyotypes were normal (Figure 1a, 1b, 1c).

Fluorescent In Situ Hybridization (FISH) was carried out on the patient using Cytocell's Prader-Willi/Angelman (SNRPN) probe (product no: LPU 005) designed for loci within the 15q11-q13 region, in addition to the 15qter subtelomere specific probe (clone 154P1), following the standard protocol. FISH analysis did not reveal any microdeletions in the Prader-Willi/Angelman region, confirming the presence of the same loci on each arm of the translocated chromosomes 15 (Figure 1d).

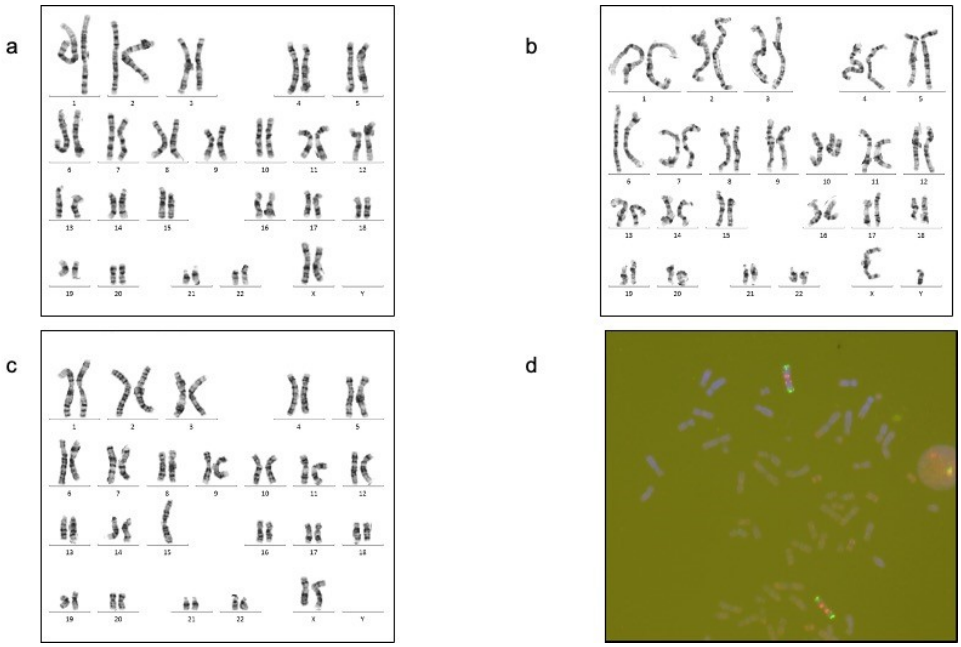


**Figure 1.** The karyotype analyses by conventional cytogenetic G-banding method (a,b,c). a) A normal karyotype of her mother. b) A normal karyotype of her father. c) A karyotype shows the case with apparently balanced *de novo* Robertsonian translocation 45,XX,der(15;15)(q10;q10)dn. d) A photograph from the Locus specific Fluorescent in situ hybridization (FISH) analysis. Locus-specific FISH analysis was carried out using Cytocell's Prader-Willi/Angelman (SNRPN) probe (product no: LPU 005), which was a red labeled specific for loci within the 15q11-q13 and the 15qter subtelomere specific probe (clone154P1) which was a green labeled. FISH detected two signals in the case for probe-specific Prader-Willi/Angelman (SNRPN) 15q11-q13 chromosomal region, indicating Robertsonian translocation of two long arms in derivative paternal chromosome 15.

To determine haplotype segregation on chromosome 15, Ion S5 system reads obtained from paired-end sequencing platforms were aligned to the human reference genome GRCh37 (hg19). Variants detected using next-generation sequencing-based methods were visualized using the Integrative Genomics Viewer (IGV) and assessed with Ion Reporter Software. To illustrate the heterodisomy, three variants within chromosome 15 were chosen: SMAD3 (RefSeq NM_005902.3) c.207-14678 G>A, ADAMTS7 (RefSeq NM_014272.3) c.744A>G(p.Val248=) and c.640T>C (p.Ser241Pro). For SMAD3 c.207-14678G>A, both the patient and the father exhibited a homozygous GG genotype, while the mother had an AA genotype. Concerning ADAMTS7 c.744A>G, the mother displayed a heterozygous genotype, whereas the father and the patient presented with a CC genotype. In the case of ADAMTS7 c.640T>C, the patient and her father were heterozygous, while the mother had the wild-type AA genotype. These findings suggest that the case inherited paternal heterodisomic UPD. Figure 2 shows the IGV view of the case, her mother, and father.

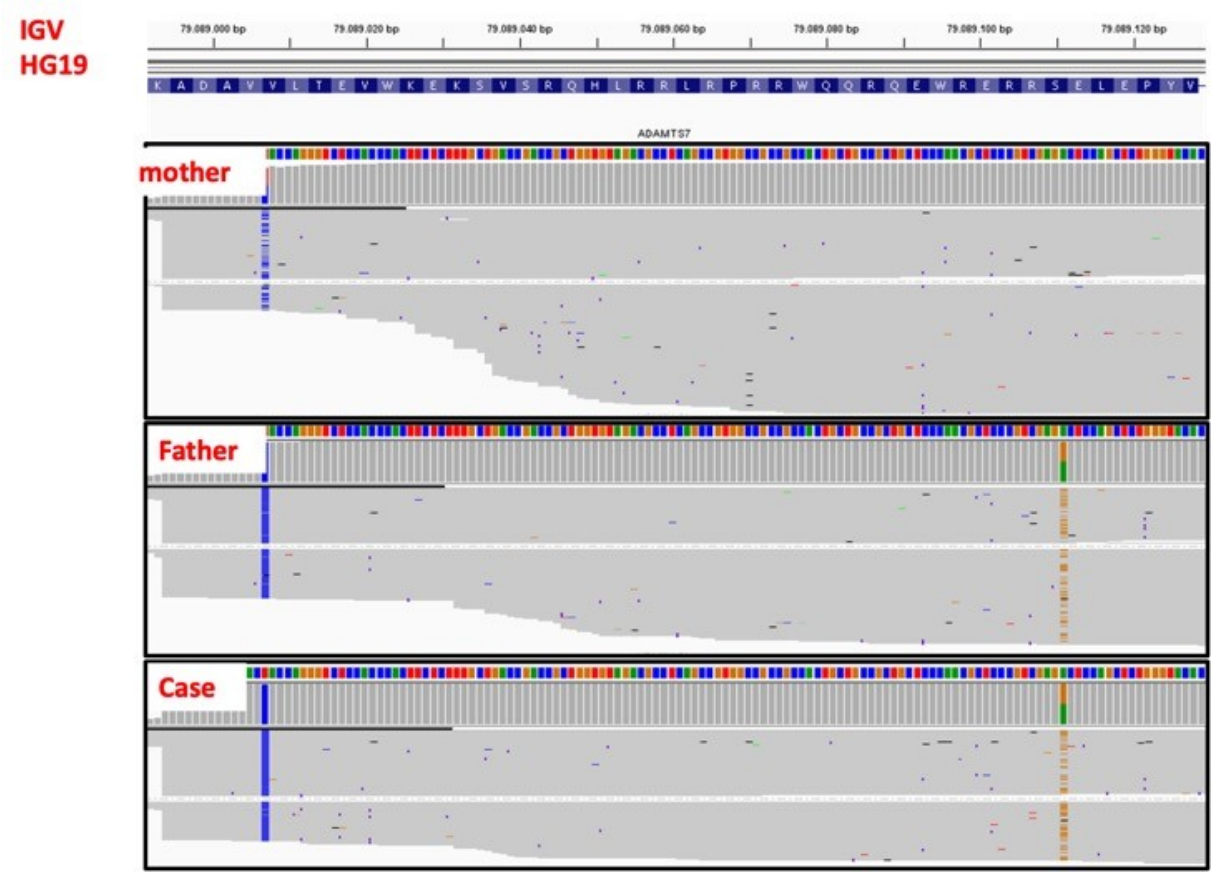


**Figure 2.** The case, her mother and father's IGV views are shown.

## Discussion

In the patient we reported, a *de novo* 15;15 Robertsonian translocation occurred, and there was no familial history of Angelman syndrome. The literature indicates a sporadic occurrence rate of UPD-induced Angelman syndrome at approximately 2-3% (4).

The reason why maternal UPD15, leading to non-disjunction during meiosis, occurs ten times more frequently than paternal UPD15 remains unclear. However, post-zygotic gain or loss of paternal chromosome 15 is more likely to occur in both paternal and maternal UPD15 cases. Robinson et al. (5) reported that in 21 AS cases caused by paternal UPD15, the mean maternal and paternal ages were 33.4 and 39.4 years, respectively, higher than in controls. In our study, the parents' ages were 40 and 39, respectively, aligning with the older parental age observed in the literature. It's believed that paternal errors predominantly occur in the post-zygotic stage. Additionally, the non-disjunction event leading to nullisomy of chromosome 15, associated with older maternal age, typically occurs in the oocyte (5).

The NGS method was used to ascertain the presence of UPD in the patient with the Robertsonian translocation of 15;15. Subsequent segregation analysis confirmed the paternal origin of the disomy, indicating paternal heterodisomic UPD in the patient. It's established that around 2-3% of AS patients carry paternal UPD. Notably, prior reports

on UPD-associated AS cases have emphasized their heterodisomic nature (6). This suggests phenotypic differences between UPD and deletional cases, with UPD-associated AS cases displaying milder symptoms on chromosome 15 (5). Consequently, AS patients with UPD may go undiagnosed due to their less typical phenotype (7).

The first documented case of AS arising from *de novo* paternal uniparental heterodisomy was reported by Ramsden et al. (6) in 1996. This patient presented with typical Angelman syndrome features at age 4, including developmental delay, ataxia, jerky movements, absent speech, and a cheerful disposition. The determination of heterodisomic uniparental disomy was made through methylation analyses, revealing both 15 chromosomes to be of paternal origin (6).

In our patient, dysmorphic features such as a protruding tongue, mental retardation, sleep disturbances, inability to speak, drooling, and ataxia were observed. These clinical manifestations align with previously published reports (8). However, specific EEG and MRI findings were not observed in our patient. Subsequently, Li et al. (9) reported two cases: one, a 3-year-old female with paternal UPD at chromosome 15, displayed EEG and MRI abnormalities; the other case, a 3.5-year-old male with paternal UPD on 15q11-13, had no history of seizures, and the MRI result was normal, similar to our patient (4).

The specific EEG pattern associated with Angelman syndrome is determined by assessing electrophysiological parameters, either individually or in combination. Although crucial for clinical diagnosis, obtaining conclusive EEG results in a single test may be challenging. It's advisable to repeat the EEG examination as findings can vary over time within the same case (4). In our reported case, the EEG test produced normal results without any observed seizure activity. Tan et al.'s (8) 2011 study noted that among 92 AS patients aged 5-60 months diagnosed through molecular testing, 84 cases displayed abnormal EEG results. However, despite these abnormalities, clinical seizures were only evident in 65% of all cases (8).

Our patient's body mass index (BMI) was found to be >85%. This observation was supported by Tan et al. (8), who reported that almost half of the children with UPD/imprinting defects had a high BMI (>85%), despite facing feeding difficulties. Tan et al. (8) also noted that the BMI of our patient was recorded as >85%, aligning with our study's results. These findings strengthen the association between paternal UPD and higher BMI.

Furthermore, Table 1 in the literature details the karyotype and clinical features of cases similar to ours. Our case, highlighted in red in Table 1, shares similarities with these cases.

Genetic counseling should be recommended to families when Angelman syndrome arises sporadically. This counseling provides information about the risk of recurrence. As UPD occurred *de novo* in our case, we informed the family that the risk of UPD recurrence in their future offspring would be <1% (28).

Thomas Liehr (29) emphasized UPD as a chromosomal disorder that always requires examination at a chromosomal level. This approach aids in understanding the biological processes in individual patients (29). We believe that starting genetic tests for AS/PWS with cytogenetic analysis is crucial. This step serves as the initial stage in identifying uniparental disomy and comprehending the biological processes underlying such cases.

## Conclusions

We reported that the case was a 4-year-old female carrying paternal heterodisomic UPD, leading to AS. In particular, since UPD carriers of AS patients have few of the phenotypic features of the syndrome, the presence of UPD in the first test will be demonstrated by conventional cytogenetics rather than molecular analysis. Also, we confirmed that AS patients with UPD have milder clinical symptoms as well as higher BMI than AS individuals with other underlying genetic abnormalities. Our data can lead to understanding phenotype-genotype correlation in AS carrying UPD.

## Acknowledgements

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## Conflict of interest statement

The authors declare that have no conflicts of interest.

**Written consent:** This study had an approval number was 2012-KAEK-20 and was obtained on 30/05/2022 from the ethics committee of Akdeniz University.

**Funding:** This study was supported by Akdeniz University Medical Faculty Genetic Diseases Diagnostic Center.

265 **Table 1.** Clinical and karyotype comparison of our case with AS cases in the literature.

| Karyotype | DD | Ataxic movement and gait | Unique behavioral features | Speech impairment | Micro-cephaly | Seizures | EEG abnormality | Hypo-pigmentation | Atypical dysmorphic features for AS | Family history | Reference number |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 45,XY,–10,–15,+t(10;15)(q26;q13)dn? (II-1) | n.d. | + | n.d. | n.d. | + | (+) ~14 y | + | n.d. | high bossed forehead, small mandible, hypoplastic maxillae, very high palate, short nose, flattened nares, short stature (<3rd centile at 15 y) | - | 10 |
| 45,XY,–13,–15,+der(13),t(13;15)(p13;q13)mat (II-1) | DQ50 (2 y) | n.d. | n.d. | n.d. | - | (+) ~ 3 mo | n.d. | n.d. | telecanthus, long upper lip, higharched palate, broad nasal bridge, full nasal tip with flare of nasal alae | - | 11 |
| 46,XX,–15,+der(22)t(15;22)(q13;q11)mat (II-2) | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | PWS in 2 relatives | 12 |
| 45,XX,–6,–15,+der(6)t(6;15)(p25.3;q11.1)pat (I-2) | + | (+) walked at 2 y | n.d. | (+) no word 4.5 y | - | - | (+) typical for AS | + | epicanthic folds | PWS in cousin (caused by *de novo* paternal deletion) | 13 |
| 45,XY,–8,–15,+der(8)t(8;15)(p23.3;q11)pat (I-2) | + | + | + | (+) no word 29 y | n.d. | (+) severe | (+) typical for AS | - | n.d. | - | 14 |
| 45,XY,–10,–15,+der(10)t(10;15)(q26;q13) (II-1) | + | + | + | (+) no word | + | (+) severe | (+) typical for AS | (+) skin, eyes and hair | short stature (<3rd centile at 24 y) | - | 15 |
| 45,XX,–3,–15,+der(3)t(3;15)(q29;q12) (II-1) | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | - | 16 |
| 46,XX,–15,+der(14)t(14;15)(q11.2;q11.2)mat (II-2) | + | + | + | (+) no word | + | + | (+) typical for AS | n.d. | extreme short stature at 10 y | - | 17 |
| 45,XY,–8,–15,+der(8)t(8;15)(p23.3;q13)mat (II-1) | + | (+) walked at 18 mo | + | began to babble at 8 mo | + | (+) ~18 mo | (+) typical for AS | (+) light red hair | protruding ears, short stature (<5th centile at 18 mo) | AS suspected in aunt | 18 |
| 45,XY,der(15;15)(q10;q10)dn (I-1) | + | + | + | + | + | + | (+) typical for AS | - | some patients showed overgrowth | - | 19 |
| 46,XX,–15,+der(14)t(14;15)(q11;q13)mat (II-2) | + | + | + | n.d. | + | + | + | n.d. | n.d. | PWS in 2 cousins | 20 |
| 45,XX,–1,–15,+der(1)t(1;15)(p36.31;q13.1)mat (II-1) | + | + | - (did not smile or pay any attention to her surroundings) | n.d. | - | (+) from 7 mo | + | (+) skin, hair, irises | frontal bossing, hypertelorism, flat nasal root, apparently low-set ears with asymmetry and cupping, small hands and feet | - | 21 |
| 45,XY,–15,–22,+der(22)t(15;22)(q13;p11) (II-1) | + | never walked alone at 30 y | n.d. | n.d. | n.d. | (+) ~ 3 y | n.d. | n.d. | n.d. | - | 22 |
| 45,XY,–13,–15,+der(13)t(13;15)(q34;q15)mat (II-1) | n.d. | contractures and increased tone | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | sloping forehead, small anterior fontanel, very prominent nasal root, cup-shaped ear deforms; abnormal palmar creases | - | 22 |

| Karyotype | DD | Ataxic movement and gait | Unique behavioral features | Speech impairment | Micro-cephaly | Seizures | EEG abnormality | Hypo-pigmentation | Atypical dysmorphic features for AS | Family history | Reference number |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 45,XY,–1,–15,+der(1)t(1;15)(q44;q13)dn (II-1) | + | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | - | 22 |
| 46,XX,–15,+der(22)t(15;22)(q13;q11.2)mat (II-2) | n.d. | gained head control but could not sit without support at 29 mo | n.d. | (+) no word: 23 mo | - | + | (+) typical for AS | n.d. | preauricular tag, preauricular pit, short stature (–2.4 SD at 23 mo) | 22q11.2 deletion syndrome in brother | 23 |
| 45,XX,–10,–15,+der(10)t(10;15)(q26;q13)mat (II-1) | DQ60 (10 mo) | - | - | n.d. | + | - | n.d. | (+) skin (-) hair and iris | n.d. | PWS in cousin | 24 |
| 45,XY,–1,–15,+der(1)t(1;15)(q44;q12)mat (II-1) | n.d. | n.d. | + | (+) no word | + | - | n.d. | (+) face, iris | older brother, thin upper lip, overhanging nasal tip, large ears | - | 25 |
| 45,XY,–1,–15,+der(1)t(1;15)(q44;q12)mat (II-1) | n.d. | n.d. | n.d. (autistic feature) | (+) no word | n.d. | - | n.d. | n.d. | younger brother, bushy eyebrows, overhanging nasal tip, bilateral low-set large ears | | 25 |
| 45,XX,–10,–15,+der(10)t(10;15)(q26.3;q11.2)mat (II-1) | Mode-rate delay | climbed stairs with support, but could not run or jump at 5 y | n.d. (autistic feature) | (+) spoke 4 disyllables at 5 y | + | - | (+) paroxysmal activity in the left and right occipital region | not hypo | low anterior hair implantation, bushy eyebrows, bilateral epicanthal folds, telecanthus, lips with absent Cupid's bow, slightly broad nasal bridge, prominent nose with a bulbous tip, short, broad, and smooth philtrum, hands with tapered fingers, broad thumbs and broad 2nd fingers | minor dysmorphic features in uncle and cousin | 26 |
| 46,XX,–15,+der(13)t(13;15)(q14.1;q12) (II-2) | + | gained head control but could not sit at 24 mo; tonic spasmlike movement | (-) no happy demeanor | (+) no word at 24 mo | + | (-) tonic spasm like involuntary movement | (+) typical for AS | - | upslanted palpebral fissures, hypertelorism, thin lips with downturned corners of mouth, bilateral clinodactyly of 5th fingers | - | 27 |
| 45,XX,der(15;15)(q10;q10)dn (I-2) | + | (+) broad-based gait, walked at 22 mo | disrupted sleep | + | - | - | - | - | (+) wide mouth with protruding tongue, flexion of both fingers and drooling | no family history to our knowledge | Present case |

DD: Developmental Delay; mo: months; y: years, + : positive for the finding;, – : negative for the finding; n.d.: not described in the report, UPiD: uniparental isodisomy; UPhD: uniparental heterodisomy; I-1: Paternal UPiD; I-2: Paternal UPhD; II-1: Deletion and monosomy by maternal translocation; II-2: Deletion and trisomy by maternal translocation. This table adapted from Niida et al., 2016 (27).

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