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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

http://dergipark.gov.tr/muskutd/page/4152

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 dili İngilizcedir. 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 alanlarda olmalıdır. İngilizce ve Türkçe başlık olmalı ve özet 250 kelimeyi geçmemelidir. Derleme içeriği 5000 kelimeyi aşmamalı ve kaynak sayısı en fazla 70 olmalıdır.

Orijinal makaleler: Orijinal makaleler temel veya klinik çalışmalar veya klinik denemelerin sonuçlarını bildirir. Makale Türkçe özet, İngilizce özet, giriş, gereç ve yöntemler, bulgular/sonuçlar, tartışma, teşekkür (gerekliyse), çıkar çatışması bildirimi, etik kurul onayı (yer/tarih/sayı), fon bildirimi, 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. Çalışma 3 - 5 anahtar kelime içermelidir. 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.

Kaynaklar: Kaynaklar metin içinde alıntılanma sırasına uygun olarak doğal sayılar kullanılarak numaralandırılmalı ve cümlenin sonunda parantez içinde verilmelidir. Kaynaklar listesinde yazar sayısı üç veya daha az ise hepsi, üçten fazla ise sadece ilk üç ismi yazılmalı ve 've ark.' ilave edilmelidir. Kaynak ve kısaltılmış dergi adları yazımları Index Medicus'a veya aşağıda verilen örneklere uygun olmalıdır. Çalışmaya yazılan kaynakların okunmuş olması ve talep edildiğinde sunulması gerekmektedir.

Dergi makaleleri için örnek

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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

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Unilateral Axillary Lymphadenopathy Frequency and Follow-up Results After Inactivated COVID-19 Vaccination

İnaktive COVID-19 Aşısı Sonrası Unilateral Aksiller Lenfadenopati Sıklığı ve Takip Sonuçları

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Öz

Aşılarının yaygınlaşmasıyla birlikte aşıya bağlı ipsilateral aksiller lenfadenopati ile karşılaşılabilmektedir. Çalışmanın amacı inaktive COVİD-19 aşısı sonrası aksiller lenfadenopati sıklığını, lenf bezlerinin sonografik özelliklerini ve takip sonuçlarını değerlendirmektir. Mart-Nisan 2021 tarihleri arasında gerçekleştirilen prospektif çalışmaya toplam 127 katılımcı dahil edilmiştir. Tanımlayıcı ve çıkarımsal istatistiksel analizler SPSS kullanılarak gerçekleştirilmiştir. İkinci doz aşılamadan sonra 10-16 günlük süreçte 127 katılımcının (39.92±8.96 yaşında, %68.5'i erkek) ultrasonografi ile aksiller lenf nodu durumu değerlendirildi. Toplam 32 katılımcıda (%25.2) ilk ultrasonda ipsilateral aksiller lenfadenopati görüldü. Bu hastalardan yalnızca birinde 30 gün sonraki kontrol ultrasonda sebat eden lenfadenopati görüldü. Lenf nodu korteks kalınlığı en kalın yerinde aşılanan tarafta (2.63±2.12 mm) karşı tarafa (1.53±1.11 mm) göre anlamlı derecede yüksekti (p<.001). Lenfadenopati sayısı aşılanan tarafta karşı tarafa göre daha yüksekti (p<.001). Ayrıca COVİD-19 öyküsü ile ipsilateral lenfadenopati bulunmaması arasında anlamlı ilişki mevcuttu (p<.001). Lokal bir yan etki olarak inaktive COVİD-19 aşısının ikinci dozu sonrasında ipsilateral aksiller lenfadenopati görülebilmekte ve genellikle 1 ay içinde gerilemektedir. Ancak daha önce COVİD-19 enfeksiyonu geçiren bireylerde aşı sonrası aksiller lenfadenopati beklenmemektedir. Aksillayı ilgilendiren radyolojik incelemelerden önce hem enfeksiyon hem de aşılama geçmişinin bilinmesi, radyoloğun lenf nodu durumunu yanlış yorumlanmasını engelleyecektir. Aşıya bağlı ipsilateral aksiller lenfadenopatinin, koronavirus enfeksiyon öyküsü olmayan bireylerde inaktif COVİD-19 aşıları sonrasında görülebileceği ve tespit edildikten bir ay sonra çoğunlukla kaybolduğu akılda tutulmalıdır.

Anahtar Kelimeler: Aşılama, Covid-19, Kortikal Kalınlık, Lenfadenopati, Ultrasonografi

Abstract

Vaccine-induced ipsilateral axillary lymphadenopathy can be encountered with the widespread application of COVID-19 vaccines. The study aims to evaluate the frequency of axillary lymphadenopathy, sonographic features of axillary lymph nodes after administration of inactivated COVID-19 vaccine, and follow-up results. Between March and April 2021, a total of 127 participants were enrolled in this prospective study. Data were analyzed using both descriptive and exploratory test techniques with SPSS. A total of 127 participants (39.92±8.96 years, 68.5% men), who were between 10-16 days after the second dose vaccination, were evaluated for axillary lymph node status by initial ultrasound. A total of 32 participants (25.2%) had ipsilateral axillary lymphadenopathy in the initial ultrasound. Only one of these patients had persistent lymphadenopathy on the control ultrasound 30 days later. The widest cortical thickness was significantly higher on the ipsilateral side (2.63±2.12 mm) compared to the contralateral side (1.53±1.11 mm) (p<.001). The number of lymphadenopathies was higher on the vaccinated side compared to the contralateral side (p<.001). A significant relationship between the history of COVID-19 infection and the absence of ipsilateral lymphadenopathy was found (p<.001). As a local adverse effect, ipsilateral axillary lymphadenopathy following the second dose of inactivated COVID-19 vaccine can be seen, and it usually regresses within a month. However, during that period, axillary lymphadenopathy is not expected in vaccinated individuals who previously experienced COVID-19 infection. Awareness and questioning of both infection and vaccination history before radiological examinations involving the axilla should help the radiologist avoid the misinterpretation of lymphadenopathy. It should be kept in mind that vaccine-induced ipsilateral axillary lymphadenopathy can be seen after inactivated COVID-19 vaccines in individuals who don't have a history of coronavirus infection, and it regresses a month later after detection.

Keywords: Vaccination, Covid-19, Cortical Thickness, Lymphadenopathy, Ultrasound

Introduction

With the widespread usage of inactivated or mRNA COVID-19 vaccines worldwide, different side effects of vaccination have been identified (1,2). One of these side effects is vaccine-induced lymphadenopathy following deltoid vaccination administration. The axilla is one of the most common regions where lymphadenitis can be observed from 1 to 3 weeks after vaccine injection. Lymph node changes typically begin on the 8th day after vaccination, with maximal antibody response reached 15 days after vaccine administration (3). Vaccine-induced ipsilateral axillary lymphadenopathy has been detected using various imaging modalities such as ultrasound, CT, MRI, or FDG PET/CT following COVID-19 vaccine administration. This phenomenon poses diagnostic challenges, complicates patient management, leads to unnecessary biopsies or treatments, and causes patient anxiety (4-7). Understanding the frequency of axillary lymph node enlargement and its imaging features after COVID-19 vaccination should assist radiologists in interpreting findings. Additionally, awareness of vaccine-induced axillary

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lymphadenopathy among both radiologists and clinicians aids in scheduling radiological examinations and evaluating results for patient management. Most cases of ipsilateral axillary lymphadenopathy following deltoid mRNA COVID-19 vaccination have been observed primarily during breast screening or imaging of oncologic patients with PET-CT. The reported lymph nodes with pathological imaging features were almost exclusively observed after vaccination with mRNA COVID-19 vaccines (8). In a study, vaccine-associated hypermetabolic lymphadenopathy in PET-CT occurs more commonly after mRNA vaccination compared to inactivated vaccination (9). In a retrospective study, it is shown that mild and diffuse lymph node cortical thickness can be detected using ultrasound following administration of inactivated COVID-19 vaccines even when there are no clinical signs of lymph node enlargement (10). The study aims to evaluate the frequency of axillary lymphadenopathy, sonographic features of axillary lymph nodes, and follow-up results following administration of the inactivated SARS-CoV-2 vaccine (CoronaVac).

Material and Method

Muğla Sıtkı Koçman University Medical Faculty Ethics Committee approved this study (Approval Reference Date and Number: 31.03.2021 and 7/II). Informed consent was obtained from all volunteers before the ultrasound examination. Study participants were selected using the quota sampling method. Between March and April 2021, a total of 127 participants were enrolled in this prospective study. The study included participants who were between 10-16 days after receiving the second dose of the CoronaVac vaccine. Exclusion criteria were as follows: participants under 18 years of age, participants with a known history of immunodeficiency or malignancy, participants with a history of rheumatological diseases that could affect axillary lymph nodes, and participants with a history of axillary surgery. Real-time ultrasound examinations of all participants were performed by the same radiologist with 12 years of experience. A second radiologist with 15 years of experience reviewed all the images, and both radiologists reached a consensus on the final results of lymph node status, whether lymphadenopathy was present or not.

Lymph nodes were classified and grouped as reactive or pathological based on the sonographic features described by Bedi et al. The cutoff value for lymphadenopathy was described as 3 mm (11). If a lymph node had cortical thickness less than 3 mm with an oval shape and visible echogenic hilum, it was described as a reactive lymph node. If the lymph node had cortical thickness more than 3 mm, didn't have a visible hilum, or was spherical shaped, it was noted as a pathological lymph node. The radiologists evaluated the lymph node status by considering both cortical thickness and morphological features of the lymph nodes. In case of disagreement between the radiologists, the lymph node was considered pathological. For both axillary regions, the short and long axis, cortical thickness and the number of reactive lymph nodes, the number and cortical thickness of spherical lymph nodes, and, also the short and long axis, cortical thickness, and the number of lymphadenopathies were separately measured. Then the widest cortical thickness of the lymph nodes containing both the reactive lymph node and lymphadenopathies for each axilla was noted for each patient. The pathological lymph node number, and the status of the axilla as positive or negative for the lymphadenopathy were also evaluated. The third radiologist (having a 3 year-experience) noted the age, weight, height of the patients, the site of the vaccination, presence, and duration of pain after vaccination, and history of a palpable mass detected by the patient.

If lymphadenopathy is detected on the initial ultrasound, participants are invited for a follow-up ultrasound a month later, following the recommendations of the Society of Breast Imaging Patient Care and Delivery Committee (12). The same radiologist performed the follow-up ultrasound without knowledge of the previous axillary status and recorded the same parameters. The contralateral axilla was used as a control group in our study.

Statistical Analysis

The distributions of the measured variables were assessed using the Shapiro-Wilk test for normality. Group-wise differences were evaluated with a t-test if the normality assumption was met, and with the Mann-Whitney U test if the normality assumption was violated. The Wilcoxon test was used to compare two related samples when the data were not normally distributed. Additionally, to explore the relationship between the number of lymph nodules, age, and BMI, the Pearson correlation coefficient was calculated. The relationship between categorical variables was examined using the Chi-square test to identify differences between sub-categories. Results were presented as mean and standard deviation for parametric data and as mean, median, and interquartile range for non-parametric data. Statistical analyses were performed using SPSS version 25 (developed by SPSS Incorporated, located in Chicago, Illinois, USA). The significance level was set at $p<.05$.

Results

A total of 127 participants, comprising 68.5% (87) men and 31.5% (40) women, participated in our study. Eleven volunteers were excluded from the study due to bilateral vaccination (n=4), history of

breast cancer (n=3), known axillary lymph node enlargement before vaccination (n=2), and a history of rheumatological diseases (n=2). The average age was recorded as 39.92±8.96 years. Among the participants, 30 (23.6%) had a history of COVID-19, while 97 (76.4%) had a negative history. Nineteen of them had mild, nine had moderate, and two had severe COVID-19 infection. For participants with a history of COVID-19, the time between the first diagnosis and the initial ultrasound for the study was found to be 96.6±20.5 days. Following the second dose of vaccination, the time between vaccination and the initial ultrasound examination ranged from 10 to 16 days, with a mean of 13.65±1.21 days. After the second vaccination of CoronaVac, the initial ultrasound revealed that a total of 38 patients (29.92%) had axillary lymphadenopathy. Of the participants, 25.2% (n=32) had only ipsilateral axillary lymphadenopathy on the injection side, while five participants had bilateral axillary lymphadenopathy, and only one participant had contralateral axillary lymphadenopathy on the initial ultrasound. For the contralateral side of the axilla, a total of six participants (4.72%) had lymphadenopathy.

The Chi-square test revealed no difference in terms of ipsilateral and contralateral lymph node status among male and female participants (p=0.487, 0.423, respectively). The relationship between age and contralateral axillary reactive lymph node cortex thickness is negative, with a low correlation coefficient of r=-0.222, and a significance level of p<0.05. Moreover, ipsilateral and contralateral axillary reactive lymph node cortex thickness exhibited a positive and moderate correlation, with a correlation coefficient of r=0.408 and a significance level of p<0.01.

According to the t-test results, the measured variables of "long axis of reactive lymph nodes and also short axis, long/short axis ratio, and cortical thickness of both the reactive lymph nodes and lymphadenopathy" were not significantly different between vaccinated ipsilateral and contralateral axilla. However, "the widest cortical thickness of all the lymph nodes in the axilla" was significantly higher in the "ipsilateral vaccinated side" group, with a mean of 2.63±2.12 compared to the "contralateral side" group, with a mean of 1.53±1.11 at p<0.001 (Table 1).

Table 1. Descriptives of parametric variables and group-wise differences in initial ultrasound

Variable	Group	Mean	SD	MD	95%CI Lower	95%CI Upper	*p*
long axis of RL (mm)	Ipsilateral	16.57	4.77	-0.79	-2.32	0.73	.306
	Contralateral	17.37	5.91				
short axis of RL (mm)	Ipsilateral	7.25	1.86	-0.01	-0.57	0.56	.984
	Contralateral	7.26	2.12				
widest cortical thickness of RL (mm)	Ipsilateral	1.96	0.58	0.15	-0.01	0.31	.061
	Contralateral	1.80	0.55				
long/short axis ratio of RL	Ipsilateral	2.34	0.63	-0.08	-0.26	0.09	.352
	Contralateral	2.43	0.60				
short axis of LP (mm)	Ipsilateral	8.06	1.85	-0.27	-2.00	1.46	.753
	Contralateral	8.33	2.32				
cortical thickness of LP (mm)	Ipsilateral	3.95	0.75	0.54	-0.09	1.17	.093
	Contralateral	3.42	0.26				
long/short axis ratio of LP	Ipsilateral	2.27	0.58	-0.05	-0.56	0.46	.841
	Contralateral	2.32	0.47				
widest cortical thickness of all the lymph nodes in the axilla (mm)	Ipsilateral	2.63	2.12	1.10	0.68	1.52	.000
	Contralateral	1.53	1.11				

RL: Reactive lymph node, LP: Lymphadenopathy, Ipsilateral: Vaccinated side, Contralateral: Non-vaccinated side, SD: Standard Deviation, MD: Mean Difference, CI: Confidence Interval

On the other hand, "the number of reactive lymph nodes" and "the long axis of lymphadenopathy" were not found to be different between the "vaccinated side" and "other side" groups according to the Mann-Whitney U test. Nevertheless, the number of lymphadenopathies was significantly higher in the "vaccinated side" (0,1) compared to the "contralateral side" (0,0) at p<0.001, as detailed in Table 2.

Table 3 presents the number of observations in each group and indicates if there is a significant relationship between the groups, with the corresponding p-value on the right-hand side. We observed that "lymphadenopathy with thickened cortex" and "spheric lymph nodes" were more commonly observed on the vaccinated side,

accounting for 84.2% and 91.7% of the total, respectively. Moreover, the "status of axilla" was predominantly characterized as "lymphadenopathy" (86%) on the vaccinated side compared to the contralateral side (only 14%). Furthermore, the presence of pain in the arm was investigated in cases of "ipsilateral lymphadenopathy with thickened cortex". The results indicated that there was no arm pain in 81% (n=77) of cases with "lymphadenopathy", but arm pain was present in 19% (n=18) of cases. Additionally, when "lymphadenopathy with thickened cortex" was present, arm pain was absent in 59.4% (n=19) of cases, and present in 40.6% (n=13) of cases (p=0.014). There was a statistically significant relationship between COVID-19 history and the absence of ipsilateral lymphadenopathy with thickened cortex, as shown in Table 4 (p<0.001).

Table 2. Descriptives of non-parametric variables and group-wise differences in initial ultrasound

Variable	Group	Mean	Median	IQR	*p*
Number of RL	Ipsilateral	1.86	2	1.5	.947
	Contralateral	1.85	2	1.75	
The long axis of LP (mm)	Ipsilateral	18.06	17	6	.559
	Contralateral	18.83	20	8.25	
Number of thickened cortex lymh node	Ipsilateral	0.34	1	1	.000
	Contralateral	0.06	1	1	
Number of LP (with spherical lymph nodes)	Ipsilateral	0.43	0	1	.000
	Contralateral	0.07	0	0	

IQR:Interquartile range, RL: Reactive lymph node, LP: Lymphadenopathy

Table 3 presents the number of observations in each group and indicates if there is a significant relationship between the groups, with the corresponding p-value on the right-hand side. We observed that "lymphadenopathy with thickened cortex" and "spheric lymph nodes" were more commonly observed on the vaccinated side, accounting for 84.2% and 91.7% of the total, respectively. Moreover, the "status of axilla" was predominantly characterized as "lymphadenopathy" (86%) on the vaccinated side compared to the contralateral side (only 14%). Furthermore, the presence of pain in the arm was investigated in cases of "ipsilateral lymphadenopathy with thickened cortex". The results indicated that there was no arm pain in 81% (n=77) of cases with "lymphadenopathy", but arm pain was present in 19% (n=18) of cases. Additionally, when "lymphadenopathy with thickened cortex" was present, arm pain was absent in 59.4% (n=19) of cases, and present in 40.6% (n=13) of cases (p=0.014). There was a statistically significant relationship between COVID-19 history and the absence of ipsilateral lymphadenopathy with thickened cortex, as shown in Table 4 (p<0.001).

The measured variables of "ipsilateral axillary reactive lymph node cortex thickness", "ipsilateral axillary lymphadenopathy with thickened cortex", "contralateral axillary reactive lymph node cortex thickness", and "contralateral axillary lymphadenopathy with thickened cortex" were found to be 2.00±0.57, 4.08±0.79, 1.79±0.55, and 3.46±0.27 for females, and 1.85±0.59, 3.63±0.54, 1.83±0.55, and 3.2±n/a for males, respectively. Moreover, the differences between the two genders in these variables were not statistically significant, with p-values of 0.231, 0.132, 0.745, and 0.429, respectively.

Table 3. Number of observations for the vaccinated ipsilateral and contralateral side

		Group		
		Ipsilateral % (n)	Contralateral side % (n)	*p*
Reactive Lymph node	No	49.2% (30)	50.8% (31)	.883
	Present	50.3% (97)	49.7% (96)	
Lymphadenopathy with thickened cortex	No	44% (95)	56% (121)	.000
	Present	84.2% (32)	15.8% (6)	
Spheric Lymh Node	No	47.9% (116)	52.1% (126)	.003
	Present	91.7% (11)	8.3% (1)	
Conclusion of status of axilla	Normal	42.7% (90)	57.3% (121)	.000
	Lymphadenopathy	86% (37)	14% (6)	

n: number of observation. Note:p value is obtained with Chi-square test and significant at 0.05

Table 4. Relationship between COVID-19 history and ipsilateral axillary lymphadenopathy with thickened cortex

		Covid 19 History		
		Negative	Positive	*p*
Ipsilateral lymphadenopathy with thickened cortex	No	68% (65)	32% (30)	.000
	Present	100% (32)	%0 (0)	

Note:p value is obtained with Chi-square test and significant at 0.05

The control ultrasound was performed 30 days later for the 38 participants who had lymphadenopathy in the initial ultrasound. Among them, only 1 of the 38 participants had ipsilateral lymphadenopathy with a thickened cortex (3 mm cortex thickness) in the control ultrasound, while the second ultrasound revealed that all the other participants had reactive lymph nodes. The mean number of ipsilateral and contralateral axillary lymphadenopathy decreased over time from 1.45 to 0.03 and 0.24 to 0.00, respectively, at p<0.001 and p=0.011, respectively. The recorded time-wise differences are detailed in Table 5. According to the Wilcoxon test results, time-wise differences were explored in the cortex thickness of ipsilateral axillary lymphadenopathy from the initial to the control ultrasound at p<0.001, since the cortex thickness of ipsilateral axillary lymph nodes significantly decreased from the initial ultrasound to the control ultrasound (4.93 to 1.97 mm). Moreover, similar results were found for the cortex thickness of contralateral axillary lymph nodes as well, with a significant reduction from 2.10 to 1.47 mm (p=0.012). The cortex thickness of axillary lymphadenopathy in the control ultrasound 30 days after was found to be 1.97±0.78 mm for the vaccinated side and 1.47±0.95 mm for the other side, with a statistically significant difference at p=0.018. The number of axillary lymphadenopathies in the control ultrasound 30 days after was recorded as 0.03±0.16 for the vaccinated side and 0.00±0.00 for the other side, and therefore, was not significantly different (p=0.317). Another ultrasound was performed one month later on the patient who had a lymph node with thickened cortex, and it was found that the cortical thickness had reduced to 2.2 mm, which is under the cut-off value.

Table 5. Time-wise differences of ipsilateral and contralateral axillary lymphadenopathy

Variable	Mean	SD	Median	IQR	Paired	p
CTIL-i	4.93	2.38	4.00	2.03	CTIL-i- CTIL-30	.000
LNIL-i	1.45	0.80	1.00	1.00	LNIL-i - LNIL-30	.000
CTIL-30	1.97	0.78	2.15	0.90	CTCL-i - CTCL-30	.012
LNIL-30	0.03	0.16	0.00	0.00	LNCL-i - LNIL-30	.024
CTCL-i	2.10	1.37	2.10	1.15		
LNCL-i	0.24	0.59	0.00	0.00		
CTCL-30	1.47	0.95	1.85	1.20		
LNIL-30	0.00	0.00	0.00	0.00		

IQR:Interquartile range. The p-value is obtained with the Wilcoxon test for two-related samples. CTIL-i: cortex thickness of ipsilateral axillary lymphadenopathy in initial ultrasound. CTIL-30: cortex thickness of ipsilateral axillary lymphadenopathy in 30 days later. LNIL-i: lymph node number of ipsilateral axillary lymphadenopathy in initial ultrasound. LNIL-30: lymph node number of ipsilateral axillary lymphadenopathy in 30 days later. CTCL-i: cortex thickness of contralateral axillary lymphadenopathy in initial ultrasound

Discussion

Unilateral axillary lymphadenopathy causing misdiagnosis after mRNA COVID-19 vaccination has been demonstrated in several case reports and studies (4-8). Additionally, it has been proven that following administration of the first and second doses of the COVID-19 vaccine, the number of visible lymph nodes, maximum diameter, and cortical thickness significantly increased statistically, and in the follow-up, none of the participants returned to baseline values before vaccination (13). Our study indicates that ipsilateral axillary lymphadenopathy can also occur as a local adverse effect after administration of the inactivated COVID-19 vaccine, like what is observed after mRNA vaccines. It was observed that approximately one-fourth of the participants developed ipsilateral axillary lymphadenopathy within 2 weeks after the second dose of CoronaVac, and 97% of these participants had reactive lymph nodes in the control ultrasound performed 1 month later. Six weeks after the second dose of the inactivated COVID-19 vaccine, unilateral axillary lymphadenopathy is not expected to be detected. One of the significant findings of our study is that axillary lymphadenopathy is not expected in vaccinated patients who had a prior COVID-19 infection. This result underscores the importance of not only considering the vaccination history but also the history of COVID-19 infection when evaluating unilateral axillary lymph nodes in the era of COVID-19 vaccination.

The mean age of the 127 participants in our prospective study was 39.92±8.96, with a 23.6% history of COVID-19 infection, and 68.5% of them were men. We did not find a difference in lymph node status and cortical thickness of lymph nodes between sexes. A study that can be compared with ours investigated the occurrence of axillary lymphadenopathy similarly. In that study, 91 participants (79.1% women) with a mean age of 44, who received the mRNA COVID vaccine, were included, and 28% of the population had previously had the infection (13). While the frequency of unilateral axillary lymphadenopathy among participants after the second dose of the inactivated vaccine was 25.2% in our study, they demonstrated that the frequency was 81.3% after the mRNA vaccine, which is much higher than in our study. This difference in frequency may arise from the different types of vaccines, which elicit different

immune responses through different mechanisms. The high percentages of vaccine-induced hyperplasia of lymph nodes after COVID-19 vaccines are indicative of a robust immune response to these vaccines (14).

The mean cortical thickness of ipsilateral lymph nodes (2.63 mm) was significantly wider compared to the contralateral side (1.53 mm) two weeks after receiving the inactivated vaccine. It has been shown that the mean cortical thickness of lymph nodes increased from 1.6 mm to 4.6 mm one week after receiving the mRNA vaccine (13). The mean contralateral cortical thickness in our study and the baseline cortical thickness in the other study are nearly the same, which can be assumed as the standard cortical thickness of normal axillary lymph nodes due to the lack of information about this issue. Taken together, it is evident that these vaccines cause cortical thickening regardless of whether they cause lymphadenopathy appearance on ultrasound or not.

One of the striking findings of our study, which correlates with the other study, is the paradoxical lymph node response in participants who experienced previous COVID-19 infection compared to naive participants (13). We believe that the localization and mechanism of the immune response are different in patients previously infected and those who are not. Further investigation into the changes due to immune response in the occurrence of COVID-19 is needed to prove this hypothesis. Several recommendations have been released in the era of COVID-19 vaccination, but the history of infection was not specifically mentioned in these recommendations (12,15). With the results of current and future studies, recommendations for patients with a history of COVID-19 infection or those requiring a booster dose may also be altered.

The cortical thickness of ipsilateral axillary lymphadenopathy significantly reduced between the initial and control ultrasounds in our study. However, cortical thickness on the ipsilateral side was still found to be statistically wider than the contralateral side in the control ultrasound. Only 1 of the 38 participants had cortex thickness in the control ultrasound that achieved normal thickness in the second control ultrasound 1 month later. In contrast, the other study showed that 53.1% of participants normalized cortex thickness within a month, and 9.4% continued presenting cortical thickness in the 4th month. Additionally, none of their participants reached basal cortical thickness in follow-ups (13). The wider cortical thickness on the ipsilateral side in the control ultrasound issued in our study, and the failure to reach basal levels in follow-ups in the other study, indicates that despite cortical thickness regressing over time, it remains thicker than before vaccination. The difference in normalization of cortical thickness between the two studies may depend on the difference in vaccine type and immune response. Monitoring the difference in normalization of lymphadenopathies among different COVID-19 vaccines closely in further studies is necessary to provide more accurate recommendations according to vaccine types.

The main limitation of our study is that we lack data on the axillary status of the participants before vaccination and after the first dose vaccination. We did not monitor the status of lymphadenopathies for short periods, and we conducted a control ultrasound only 1 month after detecting lymphadenopathies. Another limitation is that our study group received the inactivated COVID-19 vaccination, so we did not compare the frequency of axillary lymphadenopathy with other types of COVID-19 vaccines. Additionally, we did not evaluate the participants after the third dose, which may present new challenges with the widespread practice of booster doses. Furthermore, we did not assess the status of ipsilateral supraclavicular and cervical lymph nodes, where lymphadenopathy could also be observed after vaccination.

Conclusion

The widest cortical thickness of the lymph nodes and the number of lymphadenopathies are significantly higher on the ipsilateral side of vaccination compared to the contralateral side. As a local adverse effect of vaccination, ipsilateral axillary lymphadenopathy can be seen after the second dose of inactivated COVID-19 vaccine, and it mostly regresses within a month. However, during that period, axillary lymphadenopathy is not expected in vaccinated patients who previously experienced COVID-19 infection. The frequency of vaccine-induced axillary lymphadenopathy after inactivated COVID-19 vaccine is 25.2%, and it mostly regresses within a month. The timing of elective imaging such as screening containing the axillary region after vaccination would be appropriate approximately 6 weeks after receiving an inactivated COVID-19 vaccination. Ipsilateral lymphadenopathy after inactivated COVID-19 vaccine is not expected in individuals who had COVID-19 infection before the vaccination. Recommendations for individuals presenting with unilateral axillary lymphadenopathy after vaccination, with and without a history of COVID-19 infection, may vary. Before performing radiological examinations of the axilla, radiologists and clinicians need to be aware of and inquire about both COVID-19 infection and vaccination history and timing. This will help radiologists and clinicians avoid misinterpretation and mismanagement of unilateral lymphadenopathy.

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Conflict of interest statement

The authors declare that they have no known competing financial or personal relationships that could be viewed as influencing the work reported in this paper.

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Investigating the Role of IL-17A gene rs2275913 Variant in Rosacea: *In Silico* Analysis Suggests Further Studies

Rosacea'da IL-17A geni rs2275913 Varyantının Rolünün Araştırılması: *In Silico* Analiz Önerileri

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Öz

Vücudun patojenlere karşı bağışıklık tepkisinin önemli bir bileşeni olan İnterlökin-17 (IL-17), çeşitli inflamatuar süreçlerde rol oynar. Özellikle rosacea hastalarının derisi kronik inflamasyon sergiler ve IL-17'nin ek proinflamatuar kemokinler ve sitokinlerin üretimini indüklediği bilinmektedir. Bu inflamatuar kaskad, genişlemiş kan damarları, bağışıklık hücresi infiltrasyonu ve papül ve püstüllerin gelişimi dahil olmak üzere rosacea'nın ayırt edici özelliklerine katkıda bulunabilir. Çalışmada IL-17A genindeki spesifik bir genetik varyasyonun (-197 G>A; rs2275913) rosacea duyarlılığı ile ilişkili olup olmadığının incelenmesi amaçlandı. IL-17A varyantını ve rosacea riskini 31 sağlıklı bireyde ve rosacealı 25 bireyde karşılaştırdık. IL-17A varyantının genotiplemesi PCR-RFLP yöntemi kullanılarak yapıldı. Genotip ve alel frekans dağılımları gruplar arasında ki-kare testi ($\chi 2$) kullanılarak karşılaştırıldı. Ek olarak, web tabanlı araçlar kullanılarak IL-17A geninin gen ontolojisi (GO) analizi de gösterilmektedir. Bu çalışmada rs2275913 polimorfizmi ile rosacea duyarlılığı arasında anlamlı bir ilişki gözlenmedi (p=0.124), ancak *in silico* analizi IL-17A gen etkileşim ağının hastalıkta rol oynayabileceğini düşündürdü. IL-17A'nın ve ilgili genlerin, özellikle de bağışıklık savunması ve inflamatuar süreçlerdeki düzenlenmesindeki kritik işlevi göz önüne alındığında, rosacea gelişimi üzerindeki potansiyel etkisinin daha fazla araştırılması gerekmektedir.

Anahtar Kelimeler: IL-17A, İnflamatuar Sitokinler, Gen Polimorfizmi, Rosacea

Abstract

Interleukine-17 (IL-17), a crucial component of the body's immune response against pathogens, is also implicated in various inflammatory processes. Notably, the skin of rosacea patients exhibits chronic inflammation, and IL-17 is known to induce the production of additional pro-inflammatory chemokines and cytokines. This inflammatory cascade can contribute to the hallmark features of rosacea, including dilated blood vessels, immune cell infiltration, and the development of papules and pustules. The study aimed to examine whether a specific genetic variation in the IL-17A gene (-197 G>A; rs2275913) is associated with rosacea susceptibility. We compared the IL-17A variant and rosacea risk in 31 healthy individuals and 25 with rosacea. Genotyping of the IL-17A variant was performed using the PCR-RFLP method. Genotype and allele frequency distributions were compared across groups using the chi-square test ($\chi 2$). Additionally, gene ontology (GO) analysis of the IL-17A gene using web-based tools is also demonstrated. No significant association between the rs2275913 polymorphism and rosacea susceptibility was observed in this study (p=0.124) but *in silico* analysis suggested that the IL-17A gene interaction network might play a role in the disease. Given its critical function in regulating IL-17A and related genes, particularly in immune defense and inflammatory processes, further investigation into its potential influence on rosacea development is required.

Keywords: IL-17A, Inflammatory Cytokines, Gene Polymorphism, Rosacea

Introduction

Rosacea is a chronic skin condition distinguished by persistent redness, typically concentrated in the central region of the face. Predominantly affecting individuals aged 30 to 60, this dermatological disorder poses unique challenges due to its prolonged nature (1). While a definitive cure for this skin disorder remains elusive, there exist specific treatment modalities that aim to mitigate its effects. The global prevalence of rosacea currently stands at approximately 5%, and projections indicate a potential rise in this rate in the years to come (2). While the exact triggers of rosacea remain elusive, there's mounting evidence pointing towards an intricate interplay of genetics and environmental influences (3,4). Although the pathophysiology of rosacea is multifactorial, studies conducted in recent years emphasize the importance of innate and acquired immunity. The expression of genes facilitated by TH17, which encode cytokines such as IL-17A, IL22, IL6, IL20, and chemokine CCL20, is believed to be a significant contributing factor in the intricate landscape of rosacea development (5,6). IL17A is produced by CD4+ T helper (TH) cells and is an important pro-inflammatory cytokine in triggering the immune

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response. Since IL-17 is one of the triggers of the inflammatory process, it contributes to the pathophysiology of rosacea (7).

Additionally, studies have elucidated the angiogenic properties of IL-17, expanding our understanding of its multifaceted role in the pathogenesis of rosacea. In particular, while CD4+ TH cells typically dominate immune cell infiltrates, rosacea presents a distinct TH1/TH17 polarization pattern that distinguishes it from conditions such as acne and vulgaris (8). Although the connection between the active roles of IL-17A in the inflammatory response and the inflammation process in the pathogenesis of rosacea has been elucidated, more findings are needed about the contribution of IL-17A gene variants to the pathogenesis of rosacea. The study aimed to investigate the potential effect of the IL-17A gene rs2275913 variant on rosacea disease.

Material and Method

Study Groups

The study sample group was created using convenience sampling. Our study included 25 adult individuals (25 patients) who applied to Muğla Sıtkı Koçman University, Department of Dermatology and Venereology, were diagnosed with rosacea disease, were over 18 years of age, had cognitive ability, and did not have a serious immune disease. The healthy control group included 31 adults over the age of 18 (31 controls) who had not previously been diagnosed with any skin or venereal disease or serious chronic disease. The study did not include infection, heart failure, cancer, or pregnant and haematological patients. This research adhered to the ethical principles outlined in the Declaration of Helsinki and received prior approval from the Muğla Sıtkı Kocman University Faculty of Medicine Ethics Committee. Each participant actively provided written informed consent prior to their involvement in the study.

Molecular Analyses

Blood samples from participants for routine testing were stored at -40°C for DNA extraction. DNA extraction was employed using the Hibrigen Blood DNA Isolation Kit (Hibrigen Biotechnology R&D Industry and Trade Inc., Turkey). The rs2275913 polymorphism within the IL-17A gene was genotyped using PCR-RFLP analysis. The PCR reaction, performed in a 25-µL volume, included 100 ng of genomic DNA and 2X Taq Master Mix (Hibrigen Biotechnology R&D Industry and Trade Inc., Turkey). The specific primer sequences and the PCR and RFLP conditions are provided in Table 1.

Statistical analysis

Categorical data analysis was performed using Yates' corrected chi-square test and Fisher Freeman-Halton tests in SPSS (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). The percentage (%) and n (frequency) represent the category values. A p-value of less than 0.05 showed statistical significance for the results.

The power of the study was calculated using posterior power analysis and "G-Power-3.1.9.4" program. The power of the study, which included a total of 56 patients with a medium effect size, was determined to be 0.72.

In silico analyses

We leveraged the STRING database to elucidate potential interactions between IL-17A and the network proteins (https://string-db.org/). This tool provides information about how proteins interact with each other, both directly and indirectly, revealing not only their physical connections but also how they work together. Furthermore, we utilized DisGeNET (http://www.disgenet.org/home) to delve into gene-disease associations, specifically focusing on genes linked to rosacea (C0035854). By mining this curated database, we aimed to identify genes potentially involved in the pathogenesis of this skin condition.

Results

Molecular analyses

DNA samples taken from 25 patients and 31 healthy individuals over the age of 18 were analysed for IL-17A gene rs2275913 variant. The genotype and allele distributions of the IL-17A gene rs2275913 variant are shown in Table 2 and gel visualisation is shown in Figure 1.

Table 1. PCR and RFLP conditions used for polymorphisms of IL-17A gene

PCR conditions of the IL-17A gene rs2275913 variant				
Gene	Polymorphism	Primers	Temperature of annealing	Product size
IL17A	rs2275913 (-196 G>A)	P1 P2	65 °C	445 bp
RFLP conditions of the IL-17A gene rs2275913 variant				
Gene	Polymorphism	Restriction enzyme	Digestion conditions	Restriction fragment sizes
IL17A	rs2275913 (-196 G>A)	*Eco*NI	37°C, 16-18h	G allele: 445 bp A allele:148 and 297 bp

R: 5'-GCATAACTCTTCTGGCAGCTGTA-3, F: 5'-GTATTTCTGGACCGTGGGCA-3'

The control group exhibited genotype frequencies consistent with Hardy-Weinberg equilibrium (p=0.138). For the rs2275913 polymorphism, the distribution of GG, AG and AA genotypes was 12%, 4% and 84% in the case group, and 3.2%, 19.4% and 77.4% in the control group, respectively. We have detected no significant difference in the genotype frequencies and allele frequency the between two groups (p>0.999) (Table 2).

Table 2. Distribution of IL-17A genotypes and allele frequencies in Cases and Healthy controls

	Healthy controls *n* (%)	Cases *n* (%)	*p*
Genotype rs2275913 (-197 G>A)			
AA	24 (77.4)	21 (84.0)	
GG	1 (3.2)	3 (12.0)	0.138
AG	6 (19.4)	1 (4.0)	
Allele rs2275913 (-197 G>A)			
G	54 (87.1)	43 (86.0)	>0.999
A	8 (12.9)	7 (14.0)	

In silico Analyses

We obtained functional enrichment descriptions for potential STRING networks using the interactions IL17A. STRING reported pathways related to recognized networks, drawing from the Gene Ontology and Reactome Pathways databases (Table 3). The results of positive regulation of plasma cell differentiation, IL17 receptor activity and CD163 mediating an anti-inflammatory response ranked first. The prominent results were related to inflammation, consistent with the pathogenesis of rosacea. Among the genes annotated in these results were IL2, IL10, IL17RA, IL17RC and IL6 genes. A total of 41 genes associated with rosacea were identified in the DisGeNET database. In the analysis results, CAMP, TRPV4, HLA-DRA, IL17A, BTNL2, PSG2, OCA2, NHS, HLA-DRB AND CD79A genes were among the top 10 genes.

Discussion

Rosacea is a chronic inflammatory skin disorder characterized by persistent facial erythema. Its pathogenesis involves a complex interplay of genetic and environmental factors, culminating in the activation of various immune cell types, including keratinocytes, mast cells, endothelial cells, and T lymphocytes (9). Among these, Th17 cells play a pivotal role, releasing pro-inflammatory cytokines like IL-17A, which contribute to the characteristic inflammation observed in rosacea lesions (10,11). This study aimed to explore the potential association between the rs2275913 polymorphism within the IL-17A gene and rosacea susceptibility. While our analysis did not reveal a statistically significant difference in genotype or allele frequencies between rosacea patients and healthy controls, the findings warrant further discussion in the context of existing literature.

Different studies highlight the important role of IL-17A in the pathogenesis of various inflammatory skin disorders, including psoriasis, ankylosing spondylitis, and rheumatoid arthritis. It has been suggested that inhibiting IL-17 may be beneficial in the treatment of chronic inflammatory diseases such as psoriasis, ankylosing spondylitis, and rheumatoid arthritis (6). In a mouse model of imiquimod-induced psoriasis, the pro-inflammatory cytokine IL-17 was identified as a regulator of IL-25, a protein highly expressed in psoriatic skin lesions (12). Increased protein levels of IL-17F, IL-17A and IL-17C in psoriatic skin lesions demonstrated that, in addition to IL-17F, IL-17A and IL-17C also plays a potential role in pathogenesis (13). In the management of psoriasis, secukinumab has demonstrated its ability to provide long-term symptom relief by selectively neutralizing IL-17A, the primary inflammatory cytokine in this autoimmune disorder (14). Pityriasis rubra pilaris (PRP), a rare acquired inflammatory skin condition, is associated with increased levels of Th17 and Th1 cytokines, including IL-17A, IL-6, TNF, IL-22, IL-12, IL-23, and IL-17F (15). Quantitative analysis in lesional skin of Systemic Sclerosis revealed higher mRNA expression of Th17 cytokines, including IL-13, IL-17A, IL-26 and IL-22 compared to healthy controls (16).

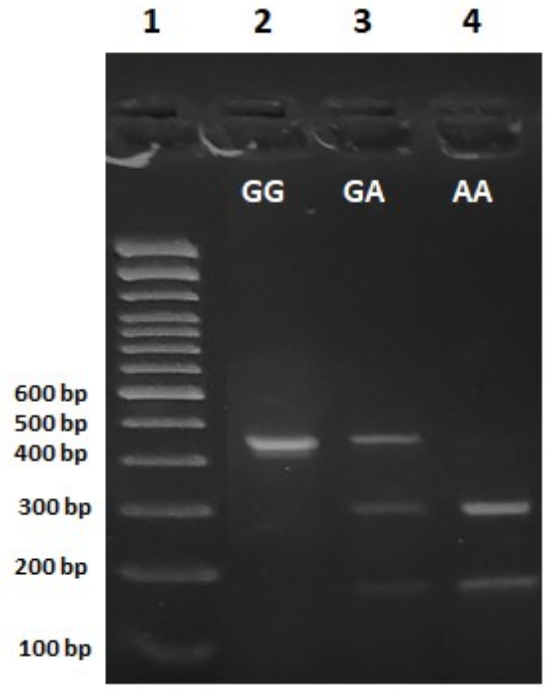
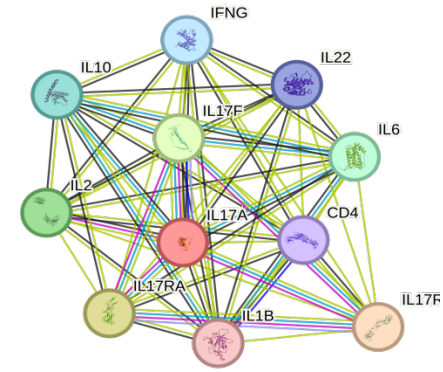
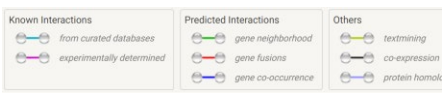


Figure 1. Polymerase chain reaction-restriction fragment length polymorphism analysis for genotyping of IL-17A rs2275913 variation. Lane 1 is a 100 bp DNA marker, lane 2 is GG genotype, lane 3 is GA genotype; lane 4 is AA genotype.

Table 3. Functional enrichments of networks based on IL-17A interactions

Network	Database	Description	Strength[1]	FDR[2]
 (number of nodes: 11, number of edges: 54, average node degree: 9.82, avg. local clustering coefficient: 0.982, expected number of edges: 23, PPI enrichment p-value: 3.24e-08)	Gene Ontology-Biological Process	1# GO:1900100 Positive regulation of plasma cell differentiation	3.08	0.00040
		2# GO:0060559 Positive regulation of calcidiol 1-monooxygenase activity	3.08	0.00040
		3# GO:2000340 positive regulation of chemokine (C-X-C motif) ligand 1 production	3.03	2.77e-06
		4# GO: 0032747 Positive regulation of interleukin-23 production	2.89	5.06e-06
		5# GO:0097400 Interleukin-17-mediated signaling pathway	2.78	7.95e-06
	Gene Ontology-Molecular Function	1# GO:0030368 Interleukin-17 receptor activity	2.65	0.0070
		2# GO:0005125 Interleukin-17 receptor activity	1.79	3.63e-10
		3# GO:0004896 Cytokine receptor activity	1.75	0.0088
		4# GO:0070851 Growth factor receptor binding	1.72	0.00053
		5# GO:0005126 Cytokine receptor binding	1.67	4.60e-08
	Reactome Pathways	1# HSA-9662834 - CD163 mediating an anti-inflammatory response	2.6	0.0032
		2# HSA-8877330 RUNX1 and FOXP3 control the development of regulatory T lymphocytes (Tregs)	2.55	0.0033
		3# HSA-6783783 Interleukin-10 signaling	2.08	0.00056
		4# HSA-448424 Interleukin-17 signaling	2.02	2.13e-05
		5# HSA-8950505 Gene and protein expression by JAK-STAT signaling after Interleukin-12 stimulation	1.99	0.0296



[1]**Strength:** Log10(observed / expected). This measure describes how large the enrichment effect is. It's the ratio between i) the number of proteins in your network that are annotated with a term and ii) the number of proteins that we expect to be annotated with this term in a random network of the same size.
[2]**False Discovery Rate:** This measure describes how significant the enrichment is. Shown are p-values corrected for multiple testing within each category using the Benjamini–Hochberg procedure.

There are studies investigating the relationship of various variants of the IL-17A gene with different dermatological diseases. It has been reported that the IL-17A rs2275913 variant does not affect the course of atopic dermatitis (17). The -152 G/A IL-17A variant AA genotype has been found to increase the risk of developing atopic dermatitis (18). It has been determined that AA+GA genotypes of the IL-17A (rs10484879) variant increase fungal growth and psoriasis susceptibility (19). A similar study determined that the IL-17A (rs10484879) G/T variant was effective in the pathogenesis of psoriasis in a North Indian population (20). Another study found no association between IL-17A rs4711998 and IL-17A rs2275913 variants and the development of vitiligo (21).

While the exact role of IL-17A in rosacea pathogenesis remains to be fully elucidated, the current understanding suggests its involvement in the inflammatory cascade. Further research is warranted to explore the functional significance of specific IL-17A variants and their potential interactions with other genetic and environmental factors in rosacea development. Additionally, investigating the broader immunological landscape, including Th17 cell subsets and other cytokine profiles, might provide deeper insights into the disease mechanisms.

The utilization of STRING and DisGeNET databases provided valuable insights into potential protein-protein interactions and gene-disease associations relevant to rosacea. Notably, genes like CAMP, TRPV4, and IL17A emerged as potential players in the rosacea network, warranting further investigation. These findings pave the way for future studies to delve deeper into the molecular mechanisms underlying rosacea and identify potential therapeutic targets.

In our study, the small number of participants is a significant limitation. Furthermore, the investigation was restricted to a single polymorphism within the IL-17A gene (rs2275913). Evaluating the influence of additional polymorphisms within this gene may offer more comprehensive insights into the potential role of IL-17A in rosacea pathogenesis.

Conclusion

This study investigated the potential association between the IL-17A gene variant rs2275913 and rosacea susceptibility. While *in silico* analyses revealed functional enrichment related to rosacea pathways and identified genes potentially involved in the disease, no significant differences in genotype or allele frequencies were observed between the rosacea patient and control groups. These findings suggest that the IL-17A rs2275913 variant may not be a direct contributor to rosacea development. However, further research with larger sample sizes and exploring additional IL-17A variants or functional studies might be necessary to definitively rule out its involvement.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Ethics Committee Approval: The study protocol was approved by Muğla Sıtkı Koçman University Faculty of Medicine Medical Ethics Committee (28/07/2021 dated and 16/II numbered decision). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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The Relationship Between Umbilical Cord Blood Interferon γ-Inducible Protein-10 (IP-10) Levels and Clinical and Laboratory Parameters in Preterm Infants

Preterm Bebeklerde Umbilikal Kord Kanı İnterferon γ ile Uyarılabilen Protein-10 (IP-10) Düzeyleri ile Klinik ve Laboratuvar Parametreleri Arasındaki İlişki

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Öz

İnterferon γ ile uyarılabilen protein-10 (IP-10) güçlü inflamatuar mediatörlerden biridir. Çalışmamızda antenatal erken membran rüptürü (PPROM), fetal inflamatuar yanıt sendromu (FIRS) ve prematüreliğe bağlı morbiditeleri olan veya olmayan prematüre bebeklerde kordon kanı IP-10 düzeylerinin karşılaştırılması amaçlanmıştır. 37. gebelik haftasının altında doğan 85 prematüre bebek çalışmaya dahil edildi. Doğum anında umbilikal korddan alınan kan örneklerinde ELİSA yöntemi ile interlökin (IL)-6 ve IP-10 düzeyleri ölçüldü. Tüm olgularda prematüreliğe bağlı gelişebilecek komplikasyonlar (respiratuar distres sendromu, erken ve geç başlangıçlı sepsis, nekrotizan enterokolit, intraventriküler kanama, prematüre retinopatisi, bronkopulmoner displazi) ve mortalite kaydedildi. Kordon kanında 11 pg/ml üzerinde olan IL-6 düzeyleri FIRS olarak kabul edildi. PPROM'lu grupta (n=27, %31.8) kordon kanında medyan IP-10 seviyesi diğer gruplara göre anlamlı derecede yüksek bulundu (IP-10=345.6 pg/ml vs. 28.3 pg/ml, p<0.001). FIRS saptanan olgularda (n=36, %42.4) kordon kanında medyan IP-10 düzeyi FIRS saptanmayanlara göre anlamlı derecede yüksek saptandı (p<0.001). Erken başlangıçlı sepsis gelişen olgularda da kordon kanında medyan IP-10 seviyesi anlamlı derecede yüksek idi (p=0.019). Prematüreliğe bağlı diğer morbiditeler ile kordon kanı IP-10 düzeyi arasında anlamlı bir ilişki bulunamadı. Çalışmamızda fetal inflamasyonu olan ve erken başlangıçlı sepsis gelişen prematüre bebeklerde kordon kanında IP-10 seviyelerinin yüksek olduğu saptanmıştır. Kordon kanında yüksek IP-10 seviyesi, neonatal sepsis gelişen/gelişecek prematüre bebeklerde intrauterin inflamasyonu göstermek için erken bir belirteç olarak kullanılabilir.

Anahtar Kelimeler: Fetal İnflamatuar Yanıt Sendromu, İnflamasyon, İnterferon γ ile Uyarılabilen Protein-10, Prematürite, Sepsis

Abstract

Interferon γ-inducible protein-10 (IP-10) is one of the potent inflammatory mediators. This research aims to compare cord blood IP-10 levels in preterm infants with or without antenatal preterm prelabor rupture of the membranes (PPROM), fetal inflammatory response syndrome (FIRS) and prematurity related morbidities. We enrolled 85 newborns with gestational age below 37 weeks. Umbilical cord blood samples were obtained at delivery and stored. Cord blood IP-10 and interleukin (IL)-6 levels measured with ELISA test. All enrolled preterm infants have been followed-up for prematurity related conditions including respiratory distress syndrome, early and late onset sepsis, necrotising enterocolitis, intraventricular haemorrhage, premature retinopathy, bronchopulmonary dysplasia and mortality. FIRS defined as IL-6 levels of umbilical cord above 11 pg/ml. Cord blood median IP-10 levels were significantly higher in PPROM group (n=27, 31.8%) than in the group without PPROM (IP-10=345.6 pg/ml vs. 28.3 pg/ml, p<0.001). Cord blood median IP-10 levels were significantly higher in preterm infants with FIRS (n=36, 42.4%) compared to infants without FIRS (p<0.001). Cord blood median IP-10 levels were also higher in preterm infants with early onset sepsis than those without early onset sepsis (p=0.019). We did not observe relationship between cord blood IP-10 levels and other prematurity-related complications. Increased cord blood IP-10 levels have been observed in preterm infants with fetal inflammation and who developed early onset sepsis. Cord blood IP-10 could be considered an early marker for intrauterine inflammation and its effect on fetal outcomes, such as the development of neonatal sepsis in preterm infants.

Keywords: Fetal Inflammatory Response Syndrome, Inflammation, Interferon γ-Inducible Protein-10, Prematurity, Sepsis

Introduction

Infants born before the 37th postconceptional week of pregnancy or before the 259th day from the mother's last menstrual period are named as "preterm infants". Approximately 10% of all births are preterm, and 1-2% of these infants are younger than the 32nd week of gestation and have a birth weight below 1500 grams (1,2). In many cases, the cause of preterm birth cannot be diagnosed exactly, but increasing risk factors increases the incidence of preterm birth. The most common pathological condition that causes preterm birth is inflammation of the maternal-fetal connection (3). Studies have shown that 40% of preterm births involve intrauterine inflammation and infection, and these infections are mostly subclinical. In particular, it has been found that more than 80% of women who give birth before the 28th week of gestation have

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intrauterine infection, and infection rates decrease as the gestational week progresses (4).

Survival rates of preterm infants have increased in recent years. This increase in survival rates has also brought about an increase in morbidity rates. In addition to the different biological structure and physiological characteristics of preterm infants, respiratory distress syndrome (RDS), necrotizing enterocolitis (NEC), intraventricular hemorrhage (IVH), retinopathy of prematurity (ROP), and in the long-term cerebral palsy (CP) and bronchopulmonary dysplasia (BPD) like serious morbidities affects the prognosis (5). These problems specific to premature infants are associated with fetal inflammation, and their incidence and severity increase in the presence of inflammation (3).

Interferon-γ inducible protein-10 (IP-10) or C-X-C motif chemokine ligand 10 (CXCL10) is a chemoattractant chemokine for T cells, exerting its effects through interaction with the cell surface chemokine (C-X-C motif) receptor 3 (CXCR3) (6-8). CXCR3, a cell-surface G protein-coupled receptor expressed mainly by T-helper (Th) 1 cells, cytotoxic T cells and natural killer cells that have a key role in immunity and inflammation. IP-10 is secreted from cells as a response to increased interferon (IFN)-γ. Expression of IP-10 is seen in many Th 1 type inflammatory diseases (e.g., multiple sclerosis, rheumatoid arthritis, psoriasis, systemic lupus erythematosus, graves disease, inflammatory bowel dissease, and type I diabetes), where it is thought to play an important role in recruiting activated T cells into sites of tissue inflammation (6). In these diseases, IP-10 levels correlate with tissue infiltration of T lymphocytes (6-9).

There are limited studies on IP-10 in newborn infants. Previous research suggests that serum IP-10 levels are significantly increased in birth-asphyxiated and perinatally infected neonates (10). In infants younger than four months (including newborns) with suspected serious bacterial infections, IP-10 assays might be predictive (11). A study in preterm lambs exposed to chorioamnionitis showed that IP-10 might contribute to lung injury and altered pulmonary vascular development (12).

Currently, cytokine levels in cord blood, placental pathological examination, clinical examination in the postnatal period, and imaging are the methods used in diagnosis to evaluate the fetal effects caused by intrauterine inflammation (2, 13). In this study, we evaluated the effects of IP-10 and interleukin (IL)-6 levels in the cord blood of preterm infants on clinical and laboratory parameters and their role in determining the severity of inflammation.

Material and Method

This prospective single-center cohort study was conducted at the Neonatology clinic of Osmangazi University Faculty of Medicine Hospital Center (a level III neonatal intensive care unit [NICU]) in Eskisehir, between 2010 June and 2011 July. All infants born less than 37 weeks of gestation and admitted to the NICU for any reason were included in the study. Infants with major congenital anomalies and those referred to other hospitals were excluded due to the potential for missing medical records. Ethical approval for the study was obtained from the institutional ethics committee (dated 21.05.2010, numbered 86).

Antenatal histories, birth weight, and demographic findings of the cases were recorded. The infants were divided into four groups according to their antenatal history (Group 1: those with preterm prelabor rupture of membranes [PPROM], Group 2: those with preeclampsia, Group 3: those with gestational diabetes, Group 4: those without any risk factors).

The infants included in the study were divided into two groups according to the presence of PPROM in antenatal history, and early onset sepsis and IP-10 levels were compared between the groups.

IL-6 and IP-10 levels were measured in cord blood as markers of fetal inflammation in all preterm infants. Infants with IL-6 levels above 11 pg/ml in cord blood were classified as having “fetal inflammatory response syndrome” (FIRS) (13). The study group was then stratified by the presence or absence of FIRS. Neonatal morbidities, mortality and IP-10 levels were compared between these two groups.

During their NICU stay, all preterm infants were monitored for complications that may develop due to fetal inflammation and prematurity (RDS, early and late onset sepsis, NEC, IVH, ROP, BPD and mortality).

Sample collection

Blood samples were taken from the umbilical cord, centrifuged at 5000 rpm for 10 minutes, and the serum was separated and stored at -80 °C until analysis.

Measurement of cytokine levels

ELISA kits (Immunoassays Quantikine kits R&D Systems for human IL-6 and IP-10) were used for IL-6 and IP-10 measurements.

Clinical description

PPROM is defined as the rupture of fetal membranes before labor begins and failure to deliver within 18-24 hours following membrane rupture (14).

RDS was defined as respiratory distress with cyanosis on room air, tachypnea (respiratory rate

>60/min), intercostal retractions, and the persistence or progression of respiratory distress for 48-96 hours of life, along with a diffuse reticulogranular appearance on chest radiography and an air bronchogram (15).

Neonatal sepsis occurs in the presence of at least three of the following clinical findings, including: tachycardia (heart rate >200/min) (except in cases such as sleep, anemia, hypo/hyperthyroidism, pain, post-feeding) or bradycardia, hypotonia, hypotension, seizure, tachypnea, apnea, respiratory distress, cyanosis, impaired skin color and perfusion, malnutrition, lethargy, irritability. These clinical findings were evaluated as high acute phase reactants and/or accompanying blood culture positivity. If sepsis findings appeared within the first 72 hours of life, it was considered "early onset" sepsis, and if it appeared after the first 72 hours of life, it was considered "late onset" sepsis (16).

Diagnosis and staging in patients with clinical and radiological signs and symptoms suggestive of NEC were made according to modified Bell scoring (17).

Risky preterms (<34 weeks of gestation) and infants who were clinically considered to have IVH were evaluated using transfontanel ultrasonography (USG) (18).

BPD was diagnosed using criteria from the American National Public Health Institute and classified as mild, moderate, or severe (19).

ROP screening was performed on infants born <1500 g or <32 weeks of gestation. The screening program started at 4-6 weeks after birth or at 31-33 weeks postconceptionally. Staging was done according to vascular proliferation (20).

Statistical evaluation

'SPSS for Windows 27.0' package program was applied in statistical evaluation of the results. Whether the quantitative variables conformed to normal distribution was examined with the Kolmogorov-Smirnov test. Independent groups were compared for non-normally distributed variables using the Mann Whitney U test or Kruskal Wallis analysis of variance. The relationship between quantitative variables was determined by Pearson or Spearman correlation analysis; The relationship between qualitative variables was examined with chi-square analysis. Descriptive statistics of quantitative variables that conform to normal distribution are shown as mean ± standard deviation, and descriptive statistics of quantitative variables that are not normally distributed are shown as median (25 th-75 th percentiles). P<0.05 values were considered statistically significant.

Results

In total, 85 preterm infants were admitted to the NICU during the study period. Of these, 49.4% were female and 50.6% were male. The majority of infants (70.6%) were delivered via cesarean section. The median gestational age of the infants was 33 (30.5-35) weeks, and mean birth weight was 1924 ± 699 g. Epidemiological data of preterm infants included in the study are shown in Table 1.

Table 1. Demographic characteristics of preterm infants.

Preterm infants (n=85)	
Gestational age (week)#	33 (30.5-35)
Birth weight (g)*	1924±699
Cesarean birth n (%)	60 (70.6)
Female n (%)	42 (49.4)
Apgar score 1th min#	5 (3-7)
Apgar score 5th min#	8 (6-9)
Maternal age (years)*	28.7±5.0

*Mean ± Standard deviation, #Median (25 th-75 th percentiles)

Antenatal history and IP-10

The maternal risk factors of the infants are shown in Table 2. There was a significant difference in IP-10 and IL-6 levels between the four groups (p<0.001 and p<0.001, respectively) (Table 2). In the post-hoc analysis, cord blood median IP-10 and IL-6 levels were found to be significantly higher in group 1 than in groups 2, 3 and 4 (Figure 1). There was no significant difference in C-reactive protein (CRP) levels among the four groups (p=0.399) (Table 2).

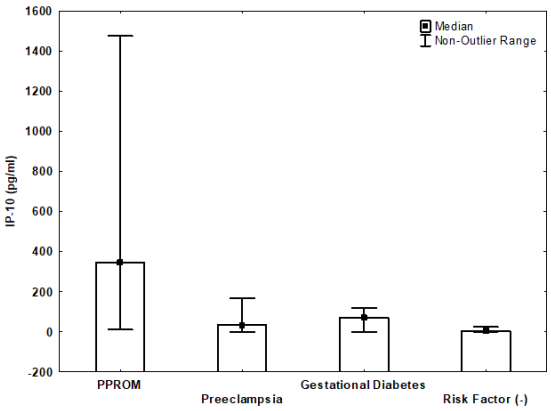

Figure 1. Cord blood IP-10 levels of the neonates according to maternal risk factors. PPROM: preterm prelabor rupture of the membranes, IP-10: interferon γ-inducible protein-10

In our study, six of 17 preterm infants who developed sepsis (five early onset sepsis, one late onset sepsis) had PPROM in their antenatal history. The median IP-10 level (345.6 pg/ml) in the group with antenatal PPROM was significantly higher than in the group without PPROM (28.3 pg/ml) (p<0.001). While the rate of early onset sepsis was twice as high in the PPROM group (18.5%) compared to the non-PPROM group (8.6%), there was no significant statistically difference between two groups (p=0.277). These findings are summarized in Table 3.

Table 2. Cord blood IP-10, IL-6 and CRP levels of the infants according to maternal risk factors

	Group 1 (n=27)	Group 2 (n=20)	Group 3 (n=15)	Group 4 (n=23)	p
IP-10 (pg/ml)	345.6 (141.2-924.2)**	33.3 (0.72-111.7)	69.7 (34.5-101.6)	4.8 (0.76-24.9)	**<0.001**
IL-6 (pg/ml)	14.6 (12.0-26.6)**	4.28 (1.1-16.7)	3.56 (1.8-6.3)	1.65 (0.6-6.4)	**<0.001**
CRP (mg/dl)	0.50 (0.2-3.08)	0.37 (0.15-16.7)	1 (0.1-3)	2.1 (0.3-3.08)	0.399

IP-10: interferon γ-inducible protein-10, IL-6: interleukin-6, CRP: C-reactive protein. Data are presented as median (25 th-75 th percentiles). **Group 1 is statistically different from group 2, group 3 and group 4 (p<0.001, p=0.008 and p<0.001 for IP-10 respectively and p=0.009, p=0.010 and p=0.010 for IL-6 respectively).

Morbidity and IP-10

Early onset sepsis developed 10 (11.8%) and late onset sepsis developed 7 (8.2%) of the infants. In our study, 20 (23.5%) of the infants had RDS; 4 (4.7%) had NEC (stage 1B in one, stage 2A in two, and stage 3A in one infant); 7 (8.2%) had IVH (stage 1 IVH in two, stage 2 IVH in four, and stage 4 IVH in one infant); 5 (5.9%) had ROP (stage 3 ROP in four, stage 4 ROP in one infant); and 9 (10.6%) had BPD (mild BPD in three, moderate BPD in four, and severe BPD in two infants).

The cord blood median IP-10 level of infants who developed early onset sepsis was significantly higher than that of infants who did not develop early onset sepsis (p=0.019), but no significant difference was found in terms of IL-6 levels (p=0.350) (Table 4). No significant statistical difference was detected between the cord blood median IP-10 and IL-6 levels of infants who developed and did not develop late onset sepsis, RDS, NEC, IVH, ROP, or BPD (p>0.05) (Table 4).

Mortality and IP-10

Ten (11.8%) of 85 preterm infants in the study group died. The cord blood median IP-10 level of these infants was 81.3 pg/ml (6.3-201), and the IL-6 level was 6.4 pg/ml (1.5-17.3). When the cord blood median IP-10 and IL-6 levels of these infants and the surviving preterm infants were compared, significant statistically difference was not detected between them (p=0.817 and p=0.859 respectively) (Table 4). Since nine out of 10 infants who developed mortality died before the 28th day, ROP and BPD were not evaluated in these infants.

FIRS and IP-10

FIRS was present in 36 (42.4%) of the preterm infants in the study group. The cord blood median IP-10 level of infants with FIRS (202.5 pg/ml [range=119.4-873.6 pg/ml]) was significantly higher than those without FIRS (11.8 pg/ml [range=0.81-62.1 pg/ml]) (p<0.001). From the perspective of perinatal morbidity, there was no statistical difference in terms of RDS, late onset sepsis, BPD, NEC, IVH, ROP frequency, and mortality between preterms with and without FIRS (p>0.05). Although the risk of early onset sepsis was higher in those with FIRS, it was not statistically significant (p=0.088) (Table 5).

Other Findings

A positive significant correlation was detected between cord blood IP-10 level and cord blood IL-6 level (r=0.80, p<0.001).

In Table 3, the resulting effect size of the posterior power analysis performed in the GPower program through the IP-10 descriptive statistics of those with and without PPROM was calculated as 2.512 and the obtained power was calculated as 99%.

Discussion

In our study, significant data were evaluated on the relationship between high cord blood IP-10 levels and morbidity, mortality, and other laboratory parameters in the neonatal period. This relationship has been recently researched, but data are limited. This is the first study to show that IP-10 levels are elevated in the cord blood of preterm infants with a history of PPROM in the antenatal period, FIRS, and early onset sepsis.

Table 3. Presence of early onset sepsis and IP-10 level according to the presence of PPROM

	PPROM (n=27)	No PPROM (n=58)	p
Early onset sepsis			
Present	5 (18.5)	5 (8.6)	0.277
Absent	22 (81.5)	53 (91.4)	
IP-10 (pg/ml)#	345.6 (141-924)	28.3 (0.92-99.1)	**<0.001**

PPROM: preterm prelabor rupture of the membranes, IP-10: interferon γ-inducible protein-10. #Median (25 th-75 th percentiles).

Table 4. Distribution of cord blood IP-10 and IL-6 levels according to neonatal morbidity and mortality

		IP-10 (pg/ml)	p	IL-6 (pg/ml)	p
RDS	Yes (n=20)	29.7 (2.1-164.2)	0.315	2.2 (1.1-13.2)	0.147
	No (n=65)	96.7 (11.8-208.7)		9.7 (1.9-18.4)	
Early onset sepsis	Yes (n=10)	173.4 (89.3-1236)	**0.019**	12.6 (5.0-18.2)	0.350
	No (n=75)	54.6 (4.8-145.6)		4.7 (1.4-14.6)	
Late onset sepsis	Yes (n=7)	11.2 (0.8-145.6)	0.350	1.6 (1.4-14.3)	0.554
	No (n=78)	77.1 (11.1-178.0)		6.4 (1.7-15.7)	
NEC	Yes (n=4)	72.3 (1.7-143.6)	0.501	7.0 (1.0-14.0)	0.638
	No (n=81)	69.8 (10.3-179.6)		6.4 (1.6-16.2)	
IVH	Yes (n=7)	9.5 (0.2-1234)	0.678	1.8 (1.0-13.1)	0.482
	No (n=78)	77.1 (11.5-171.9)		6.4 (1.6-15.7)	
ROP	Yes (n=5)	137.9 (0.6-690)	0.903	12.9 (0.6-17.7)	0.919
	No (n=71)	69.7 (9.5-168.9)		5.6 (1.4-17.3)	
BPD	Yes (n=9)	1.2 (0.1-410.7)	0.364	1.8 (0.6-17.3)	0.399
	No (n=67)	69.8 (11.7-168.9)		6.3 (1.5-17.3)	
Mortality	Yes (n=10)	81.3 (6.3-201)	0.817	6.4 (1.5-17.3)	0.859
	No (n=75)	69.8 (9.5-168.9)		6.4 (1.6-13.6)	

RDS: respiratory distress syndrome, NEC: necrotizing enterocolitis, IVH: intraventricular haemorrhage, ROP: retinopathy of prematurity, BDP: bronchopulmonary dysplasia, IP-10: interferon γ-inducible protein-10, IL-6: interleukin-6. Data are presented as median (25 th-75 th percentiles). *Since nine out of 10 infants who developed mortality died before the 28th day, ROP and BPD were not evaluated in these infants.*

Preterm births are the most significant cause of perinatal morbidity and mortality (1). The most common cause of preterm birth is inflammation and infection of the maternal-fetal junction. This inflammation and infection are mostly subclinical. Clinical and subclinical chorioamnionitis constitute 50% of preterm births, especially below the 30th week of gestation (21, 22). Similar to other studies, our study found that predisposing factors for preterm birth include pregnancy morbidities that initiate an inflammatory response, such as PPROM, preeclampsia, and gestational diabetes. It is not surprising that PPROM was found to be the most common cause in the prenatal history in our study, as it is the most common detectable factor associated with preterm birth and is present in approximately one-third of preterm births (23, 24). In our study, we found higher IP-10 and IL-6 levels in the cord blood of preterm infants with an antenatal history of PPROM compared to those without any antenatal disease.

The relationship between the increase in pro-inflammatory cytokines and preterm birth was first reported by Gomez et al. (13). IL-6 value of fetal plasma above 11 pg/ml obtained by cordocentesis in 105 pregnant women with preterm labor and 152 pregnant women with PPROM can be considered as the 'cut-off' value of the fetal inflammatory response, and IL-6 values above this figure are associated with increased neonatal morbidity. In other studies, it has been shown that there is a positive correlation between increased pro-inflammatory mediators in the amniotic fluid of pregnant women with preterm birth and amniotic fluid and fetal membrane culture results (25, 26). In our study, FIRS was more common in preterms with lower gestational age, in line with literature data. Additionally, the cord blood IP-10 level in patients with FIRS was higher than in those without FIRS. This finding suggested the pro-inflammatory role of IP-10 in the inflammation mechanism. Especially preterm infants with identified fetal inflammation face serious morbidities. RDS, sepsis, NEC, IVH and BPD are the most common causes of morbidity and mortality in these infants (27). While the incidence of early onset sepsis was higher in our cases who developed FIRS compared to those who did not, we found no significant relationship between the presence of FIRS and other neonatal morbidities, contrary to some data in the literature (27).

Neonatal sepsis is the leading cause of mortality and morbidity in preterm and very low birth weight infants (28, 29). As gestational age and birth weight decrease, the risk of developing sepsis increases (21). PPROM increases fetal infection risk, especially when chorioamnionitis is present, and the risk of neonatal sepsis rises (30, 31). In our study, six of the seven infants who developed early onset sepsis had PPROM in their antenatal history, and the rate of early onset sepsis in cases with PPROM was found to be twice as high as in cases without PPROM.

Interest in inflammatory mediators has recently focused on a group of small molecular weight cytokines known as chemokines. Chemokines are mostly secreted from inflamed or infected tissues and play significant role in different stages of the inflammatory pathways. There is a strong relationship between the amount of chemokine release and the severity of the inflammatory response (11). There are a few studies in the literature on IP-10 and other chemokine production and circulating concentrations in preterm infants (10, 11, 32, 33). In our study, the cord blood median IP-10 level of the preterm infants who developed early onset sepsis was significantly higher than those who did not develop sepsis. This finding shows that IP-10 is a valuable marker in detecting early onset sepsis. The CRP level, which is the most commonly used laboratory parameter in the early diagnosis of severe bacterial infections that cannot be detected clinically, was positive in only two of our patients

who developed early onset sepsis. Thus, it was shown once again that CRP is not a very sensitive marker of infection. Similar to our study, a study conducted on infants under four months of age, including newborns, showed that plasma IP-10 level was superior to white blood cell count and CRP levels in determining serious bacterial infections (11, 34).

In another study investigating chemokine levels to detect sepsis-induced disseminated intravascular coagulation in preterm infants at an early stage, it was found that IP-10 and other chemokines (monocyte chemoattractant protein-1 [MCP-1], IL-8, and monokine induced by interferon-γ [MIG]) were increased in infants with NEC and septicemia. This study demonstrated that preterm infants have the ability to mount strong cytokine and chemokine responses against pathogens (32).

In a large study evaluating inflammatory mediators as diagnostic markers in preterm infants with late onset bacterial infection, several cytokines and chemokines, including IP-10, IL-8, IL-6, MCP-1, MIG, regulated upon activation normal T cell expressed and secreted (RANTES), IL-1β, IL-10, and tumor necrosis factor-α (TNF-α), were studied at 0 and 24 hours. It was found that IP-10 is the best diagnostic marker of infection with the highest cut-off value at 0 and 24 hours (33). In our research, no significant difference was detected in cord blood IP-10 levels between preterms who developed late onset sepsis and those who did not. The difference between these results and ours is likely related to the very small number of infants with late onset sepsis examined.

Table 5. Distribution of neonatal morbidity, mortality and IP-10 levels according to the presence of FIRS

		FIRS (n=36)	No FIRS (n=49)	p
RDS	Yes (n=20)	7 (19.4)	13 (26.5)	0.615
	No (n=65)	29 (80.6))	36 (73.5)	
Early onset sepsis	Yes (n=10)	7 (19.4)	3 (6.1)	0.088
	No (n=75)	29 (80.6)	46 (93.9)	
Late onset sepsis	Yes (n=7)	3 (8.3)	4 (8.2)	>0.999
	No (n=78)	33 (91.7)	45 (91.8)	
NEC	Yes (n=4)	2 (5.6)	2 (4.1)	>0.999
	No (n=81)	34 (94.4)	47 (95.9)	
IVH	Yes (n=7)	3 (8.3)	4 (8.2)	>0.999
	No (n=78)	33 (91.7)	45 (91.8)	
ROP	Yes (n=5)	3 (9.4)	2 (4.5)	0.644
	No (n=71)	29 (90.6)	42 (95.5)	
BPD	Yes (n=9)	4 (12.5)	5 (11.4)	>0.999
	No (n=67)	28 (87.5)	39 (88.6)	
Mortality	Yes (n=10)	4 (11.1)	6 (12.2)	>0.999
	No (n=75)	32 (88.9)	43 (87.8)	
IP-10 (pg/ml)[#]		202.5 (119.4-873.6)	11.8 (0.81-62.1)	**<0.001**

FIRS: fetal inflammatory response syndrome, RDS: respiratory distress syndrome, NEC: necrotizing enterocolitis, IVH: intraventricular haemorrhage, ROP: retinopathy of prematurity, BDP: bronchopulmonary dysplasia, IP-10: interferon γ-inducible protein-10. Data are presented as number (%) or [#]median (25 th-75 th percentiles). *Since nine out of 10 infants who developed mortality died before the 28th day, ROP and BPD were not evaluated in these infants.*

In our study, contrary to studies in the literature, no significant relationship was found between mean cord blood IP-10 and IL-6 levels and the development of neonatal morbidities such as IVH, NEC, ROP, and BPD (12, 32, 35, 36). Studies have mostly associated IP-10 with infection and inflammation. Infection and inflammation are not the only causes of neonatal morbidity; the etiology is multifactorial. Factors such as respiratory support applications, oxygen applications, birth asphyxia, invasive interventions, mechanical ventilation, and inability to breastfeed may contribute to the development of morbidities (21, 29). For this reason, IP-10 levels may not have been found to be high in those who developed morbidity in our study.

There were some limitations regarding our study. First, the study was single-centered. Second, the sample size was small.

Conclusion

Since cord blood IP-10 levels are detected to be higher in preterm infants who had PPROM and developed FIRS during the antenatal period, it is thought that this chemokine may cause preterm birth by triggering systemic inflammation. IP-10 may be favorable as an early indicator in determining the presence and degree of inflammation in preterm infants.

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Automated External Defibrillator: Is Internet Education Reliable

Otomatik Eksternal Defibrilatör: İnternet Eğitimi Güvenilir Mi?

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Öz

Otomatik external defibrilatörler (AED) yaygınlığı her geçen gün artan ve herkes tarafından rahatlıkla kullanılabilmesi amacıyla tasarlanmış hayat kurtarıcı cihazlardır. Bu çalışmada, "YouTube" içeriğinde bulunan AED'lerle ilgili videoları doğruluk ve klavuzlara uygunluk açısından değerlendirdik. YouTube'daki AED videoları, ILCOR 2015'in AED bölümü esas alınarak değerlendirildi. Videolar değerlendirilirken, videonun yükleyicisi, video süresi, görüntülenme sayısı ve AED'nin kimin üzerinde uygulandığı da kaydedildi. Her bir video 0-9 puan arasında bir skor uygulanarak değerlendirildi. Bu çalışmada 300 video değerlendirildi. Bunlardan 215'i dışlama kriterlerine göre çalışma dışı bırakıldı ve 85'i çalışmaya dahil edildi. Bu videolar değerlendirildiğinde, 36'sının eğitim amaçlı özel şirketler tarafından yüklendiği, ortalama görüntülenme sayısının 19836 (min.-max. 7 - 254318) olduğu ve ortanca sürenin 5.46 dakika (min.-max. 0.24 - 59.1) olduğu bulundu. En fazla video 2014'te yüklenmişti (17) ve 68 videoda demonstrasyon için bir manken kullanılmıştı. Sadece 3 video (%3.5) tam puan aldı. Videoları yükleyen kurum ve görüntüleme ile güvenilirlik arasında anlamlı ilişki olduğu görüldü ($p \leq 0.05$). AED'nin uygulandığı kişi, video süresi ve yükleme zamanı ile güvenilirlik arasında bir korelasyon bulunmadı (sırasıyla $p=0.218$, $p=0.491$ ve $p=0.324$). Biz çalışmamız sonucunda YouTube'da ki 'automatic external defibrillator' adı altında yayınlanan videoların sadece 3'ünün tam puan almış olduğunu, 23 videonun da ortalama puanın üzerinde puan aldığı için eğitim açısından çok da kullanışlı olmadığını gördük.

Anahtar Kelimeler: Kardiyak Arrest, Otomatik Eksternal Defibrilatör, YouTube Video

Abstract

Automatic external defibrillators (AED) are life-saving devices whose prevalence is increasing day by day and are designed to be used easily by everyone. In this study, we evaluated the videos about AEDs on "YouTube" in terms of accuracy and compliance with the guidelines. AED videos on YouTube were evaluated based on the AED section of ILCOR 2015. While the videos were evaluated, the uploader, video duration, number of views, and who the AED was applied to were also recorded. Each video was evaluated by applying a score between 0-9 points. Three hundred videos were evaluated in this study. Of these, 215 were excluded from the study according to the exclusion criteria and 85 were included in the study. When these videos were evaluated, it was found that 36 of them were uploaded by private companies for educational purposes, the average number of views was 19836 (min. 7 – max. 254318) and the median duration was 5.46 seconds or minutes (min. 0.24 – max. 59.1). The highest number videos were uploaded in 2014 (17) and a mannequin was used for demonstration in 68 videos. Only 3 videos (3.5%) received full marks. It was observed that there was a significant relationship between the institution that uploaded the videos and the reliability of the views ($p \leq 0.05$). No correlation was found between reliability and the person to whom AED was applied, video duration and loading time ($p=0.218$, $p=0.491$ and $p=0.324$, respectively). As a result of our study, we saw that only 3 out of 85 published under the name 'automatic external defibrillator' on YouTube received full scores, and 23 videos received scores above the average score, therefore, YouTube does not appear to be a reliable source of education for AED.

Keywords: Cardiac Arrest, Automatic External Defibrillator, YouTube Video

Introduction

Cardiac arrest is defined as the state before death that will lead to death without necessary interventions. In adults, the primary cause of sudden cardiac arrest is shockable rhythms such as ventricular fibrillation (VF) and ventricular tachycardia (VT) in which adequate and speedy intervention is directly related to mortality. In the event of cardiac arrest, it has been reported that the mortality rate increases by 7% to 19% for every minute delay before initiating effective basic life support and resuscitation efforts. In shockable rhythms, the chance of survival decreases by 10-12% per minute as the rhythm persists (1-4). Therefore, the time elapsed before using a defibrillator during basic life support is extremely critical.

Automatic external defibrillators (AED) are life-saving devices whose prevalence is increasing day by day and are designed to be used easily by everyone. Designed for use by both healthcare professionals and the general public, these devices are particularly valuable for reducing time loss and correcting the rhythm in shockable rhythms (1). Lessons on the use of AED are generally available at medical schools and courses are available to improve public awareness. As with many health related topics, both healthcare professionals and the general public turn to video sharing websites, such as YouTube, for information on education on AED (5). There are many studies evaluating the availability, reliability and educational potential of text or multimedia available on the Internet. These studies have generally found inadequate information online (6–10).

YouTube is a social sharing network where videos can be easily uploaded and shared. YouTube offers ease of use and has become a popular source

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of information, the downside being that videos with wrong information are also available and this information may also spread quickly (5,11–14).

In this study we evaluated videos about AED on YouTube, using the keyword "automatic external defibrillator", in terms of accuracy and compliance with the guidelines.

Material and Method

This study was conducted by searching on the YouTube website (http://www.youtube.com) using the keyword "automatic external defibrillator" between January 13th and 15th, 2016, and then evaluating the results obtained from English pages.

The results obtained from the search using the keyword "automatic external defibrillator" on the YouTube website (http://www.youtube.com) were evaluated for inclusion to this study by two Emergency Medicine consultants with AED training. All consultants reviewed ILCOR's AED section before evaluation of videos. The document details the following steps: how AED is to be opened, where pads should be placed, waiting time for rhythm analysis, warning of bystanders, pressing button for delivery of shock, immediate restart of chest compressions and reevaluation of rhythm (1). First of all, YouTube was searched using the keyword "automatic external defibrillator" and videos found in the search results were evaluated for relevance and sufficiency. Exclusion criteria for videos were:

1. Not related to AED
2. No demonstration, just narrative
3. Language other than English
4. Not educational
5. Advertisement or announcement (course etc.)
6. Repeat video

After selection of videos, the uploading institute/person was classified as official (such as AHA, ILCOR, university etc.), healthcare worker (physician, paramedic etc.), agencies (news etc.), firms (educational courses etc.) or unknown. The video duration, views and who AED was applied to (manikin, human or both) were also noted.

All videos were evaluated by two independent emergency medicine consultants. Any discrepancy in scores was resolved by consulting a faculty member of emergency medicine. Each video was evaluated by applying a score between 0-9 points (Table 1).

Statistical analysis

Data were analyzed using the SPSS 20.0 for Windows software (SPSS Inc., Chicago, IL, USA). Normality of the quantitative data distribution was assessed using the Kolmogorov–Smirnov test. Parametric tests (independent-sample t-test and post hoc Tukey's test) were used for normally distributed data, and non-parametric tests (Mann–Whitney U-test and the Kruskal–Wallis test) were applied to data not normally distributed. Continuous data are presented as means ± standard deviations or medians and ranges, as appropriate. All tests were two-tailed, and a p-value < 0.05 was considered to indicate significance.

Table 1. Parameters used for evaluated the conformity of videos

TASK	Score
A1. Does the video show how the defibrillator should be opened?	1
A2. Were all materials within explained and introduced?	1
A3. Were the AED pads placed in the correct area?	1
A4. Did the instructor wait for rhythm analysis?	1
A5. Were bystanders warned not to touch the patient?	1
A6. Was pressing of the shock button demonstrated?	1
A7. Were chest compressions resumed?	1
A8. Was rhythm analysis re-performed?	1
A9. Were pediatric pedals or pads shown?	1

Since this study was conducted by watching videos on YouTube, which is accessible to all people, and no patient data was used, and since ethical permission is not required in similar studies in the literature, ethics committee permission was not obtained.

Results

YouTube search results for "automatic external defibrillator" were presented in pages with 15 results on each page. The first 20 pages were viewed and 300 videos were evaluated in this study. Two-hundred and fifteen videos were excluded according to the exclusion criteria. These videos were either non educational (n=62), advertisements (n=39), or did not include demonstration (n=35). Table 2 shows videos that were included for evaluation.

Table 2. Distribution of videos according to exclusion criteria.

Reason of exclusion	n	%
Not related to AED	9	3
Description but no demonstration	35	11.7
Not in English	13	4.3
Not educational	62	20.7
Primary for advertisement	39	13
Primary for entertainment	1	0.3
Non-medical video	24	8
Repeat video	32	10.7
Not excluded	85	28.3
Total	**300**	**100**

Videos were found to be uploaded by private companies (n=36), unknown (n=27) and official sources (n=22). The average number of views was 19836 (min 7 – max 254318) and the average

duration of videos was 5.46 (min.0.24 – max.59.1) minutes. The highest number videos were uploaded in 2014 (n=17) and least in 2008 (n=1). A mannequin was used for demonstration in 68 videos (Table 3). Video scores are shown in Table 3.

Only 3 (3.5%) videos received full score. Average video scores were 6.07±1.6. Score of 8 or more were accepted as above average and reliable. Only 23 videos (27.1%) were found to be reliable and It was observed that there was a significant relationship between the institution that uploaded the videos and the reliability of the views ($p \leq 0.05$). No correlation was found between reliability, the person to whom AED was applied on, video duration and loading time. (p=0,218-0,491-0,324 respectively).

The study has several limitations. Only one keyword was used and the addition of other keywords such as "AED", "external defibrillator" or "automatic defibrillator" may have led to more videos being analysed. Only English language videos were evaluated, other languages were ignored.

Discussion

A search of YouTube website (http://www.youtube.com) using the keyword "automatic external defibrillator" revealed 508 results. The first 300 videos were evaluated in this study and 215 videos were excluded from the study according to the exclusion criteria. Videos were found to be uploaded by private companies (n=36), unknown (n=27) and official sources (n=22). As a result of our study, we saw that only 27% videos related to AED received above average score and that the remaining videos were not useful for educational purposes.

Table 3. Characteristics of the videos included in the analysis.

Date	**n**	**%**
2007	2	2.4
2008	1	1.2
2009	4	4.7
2010	9	10.6
2011	13	15.43
2012	15	17.6
2013	8	9.4
2014	17	20
2015	16	18.8
Who uploaded	**n**	**%**
Official institutions (such as AHA/ERC or University...)	6	7.1
Healthcare professional (physician, emergency medical technician, nurse etc.)	13	15.3
Individual with credentials unspecified	27	31.8
News program	3	3.5
Special courses	36	42.4
Applied on whom?	**n**	**%**
Human	16	18.8
Manikin	68	80
Both	1	1.2
Score	**n**	**%**
A1 correctly applied	46	54.1
A2 correctly applied	29	34.3
A3 correctly applied	84	98.8
A4 correctly applied	85	100
A5 correctly applied	82	96.5
A6 correctly applied	62	72.9
A7 correctly applied	76	89.4
A8 correctly applied	44	51.8
A9 correctly applied	9	10.6
Total	**85**	**100**

YouTube and similar social sharing networks are commonly used and allow for fast information exchange that is generally uncontrollable. Uncontrolled, widespread and fast spread of information is useful but may also lead to the spread of misinformation. Beydilli et al. evaluated YouTube videos of pediatric resuscitation (BLS and CPR) and found that only 232 of 1200 videos were related to BLS and CPR and that only 15% of these were reliable (5). Yaylacı et al evaluated the safety and accuracy of YouTube videos on adult CPR and BLS and found that 1994 videos were uploaded, 1785 were excluded and 209 videos that were in accordance to 2010 guidelines were evaluated and very few found to be excellent with regard to educational value (11). Muragiah et al. evaluated YouTube videos of BLS and CPR and found no correlation between accuracy and the uploader and views, concluding that increased views do not mean high accuracy (13). In our study, only 28% of videos

were related to AED and only 27% of these videos were determined to be reliable. According to these two studies in the literature, although the percentage of reliable videos in our study is higher, considering that these videos are related to a highly critical issue such as the correct use of automatic external defibrillators during the performing of basic life support , we can also state based on the results of our study that the reliability of videos on this topic is low. Moreover, if all videos related to automatic external defibrillators had been examined in our study, the results might have been as low as those in these two studies

As with CPR in previous studies, AED is a topic that interests not only healthcare workers but the whole population. Therefore, both healthcare professionals and the general public may view or upload videos, with lack of validity and reliability of videos not sourced from healthcare professionals.

Guidelines published in 2015 include AED in basic life support measures and give details on: correctly opening the defibrillator, where to place pads, waiting time for rhythm analysis, should a shockable rhythm be detected the warning of bystanders followed by pressing of the shock button, immediate restart of chest compressions, reevaluation of rhythm by AED (1). When we evaluated AED videos, most scores were from the placement of pads, waiting for rhythm analysis and the warning of bystanders. The introduction and placement of pediatric pads was only seen in 10.6% of videos.

Conclusion

Defibrillators are life saving devices. It is inevitable that healthcare professionals and the general public look to the internet for information, especially on such important topics. Marking of videos from official sources may be a method of showing the general public which videos are of necessary quality, leading to the spread of reliable information.

Limitations: This is the first study to evaluate online videos regarding the use of AED. The study has several limitations. Only one keyword was used and the addition of other keywords such as “AED”, “external defibrillator” or “automatic defibrillator” may have led to more videos being analyzed. Only English language videos were evaluated, other languages were ignored. We evaluated videos uploaded before the beginning of the study, so our results may change over time. The quality and characteristics of videos may change over time.

Conflict of interest statement

The authors declare no conflict of interest.

Ethics Committee Approval: Since this study was conducted by watching videos on YouTube, which is accessible to all people, and no patient data was used, and since ethical permission is not required in similar studies in the literature, ethics committee permission was not obtained.

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Should Stabilization be Added to Decompression in Lumbar Spinal Stenosis Surgery?

Lomber Spinal Stenoz Cerrahisinde Dekompresyona Stabilizasyon Eklenmeli Mi?

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Öz

Lomber spinal stenozun cerrahi tedavisinde sadece dekompresyon yapmak veya dekompresyona stabilizasyon eklemek seçenekleri hep bir tartışma konusu olmuştur. Biz de lomber spinal stenoz cerrahisinde dekompresyona stabilizasyon eklenmesinin klinik sonuçlara etkisini araştırmayı amaçladık. Altı yıl boyunca total laminektomi ile lomber stenoz ameliyatı geçiren hastalar klinik sonuçlar açısından değerlendirildi. Hasta memnuniyeti birincil başarı kriteri olarak kabul edildi. Tekrar ameliyat edilen hastalar cerrahi sonuçlardan memnun olduklarını belirtseler bile başarısız olarak değerlendirildiler. Çalışmaya toplam 73 hasta dahil edildi. Bir veya iki segment stabilizasyonu olan hastaların, hiç stabilize olmayanlara (p=0,019) göre daha tatmin edici sonuçlara sahip olduğu görüldü. Ancak üç veya daha fazla segment stabilizasyon grubu ile sadece laminektomi (p=1.0000) ve bir veya iki segment stabilizasyon (p=0.0667) grupları arasında başarı açısından fark yoktu. Ayrıca ameliyat sırasında dura yaralanması olmamasının (p=0.02148) başarıyı arttırdığı belirlendi. Bir veya iki seviyeli stabilizasyon ile lomber dekompresyonun, tek başına dekompresyon grubuna göre daha tatmin edici sonuçlara sahip olduğu bulundu. Ancak stabilize segment sayısı arttıkça bu farkın kaybolduğu göz önünde bulundurularak stabilizasyon endikasyonu dikkatle değerlendirilmeli ve stabilize segmentin gereksiz yere uzatılmaması için azami çaba gösterilmelidir. Stabilizasyonun etkilerinin daha fazla araştırılması için daha geniş hasta serileriyle yapılacak prospektif çalışmalar faydalı olacaktır.

Anahtar Kelimeler: Dekompresyon, Laminektomi, Lomber Vertebra, Spinal Stenoz, Stabilizasyon

Abstract

In the lumbar spinal stenosis surgery, the options of performing only decompression or adding stabilization to decompression have always been a matter of debate. We aimed to investigate the effect of adding stabilization to decompression on clinical outcomes in lumbar spinal stenosis surgery. Patients who underwent lumbar stenosis surgery with total laminectomy over six years were evaluated for clinical outcomes. Patient satisfaction was accepted as the primary success criterion. Patients who underwent reoperation were considered unsuccessful even if they stated that they were satisfied with the surgical results. A total of 73 patients were included in the study. Notably, patients who underwent stabilization of one or two spinal segments had more satisfying results than those without stabilization (p=0.0195). However, no significant differences in satisfaction were observed between patients with three or more stabilized segments and either the laminectomy-only group (p=1.0000) or the one-to-two segment stabilization group (p=0.0667). It was also determined that no dural injury during surgery (p=0.02148) increased success. Lumbar decompression with one- or two-level stabilization was found to have better satisfying results than the decompression-only group. However, considering that success decreases as the number of stabilized segments increases, the indication for stabilization should be carefully evaluated and maximum effort should be made to prevent unnecessary extension of the stabilized segment. Prospective studies with more extensive patient series will help investigate the effects of stabilization further.

Keywords: Decompression, Laminectomy, Lumbar Vertebrae, Spinal Stenosis, Stabilization

Introduction

Lumbar spinal stenosis (LSS) is characterized by radicular pain and neurogenic claudication caused by compression of nerve elements due to narrowing of the spinal canal (1,2). Narrowing of the spinal canal is caused by bony and ligamentous hypertrophy, disc protrusion, spondylolisthesis, or their combination. Surgical decompression is generally accepted treatment for progressive lumbar spinal stenosis cases that do not respond to conservative treatment. Wide laminectomy and flavectomy at the stenotic levels are the standard procedures for the surgical decompression of LSS. However, the extensive removal of posterior spinal elements in advanced stenosis carries a significant risk of spinal instability. On the other hand, it may not always be possible to preserve the lateral half of facet joints and pars interarticularis. Post-decompression instability can be prevented with instrumented or non-instrumented spinal fusion (3).

Recently, surgical techniques involving minimal decompression, such as fenestration, laminotomy, laminoplasty, and split laminectomy, are increasingly used techniques to maintain post-decompression spinal stability and eliminate the need for fusion. Nevertheless, some studies reported higher restenosis rates with these minimal decompression techniques (4,5). While stabilization performed due to concerns of instability has advantages, it also has disadvantages, such as the possibility of causing complications like pseudoarthrosis and adjacent segment disease.

Decisions of adding stabilization to decompression surgery can be made on a case-by-

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case basis. This study investigates whether adding stabilization to decompression surgery in lumbar spinal stenosis affects clinical outcomes and, if so, in which subgroups it positively influences the clinical results.

Material and Method

Approval was obtained from the local ethics committee for the study. Patients who underwent surgery due to LSS within six years at our hospital were identified from the surgical records. Patient information was retrieved from the hospital's Probel data recording system, and their images were examined from the hospital's PACS system. Additionally, images taken at other hospitals were accessed through the national e-Nabız data recording system if available.

Inclusion criteria:

a) At least one level of total laminectomy

b) At least six months follow up

Exclusion criteria:

a) Preoperative functional imaging proving lumbar instability

b) History of fracture or surgery at the laminectomy levels

c) Syndromic patients (achondroplasia, etc.)

All patients who met the above-mentioned criteria and whose records were accessible were included in the study. Patients included in the study were called for polyclinic control. Face-to-face patient satisfaction and Roland Morris Disability Questionnaire (RMDQ) surveys were conducted for those who could attend clinic follow-up, and telephone surveys were conducted for those who could not. In addition, the presence of neurogenic claudication was questioned. Preoperative independent variables were gender, age, height, weight, body mass index (BMI), walking distance, and degenerative spondylolisthesis. Perioperative independent variables were the number of stabilized segments and total laminectomies, dural injury, blood usage, and length of the surgery. Dependent variables included success (reoperation/satisfaction), RMDQ, and postoperative neurogenic claudication. Patient satisfaction was divided into three groups: satisfied, unsure and unsatisfied. For surgical outcomes, patients who did not explicitly state their satisfaction (the sum of those who said they were unhappy and those who were unsure) were considered unsuccessful. Even if the patient was satisfied with their current state, the initial surgery was deemed unsuccessful if they had reoperation due to lumbar spinal stenosis. Patients who underwent lumbar spinal stenosis surgery only once and explicitly stated their satisfaction was considered successful. Neurogenic claudication was defined as the patient being able to walk less than 1000 meters before needing to sit down. Outcomes of cases that received only decompressive laminectomy were compared with those of stabilization-added ones.

Statistical analysis

Power analyses were conducted to evaluate the test power for a sample size of 73 using both medium and large effect sizes. According to the post-power analysis, statistical power was obtained as 0.727 and 0.908, for a medium effect size (Cohen's w=0.3) and large effect size (Cohen's w=0.5) respectively, both with a type 1 error of 0.05. Then the numerical data for 73 patients were initially examined for minimum, maximum, mean, and standard deviation values. Subsequently, the Shapiro-Wilk Normality Test was used to analyze whether the numerical variables followed a normal distribution. Variables found to be normally distributed were analyzed in subsequent steps using the parametric test ANOVA. While comparing numerical variables to each other, Spearman's Rank Correlation analysis was used to examine whether there is an association between continuous variables. For variables that did not follow a normal distribution, non-parametric tests such as the Mann-Whitney U Test and Kruskal-Wallis Rank Sum Test were used. Specifically, the Mann-Whitney U Test was applied for variables with two groups, while the Kruskal-Wallis Rank Sum Test was used for variables with three or more groups. Additionally, for comparing statistical significance between two categorical variables, Pearson's Chi-squared test and Fisher's Exact Test were applied. For comparing categorical variables with more than two groups, Pairwise Fisher's Exact Test was utilized to assess statistical significance between groups. All analyses were conducted using R Studio and the R programming language. P-values less than 0.05 were considered statistically significant.

Results

General findings

Seventy-three patients who underwent lumbar spinal canal surgery were investigated. Summary of the continuous variables are shown in the Table 1.

Table 1. Data regarding the general distribution of data in continuous variables

	Number of samples (N)	Min. - Max.	Mean∓SD	25th percentile	50th percentile (median)	75th percentile	Shapiro-Wilk Normality Test
Age	73	36 - 80	61.3∓8.7	56	62	68	W=0.97325, p=0.1227
Height (cm)	73	148 - 198	165.7∓10.1	159	165	171	W=0.96155, p=0.02546
Weight (kg)	73	45 - 105	81∓12.8	70	82	90	W=0.95779, p=0.01555
BMI	73	17.6 - 37.6	29.5∓3.9	27.78	29.97	31.53	W=0.97279, p=0.1154
Preoperative walking distance (m)	73	5 - 2000	219.3∓355.9	30	100	200	W=0.59327, p=6.515e-13
Postoperative walking distance (m)	73	2 - 1500	812.9∓371.2	1000	1000	1000	W=0.60374, p=9.812e-13
RMDQ	67*	0 - 24	10.4∓6.5	6	9	15.5	W=0.962, p=0.0387
Number of Laminectomies	73	1 - 6	2∓1	1	2	2	W=0.81677, p=4.26e-08
Duration of surgery (min)	73	85 - 420	209.4∓72.2	165	195	230	W=0.93612, p=0.001111
Blood Used in Surgery (cc)	73	0 - 1000	169.9∓291.9	0	0	400	W=0.6313, p=2.993e-12

*missing values were removed prior to calculating the percentiles. Note: Age and BMI exhibit a normal distribution

The average follow-up period was 45.5±21.8 months (minimum 6.5, maximum 84.8). There were 32 male and 41 female patients with an average age of 61.3±8.7 (minimum 36, maximum 80). Six patients (2 male and four female) underwent reoperation, and even though 4 of these six patients stated they were satisfied, they were considered unsuccessful. Of the remaining 67 patients, four reported being dissatisfied with the outcome, while 11 were unsure whether they were satisfied. All 15 of these patients were considered unsuccessful. Thus, 52 patients who had not undergone reoperation and were happy with the surgery were deemed successful. Neurogenic claudication (needing to sit down after walking distances shorter than 1000 meters) was present in 66 out of 73 preoperatively and 17 postoperatively.

Comparative findings

Outcome Parameters according to the operation methods (Table 2): A total of 45 patients in the study had only total laminectomy, while the remaining 28 patients had stabilization in addition to total laminectomy. The success rate in the whole stabilization group (24 patients, 85.7%) was not different statistically from those in the non-stabilized group (29 patients, 64.4%) (p=0.06098). However, when patients were grouped according to the number of stabilized segments, the stabilization group showed significant internal differences. Of the 28 patients who underwent stabilization, 22 had 1-2 segments stabilized, while six had three or more segments stabilized. Patients with one or two segments stabilization (Group 2) were found to be more successful than patients without any stabilization (Group 1) (p=0.0195).

Representative cases illustrating the successful outcomes of laminectomy with and without stabilization are provided in Figure 1 and Figure 2, respectively. No difference was found in terms of success between the group with only laminectomy without stabilization (Group 1) and the group with laminectomy plus three or more segment stabilization (Group 3) (p=1).

Roland-Morris scores also differed between these three groups (Roland Morris median for Group 1: 9, Group 2: 11, Group 3: 16; p=0.02). In pairwise comparisons, it was found that Roland Morris disability questionnaire scores were lower in Group 1 than in Group 3 (p=0.0026). However, there was no statistically significant difference between Group 1 and Group 2 and Group 2 and Group 3.

The incidence of postoperative neurogenic claudication differed among the groups (11/45, 1/22, 5/6 for Group 1, Group 2, and Group 3, respectively; p<0.001). This difference was present between Group 2 and Group 3 (p=0.0011), between Group 1 and Group 3 (p=0.0268), but not between Group 1 and Group 2 (p=0.2580).

Outcome Parameters according to the other independent variables (Table 3):

Table 2. Dependent variables (outcome parameters)according to the operation methods

Outcome parameter and compared groups	P value	Groups (operation methods)					
Success: Gr. 1 vs Gr. 2 & Gr. 3	0.06098*	Gr. 1			Gr. 2 & 3		
		Suc.		Uns.	Suc.		Uns.
		29		16	24		4
Success: Gr. 1 vs Gr. 2 vs Gr. 3	**0.005264***	Gr. 1		Gr. 2		Gr. 3	
		Suc.	Uns.	Suc.	Uns.	Suc.	Uns.
		29	16	21	1	3	3
Success: Gr. 1 vs Gr. 2	**0.0195***	Gr. 1			Gr. 2		
		Suc.		Uns.	Suc.		Uns.
		29		16	21		1
Success: Gr. 1 vs Gr. 3	1.0000*	Gr. 1			Gr. 3		
		Suc.		Uns.	Suc.		Uns.
		29		16	3		3
Success: Gr. 2 vs Gr. 3	0.0667*	Gr. 2			Gr. 3		
		Suc.		Uns.	Suc.		Uns.
		21		1	3		3
RMDQ: Gr. 1 vs Gr. 2 vs Gr. 3	**0.02****	Gr. 1 RMDQ median		Gr. 2 RMDQ median		Gr. 3 RMDQ median	
		9		11		16	
RMDQ: Gr. 1 vs Gr. 2	0.0750*	Gr. 1 RMDQ median			Gr. 2 RMDQ median		
		9			11		
RMDQ: Gr. 1 vs Gr. 3	**0.0026****	Gr. 1 RMDQ median			Gr. 3 RMDQ median		
		9			16		
RMDQ: Gr. 2 vs Gr. 3	**0.0270****	Gr. 2 RMDQ median			Gr. 3 RMDQ median		
		11			16		
PO WD: Gr. 1 vs Gr. 2 vs Gr. 3	**<0,0001 ****	Gr. 1 WD mean		Gr. 2 WD mean		Gr. 3 WD mean	
		796.22		972.73		352.5	
PO WD: Gr. 1 vs Gr. 2	**0.0324****	Gr. 1 WD mean			Gr. 2 WD mean		
		796.22			972.73		
PO WD: Gr. 1 vs Gr. 3	**0.0013****	Gr. 1 WD mean			Gr. 3 WD mean		
		796.22			352.5		
PO WD: Gr. 2 vs Gr. 3	**0.0001****	Gr. 2 WD mean			Gr. 3 WD mean		
		972.73			352.5		
PO NC: Gr. 1 vs Gr. 2 vs Gr. 3	**0.0005481***	Gr. 1		Gr. 2		Gr. 3	
		NC+	NC-	NC+	NC-	NC+	NC-
		11	34	1	21	5	1
PO NC: Gr. 1 vs Gr. 2	0.2580*	Gr. 1			Gr. 2		
		NC+		NC-	NC+		NC-
		11		34	1		21
PO NC: Gr. 1 vs Gr. 3	**0.0268***	Gr. 1			Gr. 3		
		+		-	+		-
		11		34	5		1
PO NC: Gr. 2 vs Gr. 3	**0.0011***	Gr. 2			Gr. 3		
		+		-	+		-
		1		21	5		1

Gr. 1: only decompressed; Gr. 2 decompressed and additionally 1-2 segments stabilized, Gr. 3: decompressed and additionally 3 or more segments stabilized, PO: Postoperative Gr.: Group, Suc.: Successful, Uns.: Unsuccessful, NC: Neurogenic Claudication, WD: Walking Distance. * Fisher's Exact Test. ** Kruskal-Wallis rank sum test

There was no difference in success rate and Roland Morris scores between the sexes. The occurrence of neurogenic claudication in the postoperative period was more frequent in women (14/41) compared to men (3/32) (p=0.02374). No association was found between age and success, Roland Morris scores, or walking distance after surgery.

When examining the relationship between the number of laminectomies and success, the average number of laminectomies in unsuccessful patients (2.4±1.2) was slightly higher than those in successful patients (2.0±0.9); however, this was not statistically significant (p=0.129).

Our study also considered the presence of preoperative degenerative spondylolisthesis in patients. Degenerative spondylolisthesis was detected in 48,9% (22 patients) who underwent only decompression and 85.7% (24 patients) who also had stabilization. However, degenerative spondylolisthesis did not affect success rates, Roland Morris disability questionnaire scores, or postoperative neurogenic claudication rates.

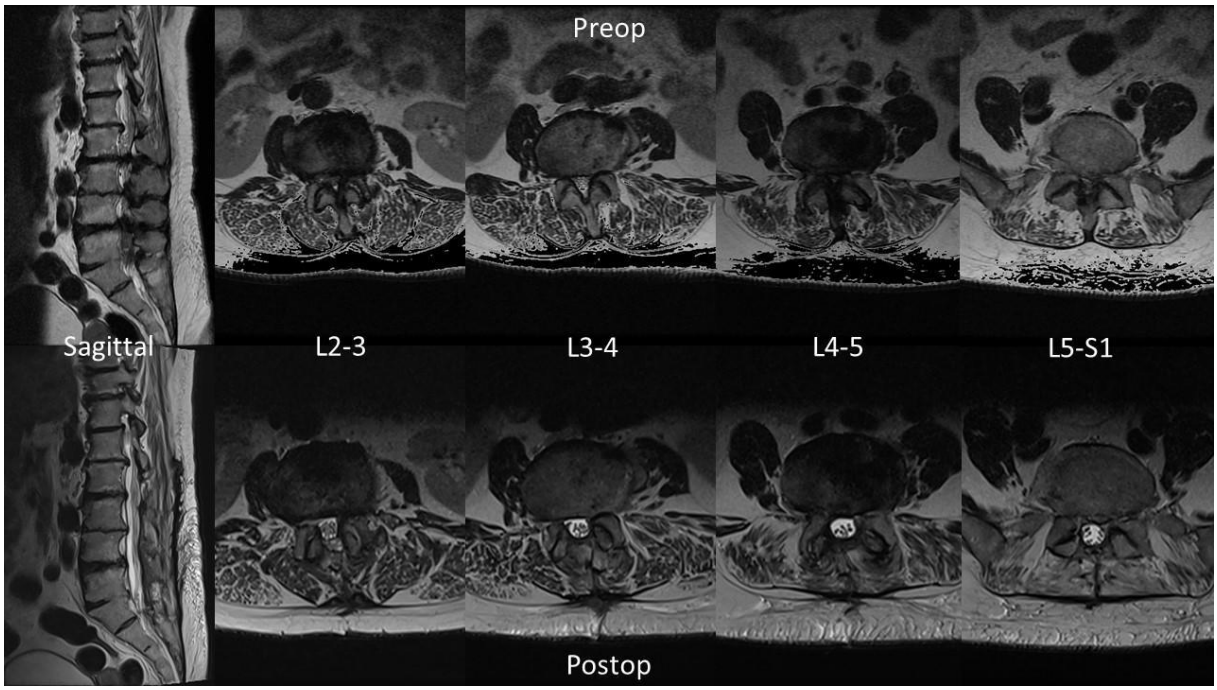

Figure 1. Pre and postoperative lumbar T-2 weighted MRI images of patient #74, a 68-year-old female who presented with a complaint of neurogenic claudication after walking 100 steps. A total of 4 levels of laminectomy were performed at L2-3-4-5. The walking restriction was eliminated postoperatively. The Roland Morris score was 18. The patient was satisfied with the surgery, and there was no need for reoperation; the case was considered "successful" in the study.

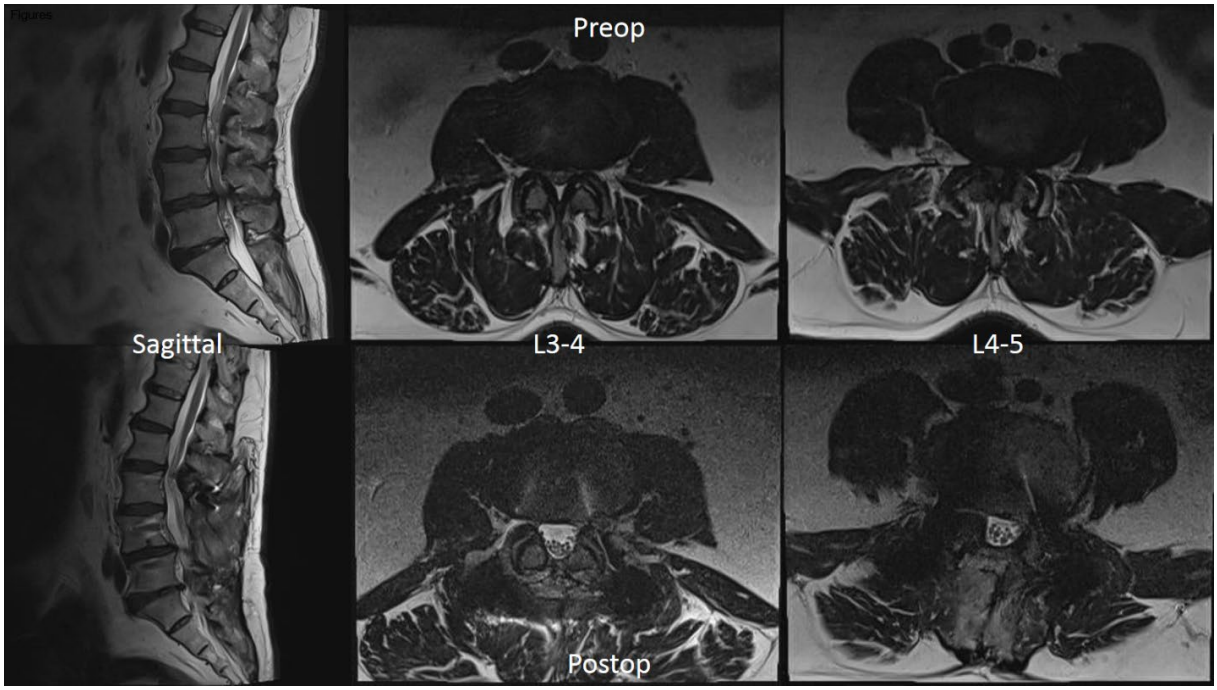

Figure 2. Pre and postoperative lumbar T-2 weighted MRI images of patient #50, a 64-year-old male patient who described neurogenic claudication after 100 meters. Radiologically, severe stenosis was present at L3-4 and L4-5, L4 grade I spondylolisthesis, and minimal degeneration at L5-S1. L3 and L4 total laminectomy and stabilization of L3-4-5 were performed, after which the walking distance became unlimited. The Roland Morris score was 14. The patient was satisfied with the surgery, and there was no need for reoperation; the case was considered "successful" in the study

Table 3. Dependent variables (outcome parameters) according to the other independent variables

Dependent Variable: Independent Variable	P value	Independent Variable Groups			
Success: Sex	0.06459*	Male		Female	
		Suc.	Uns.	Suc.	Uns.
		27	5	26	15
Success: Age	0.6***				
Success: DS	0.4235*	DS +		DS -	
		Suc.	Uns.	Suc.	Uns.
		35	11	18	9
Success: NoL	0.1295**				
Success: DT	**0.02148***	DT +		DT -	
		Suc.	Uns.	Suc.	Uns.
		4	6	49	14
RMDQ: Sex	0.2085**	M RMDQ median		F RMDQ median	
		9		11	
RMDQ: Age	0.9023****				
RMDQ: DS	0.6988**	DS + RMDQ Median		DS - RMDQ median	
		9		10.5	
RMDQ: NoL	0.6437****				
RMDQ: DT	**0.02896****	DT + RMDQ median		DT - RMDQ median	
		16		9	
PO WD: Sex	**0.02854****	M WD mean		F WD mean	
		925.78		724.88	
PO WD: Age	0.2736****				
PO WD: DS	0.4411**	DS+ WD mean		DS - WD mean	
		842.22		763.07	
PO WD: NoL	**0.01186****				
PO WD: DT	0.1493**	DT + WD mean		DT - WD mean	
		656.5		837.78	
PO NC: Sex	**0.02374***	M		F	
		NC +	NC -	NC +	NC -
		3	29	14	27
PO NC: Age	0.052***				
PO NC: DS	0.3941*	DS +		DS -	
		NC +	NC -	NC +	NC -
		9	8	37	19
PO NC: NoL	**0.02406****				
PO NC: DT	**0.04629***	DT +		DT -	
		NC +	NC -	NC +	NC -
		5	5	12	51

NS: Not significant relationship PO: Postoperative, NC: Neurogenic Claudication, WD: Walking Distance, DS: Degenerative Spondylolisthesis, M: Male, F: Female, DT: Dural Tear, NoL: Number of Laminectomies. * Fisher's Exact Test. ** Mann-Whitney U Test. *** ANOVA Test. **** Spearman's Rank Correlation Analysis

In the study, dural tears occurred in 7 patients in the group who underwent only decompression and in 3 patients who underwent decompression with added stabilization. No significant difference was detected between patients who underwent only decompression and those who underwent decompression with added stabilization regarding dural tear occurrence. However, it was observed that the absence of dural injury during surgery increased the success rate in both groups (p=0.021, Fisher's Exact Test). Patients who experienced dural tears had higher Roland Morris disability questionnaire scores (p=0.02896). The incidence of neurogenic claudication in the postoperative period was found to be higher (5/10) in those with dural tears compared to those without dural tears (12/63) (p=0.046).

No statistically significant effect of the other investigated independent variables on the postoperative dependent variables was found.

Discussion

Lumbar spinal stenosis (LSS) is a common problem in older people that frequently results in significant impairment of life comfort, causing low back pain, neurogenic claudication, and radiculopathy. For patients who do not improve with nonsurgical treatments, several surgical treatment options (such as laminectomy, the addition of fusion surgery to laminectomy, interspinous spacer, minimally invasive lumbar decompression, and trans-spinous split laminectomy) are available. The rare occurrence of rapid deterioration in LSS and periods with mild fluctuations in symptoms over time have made the surgical option an almost elective procedure. The surgical procedure has significantly varied from clinic to clinic (6). The number of operations in LSS cases has increased nearly eight-fold between 1979 and 1992 relative to the total diagnosed cases and has plateaued since

then (7,8). In recent years, although the surgical rate (1-2 per 1000 patients) remains stable and unchanged relative to all patients with spinal stenosis, lumbar fusion as a surgical preference has increased dramatically (9,10). This increase varies according to the geographical areas in the studies, with a 14-20 fold increase in fusion surgery rates in addition to the eight-fold increase in decompression surgery (9,10). Possible reasons for this variation include difficulties in reaching a consensus among surgeons on indications for surgery and differences in surgeons' experience and training (11).

While the success rate was notably higher in men (84.3%, 27/32) than in women (63.4%, 26/41), this difference did not reach statistical significance (p=0.0645) Maclean et al. analyzed the relationship between gender and postoperative pain scores, disability scales, and quality of life assessments in a review of 30 studies involving 32,951 patients. (12) They reported that the female gender had worse values on most scales, and in the remaining tests, the male gender did not have worse values in any of them. These findings provide compelling evidence that postoperative satisfaction may indeed vary according to gender.

In our study, we did not find a significant relationship between age and postoperative success. However, conflicting results are present in the literature. For example, Katz et al. (13) found no significant relationship between age and patient satisfaction, while Mariconda et al. (14) reported worse outcomes in terms of neurological deficit at 1-year follow-up and demonstrated that advanced age was associated with a poorer prognosis. Amundsen et al. (15) argued that advanced age or degenerative changes are unrelated to poor prognosis. Athiviraham et al. (16) reported that age and gender did not significantly alter the outcomes based on postoperative Roland Morris scores. All these results indicate that the impact of age on postoperative success can be interpreted differently. Therefore, further research is needed to understand age's effect on postoperative success. Our study results show that the effect of age on postoperative success is limited.

Our analysis revealed no statistically significant association between the number of laminectomies performed and success. Conflicting results have been published in the literature on this subject. Ulrich et al. (17) reported that multilevel decompression was associated with worse outcomes when comparing patients who underwent single-level decompression with those who underwent multilevel decompression. However, some studies show no relationship between the amount of decompression and patient outcomes (15,18). Park et al. (19) found no difference in the 2-year results when comparing one-level and more-than-one-level decompression in patients with pure spinal stenosis (without degenerative spondylolisthesis).

In our series, the presence of degenerative spondylolisthesis in addition to lumbar spinal stenosis did not change the success rate. Different results have been reported in the literature in cases of degenerative spondylolisthesis accompanying lumbar stenosis. Försth et al. found that "adding fusion surgery to decompression did not provide additional benefits for patients with degenerative spondylolisthesis" (20). Park et al. (19) reported that the 2-year outcomes of patients who underwent single-level decompression were better than those who underwent multilevel decompression in patients with spinal stenosis and degenerative spondylolisthesis.

This study's data highlights despite 1-2 level stabilization being generally more successful, the sudden decrease in success when the stabilized segment exceeded two levels indicates the importance of planning the operation to include as few segments as possible if stabilization is required in LSS surgeries. Different results have been reported on this subject in the literature. For example, when examining whether or not fusion is added to decompression, Försth et al. claimed that adding fusion surgery to decompression did not provide any additional benefits for all patients (with and without listhesis) (20). In addition, Chang et al. conducted a meta-analysis of 17,785 cases. They concluded no difference in the assessed pain scores, ODI, and EQ-5D quality of life scale when adding fusion to decompression. They felt adding fusion in spinal stenosis surgery was not very positive since it increased operation time, blood loss, and hospital stay (21). On the other hand, some studies show that adding fusion to decompression helps achieve better outcomes than decompression surgery alone. For example, Ghogawala et al. found that laminectomy plus fusion provided better functional results and lower revision surgery rates than laminectomy alone in LSS and stable degenerative spondylolisthesis (22). Austevoll et al. showed that ODI outcomes were similar at the 12-month follow-up, but the fusion group had less back and leg pain. However, they did not attribute superiority to any method since the operation time and length of hospital stay were more prolonged (23).

In the literature, there are various findings regarding the relationship between the length of stabilization in lumbar spinal stenosis surgery and clinical outcomes. Sun et al. found no significant difference in scores when comparing long and short stabilization in lumbar spinal stenosis treatment (24). In contrast, Lee et al. showed that in patients with lumbar spinal stenosis, those who underwent short segment fusion (1-2 segments) with decompression had better results in scores evaluated at 10-year follow-up compared to those who underwent long segment fusion (3 or more sections) (25).

The discrepancies in the literature may be due to differences in study methods, measurement tools,

and patient populations. For example, Försth et al. (20) used Oswestry and VAS scales in their study. Chang et al. (21) used ODI and EQ-5D scales. As mentioned above, in our research, fusions with fewer segments were observed to be more successful when patient satisfaction was considered. It cannot be said that the research results using different evaluation tools contradict each other.

Our findings reveal that the success rate is lower for patients experiencing dural tears during surgery. This finding is consistent with the literature. For example, in a study conducted by Alhaug et al. (26), they found that, during the 12-month follow-up of 8,919 patients, those with dural tears had lower ODI scores, and a higher number of patients worsened. Therefore, it can be concluded that a dural tear negatively affects surgical outcomes. The consistency of our findings with the literature increases the importance and reliability of this conclusion.

As expected, there was a statistically significant association between postoperative Roland-Morris score and success ($p=0.003$). Regarding statistical significance, the most meaningful threshold was found to be 17. That is, while successful cases were the majority (89.2% successful) in the 0-17 range, unsuccessful cases were the majority (56.3% unsuccessful) in the 18-24 range ($p<0.0001$, chi-square test). Although both are dependent variables, investigating the relationship between Roland Morris and success will demonstrate the likelihood of patient satisfaction or, by our standards, the possibility of the surgery being successful based on the RMES score.

The lack of a statistically significant relationship with success for some independent variables may be due to the small size of our series. Therefore, investigating the relationship between these factors and success in a more extensive series is worth exploring.

Conclusion

Based on the results of our study, we have concluded that lumbar stabilization, when limited to 1-2 segments, is associated with higher patient satisfaction. However, as the number of stabilized segments increases, the positive impact on patient satisfaction may decrease. Our study highlights the importance of exercising utmost care and employing meticulous techniques during surgery to avoid dural tears, as our results indicate that the occurrence of dural tears significantly impacts postoperative success. We believe future prospective studies with larger sample sizes will provide more insight into this matter.

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None.

Conflict of interest statement

The authors declare no conflicts of interest.

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Effect of Blood Sample Tubes on Diagnosis of Gestational Diabetes in Pregnant Women Undergoing OGTT

OGTT Yapılan Gebelerde Kan Örnek Tüplerinin Gestasyonel Diyabet Tanısına Etkisi

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Öz

Gebelikte diagnostik oral glukoz tolerans testi (OGTT) yapılması gestasyonel diyabetin erken tanısı için önemlidir. Hiperglisemi hızlı bir şekilde saptanabilir, böylece anne ve fetüs korunabilir. Bu çalışmada OGTT uygulanan gebelerde kan örneği tüpleri ile glukoz stabilitesi arasındaki ilişkinin araştırılması amaçlanmıştır.Yöntemler: OGTT (75g) yapılan 20 gönüllü gebenin glukoz yüklemesi sonrası 120. dakikada kan örnekleri alındı. Serum için VACUETTE CAT Serum Separator (Clot Activator Tube) kullanıldı; Plazma için VACUETTE FC Mix tüpü (Na2 EDTA, sodyum florür, sodyum sitrat) ve VACUETTE FE (Sodyum Florür/K3 EDTA) kullanıldı. Bu tüplerin üçü de 0. saat, 2. saat ve 4. saat olmak üzere üç farklı zamanda santrifüjlendi. Tüm örnekler 0., 2., 4. saatlerde santrifüj edilerek glukoz değerleri ölçüldü. Sadece VACUETTE FC Mix tüplerinde glukoz değerleri arasında istatistiksel olarak anlamlı fark bulunmadı ($p>0.05$). Ancak, bu farklı zaman aralıklarında serum ve florürlü tüplerde istatistiksel olarak anlamlı fark bulundu ($p<0.01$). VACUETTE FC Mix'e alınan kanların 4. saatte bile santrifüj edilmeden bekletildiğinde glukoz değerlerinin düşmediği ve bu nedenle rutin kullanımının faydalı olacağı gösterildi. Ancak VACUETTE CAT serum ayırıcı ve VACUETTE FE'de glukoz değerlerinin stabil olmadığı saptanmıştır.

Anahtar Kelimeler: Gebelik, Gestasyonel Diyabet, Glikolizis, Glukoz, Oral Glukoz Tolerans Testi

Abstract

Performing a diagnostic oral glucose tolerance test (OGTT) during pregnancy is important for early diagnosis of gestational diabetes mellitus. Hyperglycemia can be detected quickly, and mother and the fetus can be protected. This study aimed to research the relationship between blood sample tubes and the stability of glucose in pregnant women undergoing OGTT. Blood samples were taken at the 120th minute after glucose loading from 20 pregnant volunteers who underwent OGTT (75g). VACUETTE CAT Serum Separator (Clot Activator Tube) was used for serum; VACUETTE FC Mix tube (Na2 EDTA, sodium fluoride, sodium citrate) and VACUETTE FE (Sodium Fluoride/K3 EDTA) were used for plasma. All three of these tubes were centrifuged at three different times i.e. 0. hour, 2. hour, and 4. hour. All samples were centrifuged at 0., 2., and 4. hours, and glucose values were measured. No statistically meaningful difference was found between their glucose values in only VACUETTE FC Mix tubes ($p>0.05$). However, a statistically meaningful difference was found at these different time intervals in serum and fluoride tubes ($p<0.01$). It was shown that when the blood sampled into VACUETTE FC Mix was kept without centrifugation even at the 4. hour, their glucose values did not decrease and therefore the routine use of them would be beneficial. However, it was found that the glucose values were not stable in VACUETTE CAT Serum Separator and VACUETTE FE.

Keywords: Pregnancy, Gestational Diabetes, Glycolysis, Glucose, Oral Glucose Tolerance Test

Introduction

Gestational diabetes mellitus (GDM) is glucose intolerance with first recognition or diagnosis during pregnancy. Blood glucose levels are low or normal in general after giving birth (1). GDM occurs in 7% of pregnant women. Usually, GDM develops in connection with the placenta hormones blocking the insulin's effects (increasing the insulin's resistance) after the 24th week of pregnancy (2). If the regulation of blood glucose is disrupted in pregnant women, it might cause negative outcomes to emerge in both them and their children, especially in mothers who had suffered diabetes before getting pregnant.

Diabetes or prediabetes is diagnosed by performing fasting plasma glucose (FPG), oral glucose tolerance test (OGTT), and glycosylated hemoglobin Alc (HbAlc) measurement (2). As a principle, OGTT is used for diagnostic purposes during pregnancy or in epidemiologic studies where the blood glucose levels are uncertain (4). Thus, the high glucose level in mothers' plasma is found out on time and precautions can be taken to prevent the harmful effects of hyperglycemia on the fetus (3). International Association of the Diabetes and Pregnancy Study Groups suggests performing OGTT with 75g glucose for two hours (2). At present, there is no full consensus to employ the single-stage diagnosis approach instead of the two-stage test to diagnose GDM. Any of the two-stage diagnosis approach (preliminary screening test with 50g glucose followed by OGTT using 100g glucose for three hours) or the single-stage diagnosis approach (OGTT using 75g glucose) can be employed (3).

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In this study, a 75g oral glucose tolerance test was performed on the volunteer pregnant women, and samples were taken at the 120th minute. A plasma glucose test performed in a laboratory is the basic tool to diagnose diabetes mellitus, impaired fasting glucose (IFG), and/or impaired glucose tolerance (IGT), especially for screening, and to diagnose GDM in cases where HbAlc cannot be used (5). So, according to international guidelines, plasma glucose measurement must be accurate precise, and final in terms of patient classification following the international guidelines (5).

For glucose measurements, plasma must be separated as soon as possible from the cells. Sometimes this may not be possible, in which case a tube containing a glycolysis inhibitor such as sodium fluoride should be used for sampling (6). If a sample is processed after a delay, determination of glucose may not be accurate (7). It is a known fact that if blood samples are kept without centrifugation, their glucose concentration will gradually reduce (8). It was reported that plasma glucose samples which are not centrifuged immediately, reduce 5% to 7% in vitro per hour due to glycolysis (5). American Diabetes Association (ADA) and the National Academy of Clinical Biochemistry (NACB) recommend that to minimize in vitro glycolysis, the sample tube should be immediately placed in ice water and the plasma should be separated from the cells within 30 minutes, or if this is not possible, a sample tube containing a rapid glycolysis inhibitor should be used, e.g. citrate buffer (5).

In conclusion, measured concentrations of glucose highly depend on such pre-analytic variables as the type of phlebotomy tubes used and the time between phlebotomy and analysis (9). This study focused on vacuum tubes containing different anticoagulants for collecting venous blood samples, and their effect on the stability of glucose.

Material and Method

Twenty pregnant women who were loaded with 75g glucose for OGTT to assess gestational diabetes at Istanbul Bakırkoy Dr. Sadi Konuk Training and Research Hospital were included. This study was performed after the Ethics Committee approved it (protocol no: 2022/170, decision no: 2022-09-23) and informed consent was obtained from each subject. Research involving human subjects complied with all relevant national regulations, and institutional policies and is following Helsinki Declaration (as revised in 2013).

The same personnel collected nine venous blood samples from each subject 120 minutes after loading 75g glucose. 3 VACUETTE CAT Serum Separator Clot Activator Tubes (SST) were used to obtain a serum sample; 3 VACUETTE FC Mix tubes (Na2 EDTA, sodium fluoride, sodium citrate) and 3 VACUETTE FE Sodium Fluoride/K3 EDTA tubes were used for plasma. In VACUETTE FC Mix tubes; to ensure optimal glucose stabilization, the tubes were inverted 10x directly after blood collection. 3 tubes (SST, Mix and EDTA) were centrifuged at 1,800g and 20°C for 10 minutes at the 0. hour, 3 tubes at 2. hour, and 3 tubes at 4. hour.

Glucose was measured with hexokinase glucose-6-phosphate dehydrogenase method using AU5800 Series Chemistry Analyzers (Beckman Coulter, USA). Internal quality control was performed on a minimum of two levels of glucose by using Beckman Coulter control materials.

VACUETTE CAT Serum Separator Tube-454243, VACUETTE FC Mix Tube-454513, and VACUETTE FE Sodium Fluoride/K3 EDTA-454221 tubes are manufactured by Greiner Bio-One (Kremsmünster, Austria).

Statistical review

The NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA) software was used for statistical analyses. Descriptive statistical methods (mean, standard deviation, median, frequency, minimum, maximum) were utilized to evaluate study data. The normality of distribution for quantitative data was assessed using the Shapiro-Wilk test and graphical examinations. Fasting blood glucose measurements were evaluated based on time and application types using the Repeated Measures with Linear Mixed Model. For within-group comparisons of normally distributed quantitative variables, repeated measures ANOVA was applied, and Bonferroni-corrected pairwise comparisons were used for dual comparisons. For non-normally distributed quantitative variables, within-group comparisons were conducted with the Friedman test, and Bonferroni-corrected Wilcoxon signed-ranks tests were used for pairwise comparisons. Dependent t-tests were used for normally distributed within-group comparisons of quantitative variables, and Wilcoxon signed-ranks tests were used for non-normally distributed variables. Statistical significance was set at $p<0.05$.

Results

At the end of the study, glucose levels were measured in VACUETTE FC Mix plasma, VACUETTE CAT Serum Separator, and VACUETTE NaF (sodium fluoride) /EDTA plasma tubes, in which blood samples were collected 120 minutes after loading glucose (Table 1).

To examine the effects of application types and timing on postprandial blood glucose measurements, GLM modeling was used in repeated measures. The model obtained was found to be statistically significant ($F=22.758$, $p=0.000$). In the model, the effects of both applications and the Time × Application interaction were statistically significant ($p=0.000$). The effect size was determined as 0.577,

and post hoc power was 98.2%. Therefore, all evaluations were conducted in detail within groups, and the results are presented in Table 2.

The average difference of 3.89±2.25 units between the postprandial FC Mix measurement and the gray measurement at baseline (0 hour) in the subjects participating in the study was found to be statistically significant (p=0.001).

The average difference of 6.39±3.01 units between the postprandial FC Mix measurement and the gray measurement at the 2nd hour was also statistically significant (p=0.001).

Similarly, the average difference of 7.47±3.13 units between the postprandial FC Mix measurement and the gray measurement at the 4th hour was statistically significant (p=0.001).

The average difference of 8.61±4.39 units between the postprandial FC Mix measurement and the serum measurement at baseline (0 hour) was found to be statistically significant (p=0.001). The average difference of 18.66±4.66 units between the postprandial FC Mix measurement and the serum measurement at the 2nd hour was statistically significant (p=0.001).

The average difference of 27.85±8.75 units between the postprandial FC Mix measurement and the serum measurement at the 4th hour was also statistically significant (p=0.001).

The average difference of 4.72±3.62 units between the gray measurement at baseline (0 hour) and the serum measurement was statistically significant (p=0.001).

The average difference of 12.27±5.03 units between the gray measurement at the 2nd hour and the serum measurement was statistically significant (p=0.001).

Table 1. Investigation of factors affecting postprandial blood glucose measurements

	Type III Sum of Squares	Mean Square	F	*p*
Application	487.62	487.62	28.55	***0.000*****
Time X Application	1850.89	1850.89	108.37	***0.000*****

*Application: FC mix, Gray tube, and Serum, Time: Baseline (0 hour), 2nd hour, and 4th hour, Repeated Measures with Lineer Mix Model, **p<0.01*

The average difference of 20.37±8.50 units between the gray measurement at the 4th hour and the serum measurement was also statistically significant (p=0.001).

No statistically significant difference was found among the changes in postprandial blood glucose measurements at 0, 2, and 4 hours in the FC Mix group (p>0.05).

In the gray group, statistically significant differences were found among the changes in postprandial blood glucose measurements at 0, 2, and 4 hours (p=0.001). According to the results of pairwise comparisons conducted to determine the differences, the average decrease of 2.57±1.80 units in postprandial blood glucose measurements at the 2nd hour compared to baseline was statistically significant (p=0.001). The average decrease of 4.55±2.45 units in postprandial blood glucose measurements at the 4th hour compared to baseline was statistically significant (p=0.001). The average decrease of 1.97±1.61 units in postprandial blood glucose measurements at the 4th hour compared to the 2nd hour was also statistically significant (p=0.001).

In the serum group, statistically significant differences were found among the changes in postprandial blood glucose measurements at 0, 2, and 4 hours (p=0.001; p<0.01). According to the results of pairwise comparisons conducted to determine the differences, the average decrease of 10.12±4.74 units in postprandial blood glucose measurements at the 2nd hour compared to baseline was statistically significant (p=0.001; p<0.01). The average decrease of 20.20±7.86 units in postprandial blood glucose measurements at the 4th hour compared to baseline was statistically significant (p=0.005; p<0.01). The average decrease of 10.08±6.15 units in postprandial blood glucose measurements at the 4th hour compared to the 2nd hour was also statistically significant (p=0.005; p<0.01) (Figure 1).

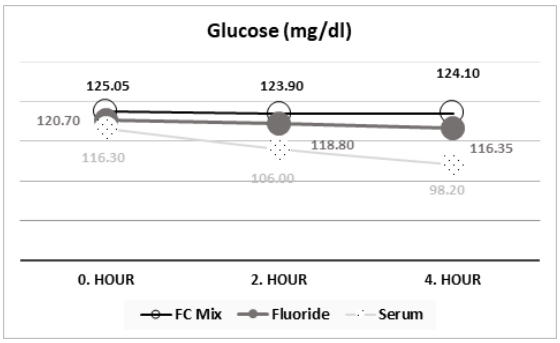

Figure 1. Glucose median levels of three different tubes at 0., 2., and 4. hours

A statistically significant relationship was found among the samples taken at the 120th minute after a 75 g loading dose (p=0.001). According to the results of pairwise comparisons conducted to determine differences, postprandial blood glucose measurements in the serum group were significantly lower than those in the FC Mix and Capillary groups (p=0.003; p=0.026) (Table 3).

The average difference of 3.85±2.68 units between the postprandial FC Mix measurement and the gray measurement was found to be statistically significant (p=0.003).

Table 2. Evaluation of the difference between fc mix and serum measurements over time

Satiety Measurements		**[1]FC Mix**	**[2]Gray tube**	**[3]Serum**	**Test Value**	**[1,2]Difference**	**[1,2]Test Value; *p***	**[1-3]Difference**	**[1-3]Test Value; *p***	**[2,3]Difference**	**[2,3]Test Value;*p***
0. Hour	*Min-Max (Median)*	65.6-231.8 (125.05)	57.9-229.8 (120.7)	56.6-227.8 (116.3)	***F:57.23***	3.89±2.55	t:6.810	8.61±4.39	Z:-3.920	4.72±3.62	Z:-3.921
	Ort±Sd	132.45±40.93	128.56±40.62	123.84±40.24	***[b]0.001***		***[a]0.001***		***[d]0.001***		***[d]0.001***
2. Hour	*Min-Max (Median)*	65.6-237.3 (123.9)	55.8-227.3 (118.8)	23.3-217.2 (106.0)	F:192.16	6.39±3.01	t:9.498	18.66±4.66	t:17.878	12.27±5.03	t:10.905
	Ort±Sd	132.38±41.61	125.98±41.03	113.71±38.92	***[b]0.001***		***[a]0.001***		***[a]0.001***		***[a]0.001***
4. Hour	*Min-Max (Median)*	62.4-233.5 (124.1)	53.6-223.4(116.35)	48.5-202 (98.2)	F:156.96	7.47±3.13	t:10.672	27.85±8.75	t:14.223	20.37±8.50	t:10.712
	Ort±Sd	131.48±41.36	124.01±40.91	103.63±38.36	***[b]0.001***		***[a]0.001***		***[a]0.001***		***[a]0.001***
Test Value		F:1.458	F:52.590	Chi.Square:40.000							
	p	***[b]0.245***	***[b]0.001***	***[c]0.001***							
0-2 Hour		***1.000***	***0.001***	***0.001***							
0-4 Hour		***0.613***	***0.001***	***0.005***							
2-4 Hour		***0.445***	***0.001***	***0.005***							

*[a]Paired Samples Test, [b]Repeated Measures Test, [c]Friedman's Test, [d]Wilcoxon Test, **p<0.01*

Table 3. Evaluation of measurements on 11 individuals

N=11	**Samples taken at 120 minutes after 75 g loading (0th hour)**	
	Median ±Sd	*Min-Max (Median)*
FC Mix	126.60±51.70	65.6-231.8 (112.60)
Gray	122.75±51.36	57.9-229.8 (108.3)
Serum	119.09±50.81	56.6-227.8 (107.0)
Capillary	136.59±44.34	97-221 (111.5)
Test Value	F:17.945	
p	*[c]0.001***	
Difference	*Median ±Sd*	*P*
FC Mix- Gray	3.85±2.68	*[d]0.003***
FC Mix-Serum	7.51±3.84	*[d]0.003***
Gray-Serum	3.66±3.21	*[d]0.003***
FC Mix- Capillary	9.98±22.17	*[d]0.374*
Gray-Capillary	13.83±22.28	*[d]0.075*
Serum- Capillary	17.50±22.15	*[d]0.026**

*[c]Friedman's Test, [d]Wilcoxon Test,*p<0.05, **p<0.01*

The average difference of 7.51±3.84 units between the postprandial FC Mix measurement and the serum measurement was also statistically significant (p=0.003; p<0.01).

The average difference of 3.66±3.21 units between the postprandial serum measurement and the gray measurement was statistically significant (p=0.003; p<0.01).

The difference between the postprandial capillary measurement and the FC Mix and gray measurements was not statistically significant (p>0.05).

The average difference of 17.50±22.15 units between the capillary measurement and the gray measurement was statistically significant (p=0.026; p<0.05).

Discussion

Although OGTT for pregnant women has been argued in recent years, laboratories must focus only on performing an accurate glucose assay.

The laboratory process is divided into the pre-analytic, analytic, and post-analytic steps. At the pre-analytic phase, it is essential to ensure blood stable for an accurate analysis of glucose concentration. This process involves centrifugation quickly after specimen collection.

It is observed that today certain negative circumstances of the health sector delay the time to centrifuge blood collection tubes after venipuncture and therefore cause the decreased blood glucose levels.

With the increase in the number of city hospitals, daily patient admission is increased and distances between blood collection departments and laboratories are also increased. And in some cases, the use of a pneumatic system becomes almost mandatory. The faults in the pneumatic system used in these large hospitals can cause delays in getting samples to the laboratory.

In our country, family health centers send blood samples to the central laboratories. However, in some family health centers, lack of centrifuge or adjustment problems in the existing centrifuge cause problems that affect sample quality. Incorrect results are obtained from samples that are not centrifuged and not properly centrifuged.

Furthermore, it is a well-known fact that since glucose concentration is reduced in non-centrifuged blood specimens, the risk of incomplete diagnosis of diabetes is limited and values lower than actual values are measured (10).

The purpose of this study was to assess the stability of glucose and to study the glycolysis inhibition in serum or plasma samples using different test tubes for blood samples from volunteer pregnant subjects who underwent OGTT. Blood samples were stored in test tubes containing NaF/EDTA or EDTA, fluoride and sodium citrate and SST, which were centrifuged after a delay or in an insufficient way under controlled delay conditions (up to 240 minutes after the collection) to take under control the pre-analytic step of the glucose test.

Diabetes is a disease characterized by high blood glucose levels and developing chronically. Any kind of diabetes might lead to complications in various parts of the body and increase the risk of early death in general (11). GDM is one of the types of diabetes and is defined as glucose intolerance arising or diagnosed during pregnancy (1). Performing oral glucose tolerance tests on pregnant women for diagnostic purposes is necessary for early diagnosis of gestational diabetes (3).

To diagnose diabetes, measurement of blood glucose must be available in the primary healthcare services. The role played by the control of blood glucose to prevent the development and progression of complications was proven (5).

Glucose concentration may be measured in whole blood, serum, or plasma, but plasma is suggested for diagnosis (6). When glycolysis is not quickly inhibited or blood cells cannot be quickly separated from plasma, the measurement of fasting

glucose in plasma is influenced by the decrease of glucose over time in blood sample tubes (10).

Organizations such as the American Diabetes Association and the World Health Organization have made the following recommendations: glycolysis should be minimized by centrifugation of the sample or by placing the tubes on ice immediately after blood collection and centrifuging within 30 minutes (9). In many laboratories, samples cannot be processed or analyzed within one hour after they are collected.

The initial decrease of glucose concentration can be limited by centrifuging or storing the blood sample tubes in an ice/water medium, but blood samples collected in a department or separate institution relatively far from the laboratory lie outside the range of the laboratory. Furthermore, when blood tubes are placed in ice water, the tube label may become unreadable, which may lead to patient identification problems (10).

Md Nahidul Islam and et al. reported that glucose analysis using FC-Mix tube demonstrated a strong correlation WHO specifications when stored at 4°C. When FC-Mix tubes were stored at room temperature, glucose was stable for 4 days. These findings suggest that the FC-Mix effectively inhibits glycolysis and should be introduced into routine clinical practice (12).

Van den Berg and et al. published that, addition of citrate almost completely prevented in vitro glycolysis, but showed a positive bias (0.2 mmol/l) compared to control. This is partly due to a minor decrease in glucose level in control blood, drawn according to the current guidelines. This decrease occurs within 15 minutes, in which glycolysis has been described to be minimal and acceptable. NaF-EDTA-citrate based test tubes provide the best pre-analytical condition available (13).

Nowadays, considerable efforts have been spent to find out efficient protectors against glucose decrease through glycolysis (14).

A conventional approach is to add NaF in combination with anticoagulant potassium oxalate (KOx) to stabilize the glucose concentration in a blood sample tube. The mechanism of the effect of fluoride is based on inhibition of enzyme enolase applying a late effect in glycolytic terms. Therefore, the activity of the glycolytic enzymes located upstream of the enolase is not considerably affected, so they remain active and continuously metabolize the glucose. This explains why fluoride's effect on glycolysis inhibition lasts up to four hours. (10).

The efficiency of such glycolysis inhibitors as sodium fluoride (NaF) combined with KOx in stabilizing blood glucose is limited (10).

Van den Berg and et al. used a new protocol including a new phlebotomy tube type containing a NaF-EDTA-citrate additive and published glucose results, that are 100% identical to the gold standard in laboratory and clinical diagnosis, without the need to further adapt current procedure characteristics such as pre-analytical turn-around time (TAT) (15).

Regulations on gestational diabetes mellitus suggest using only the blood sample tubes that meet the pre-analytic needs of glycolysis inhibition. Regulations on GDM cover possible errors in pre-analytic needs and focus on fluoride's incomplete inhibition of glycolysis. Inhibition of glycolysis only by NaF, in the absence of other additives, begins two hours after blood is collected and will not be completed for four hours (16).

The study performed by Bonetti et al. indicates that blood samples collected into tubes containing a clot activator, lithium-heparin or sodium fluoride are not suitable for glucose measurements (17).

We evaluated the effect of three routinely used collection tubes (SST, mix and EDTA) on the stability of glucose in blood, and found that initial glucose concentration was not significantly different among three tube types. It is reported, that immediate glycolysis inhibition was not achieved in any tube type, and only sodium fluoride was efficient in inhibiting glycolysis in the settings of delayed sample processing (18).

In stabilization studies of blood glucose using not centrifuged blood samples, hexokinase enzyme found to become active only at pH 5.9 or higher, which is located at the start of the glycolytic way (10).

Therefore, acidifying the blood utilizing a citrate buffer will prevent glucose from catabolizing due to glycolysis starting to happen at a much earlier stage and proceeding at a speed faster than that of fluoride (10).

The new generation blood sample tests designed contain three different additives: the first additive lowers the pH level of the blood and therefore helps to inhibit hexokinase enzyme (i.e. citrate): the second additive directly inhibits enolase enzyme (i.e. NaF), and the third additive irrevocably inhibits the coagulation of blood (i.e. ethyl diamine tetra acetic acid-EDTA) (10).

Regulations issued by ADA and NACB suggest that plasma glucose should be analyzed by using gold standard treatment tubes or blood sample tubes containing such quick effect glycolysis as citrate buffer (19). The use of granular glycolysis inhibitor provides a pre-analytic benefit by removing the dilution effect which might be caused by insufficient filling of a tube with a liquid additive (20).

Bonetti et al administered 75g OGTT to 147 volunteer subjects, 83 of whom were pregnant, and collected samples into tubes with NaF/K3EDTA, NaF/Na2EDTA/citrate liquid form, NaF/K2Ox, and NaF/Na2EDTA/citrate granule form. It was shown that measurement of glucose in tubes containing citrate is more efficient in terms of diagnosing carbon hydrate disorders than in tubes containing NaF (21).

This was confirmed in 2019 by Jamieson et al., who compared plasma glucose stability over time in 501 samples taken during OGTT after 24 weeks of gestation and found that the samples containing citrate as a glycolytic inhibitor offered the best short and long-term stability for glucose levels even compared with fluoride samples placed immediately on ice (22).

In a study, by using tubes containing citrate and NaF, Yağmur et al found that in NaF plasma tubes, the concentration of glucose decreased from 90 mg/dL to 87 mg/dL (–3.3%) within the first four hours and decreased significantly to 82.6 mg/dL (–8.2%) 12 hours later. They also found that the citrate buffer in the glucose tubes prevent the glucose concentration from falling (23).

In a study on volunteer pregnant women, Daly et al used fluoride/EDTA plasma tubes and citrate-added plasma tubes. They kept fluoride/EDTA(FE) tubes at room temperature and in icy water. As a result, they suggest replacing FE tubes with citrate-fluoride-EDTA tubes to measure maternal glucose for diagnosing GDM in the absence of an ice mixture, early cell separation, and analysis (24).

Zhang et al collected venous blood samples from 58 fasting volunteers into NaF/citrate tubes and no additive tubes. They found that after keeping for 10 hours at room temperature, glucose was higher (13.4%) in no additive tubes than in NaF/citrate tubes (2%) (25).

In 2013, Garcia del Pino et al. determined that citric acid immediately inhibits glycolysis. These authors showed that glucose levels in samples with sodium fluoride was significantly lower than with temporally paired citrate tubes (26).

Stapleton et al. performed a study on volunteer pregnant women, and used tubes containing citrate, fluoride/EDTA tubes, and lithium-heparin. They reported that the average concentration of glucose in samples with fluoride/citrate remained stable for 2.5 hours. In addition, that there was a statistically significant difference between glucose levels of samples at hours 0. and 2.5 with fluoride-EDTA and lithium heparin kept at room temperature (27).

Comparable results were reported by Norman et al. evaluating paired fasting plasma glucose samples collected into sodium fluoride and citrate tubes and found higher glucose levels in the samples collected into the citrate tubes (28).

Gambino et al compared tubes containing NaF and sodium oxalate with tubes containing citrate buffer, NaF, and EDTA. As a result, they found that the average concentration of glucose in blood samples with NaF and sodium oxalate decreased 4.6% at 2. hour and decreased 7.0% at 24. hour. Glucose levels decreased 0.3% at 2. hour and decreased 1.2% at 24. hour in samples with citrate buffer, NaF and EDTA. They commented that acidification should singularly replace NaF to obtain an accurate concentration of glucose (29).

Serum data showed that the recommended clotting time of 30 minutes was sufficient to cause significant changes and that prolonged contact with cells triggered glucose consumption. This simply shows that under usual laboratory operating conditions, routine serum tubes are not suitable for estimation of glucose levels accurately. Also, a similar situation applies to NaF/EDTA tubes.

Conclusion

Our results indicate that VACUETTE FC Mix tubes are the most efficient ones in terms of preventing the concentration of glucose from undergoing clinically meaningful changes at room temperature.

EDTA, fluoride, and citrate/citric acid buffer contribute to reliable measurement of the glucose concentration, in cases where the time until centrifugation is prolonged, including transportation and/or storage periods. Stability of glucose is necessary at the pre-analytic phase for diagnosing and treating diabetes. From a clinical point of view, unreliable blood glucose level measurements can cause misdiagnosis, delay in diagnosis and complications Maximum care and supervision are required for the material used in the laboratory, sample stability in all phases (pre-analytic, analytic, etc.).

Stability of glucose is important at the pre-analytic phase, and studies on stability need to be performed in larger numbers at different centers and on more varied groups of patients. The use of tubes containing EDTA, fluoride, and citrate/citric acid buffer looks promising in terms of protection of the stability of glucose.

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Conflict of interest statement

Authors state no conflict of interest.

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The Identification of Methylation Profiles of FTO and PPARG Genes in Type 2 Diabetes Mellitus Patients

Tip 2 Diyabet Mellitus Hastalarında FTO ve PPARG Genlerinin Metilasyon Profillerinin Belirlenmesi

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Öz

Tip 2 diyabet (T2DM), genetik yatkınlıklar, çevresel etkileşimler ve çeşitli genler tarafından yönlendirilen karmaşık bir metabolik hastalıktır. Günümüzde, giderek artan sayıda çalışma diyabetes mellitus (DM) ile epigenetik, özellikle DNA metilasyonu arasındaki ilişkiyi göstermektedir. Bu çalışmada, klinik olarak T2DM tanısı almış hastaların periferik kan örneklerinde yağ kütlesi ve obezite ilişkili (FTO) ve peroksizom proliferatör aktive reseptör gama (PPARG) metilasyon düzeylerini ölçmeyi amaçladık. Çalışmamızda, Endokrinoloji Polikliniğine başvuran T2DM hastalarından (n=43) ve yaş-cinsiyet eşleştirilmiş sağlıklı bireylerden (n=42) tam kan alındı. Tam kan örneklerinden izole edilen genomik DNA'ların bisülfit dönüşümünden sonra hedef genlerin metilasyon profilleri metil-spesifik PCR ve jel elektroforezi yöntemleri ile analiz edildi. İstatistiksel analizler sonrası, T2DM ve kontrol grupları arasında FTO metilasyon durumu açısından anlamlı bir fark bulunmadı. T2DM'de PPARG geninin metilasyon seviyesi kontrol grubuna kıyasla önemli ölçüde daha yüksekti. PPARG'nın insülin duyarlılığını artırıcı etkileri göz önüne alındığında, bulgularımız metilasyon aracılı PPARG gen ekspresyonunun baskılanmasının T2DM hastalarında insülin direncinin yükselmesine yol açabileceği olasılığını doğrulamaktadır. T2DM hastalarında PPARG genindeki metilasyonun etkilerini ve hastalıkla ilişkisini daha iyi anlamak için daha fazla hasta ve kantitatif yöntemlerle yürütülen daha fazla gen ekspresyonu çalışması gerekecektir.

Anahtar Kelimeler: DNA Metilasyonu, FTO, PPARG, Tip 2 Diyabet Mellitus

Abstract

Type 2 diabetes (T2DM) is a complex, metabolic disease driven by genetic susceptibilities, environmental interactions, and various genes. Nowadays, increasing number of studies show the relationship between diabetes mellitus (DM) and epigenetics, especially DNA methylation. In this study, we aimed to measure the methylation levels of fat mass and obesity associated (FTO) and peroxisome proliferator activated receptor gamma (PPARG) in the peripheral blood samples of patients with clinical diagnosis of T2DM. In our study, whole blood was taken from T2DM patients (n=43) who applied to the Endocrinology Outpatient Clinic and from age-gender-matched healthy individuals (n=42). After the bisulfide conversion of isolated genomic DNAs from whole blood samples, the methylation profiles of target genes were analyzed with methyl-specific PCR and gel electrophoresis methods. Post-statistical analyses, no significant difference was found between the T2DM and control groups regarding FTO methylation status. The methylation level of PPARG gene in T2DM was significantly higher compared to the control group. Given the insulin sensitizing effects of PPARG, our findings confirm the possibility that methylation-mediated suppression of PPARG gene expression may lead to elevation of insulin resistance in T2DM patients. Further gene expression studies with more patients and quantitative methods will be required to better understand the effects of methylation in the PPARG gene in T2DM patients and its relationship to the disease.

Keywords: DNA Methylation, FTO, PPARG, Type 2 Diabetes Mellitus

Introduction

Type-2 diabetes mellitus (T2DM) is a complex, chronic, and progressive disease which arises due to insufficiency of insulin secretion and/or efficiency and is accompanied with hyperglycemia (1). T2DM is characterized with disruption of diet regulation as a result of dysfunctional carbohydrate, lipid, and protein metabolisms together with controllable or uncontrollable risk factors (2).

Epigenetic is defined as inherited alterations in gene activity and function independent from DNA sequence changes (3). Epigenetic mechanisms play crucial roles in cellular differentiation and maintenance of cell viability, which is essential for a healthy developmental process yet, any deviation from these regulations can lead to propensity for severe pathologies (4). Understanding the underlying mechanism of association between epigenetic alterations and disease progression contributes to enlighten the intricate combinatory effect of genetic and environmental factors in pathogenesis of several complex diseases (5). The most prevalent epigenetic modification in mammals is methylation of DNA from cytosine residues within CpG dinucleotides (6).

Fat mass and obesity-associated (FTO), also called alpha-ketoglutarate dependent dioxygenase, is one of the first genes contributing to polygenic obesity which has been identified with genome-wide association studies (GWAS) (7). FTO is commonly expressed in energy homeostasis and food-intake related regulatory regions in the body (1,8). Previous studies reported that FTO level was upregulated in skeletal muscle and adipose tissues of T2DM

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patients and it showed a strong correlation with T2DM disease that is characterized with dyslipidemia and hyperglycemia (9). Besides, in a clinical epigenetic study conducted with patients bearing impaired glucose metabolism, hypomethylation was detected within a single CpG region of FTO gene's first intron (10).

Peroxisome proliferator activated receptor gamma is a transcription factor which is a member of hormone receptors super family (11). PPARG regulates the expression of various genes involved in the pathogeneses of some metabolic diseases such as hyperlipidemia, diabetes, and obesity (11). PPARG is a type-II nuclear receptor and was initially identified in adipose tissue due to its prominent roles in fatty acid storage and glucose metabolism (12). PPARG supports lipid uptake and adipogenesis by increasing insulin sensitivity and adiponectin secretion (12). Certain mutations and epigenetic alterations in PPARG gene were correlated with obesity and dysfunctional lipid and glucose homeostasis which eventually leads to T2DM (12). In previous clinical case-control cohort studies performed with T2DM patients, various epigenetic alterations including DNA methylation were detected in several candidate genes like PPARG in target tissue and organs for insulin such as skeletal muscle, adipose tissue, and liver (13-16).

In this context, DNA methylation emerges as a promising indicator for complex diseases of which pathogeneses cannot be solely explained with traditional genetic traits (4). The aim of the present study is to investigate the DNA methylation statuses of certain regions containing CpG islands within promoters of PPARG and FTO genes in T2DM patients and healthy controls by using methylation specific PCR method in bisulfite-converted peripheral blood genomic DNA.

Material and Method

Study Population

An approval was obtained for the present study from Hatay Mustafa Kemal University Clinical Research Ethical Board (date 06/09/2021, issue no. 4298783/05045), and all experimental procedures involving human subjects adhered to the Helsinki Declaration. Biological materials were collected after verbal and written consent was obtained from participants who visited the endocrinology polyclinic at Hatay Mustafa Kemal University Research and Training Hospital. We included 43 patients with type-2 diabetes mellitus (T2DM), diagnosed by an expert clinician according to the American Diabetes Association criteria, and 42 age- and gender-matched healthy control subjects without insulin resistance or fasting blood glucose levels exceeding 100 mg/dL. The total sample size was calculated using G-Power analysis software (17) based on a previous study (18). Volunteers who are pregnant, under 18 years of age, or had inflammatory, chronic kidney, oncological, and acute or chronic infectious diseases were excluded from the study.

Sample Collection

Ten mL whole blood samples were collected into EDTA-containing tubes via venipuncture and stored at -20°C until the day of experiments which were performed between September-December 2022 at Hatay Mustafa Kemal University, Department of Medical Biology and Genetics.

DNA Extraction and Concentration Assessment

DNA isolation was performed following instructions of a commercial kit (Thermo Scientific GeneJET Genomic DNA Purification Kit, #K0721). Briefly, a 200 µL whole blood sample was mixed with a lysis solution containing proteinase K. After short vortex, the mixture was incubated at 56°C for 10 min with vortexing every 2 min. Then, pure ethanol was added to the mixture, and after brief vortex it was spun down through a column at 6000×g for 1 min. After two washing steps, the DNA sample was eluted from the column with 200 µL of elution buffer and kept at -20°C for long-term storage. DNA concentration and purity were measured using the µ-Drop tool with a spectrophotometer (MultiScan Go, Thermo Scientific).

Bisulfite Modification

In order to detect methylated cytosine residues with methylation specific PCR, bisulfite conversion of genomic DNA was performed following the manufacturer's instructions of a commercial kit (EpiJET Bisulfite Conversion Kit, Thermo Fisher, #K1461). For this purpose, 400 µg purified genomic DNA was mixed with modification reactive reagent and incubated in a thermal cycler (BioRad Thermal Cycler) with the following settings: 10 min at 98°C, 150 min at 60°C. The converted DNA sample was pipetted into the binding reagent-containing column and spun down for 30 s. After a single wash, the sample on the column was incubated with desulfonation reagent for 30 min and centrifuged briefly. After two washing steps of the column, the bisulfite-converted DNA sample was eluted with 10 µL elution buffer and stored at -20°C.

Methylation Specific Polymerase Chain Reaction (MSP)

We utilized "UCSC Genome Browser" database (UC Santa Cruz California University, http://genome.ucsc.edu) to determine the promoter regions of FTO and PPAR genes (19). Following detection of promoter sequences of FTO (chr16:53,703,706-53,704,323) and PPAR (chr3:12,287,496-12,288,834) genes, we used "Methprimer 2.0" database (http://www.urogene.org/methprimer2) to predict

CpG islands within the marked regions and to design specific primer pairs targeting methylated and unmethylated sequences (20).

The designed primer sequences (Macrogen Europe, Amsterdam, Netherlands) targeting methylated and unmethylated promoter regions of FTO and PPAR genes were listed in Table 1.

For MSP reactions, a commercial PCR master mix (Thermo Fisher PCR Master Mix 2X #K0171) and methylated and unmethylated control DNA kit (EpiTect PCR Control DNA kit, Thermo Fisher, #59695) were used. MSP reaction cycling conditions were summarized in Table 2. Agarose gel electrophoresis (2.5%) was run to visualize the PCR products with a computerized imaging system under ethidium bromide imaging channel (BioRad ChemiDoc XRS+).

Statistical Analysis

Data analysis was performed with Graphpad 8.0.2 package program. Non-categorical variables were expressed as n (sample size) and mean±standard deviation. For categorical data, we reported n (sample size) and percentage (%). To assess the normal distribution of non-categorical data, we employed Shapiro-Wilk test. The comparison of two individual groups was performed with either the independent t-test (for normally distributed data) or the Mann-Whitney U test (for non-normally distributed data). For comparisons involving categorical values, we applied either the Fisher's exact test or Chi-square test for trend.

Results

Demographic Features of Subjects

The demographic characteristics of the participants were summarized in Table 3. The priori test of the power analyses using two-sided Fisher exact test assumption gave the following outputs: Total sample size: 36, actual power: ~0.83, actual α: ~0.03 (with α error probability: 0.05).

We found no significant differences between the subjects in T2DM and control groups concerning age, gender, and smoking habits ($p>0.05$). However, there was a notable difference in BMI values between two groups ($p<0.05$).

Table 1. Methylated and unmethylated primer sequences designed based on the promoter regions of *FTO* and *PPARG* genes.

Gene	Primer type	Primer sequence	Amplicon size (bp)
FTO	Methylated	**F:** 5'-GTTGAGAGAATTATATGTAGGAGGCG-3' **R:** 5'-GTTCCTCGACAATCGAAATACG-3'	**112**
	Unmethylated	**F:** 5'-GTTGAGAGAATTATATGTAGGAGGTGG-3' **R:** 5'-CATTCCTCAACAATCAAAATACACTT-3'	**113**
PPARG	Methylated	**F:** 5'-ATTGACGGGGTTTTAGACGGAT-3' **R:** 5'-CGTCAATCCGAATCCTACCG-3'	**102**
	Unmethylated	**F:** 5'-GGGAATTGATGGGGTTTTAGATG-3' **R:** 5'-CCATCAATCCAAATCCTACCAAAC-3'	**107**

FTO: Fat mass and obesity-associated, PPARG: Peroxisome proliferator activated receptor gamma, bp: base pair.

Modified Profile of Methylated DNA Pattern in PPARG but Not FTO in T2DM Patients

MSP analyses revealed that the designed primers targeting the methylated (M) and unmethylated (UM) promoter regions of FTO and PPAR genes yielded amplicons at expected sizes based on agarose gel monitoring (Figures 1-2).

In figure 1, a representative 26-well gel image of FTO-gene specific methylation reactions is depicted, which consists of 2 DNA markers (1. and 13. wells), 3 control samples (M-DNA, UM-DNA, and non-bisulfite DNA) that were treated with either methylated (wells 2-4 respectively) or unmethylated primers (wells 14-16 respectively). The rest of the wells corresponds to the representative control (K1-K4) and patient (H1-H5) samples treated with M- and UM-primers. As expected, the positive control samples in 2. and 14. wells gave a positive signal with 112 and 113 bp amplicons corresponding to M- and UM-primers respectively. After analyses of all samples regarding FTO methylation status we found that, ~93% of control samples and ~98% of T2DM patient samples were unmethylated and there was so significant difference between the two groups ($p>0.05$, Table 4). The rest of the samples was heterozygous for the methylation status.

Table 2. PCR reaction conditions.

Step	Temperature (°C)	Duration (s)	Cycle (times)
Initial denaturation	95	180	1×
Denaturation	95	45	
Annealing	62^a, 64^b, 63^c, 56^d	30	35×
Extension	72	30	
Final extension	72	300	1×

Annealing temperatures for; a: FTO unmethylated primer, b: FTO methylated primer, c: PPARG unmethylated primer, d: PPARG methylated primer. FTO: Fat mass and obesity-associated, PPARG: Peroxisome proliferator activated receptor gamma.

Table 3. Demographic features of T2DM patients and healthy controls.

Parameters	HC (n=42)	T2DM (n=43)	*p* value
Age (Years, mean±SD)	59.8±10.5	58.1±11.8	0.46
Gender n (%)			
Male	23 (54.8)	17 (39.5)	0.19
Female	19 (45.2)	26 (60.5)	
BMI (kg/m^2, mean±SD)	26.7±4.0	29.6±5.0	***0.0034**
Smoking n (%)			
Smoker	13 (31.0)	14 (32.6)	>0.9999
Non-smoker	29 (69.1)	29 (67.4)	
HA1C (%)	N/A	9.3	N/A

BMI: Body mass index, HC: Healthy control, T2DM: Type-2 Diabetes mellitus, N/A: Not applicable

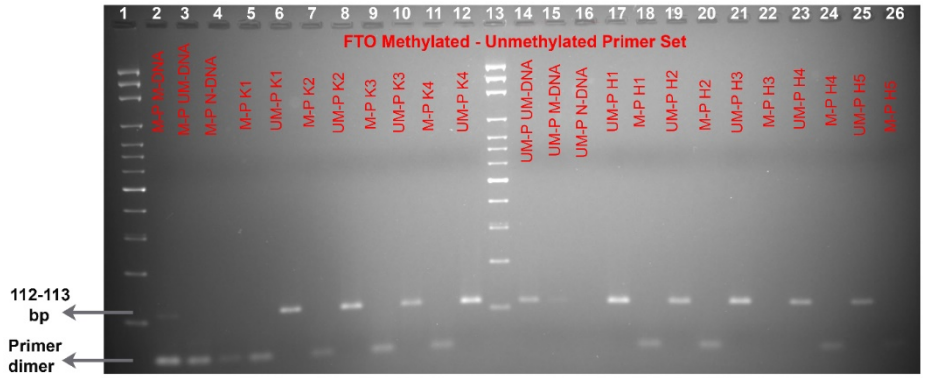

Figure 1. Representative gel images of MSP amplicons amplified with M- and UM-primers targeting promoter region of FTO gene. 1. and 13. wells: DNA marker, 2.-4. and 14.-16. wells: M-DNA, UM-DNA, and non-bisulfite-DNA controls amplified with M- and UM-primer sets respectively, 5.-12. wells: K1-K4 control samples amplified with M- and UM-primer sets, 17.-26. wells: H1-H5 T2DM patient samples amplified with M- and UM-primer sets. MSP: Methyl specific PCR, M: Methylated, UM: Unmethylated, K: Control, H: Patient, FTO: Fat mass and obesity-associated, bp: base pair.

In figure 2, representative gel images are depicted which shows MSP reactions conducted with M- and UM-primer set that is specific to PPAR gene promoter region. In gel image at upper panel indicating M-primer specific reactions, DNA marker at 1. well, positive control (M-DNA) at 2. well, and the rest of the wells with control (K27, K38-40, K50) and patient (H1-H13, H15-H20) samples were located. Methylated samples had a clear band size at 102 bp as the positive control sample. At the lower panel of figure 2, there are MSP reactions conducted with UM-primer set which consists of DNA marker (1. well), positive control (UM-DNA, 2. well), and the other samples between 3-23 wells (K28-K30, K32-K37, K39-50). The positive reactions had a clear band size at 107 bp. Further analyses revealed that (Table 4), the ratio of unmethylated samples was ~86% in the control group while ~67% in T2DM patient group. The heterozygous (M+UM) ratio in control and patient groups was ~12% and ~30% respectively. We found a significant difference between the two groups regarding methylation status ($p<0.05$, Table 4).

Table 4. Methylation status of FTO and PPARG genes in T2DM patients and healthy controls

Genes	HC (n=42)	T2DM (n=43)	*p* value
FTO n (%)			
Methylated	0 (0.0)	0 (0.0)	
Unmethylated	39 (92.9)	42 (97.7)	0.36
Hetero	3 (7.1)	1 (2.3)	
Non-detected	0 (0.0)	0 (0.0)	
PPARG n (%)			
Methylated	1 (2.4)	0 (0.0)	
Unmethylated	36 (85.7)	29 (67.4)	***0.01**
Hetero	5 (11.9)	13 (30.2)	
Non-detected	0 (0.0)	1 (2.3)	

*$p<0.05$. Chi-square test for trend for PPARG. Fisher's exact test for FTO. HC: Healthy control, T2DM: Type 2 diabetes mellitus, FTO: Fat mass and obesity-associated, PPARG: Peroxisome proliferator activated receptor gamma.

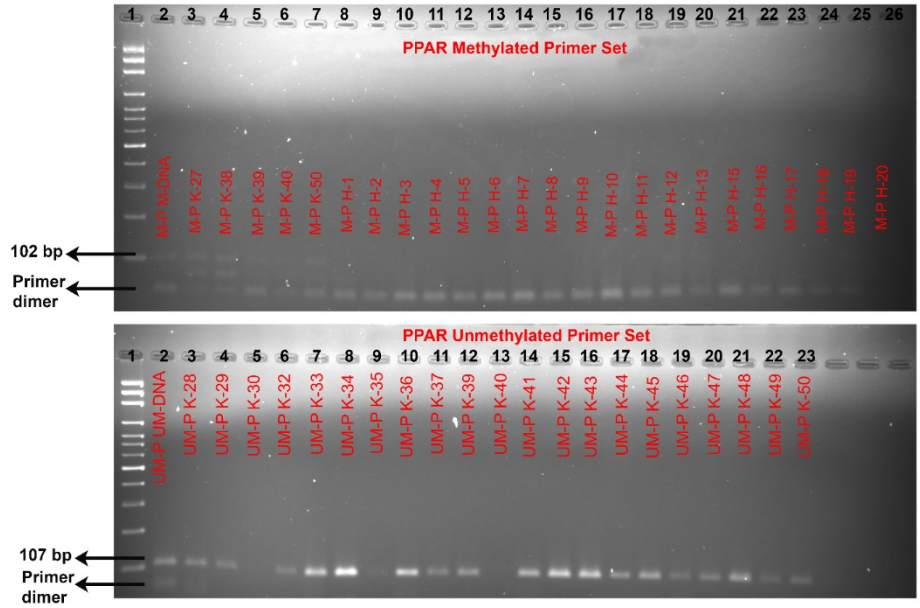

Figure 2. Representative gel images of MSP amplicons amplified with M-primer (upper panel) and UM-primer (lower panel) targeting promoter region of PPARG gene. In the upper panel (M-primer); 1. well: DNA marker, 2. well: Positive control (M-primer + M-DNA), rest of the wells: K27, K38-40, K50, H1-H13, H15-H20. In the lower panel (UM-primer); 1. well: DNA marker, 2. well: Positive control (UM-primer + UM-DNA), 3.-23. wells: K28-K30, K32-K37, K39-K50. MSP: Methyl specific PCR, M: Methylated, UM: Unmethylated, K: Control, H: Patient, PPARG: Peroxisome proliferator activated receptor gamma, bp: base pair.

Discussion

T2DM, which is a metabolic complex disease progressing due to life style, environmental, and genetic factors, is the primary reason of various diseases and disabilities worldwide (21). Since the physiological onset of T2DM occurs far earlier than the emergence of clinical symptoms, seeking early diagnostic and prognostic biomarkers, which would facilitate interference strategies concerning prevention or delay of the disease progression, has lately being gained a pivotal notice (21,22). As a result of gene-environment interaction, the modulation of gene expression can take place through chemical alterations around the genome so called epigenetic mechanisms such as DNA methylation and histone modifications (23). In this manner, the early diagnosis of T2DM via epigenetic indicators can contribute to disease management as well as can prevent the disease progression in people under high risk (24). DNA methylation is one of the most studied epigenetic modifications in several diseases including T2DM due to its stable chemical structure and easy profiling (6,21).

In the present study, we analyzed the methylation statuses of FTO and PPARG genes at their certain promoter regions in peripheral blood genomic DNA samples of T2DM patients and heathy controls using MSP method. Based on our findings, there was a significant difference regarding DNA methylation profile in PPARG between patient and control samples whereas there was no significant alteration in FTO gene.

FTO encodes a 2-oxoglutarate-dependent nucleic acid demethylase and several studies have reported that variants within FTO locus showed a strong correlation with obesity and can be used as a risk predictor for T2DM and cardiovascular diseases (25-27). A previous genome-wide DNA methylation profiling study conducted by Dayeh and Ling in pancreatic islets of T2DM patients analyzed 1649 CpG regions of 853 genes including FTO and PPARG (28). They ascertained that there was a close association between some of the GWAS-analyzed T2DM and obesity-linked candidate genes (CDNK1A, PDE7B, EXOC3L2, HDAC7, FTO) and the level of deteriorated β-cell function. In another clinical study performed with peripheral leucocytes of 25 T2DM patients and 11 healthy controls, a hypermethylation of a CpG region within FTO gene's promoter sequence has been identified and correlated with T2DM and metabolic syndrome (29). More recently, a strong association has been revealed between the methylation profile in a CpG region of FTO gene and T2DM disease (7). The association between FTO methylation status and T2DM disease was analyzed previously with a differential manner comprising 1169 cases and controls in total (10). First, in this study, a pool-based scanning was performed to differentially detect methylated DNA sequences among T2DM-related genomic regions and then a microarray method was applied to confirm and measure methylation status of the regions at upper levels of the list. Finally, it was reported that a CpG region within the first intron of FTO was found to be mildly

(~3.4%) but significantly () hypomethylated in T2D patients compared to controls.

FTO is the first gene that was shown to contribute to non-syndromic human obesity (7). The recent findings demonstrated that fatty diet leads to lipid deposition in several organs and thereby it affects the risk ratios of metabolic diseases namely obesity, insulin resistance and T2DM, and cardiovascular disorders (30). In a previous clinical study conducted by Perfilyev et al. it was ascertained after analyzing 4875 CpG regions that diet can affect DNA methylation profile (30). In the same study, the saturated fatty acid diet altered the DNA methylation status of 1797 genes including FTO whereas polyunsaturated fatty acid diet affected the methylation status of 125 genes excluding FTO. In light of these outcomes, the unaffected methylation profile of FTO in our study could arise from the diet habits of the participants in our study.

The recent multidirectional studies seeking the complex diseases revealed solid evidences regarding how genetic and epigenetic factors are implicated in the etiopathogenesis of these multifactorial diseases. FTO was claimed to be involved in T2DM genotype-epigenotype interactions on the basis of propensity for obesity by increasing its DNA methylation status (31). In the present study, we found no significant difference between the FTO methylation profiles of patient and control groups. The contradicting results between our findings and previous studies can likely stem from the scanning of different regions of FTO gene for possible DNA methylation process. Besides, we analyzed peripheral blood leukocytes for methylation analysis rather than adipose or pancreatic tissue, which can be the main reason for such contrasts between the studies (7). On the other hand, parallel to our findings, in a case-control study, no marked correlation was detected between FTO methylation profile and T2DM disease (29). The contradictions between these studies can rise from the multiple factors such as genetic diversity, life style, environmental effects, and differences in analyzed CpG regions (32). Besides, the DNA methylation variations among the subjects were asserted to change the individual predisposition to T2DM (7,10).

PPARG is another key player involved in metabolic pathways, which is commonly expressed in adipose tissue and acts as a hormone receptor (33). Basically, PPARG is activated upon binding to its ligand that results in its heterodimerization with retinoid X receptor and finally induction of transcriptional activation which leads to adipocyte differentiation and increasing of insulin sensitivity (31,34). Moreover, PPARG agonists were suggested to be utilized as anti-diabetic agent in clinics owing to its adipogenic and insulin-sensitivity actions (29). In the present study, we showed that the DNA methylation level of PPARG in T2DM patients was notably higher compared to the one in the control group. It has been previously reported in line with our findings that, the altered methylation profile in PPARG, KCNQ1, TCF7L2, and IRS1 genes were detected in adipose and pancreatic tissues of T2DM patients (23,24). In addition, a hypermethylated CpG region was detected in promoter sequence of PPARG gene of T2DM patients compared to healthy controls (29).

Epigenetic mechanisms play pivotal roles in gene expression regulations and thus they are crucial for normal and healthy development. Therefore, any deviations in epigenetic modulations can result in severe irregularities in gene expressions and eventually diseases (4). It is of primary importance to elucidate epigenetic modifications with respect to eliciting the intricate nature of gene-environment interactions in complex multifactorial diseases namely diabetes and cancer. In this regard, DNA methylation steps forward as a valuable indicator for complex diseases that cannot solely be explained with genetic traits of individuals (5). For future studies, populations with larger cohort size should be analyzed to find out promising DNA methylation signatures for T2DM pathogenesis by taking into account other confounder factors such as ethnicity and diet (21,24).

Conclusion

In the present study, we analyzed the methylation profile of metabolism-related FTO and PPARG genes' promoter regions in T2DM patients. We observed a marked increase in methylation levels of PPARG gene in patient group compared to healthy controls whereas there was no significant difference in FTO gene's methylation pattern. We suggest that, the methylation profile at the selected target promoter region of PPARG can be a promising indicator for T2DM pathogenesis. Still, further studies with larger population size together with longitudinal research strategy are warranted for implementation of peripheral blood-based DNA methylation profiling into clinical bed-side.

Study limitations

The total number of participants is relatively fewer in our study compared to similar studies in the literature. The current study limited to the methylation profile analysis of a single region within the promoter of target genes. We used a qualitative method for the analysis of methylation status of target genes however, quantitative methods are required for analytical inferences. The present study has also missing data concerning the alterations in mRNA expression levels of target genes depending on methylation patterns.

Acknowledgements

Regarding the limitations of our study, this was a retrospective review with a small sample size in a single center in a restricted region.

Conflict of interest statement

The authors of the present study disclose no financial or non-financial conflict of interest.

Ethics Committee Approval: The present study was approved by Hatay Mustafa Kemal University Clinical Research Ethical Board (Date: 06/09/2021, issue No: 4298783/05045).

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Comparison of Corneal Keratometry Measured by Three Different Methods

Farklı Üç Yöntemle Ölçülen Korneal Keratometrik Verilerin Karşılaştırılması

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Öz

Kısmi koherens interferometri reflektometri optik biyometri (Nidek AL Scan, 2.4-3.3 mm zonlar, Nidek Teknoloji, Gamagori, Japonya), korneal aberrometre/topograf (Nidek OPD Scan II, Nidek Teknoloji, Gamagori, Japonya) ve standart otorefraktokeratometre (Topcon KR 8900, Topcon, Tokyo, Japonya) cihazları kullanılarak elde edilen korneal keratometri ölçümleri (en düz-en dik keratometri, ortalama keratometri ve korneal astigmatizma) arasındaki değiştirilebilirliği ve uyumu test etmek için yapılan bu prospektif karşılaştırmalı çalışmaya yaş ortalaması 24.37±3.91 yıl olan 360 sağlıklı gönüllünün 360 sağ gözü dahil edildi. İkili karşılaştırmaları değerlendirmek için eşleştirilmiş t-testi kullanıldı. Üç cihaz arasındaki uyumu değerlendirmek için %95 uyum sınırları ile Bland-Altman testi kullanıldı. Nidek AL Scan'in 2.4 ve 3.3 mm bölgelerinde elde edilen tüm keratometrik değerleri arasında istatistiksel olarak anlamlı bir fark yoktu (p>0.05). Nidek AL Scan (2.4 -3.3 mm bölgesi) ve Nidek OPD Scan II in ikili karşılaştırmaları arasında AstK değerleri açısından istatistiksel olarak anlamlı bir fark saptanmadı (p>0.05). Nidek OPD Scan II ve Topcon KR 8900 ile ölçülen K1, K2 ve ortalama K değerleri Nidek AL Scan (2.4 -3.3 mm bölge) ile ikili karşılaştırıldığında, istatistiksel olarak anlamlı bir fark olduğu görüldü (p<0.05). AstK değerleri de Topcon KR 8900 ile Nidek AL Scan (2.4 -3.3 mm zonlar) ve Nidek OPD Scan II arasında istatistiksel olarak farklıydı (p<0.05). Sadece Nidek AL Scan, kendi içinde 2.4 ve 3.3 mm korneal zonlarda elde edilen tüm keratometrik parametreler için karşılaştırılabilir ölçümler sağlamıştır. Her bir cihazlar arası uyum için elde edilen %95 uyum sınırlarının geniş olması (>1.0 D) bu üç cihazın birbirinin yerine kullanılamayacağını düşündürmektedir.

Anahtar Kelimeler: AL Scan, Biyometri, Keratometri, OPD Scan, Topcon Otorefraktometre

Abstract

To compare and evaluate the interchangeability and agreement between corneal keratometry measurements (flattest-steepest keratometry, mean keratometry and corneal astigmatism) using partial coherence interferometry reflectometry optical biometry (Nidek AL Scan, 2.4–3.3 mm zones, Nidek Technologies, Gamagori, Japan), corneal aberrometer/topographer (Nidek OPD Scan II, Nidek Technologies, Gamagori, Japan) and standard autorefractokeratometer (Topcon KR 8900, Topcon Inc., Tokyo, Japan) a total of 360 right eyes of 360 healthy volunteers with a mean age of 24.37±3.91 years were enrolled in this prospective comparative study. Paired t-tests were used to evaluate pairwise comparisons. The Bland–Altman test with 95% limits of agreement was used to evaluate the agreement between the three devices. There were no statistically significant differences between all keratometric values of the Nidek AL Scan obtained in the 2.4 and 3.3 mm zones (p>0.05). There were no statistically significant differences in AstK values between the Nidek AL Scan (2.4 -3.3 mm zone) and the Nidek OPD Scan II pairwise comparisons (p>0.05). When the K1, K2, and Kmean values measured with the Nidek OPD Scan II and Topcon KR 8900 were compared with the Nidek AL Scan (2.4 -3.3 mm zone), a statistically significant difference was found (p<0.05). AstK values were also statistically different between Topcon KR 8900 versus Nidek AL Scan (2.4 -3.3 mm zone) and Nidek OPD Scan II (p<0.05). Only the Nidek AL Scan provided comparable measurements for all keratometric parameters analyzed in the 2.4 and 3.3 mm zones. The LoA obtained for each inter-device agreement should be analyzed carefully to consider the interchangeability of these three devices.

Keywords: AL Scan, Biometry, Keratometry, OPD Scan, Topcon Autorefractokeratometre

Introduction

The cornea accounts for approximately two-thirds of the total refractive capacity of the optical system in the eye (1). Measuring the refractive capacity and curvature of the cornea is known as keratometry (2). The most accurate and precise measurement of keratometry is vital for calculating the power of the intraocular lens to be used in modern cataract surgery, refractive surgery, contact lens applications, diagnosis, and follow-up of ectatic diseases, such as keratoconus (3-6). Abnormal values of corneal keratometry can result in serious ametropia and amblyopia at a tender age. Moreover, it is crucial to consider the difference between keratometry values in the principal meridians when determining corneal astigmatism. Hence, accurately measuring keratometry is essential to comprehend the state of refractive errors (7,8).

The gold standard method for keratometry measurement is manual keratometry; Helmholtz and Javal keratometry (9). Since this method is practitioner dependent and time consuming, it has been replaced by computerized automated systems (10). Currently, corneal topography/tomography devices, optical biometry devices, optical coherent tomography devices and standard autorefractokeratometers are the most preferred devices for keratometric measurements worldwide. The different optical and technical principles of each

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of these devices have led to frequent testing of their reliability and interchangeability (11-15).

The aim of this study is to compare the corneal keratometric data obtained from Nidek AL Scan optical biometry (Nidek Technologies, Gamagori, Japan), Nidek OPD Scan II aberrometer/topographer (Nidek Technologies, Gamagori, Japan) and Topcon KR 8900 autorefractokeratometer (Topcon Inc., Tokyo, Japan) devices which are frequently used in daily clinical practice and to determine whether the keratometric data of these devices can be used interchangeably.

Material and Method

This prospective comparative study included keratometric data from the right eyes of 360 participants (180 females, 180 males) aged 20-30 years who were recruited for a routine ophthalmologic examination at our clinic between January 2024 and May 2024. The study was granted approval by the Samsun Ondokuz Mayıs University ethics committee (Date: 14/12/2023, Decision no: 2023/401) and was executed in compliance with the principles outlined in the Declaration of Helsinki. All participants underwent a comprehensive ophthalmologic examination, including refraction and biomicroscopy, fundoscopy, and intraocular pressure measurements. Patients who had undergone any previous ocular surgery (cataract, refractive, pterygium, glaucoma, vitrectomy, etc.), had any corneal pathology, active ocular surface infection, nystagmus or albinism, or were unable to cooperate with any of the devices were excluded. Patients who had worn contact lenses within 24 h prior to the examination were also excluded. All participants provided informed consent to participate in this study.

Prior to the ophthalmologic examination, the same technician utilized Nidek AL Scan optical biometry (Nidek Technologies, Gamagori, Japan), Nidek OPD Scan II aberrometer/topographer (Nidek Technologies, Gamagori, Japan), and a Topcon KR 8900 standard autorefractokeratometer (Topcon Inc., Tokyo, Japan) to measure keratometric data. The results obtained from each device (including the flattest-K1 and steepest-K2 keratometry, mean keratometry, and corneal astigmatism) were subsequently compared. The Nidek AL Scan device was utilized to obtain data in two distinct corneal zones, measuring 2.4 mm and 3.3 mm. The data obtained from the two zones of the Nidek AL Scan device were compared with each other and with the data from other devices separately. The study aimed to determine whether the corneal keratometric data from the devices were interchangeable and whether they were compatible with one another.

The following formulas were used to calculate mean corneal keratometric value (Kmean) and corneal astigmatism (AstK).

Mean corneal keratometric value (Kmean) = (K1+K2)/2

Corneal astigmatism (AstK) = K2-K1

Devices

Nidek AL Scan

Nidek AL Scan (Nidek Technologies, Gamagori, Japan) is an optical biometer that measures the axial length without contact with the eye using partial coherence interferometry (830 nm). It projects a double ring with diameters of 2.4 and 3.3 mm on the cornea and calculates corneal keratometry using images obtained from 360 points in each ring. The device also measures anterior chamber depth, central corneal thickness, pupil diameter and corneal white-to-white distance, and provides data on the anterior segment and axial length of the eye in six different parameters within 10 seconds. Using these data it calculates the power of the intraocular lens to be used in cataract surgery.

The Nidek OPD- Scan II

The Nidek OPD-Scan II (Nidek Technologies, Gamagori, Japan) combines a wavefront aberrometer, Placido disc topographer, autorefractometer, and pupillometer into a single unit manufactured by Nidek Technologies in Gamagori, Japan. This device is capable of measuring wavefront errors through dynamic skiascopy, which involves sending 1.440 individual beams of light through the pupil and on to the retina. The time it takes for the beams to return to the instrument's sensors, with a resolution of 0.4 seconds or less, is analysed to determine the wavefront errors of the visual system, including lower-order aberrations such as sphere and astigmatism, which are measured in a 2.6-mm zone in the pupil, similar to a traditional autorefractor. Additionally, the OPD-Scan II measures the low-order wavefront error across a 4-6 mm zone in the pupil and calculates the spherical, cylindrical, and axes values using Zernike vector analysis. The root-mean-square wavefront error is then calculated.

Topcon KR 8900 Autorefractokeratometer

The Topcon KR 8900 autorefractometer (Topcon Inc., Tokyo, Japan) is a versatile device that assesses the refractive status of the eye through rotary prism evaluations. It measures objective spherical refractive power (ranging from -25 D to +22 D), cylindrical refractive power (between -10 D and +10 D), astigmatic axis (varying from 0° to 180°), corneal curvature, principal meridian direction, and corneal refractive power. To ensure accurate results, the device requires a minimum pupil size of 2 mm and employs a three-dimensional auto-alignment mechanism. The KR 8900 also incorporates the Scheiner double-pinhole principle for data collection, which involves projecting two light

sources onto the plane of the pupil to simulate the Scheiner pinhole apertures.

Statistical analysis

The obtained data were analysed using SPSS (version 21.0, SPSS, Inc., Chicago, IL, USA). First, the data distribution was evaluated using the Kolmogorov–Smirnov test. Normally distributed data are expressed as the mean±standard deviation, and non-normally distributed data are expressed as the median and maximum–minimum values. Categorical data are expressed as numbers and percentages. A paired t-test was employed to evaluate the measurements taken from the devices. The methodology put forth by Bland and Altman was utilized to determine the level of agreement between the devices. The Bland–Altman test with 95% limits of agreement (LoA; calculated as: the mean difference of two methods ±1.96 S.D.) was used to evaluate the differences between the individual measurements for each subject and illustrated using the Bland-Altman plot.

Results

The mean age of the 360 patients (180 males, 180 females) included in the study was 24.37±3.91 years. The flattest keratometry values (K1), steepest keratometry values (K2), mean keratometric value (K mean), corneal astigmatism values (AstK) obtained with Nidek AL-Scan biometry (2.4 and 3.3 mm zones), Nidek OPD Scan II aberrometer/topography and Topcon KR 8900 device are summarized in Table 1.

Table 1. Keratometric values measured with three different devices.

Parameter	Nidek AL Scan-2.4mm	Nidek AL Scan-3.3mm	Nidek OPD Scan II	Topcon KR 8900
K1 (D)	42.22±1.57	42.27±1.59	42.74±1.55	42.66±1.59
K2 (D)	43.76±1.46	43.79±1.51	44.22±1.45	43.97±1.47
Kmean	42.99±1.44	43.03±1.46	43.48±1.42	43.31±1.46
AstK	1.53±1.00	1.52±1.00	1.48±0.99	1.30±0.90

When comparing the K1, K2, Kmean, and AstK values obtained with the Nidek AL Scan biometry in the 2.4 and 3.3 mm zones, no statistically significant differences were observed among the two different zone measurements ($p>0.05$). There were no statistically significant differences in AstK values between the Nidek AL Scan (2.4 -3.3 mm zone) and the Nidek OPD Scan II pairwise comparison ($p>0.05$). However, significant differences were identified among the K1, K2 and Kmean measurements obtained with the Nidek AL Scan biometry (2.4-3.3 mm zones) compared with the Nidek OPD Scan II and Topcon KR 8900 devices ($p<0.05$). Significant differences were also observed between Nidek OPD Scan II and Topcon KR 8900 in whole keratometric values. All p-values are summarized in Table 2.

Table 2. Pairwise comparisons of K1, K2, Kmean and AstK among three devices.

Pair of devices	K1	K2	Kmean	AstK
AL Scan-2.4 / AL Scan-3.3	**0.074***	**0.449***	**0.074***	**0.744***
AL Scan-2.4 / OPD Scan II	0.000*	0.000*	0.000*	**0.092***
AL Scan-2.4 /Topcon KR 8900	0.000*	0.000*	0.000*	0.000*
AL Scan-3.3 / OPD Scan II	0.000*	0.000*	0.000*	**0.337***
AL Scan-3.3 /Topcon KR 8900	0.000*	0.000*	0.000*	0.000*
OPD Scan II /Topcon KR 8900	0.002*	0.000*	0.000*	0.000*

*Paired t-test, $p<0.05$.

Bland-Altman analysis identified the lowest mean differences (95% CI of limits of agreement) -0.04±0.32 in K1, -0.03±0.49 in K2, -0.04±0.26 in Kmean, 0.02±0.64 in AstK for Nidek AL Scan between 2.4 and 3.3 mm zones values. The mean difference was highest in K1 -0.51±0.24, K2 -0.46±0.39, Kmean -0.49±0.24 between Nidek AL Scan (2.4 mm zone) and Nidek OPD Scan II respectively. The mean difference was highest in AstK 0.24±0.43 between Nidek AL Scan (2.4 mm zone) and Topcon KR 8900. Bland-Altman plots showing differences between Nidek AL Scan (2.4 and 3.3 mm zones), Nidek OPD Scan II and Topcon KR 8900 at K1, K2, Kmean, AstK values were presented in Fig.1 and Fig.2.

Although the mean difference was below 0.50 D in pairwise comparisons between all three devices, the 95% LoA agreement range was wider than 1.0 D, suggesting that these three devices are not interchangeable in a clinical setting.

Discussion

This study investigated corneal keratometry data obtained using three different methods in a sample of 360 healthy volunteers who underwent routine ophthalmic examination at our clinic. The Nidek AL Scan keratometric data did not show significant differences, and exhibited good agreement in two different corneal zones. A statistical difference was found in all keratometric data obtained from the Topcon KR 8900 between the Nidek AL Scan device (in two different corneal zones) and Nidek OPD Scan II. There was no statistically significant

distinction observed between the Nidek OPD scan II and Nidek AL Scan (2.4-3.3 mm zones) solely with regards to the AstK value. Rather than assessing repeatability, this study assessed the mean differences and used pairwise comparisons to better understand whether the three devices were comparable and interchangeable.

Schultz et al. compared keratometry and astigmatism measurements provided by the Verion Reference Unit (an image-guided system) with the Tonoref II automated tonometer–refractometer, AL-Scan optical biometer, IOL Master 500 biometer, Pentacam rotating Scheimpflug camera and OPD Scan III wavefront aberrometer (16). The similar result between the keratometric data of the Nidek AL Scan in the 2.4 and 3.3 mm zones is consistent with the findings of the current study. On the other hand, the absence of a difference between the Nidek OPD Scan III, Tonoref II, and AL Scan biometry is in contrast. This disparity may be attributed to the fact that we employed OPD Scan II and Topcon KR 8900 autorefractometer in the current study and their study population comprised older patients.

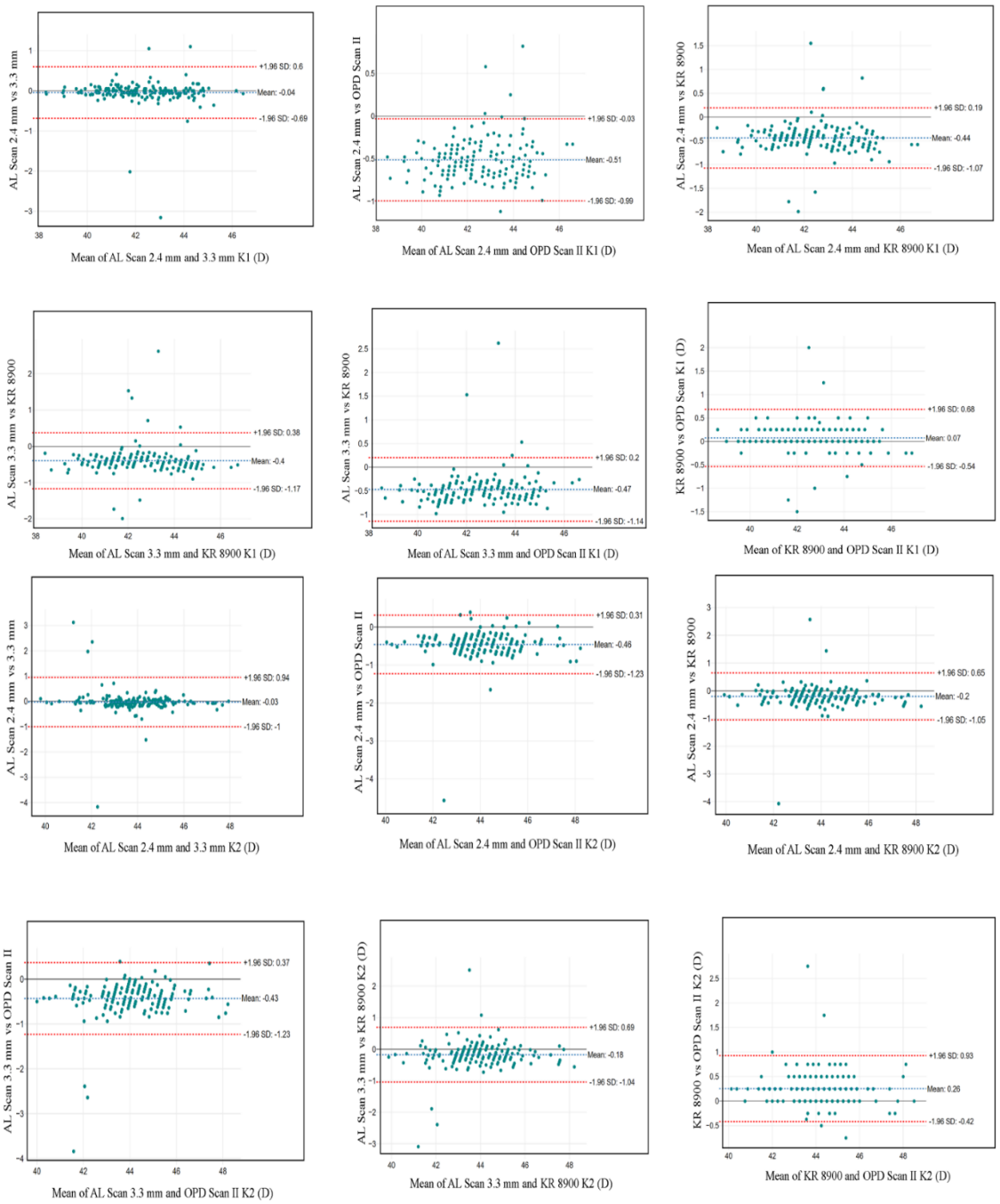

Figure 1. Bland-Altman plots showing agreement between Nidek AL Scan (2.4 and 3.3 mm), Nidek OPD Scan II, Topcon KR 8900 at K1 and K2 keratometric measurements. The middle line presents the mean difference, the bottom and the top dashed lines show the lower and upper 95% limits of agreement.

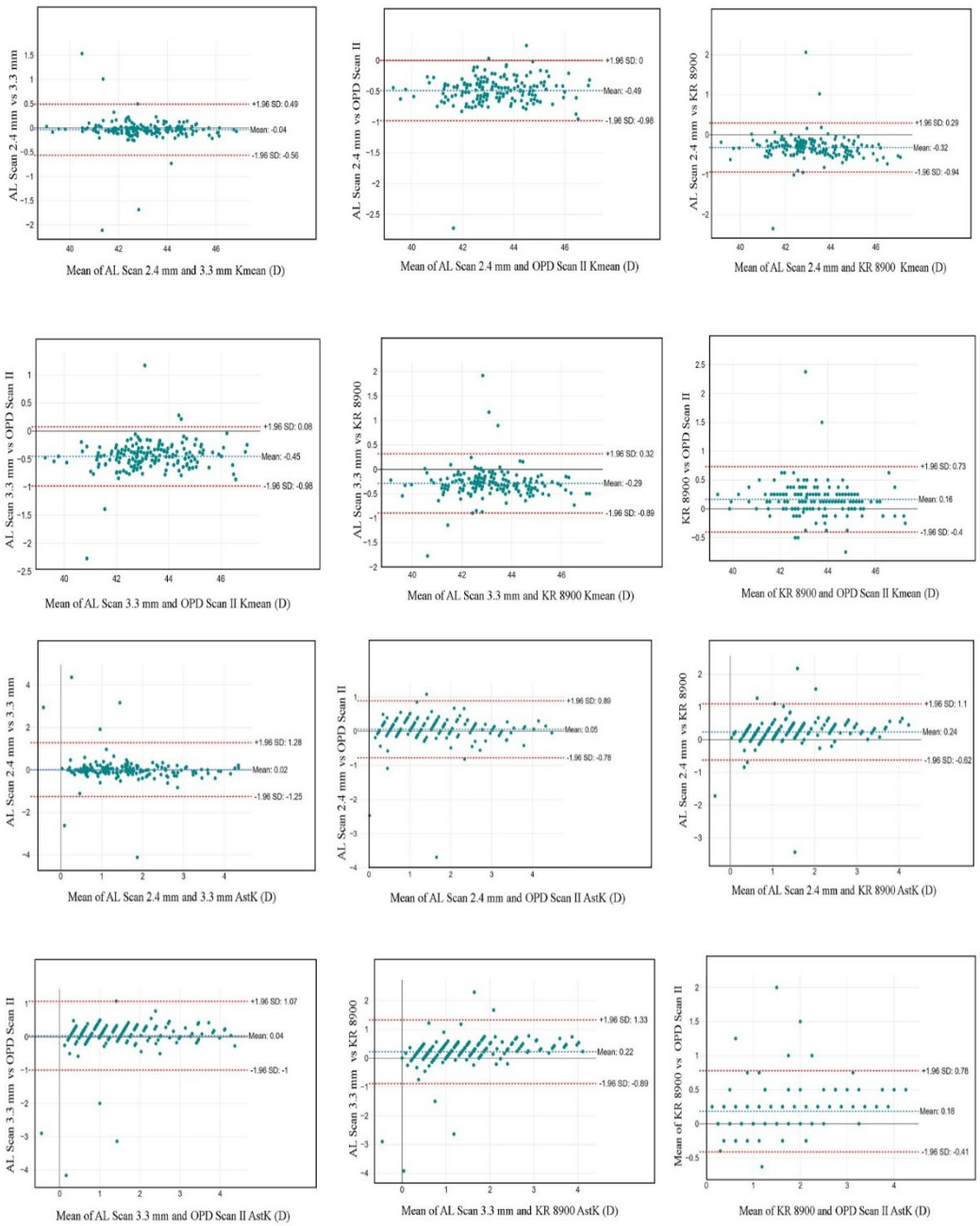

Figure 2. Bland-Altman plots showing agreement between Nidek AL Scan (2.4 and 3.3 mm) Nidek OPD Scan II, Topcon KR 8900 at Kmean and AstK keratometric measurements. The middle line presents the mean difference, the bottom and the top dashed lines show the lower and upper 95% limits of agreement.

Shirayama et al. investigated the reproducibility and comparability of anterior corneal power measurements obtained using the Humphrey Atlas corneal topographer, Galilei Dual Scheimpflug Analyzer, IOL Master, and a manual keratometer (17). The study found that the intraclass correlation coefficients (ICCs) for all the devices tested were higher than 0.99, indicating a high degree of agreement. The 95% limits of agreement (LoAs) for the mean keratometry values were less than 0.5 D for each pair of devices. Based on these findings, the authors concluded that the corneal power measurements from the four devices were highly reproducible and comparable. While the study did not make any specific recommendations regarding the interchangeability of the devices, the reported 95% LoAs suggest that the measurements could be considered interchangeable, given the clinical relevance implied by a 0.50 D difference.

In a study conducted by Çağlar et al., the authors evaluated Nidek AL Scan biometry, Sirius topography (CSO, Florence, Italy) and ultrasound biometry (Aviso A/B, Quantel Medical, MT, USA) in a population with a mean age of 39.24±14.37 years (18). The researchers reported no statistically significant difference in average keratometry

between the 2.4 and 3.3 mm zones of the AL Scan and SimK of the Sirius topography device, and they found a very high correlation coefficient between the devices (0.977). The highest mean difference between the parameters was 0.059 D and the widest LoA was-0.715 to 0.730 D. The authors claimed that AL Scan biometry and Sirius Scheimpflug/Placido photography-based topography could be used interchangeably in terms of keratometry. The finding of no difference in average keratometry measurement between the two zones of AL Scan biometry is consistent with current research. However, their study's finding of compatibility with Sirius topography differs from our results, which may be attributed to the use of the Nidek OPD Scan II as the topography device. Duman et al compared Nidek AL Scan with Sirius topography system in a population with a mean age of 71.79±7.91 years with cataract (19). Opposite to the Çağlar et al.'s study they only found good agreement in keratometric values with Sirius and AL Scan in 2.4 mm zones. Keratometric measurements of AL Scan in 3.3 mm zones were statistically different from Sirius device. The researchers suggested that 2.4 mm corneal zone measurements of AL Scan could be more appropriate for determining the lens power in clinical settings. The reason for this disparity, as they perceived it, was attributed to the dissimilar age range of patients with cataracts encompassed.

Hashemi et al. conducted a study comparing the Nidek ARK-510A autorefractokeratometer to rotating Scheimpflug imaging with Pentacam and Lenstar LS 900 biometry in a population of children aged 6-12 years old (20). The results of the study indicated that these three devices are not interchangeable in the evaluation of corneal astigmatism in children. The authors determined that the difference between the devices may be due to the fact that targets used by the devices stimulate the accommodation at different levels and affect the corneal curvature and that the amplitude of accommodation is higher in the paediatric age group.The young age of the participants in our current investigation might have also contributed to the occurence of notable variations between the devices.

Six different keratometers—Javal-Schiotz, IOL Master, Pentacam, OPD Scan III, Medmont and TMS-5—were evaluated in a population with a mean age of 36±11.4 years, as reported by Hamer et al (21). According to the study, OPD Scan measurements were found to be significantly different from those obtained using the other devices with lower results being observed. Additionally, Javal-Schiotz was found to produce significantly higher results compared to the other devices. The study also revealed a weaker correlation between OPD Scan and IOL Master measurements. The researchers observed that Placido disc systems generated a broader distribution of data with a higher incidence of outliers in comparison to both Scheimpflug and automated keratometric methods. The possibility of an unstable tear film was cited as a contributing factor, and it was suggested that the use of ocular lubricant before measurement with Placido disk systems may be beneficial.

Mehravaran et al. compared min-K, max-K and mean-K values obtained by using Topcon 8800 autorefractokeratometer, IOL Master, EyeSys 3000 Corneal Analysis System (EyeSys Vision), and Pentacam HR with a manual Javal keratometer in 42 eyes of 21 patients aged 31.74±6.82 years old (22). For both values, Topcon 8800 and IOL Master generated higher readings than Javal, while EyeSys 3000 and Pentacam showed lower results. Compared to Javal, the smallest difference in measuring min-K was observed with the IOL Master, with a mean inter-device difference of -0.09±0.24 D. However, in terms of inter-device agreement, the IOL Master and Topcon yielded comparable results. When comparing to Javal, the smallest difference for max-K readings was seen between Javal and Pentacam, but the 95% LoA suggested better agreement for Topcon. For mean-K readings, the smallest difference was observed between Javal and Topcon, while the IOL Master showed slightly better agreement. Researchers have reported that Topcon and IOL Master were safe to be interchanged with Javal keratometry in a clinical setting.

The discrepancies among keratometers are attributable to the fact that manufacturers do not employ a uniform index of refraction or measurement area or method. Placido-based systems gauge the cornea paracentrally rather than centrally, thereby creating a blind spot that may neglect from 1.3 to 2.1 mm of the central zone. As the measurement area approaches the center, the corneal curvature increases in steepness. Although some differences among keratometers may be negligible, others may hold clinical significance, particularly when determining the intraocular lens power for cataract surgery. In fact, a 0.25 D error in measuring the corneal refractive power can result in an approximate correction error of 0.28±0.04 to 0.31±0.05 D (23). Norrby et al. also demonstrated that inaccurate corneal power constitutes a significant source of error in intraocular lens (IOL) power calculations. A 1 D error in the corneal power measurement results in an approximately 1 D error in the calculation of the IOL power (24). The authors asserted that inaccurate keratometry measurement constitutes one of the primary sources of postoperative refractive surprise following intraocular lens implantation.

Conclusion

In conclusion, various studies have documented differences and similarities in keratometric measurements among different age groups using

different devices. Despite the mean difference of 0.5 D or less between the three devices in our study, the 95% LoA range between three devices was over 1.00 D, highlighting that Nidek AL Scan, Nidek OPD Scan II and Topcon KR8900 devices cannot be used interchangeably in terms of corneal keratometric measurements.It is important to note that these devices may yield different results when utilized interchangeably in clinical practice. Further studies are needed to evaluate these three devices' keratometric data on different age populations with without corneal diseases such as kerakonus.

Study Limitations

Limitations of the study include the absence of a manual keratometer and the fact that only healthy corneas were included. An additional limitation is the lack of postoperative outcomes from refractive surgery procedures conducted based on keratometric data obtained from these three distinct keratometric devices.

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Conflict of interest statement

The authors declare that they have NO affiliations with or involvement in any organization or entity with any financial interest in the subject matter or materials discussed in this manuscript.

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Study on The Frequency of Antibiotic Use Before Diagnosis in Granulomatous Mastitis

Granülomatöz Mastitte Tanı Öncesi Antibiyotik Kullanım Sıklığı Üzerine Bir Çalışma

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Öz

Bu retrospektif kohort çalışmasında 2015-2020 yılları arasında granülomatöz mastit tanısı almış 69 hastaya ait veriler derlenmiştir. Hastalara ait demografik veriler, tanıdan önce antibiyotik alıp almadıkları, antibiyotik kullanımının türü ve süresi, patolojik tanı ile tedavi başlangıcı arasında geçen süre, mikrobiyolojik testlerde belirli bir faktörün varlığı, tanıdan sonra tedavi türü ve iyileşme süresi kaydedildi. Ortalama yaş 34.58±7.30 yıldı. Hastaların %94.2'sinin (n=65) tanıdan önce ampirik antibiyotik aldığı bulundu. Hastaların %71'i (n=53) en az iki farklı antibiyotik almıştı. Patolojik tanıya kadar geçen ortalama süre 12.7 aydı. Genellikle granülomatöz mastit tanısı patolojik olarak konulana kadar verilen ampirik antibiyotik tedavileri tanı sürecini uzatır. Bu hastalarda gereksiz ve uzun süreli antibiyotik kullanımının önüne geçmek için tanısal testlere öncelik verilmesi daha uygun olacaktır.

Anahtar Kelimeler: Ampirik Tedavi, Antibiyotik, İdiyopatik, Granulomatöz Mastit, Tedavi Yönetimi

Abstract

This retrospective cohort study compiled data from 69 patients pathologically diagnosed with granulomatous mastitis between 2015 and 2020. Patient demographics, whether they were taking antibiotics before diagnosis, the type and duration of antibiotic use, the time elapsed between pathologic diagnosis and initiation of treatment, the presence of a specific factor in microbiologic tests, the type of treatment after diagnosis, and the recovery period were recorded. The mean age was 34.58±7.30 years. It was found that 94.2% (n=65) of patients had taken empirical antibiotics before diagnosis.Seventy-one percent of the patients (n=53) had taken at least two different antibiotics.The mean time to pathological diagnosis was 12.7 months. Empiric antibiotic treatments, often given until the diagnosis of granulomatous mastitis is made pathologically, prolong the diagnostic process. It would be more appropriate to prioritize diagnostic testing to avoid unnecessary and long-term antibiotic use in these patients.

Keywords: Empiric Treatment, Antibiotic, Idiopathic, Granulomatous Mastitis, Treatment Management

Introduction

Granulomatous mastitis is a chronic inflammatory disease of the breast. This disease is divided into idiopathic and secondary. Secondary mastitis occurs due to a specific factor (1). Both infectious and non-infectious agents are found in the secondary group. In addition to factors involved in the etiology of granuloma, such as sarcoidosis, Wegener's disease, and foreign bodies, rare pathogens such as tuberculosis, corynebacteria, bacteria, fungi, and parasites are among the causes of secondary granulomatous mastitis (2). To speak of idiopathic granulomatous mastitis, it must be confirmed that there is no other etiologic cause (3).

The etiology of idiopathic granulomatous mastitis is not yet fully understood, and its association with autoimmune diseases and some rare/difficult-to-detect infectious agents is under investigation. Since the etiologic cause has not been fully elucidated, there is no consensus on treating the disease.

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Findings in patients often mimic simple mastitis symptoms. The most common findings include painful induration, redness, discharge, and temperature elevations in the breast. As these findings primarily indicate an inflammatory process, empiric antibiotic therapy is initiated, and drainage is placed in most patients before diagnosis (4). In particular, patients with idiopathic granulomatous mastitis are treated unsuccessfully with a provisional diagnosis of bacterial breast infection.

In addition to the unnecessary use of antibiotics, long-term and different types of antibiotic treatments lead to prolongation of the process and secondary damage in patients who do not benefit from treatment (5). Although granulomatous mastitis is a benign and inflammatory breast disease, morbidity can be severe.

Empiric antibiotic therapy is commonly used for many infectious diseases, considering potential factors. However, repeated use of antibiotics without differentiation between granulomatous mastitis and simple mastitis delays the diagnosis of these patients and often complicates the process. Therefore, rapid radiologic evaluation and non-delayed breast biopsy for clinical suspicion in this patient population may minimize treatment duration with rapid diagnosis and reduce recurrence and treatment resistance. It is important to initiate antibiotic therapy when an infectious agent is detected after a microbiological examination. In this study, we aimed to determine the frequency and duration of antibiotic use before

diagnosis in patients with granulomatous mastitis. We also aimed to investigate the impact of antibiotic use on the diagnostic treatment process.

Material and Method

In this retrospective cohort study, data from 90 patients with a pathologic diagnosis of granulomatous mastitis were analyzed at two different centers between 2015 and 2020. One of the centers was a 3rd-level hospital; the other was a 3rd-level hospital, and the same physicians performed all patients' diagnostic and treatment processes. Data were obtained from medical history reports and records of the physicians who led the diagnosis and treatment process. Twenty-one patients in whom the diagnosis of granulomatous mastitis could not be confirmed pathologically, whose data were not accessible, or who did not receive the recommended treatment were excluded from the study.

Patient's demographic data, whether they had taken antibiotics before diagnosis, the type and duration of antibiotic use, the type of disease onset, the time elapsed between the pathological diagnosis and the start of treatment, the presence of a specific factor in microbiological tests, the type of treatment given after diagnosis, and the recovery time were recorded. In addition, comorbidity, history of chest trauma, smoking, body mass index, menopausal status, contraceptive use, pregnancy, and breastfeeding status were recorded.

After the clinical assessment of patients, CRP, WBC, prolactin, TFT, tissue and abscess culture results, and radiological assessment results were recorded.

Only patients with a pathologically confirmed diagnosis of granulomatous mastitis were included in the study. Patients whose pathogen could not be detected in the tests performed after diagnosis for etiologic investigation were classified as having idiopathic granulomatous mastitis. This situation was also recorded in patients with an etiologic cause of granuloma.

All patients' presence, duration, and type of antibiotic therapy before diagnosis were compared, and their impact on the diagnostic and treatment process was investigated.

Statistical Analysis

The sample size for this study was calculated using G*Power software version 3.1.9.2 (Institute of Experimental Psychology, Heinrich Heine University, Düsseldorf, Germany). Due to the lack of previous research on this topic, a preliminary study was conducted to determine the sample size. The calculation was based on the Mann-Whitney U test, which was used to evaluate the central hypothesis of this study. Specifically, the sample size was derived from the treatment durations of 10 patients who received antibiotics for 7 to 14 days and 14 patients who were treated with antibiotics for more than 14 days. With a two-tailed test, type I error of 0.05, power of 80% (1-β = 0.8), and effect size (d) of 1.056, it was determined that the study should include a minimum of 16 patients in each group, totaling at least 32 subjects.

Data analyses were performed by using SPSS for Windows, version 22.0 (SPSS Inc., Chicago, IL, United States). Whether the distribution of continuous variables were normal or not was determined by the Kolmogorov-Smirnov test. The Levene test was used for the evaluation of homogeneity of variances. Unless specified otherwise, continuous data were described as mean±SD (standard deviation) for normal distributions, and median (Q1-Q3) for skewed distributions. Categorical data were described as number of cases (%).

Statistical analysis differences in normally distributed variables between two independent groups were compared by Student's t-test, Mann Whitney U test were applied for comparisons of the not normally distributed data.

Categorical variables were compared using Pearson's chi-square test or Fisher's exact test.

Univariate and multiple logistic regression analyses were performed to assess the association between recurrence and risk factors findings.

First of all it was used one variable univariate logistic/lineer regression with risk factors that are thought to be related with risk factors that has p-value<0.05 univariate variable logistic/lineer regression were included to model on multivariable logistic/lineer regression. Enter model used in multivariable logistic/lineer regression. Whether all independent variables were significant in the model was analyzed with Wald statistic on multivariable logistic regression. Whether every independent variable was significant on the model was analyzed with t statistic on multivariable lineer regression.

Receiver operating characteristic (ROC) curve analysis was used to determine the cutoff value of the time to diagnosis associated with the risk of antibiotic treatment time.

It was accepted p-value <0.05 as significant level on all statistical analysis.

Results

Data were analyzed from 69 patients with histopathologically confirmed granulomatous mastitis between 2015 and 2020. The characteristics of all patients included in the study are listed in Table 1. The mean age was 34,58±7,30 years (range 22-59 years). All patients were women, and only two patients were diagnosed with post-menopause.

Table 1. The characteristics of all patients included in the study are listed in the table below.

	n	%
Age. $\acute{X}$ ± SD (Standart deviation)	34.58±7.30	
Size of lesion. Med(Q_1-Q_3)	35.00(30.00-50.00)	
Antibiotic treatment duration		
7-14 days	16	24.6%
>14 days	49	75.4%
Time. months. Med(Q_1-Q_3)	5.00(3.00-12.00)	
Symptoms		
Mass	35	50.7%
Drainage	2	2.9%
Fistule	7	10.1%
Pain	9	13.0%
Abscess	16	23.2%
Side		
Right	34	49.3%
Left	34	49.3%
Bilateral	1	1.4%
Localization		
Retro-areolar	18	26.5%
Upper-outer quandrant	17	25.0%
Upper-inner quadrant	19	27.9%
Lower-outer quadrant	4	5.9%
Lower-inner quadrant	5	7.4%
Whole breast	5	7.4%
Surgery		
None	0	0.0%
Biopsy	57	83.8%
Excision	10	14.7%
Mastectomy	0	0.0%
Breast-conserving	1	1.5%
Recurrence		
No	49	71.0%
Yes	20	29.0%
Antibiotic use		
No	4	5.8%
Yes	65	94.2%

Examination of lesion locations revealed they were mostly located in the upper inner quadrant and retro areolar area. The first finding in most patients was a painful mass (50.7%).

Sixty-five (94.2%) patients had received antibiotic treatment before diagnosis. Patients were grouped according to antibiotic treatments received in two groups of 7-14 days and multiple antibiotic treatments of more than 14 days. Only four patients (5.8%) had not received empiric antibiotic treatment before diagnosis. Most patients (68.8%) who received antibiotic therapy of less than 14 days received amoxicillin-clavulanic acid and ciprofloxacin, and 46.9% received antibiotic treatment longer than 14 days. It was found that 17 patients also received multiple antibiotics, such as 2nd and 3rd line cephalosporins. The recurrence rate was found to be statistically significantly higher in patients whose antibiotic intake duration was more than 14 days ($p<0.05$). The rate of amoxicillin-clavulanic acid with ciprofloxacin and 2nd and 3rd line cephalosporin use was found to be statistically significantly higher in patients whose antibiotic intake duration was more than 14 days ($p<0.05$) (Table 2). Cultures were taken from 52 patients, and specific microorganisms were grown in only 3. One of these patients grew *Staphylococcus spp*., one grew *Candida albicans*, and the other grew *E. Coli* producing extended-spectrum beta-lactamase (ESBL). The culture of the re-sampled aspirate material showed no growth in all three patients.

It was noted that 13 patients were tested with PPD (Purified Protein Derivative) and 56 with IGST (Interferon-gamma release test). While the result of the PPD test was positive in 9 patients (>15mm), the IGST test was positive in 18 patients. In addition, no patient was found to stain with ARB (Asido-resistance staining) in tissue culture, and no tuberculosis culture positivity was detected.

The mean duration of symptoms before diagnosis was 12.57 months. It was observed that this period passed with various hospitalizations and empiric antibiotic treatments.

Table 2. The comparison situation in terms of other variables according to the duration of antibiotic intake is given in the table below.

	Antibiotic treatment time				p
	7-14 days		>14 days		
	n	%	n	%	
Age. $\acute{X}$ ± SD	35.69±8.33		34.46±7.17		0.573*
Size. Med(Q_1-Q_3)	40.00(30.00-50.00)		35.00(25.00-50.00)		0.267 $^{\Phi}$
Treatment time. Med(Q_1-Q_3)	3.00(2.00-7.00)		6.00(3.00-18.00)		0.060 $^{\Phi}$
Symptoms					
Mass	6	37.5%	27	55.1%	0.162 $^{\beta}$
Drainage	2	12.5%	0	0.0%	
Fistule	2	12.5%	4	8.2%	
Pain	2	12.5%	7	14.3%	
Abscess	4	25.0%	11	22.4%	
Recurrence					
No	15	93.8%	30	61.2%	**0.014** $^{\beta}$
Yes	1	6.3%	19	38.8%	
Type of antibiotics					
Amoxicillin-clavulanate(1)	11	68.8%	9	18.4%	**0.001** $^{\beta}$
(1) + Ciprofloxacin	4	25.0%	23	46.9%	
Additional antibiotics (cephalosporins etc.)	1	6.3%	17	34.7%	
Steroids					
No	10	62.5%	32	66.7%	0.761 $^{\beta}$
Yes	6	37.5%	16	33.3%	
Anti-tuberculosis					
No	10	66.7%	42	85.7%	0.132 $^{\beta}$
Yes	5	33.3%	7	14.3%	

*Continuous variables are expressed as either the mean±standard deviation (SD) or median (Q_1-Q_3) and categorical variables are expressed as either frequency (percentage). Student t Test *, mann whitney u Test $^{\Phi}$, Chi square Test or fisher's exact test $^{\beta}$, p=Level of Significance, p<0,05*

Steroid therapy was administered to 23 (33.8%) patients diagnosed with idiopathic granulomatous mastitis. None of the patients received immunosuppressive treatment such as methotrexate. However, patients with positive IGST and/or PPD values among those who did not accept steroid treatment or who relapsed after steroid treatment received anti-tuberculous treatment. No relapse was observed in 7 of 13 patients treated this way during the one-year follow-up period.

It was noted that recurrent seizures and relapse occurred in 20 patients during the 1-year follow-up period. Although recurrence was not associated with antibiotic use alone, it is noteworthy that the number of exacerbations and course duration increased when the process was prolonged. The diagnosis of the disease was delayed until diagnosis by using empiric antibiotics (p=0.014).

Univariate logistic regression analysis was applied to determine factors that predict recurrence in patients. It was understood that variables with a p-value below 0.05 predicted or predicted relapse. Increased duration of antibiotic intake and taking multiple types of antibiotics predicts recurrence (p=0.036 and 0.042 respectively).

Variables with a p-value <0.05 in the univariate logistic regression analysis were included in the multiple logistic regression analysis. In multiple logistic regression analysis, no variable was detected that predicted recurrence (Table 3).

Table 3. Logistic Regression Analysis Applied to Determine Factors Affecting Relapse

	Univariate Logistic Regression					Multiple Logistic Regression				
	Wald	p	OR	95% C.I.for OR		Wald	P	OR	95% C.I.for OR	
Age	1.783	0.182	0.944	0.868	1.027					
Antibiotic treatment time	4.397	**0.036**	9.500	1.158	77.908	3.419	0.064	7.494	0.886	63.36
Type of antibiotics										
Amoxicillin-clavulanate (1)	0.008	0.929	0.949	0.302	2.985					
(1) + Ciprofloxacin	3.125	0.077	0.348	0.108	1.121					
Additional antibiotics (cephalosporins etc.)	4.123	**0.042**	3.273	1.042	10.278	2.007	0.157	2.358	0.720	7.725
Steroids	0.657	0.417	0.615	0.190	1.991					
Antituberculosis treatment	2.928	0.087	0.158	0.019	1.308					

Wald: test statistics, OR: odds radio, CI: Confidence interval. hosmer-lemeshow: p>0,05. Statistically significant p-values are in bold.Variables with a p value below 0.05 were included in multiple analysis.

Univariate lineer regression analysis was applied to determine the factors affecting the time to diagnosis. It was understood that variables with a p-value below 0.05 predicted the time until diagnosis. It was observed that the use of additional antibiotics (p=0.035), other than amoxicillin clavulanate, and consequently, an extended duration of antibiotic use, increases the time until diagnosis. Variables with a p-value <0.05 in the single variable lineer regression analysis were included in the multiple variable lineer regression analysis. No variable predicting the treatment duration was identified in the multiple variable lineer regression analysis (Table 4).

Discussion

Many patients on GM present with a clinical picture accompanied by inflammatory skin lesions and chest pain. Since there is no consensus on the treatment of GM, most patients are treated with antibiotics used empirically during the diagnostic investigation phase (6). Although there is limited data on antibiotic therapy in the treatment of IGM, 7-10 days of empiric antibiotic therapy is initially recommended in patients who have developed abscesses and cellulitis (7-10).

Despite antibiotic therapy, most clinicians tend to continue antibiotic therapy with different antibiotic groups or combinations if abscesses or infectious processes do not resolve and ulcers recur. GM is, by definition, a sterile inflammatory disease, and therefore antibiotic therapy often fails (11).

Recently, attention has been drawn to Corynebacterium species as a specific pathogen in GM. As Corynebacteria are part of the normal skin flora, it can be difficult to distinguish between infection, colonization, and contamination with these organisms. When isolated as pure or dominant growth of >104 cfu/ml in abscess culture, it is important to consider Corynebacterium species as causative Corynebacterial breast infections are typically characterized by abscess formation, granulomatous inflammation, and progression to sinus/fistula formation (12). In general, the lipophilic species of Corynebacterium cause mastitis (13). Of these species, *Corynebacterium kroppenstedtii* is frequently detected. Therefore, it may play a significant role in causing this disease (14). Due to the frequent detection of this lipophilic gram-positive bacillus, there is also increasing evidence of a link between Corynebacterium infection and another clinical picture called cystic neutrophil granulomatous mastitis (CNGM), which is characterized by lipo granulomas consisting of open spaces surrounded by neutrophils and granulomatous inflammation. Indeed, GM has also been referred to as "cystic neutrophil granulomatous mastitis" by some authors (15,16).

Table 4. Univariate lineer regression analysis to determine the factors affecting the time to diagnosis.

	Univariate Lineer Regression					Multiple Lineer Regression				
	β	t	p	95.0% Confidence Interval for B		β	t	p	95.0% Confidence Interval for B	
Age	-0.033	-0.264	0.792	-0.780	0.598					
Size	-0.053	-0.435	0.665	-0.327	0.210					
Symptoms										
Mass	-0.022	-0.179	0.858	-10.407	8.692					
Abscess	-0.139	-1.146	0.256	-17.637	4.774					
Others	0.158	1.311	0.194	-3.686	17.790					
Type of antibiotics										
Amoxicillin-clavulanate (1)	-0.265	-2.183	**0.033**	-22.057	-0.976	-0.189	-1.429	0.158	-19.702	3.276
(1) + Ciprofloxacin	0.010	0.080	0.936	-9.828	10.648					
Additional antibiotics (cephalosporins etc.)	0.262	2.159	**0.035**	0.874	22.634	0.184	1.393	0.169	-3.591	20.109
Antibiotic treatment time	0.051	0.405	0.687	-9.329	14.066					

β: Standardized Coefficients, t: test statistic, CI: Confidence Interval. p=Level of Significance, p<0,05. Statistically significant p-values are in bold. VIF values were found below 10 and there was no multicollineerity problem.

Considering the role of corynebacterial infections and C. kroppenstedtii in the pathogenesis of the disease, the response to antimicrobial treatments has been studied (17). It was suggested that lipophilic antibiotics with a high volume of distribution are more likely to reach adequate concentrations in lipo granulomas. Potential antimicrobial options include doxycycline and trimethoprim-sulfamethoxazole, as well as the antibiotics clarithromycin and rifampicin, which are also useful in the treatment of other granulomatous infections, including nontuberculous mycobacteria. Other treatment options, such as oral corticosteroids and immune-modulatory therapies that can suppress the inflammatory response and subsequent development of granulomatous disease, can be found at GM. The choice of the most effective antibiotic and the treatment regimen that should be used in combination remains unclear, and the optimal duration of treatment also requires further investigation. Although short-term antibiotic treatment (5-7 days) did not appear to improve

clinical outcomes in one study, repeated short-term treatments are generally preferred (18).

Information on the use of antibiotics in the treatment of IGM is limited. Nevertheless, 7-10 days of antibiotic treatment may be recommended in cases with cellulitis, abscess, and sinus formation (19).

Granulomatous mastitis is among the most critical subgroups in patients with mastitis findings. Empiric antibiotic treatments, which are often preferred in patients who are not lactating and are known to have recurrent episodes of mastitis, lead to a delay in diagnosis.

It is important to prioritize investigations in the differential diagnosis to identify the etiology and make the pathologic diagnosis of the patient and initiate pathogen-specific treatment. However, as evidenced by the data obtained, most patients waste time with repeated outpatient applications and unnecessary empiric antibiotic treatments. With each infestation, the extent of affected tissue in the breast increases, and the likelihood of local recurrence increases in this disease, for which there is no standard treatment regimen.

This study has several limitations. As it is a retrospective study, the same treatment algorithm could not be applied to all patients. This drawback could be eliminated if a prospective study and a standard algorithm were applied to IGM.

Conclusion

In conclusion, unnecessary repeated antibiotic treatment is performed in granulomatous mastitis. The unnecessary use of antibiotics is a global problem contributing to unnecessary costs, side effects, and antimicrobial resistance (20). We believe unnecessary antibiotic treatment should be avoided in patients with appropriate history and clinical features. That pathologic diagnosis should be made, and etiologic investigations should be given priority. In most cases, empiric antibiotic treatment is protracted because the necessary infrastructure and procedures are not clear to make a differential diagnosis in these patients. Since most IGM patients live in rural and third-world countries, access to diagnostic facilities and infrastructure is also difficult.

The lack of universal algorithms for diagnosis and treatment also creates difficulties. In the future, it will be beneficial to develop specific testing protocols, such as molecular methods, to distinguish infectious agents to expedite the diagnostic process for these patients. By improving the strategy for antibiotic use in these patients, it will be possible to reduce the unnecessary use of antibiotics.

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Conflict of interest statement

The authors declare no conflict of interest

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A Giant Left Atrial Myxoma Inducing Mitral Stenosis

Mitral Stenozu İndükleyen Dev Sol Atrial Miksoma

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Öz

Miksomalar kalbin iyi huylu tümörleridir, sıklıkla sol ve sağ atriumdan sırasıyla %75 ve %18 oranında köken alır. Boyutlarına ve tümörün lokalizasyonuna göre semptomlar geniş bir spektrum şeklinde görülebilir. Atrium çıkım yolunu ciddi şekilde tıkayabilir. Olgu sunumumuzda 63 yaşında kadın hastada mitral stenoz semptomları oluşturan, literatürdeki ikinci dev sol atrial mixomayı sunmayı amaçlamaktayız.

Anahtar Kelimeler: Kalp Tümörü, Miksoma, Sol Atrium

Abstract

Myxomas are considered benign cardiac tumors, most commonly originating in the left atrium (75%) and less frequently in the right atrium (18%). The presenting symptoms can vary widely depending on the size and location of the tumor, and they may seriously occlude the atrial outflow. We herein report a case involving a giant left atrial myxoma in a 63-year-old woman presenting with symptoms of mitral stenosis.

Keywords: Cardiac Tumor, Myxoma, Left Atrium

Introduction

Cardiac myxoma is a benign neoplasm and the most common primary cardiac tumor in adults. Cardiac myxomas have a wide range of manifestations, mostly obstructive, but can also cause embolic events in severe cases. We herein present a case involving a 63-year-old woman who presented with dyspnea. After an echocardiographic evaluation, she was diagnosed with a giant left atrial myxoma. She underwent successful tumor resection with a favorable postoperative outcome.

Case

A 63-year-old woman was admitted to our hospital with dyspnea and symptoms of New York Heart Association class III heart failure. A large mass in the left atrium was detected by transthoracic echocardiography (Figure 1A). The mass was attached via a pedicle originating from the interatrial septum. It was mobile and measured 7.0 × 5.5 cm in size. In addition, the mass exhibited mobility that allowed it to prolapse into the mitral valve orifice. The mass had a dense, solid characteristic. The left ventricular ejection fraction was 55%. Preoperative transesophageal echocardiography was performed, and the findings were similar to the previous echocardiogram. Because of a technical problem involving the imaging device, however, we were unable to obtain a picture. The patient provided informed consent and permission for publication of this report.

The patient underwent an operation. After careful dissection around the heart, cardiopulmonary bypass was established. Exposure was obtained through a superior septal approach. The tumor was found to be filling most of the left atrium and had a close relationship with the superior aspect of the fossa ovalis. Optimal resection of the interatrial septum along with a limited superior part of left atrial wall was performed to prevent tumor recurrence. The tumor was 7.0 × 5.5 cm in size (Figure 1B, 1C). Patch closure for septal repair was not required. The residual interatrial septum was adequate in size; therefore, primary closure of the defect was performed. The patient had a favorable postoperative outcome and was discharged with no complications. Histological evaluation confirmed an atrial myxoma.

Discussion

Myxoma is the most common primary cardiac tumor in adults. It is generally found in the left atrium, and its prevalence ranges from 0.001% to 0.3% (1). Myxomas generally exhibit benign characteristics. However, they can cause constitutional symptoms or serious complications due to thromboembolic events of tumor fragmentation or a thrombus that forms on the tumor surface (2–4). Although rare, death may occur secondary to heart failure or mechanical obstruction of cardiac valves, mimicking the signs and symptoms of valve disease (4). In our case, a giant left atrial myxoma mimicked mitral stenosis.

Myxomas can be found as a component of Carney complex (5). Carney complex is a familial autosomal dominant disorder characterized by the development of atypical myxomas in different cardiac chambers, such as the ventricles, or multiple myxomas in approximately 45% of cases. It can occur sporadic

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manner in some of cases. Tumors related to Carney complex can be detected in patients of young ages, and the recurrence rate is 15% to 22%. It is also referred to multiple endocrine neoplasia syndrome with distinctive pigmented lesions of skin and mucosal membranes. A wide spectrum of endocrine abnormalities can occur in Carney complex. Additional tumors include myxomas affecting the skin, schwannoma, primary pigmented nodular adrenocortical disease (PPNAD), pituitary adenomas, thyroid tumors, testicular tumors, and ovarian lesions. Skin pigment abnormalities include small flat brown spots (multiple lentigines) and small, bluish-black spots (blue nevi). The most common symptoms and clinical manifestation of Carney complex can vary in population. In many cases, Carney complex is due to mutations of the PRKAR1A gene (5). The myxoma in our case was not related to Carney complex.

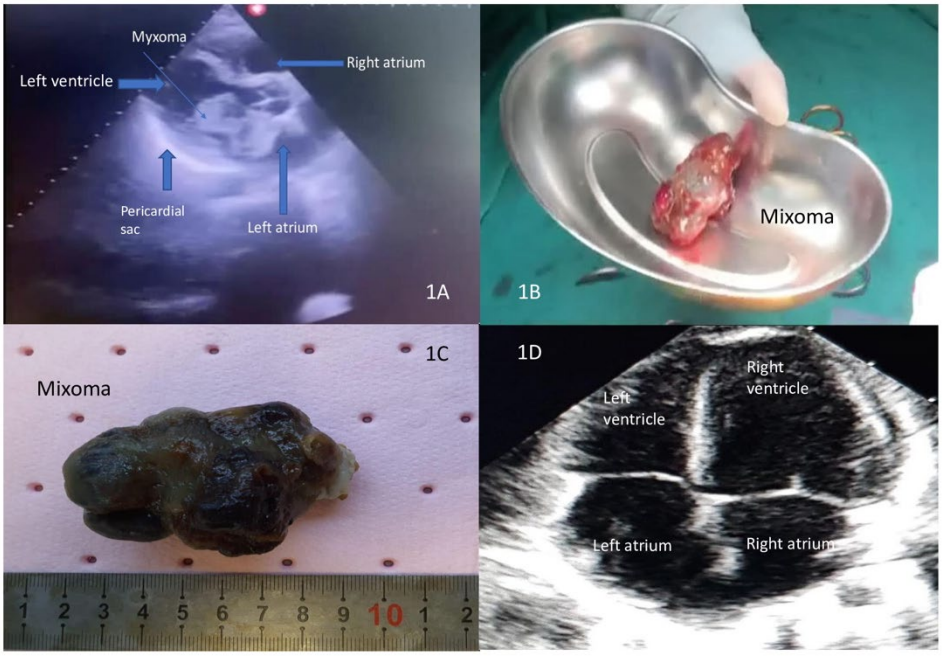

Figure 1. A. Preoperative view of giant left atrial myxoma in echocardiogram. **B.** Intraoperative view of excised mixoma. **C.** Macroscopic view and the size of giant myxoma. **D.** Postoperative view in echocardiogram.

Myxomas should be excised surgically. To obtain adequate exposure for large or multifocal tumors, a biatrial or superior septal incision is sometimes required (6,7). In addition to tumor resection, adjacent valve repair may be needed due to annular enlargement, a valve defect after tumor resection, or septal defect patch closure. No valvular structures required repair in the present case.

A myxoma may recur if the resection was not complete or the pedicle was not totally excised. Another important point is that before the aortic cross clamp is applied, manipulation of the heart should be avoided to prevent distal embolism due to tumor fragmentation or thrombus formation on the surface of the tumor (8). In our case, we also resected septal tissue around the pedicle along with part of the left atrial wall due to its adhesion to the tumor.

Many studies have recommended periodic echocardiography to detect any recurrence (8) (Figure 1D).

Conclusions

Our patient had developed only dyspnea and symptoms of mitral stenosis. Echocardiography revealed a giant left atrial myxoma mimicking mitral stenosis, and surgical resection was successfully performed.

Resection with adequate margins and careful intraoperative manipulation are crucial to prevent recurrence and reduce the risk of emboli.

Even when adequate septal resection has been performed, echocardiography is crucial for detecting recurrence during long-term postoperative follow-up.

We believe this case report represents one of the largest left atrial myxomas described in the literature to date.

Conflict of interest statement

The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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Oxybutynin Abuse in An Adolescent: A Case Report

Ergen Bir Olguda Oksibutinin Kötüye Kullanımı: Bir Olgu Sunumu

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Öz

Oksibutinin klorür, çocuklarda aşırı aktif mesane tedavisinde kullanılabilen Amerikan Gıda ve İlaç Dairesi tarafından onaylanmış tek antikolinerjik ilaç olup nörojenik mesane ve enürezis durumlarında kullanılabilmektedir. Yapılan araştırmalarda antikolinerjik ilaçların sıklıkla kötüye kullanılabileceği bildirilmekte olup oksibutininin öforizan ve halüsinojenik etkilerinden dolayı bağımlılık potansiyeli olduğu düşünülmektedir. Ergenlik madde kullanım bozukluğu açısından riskli bir dönem olup son yıllarda oksibutinin kötüye kullanımı ile ilgili olgu sunumları bildirilmektedir. Bu olgu sunumunda pediatrik popülasyonda oksibutinin sık kullanımı göz önünde bulundurulduğunda klinisyenlerin oksibutinin kötüye kullanımı açısından dikkat etmelerinin önemi vurgulanmak istenmiştir.

Anahtar Kelimeler: Adolesan, Antikolinerjik, Oksibutinin, Reçeteli İlaçların Kötüye Kullanımı

Abstract

Oxybutynin chloride is the only anticholinergic drug approved by the U.S. Food and Drug Administration for the treatment of over active bladder in children and can be used in neurogenic bladder and enuresis. Studies have reported that anticholinergic drugs can be frequently abused and oxybutynin is thought to have a potential for addiction due to its euphoric and hallucinogenic effects. Adolescence is risky in terms of substance use disorder and case reports on oxybutynin abuse have been reported in recent years. In this case report, considering the frequent use of oxybutynin in the pediatric population, the importance of clinicians being careful about oxybutynin abuse was emphasized.

Keywords: Adolescent, Anticholinergic, Oxybutynin, Prescription Drug Abuse

Introduction

Oxybutynin chloride is the only anticholinergic drug approved by the FDA (U.S. Food and Drug Administration) for the treatment of overactive bladder in children and can be used for neurogenic bladder and enuresis (1). Apart from the neurological pathways related to addiction, the cholinergic system is also thought to play a role in addiction (2). Studies have reported that anticholinergic drugs are also commonly abused, and oxybutynin is thought to have addictive potential due to its euphoric and hallucinogenic effects (3). In addition to anticholinergic drugs, abuse of some other prescription drugs used in medical illnesses and psychiatric disorders has also been reported. Frequently abused drugs are modafinil, venlafaxine, quetiapine, tianeptine, gabapentin and pregabalin (4). In a study conducted in our country in which drugs abused in prisons were investigated, pregabalin and gabapentin were reported to be in the first place and two case reports related with the abuse of oxybutynin were reported (5).

Adolescence is a vulnerable period for substance use disorders, and case reports of oxybutynin abuse have been reported in recent years (6,7). Overactive bladder in children and adolescents is a condition that, although not as common as in adults, is seen in paediatric practice, particularly in the form of urinary incontinence, and can have a negative psychosocial impact on children and their families (8). It is known that oxybutynin is the most commonly prescribed drug for its treatment (9). Given the frequency and ease with which it can be prescribed in the pediatric age group and the ease of access to the drug, it is important to be vigilant for abuse in vulnerable groups. In this case report, a case with a long history of depressive symptoms and no regular psychiatric follow-up found that oxybutynin, which had been started for overactive bladder, was good for her depressive symptoms and abused the drug. The aims of the study include; physicians should be careful when starting this drug, the risk of addiction should be considered and evaluated in patient interviews, and it can be emphasized that patients with depressive symptoms should be more careful.

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Case

A 16-year-old female patient was brought to the emergency department with multiple transverse incisions on the left forearm and was consulted. In the interview with the case, she stated that she had been self-harming for the last three years, that it started six months after her parents' divorce, that it helped her to relax, that it calmed her down when she felt bad or angry. She stated that she felt sad and tired in general for the last three years, constantly needed sleep, spent most of her time in her room and her academic success decreased. In the interview with the father, it was noted that she had been withdrawn for three years, did not want to leave her room, had

disrupted her self-care, had limited communication at home and had started smoking for one year.

When the case history was evaluated, it was stated that the neuromotor developmental stages were normal, there were no academic difficulties, there were no learning and attention problems, and there were no complaints from the school. It was stated that she had applied to our polyclinic twice before, that she had not applied to any psychiatric clinic for the last three years, and that the patient wanted to continue the interviews in her previous applications, but her father did not want her to. It was previously stated that her first presentation to us was six years ago with complaints of forgetfulness, curiosity and lying, no psychiatric disorder was considered at that time, and recommendations were given. It was learned that her second admission was three years ago with complaints of feeling sad, lack of social environment and friends, constant desire to sleep, fatigue, aripiprazole was started and gradually increased to a dose of 2.5 mg/day due to a family history of bipolar for the diagnosis of depression, and she did not come to the control interview.

When the family history of the patient was evaluated, it was learned that the parents divorced four years ago due to violence, the custody of the children was given to the father, and the mother continued to see the children face to face once a month. When the family history of our patient was examined, it was learned that she had two brothers, aged 27 and 26, the middle brother was diagnosed with bipolar disorder when he was in the 9th grade, he received lithium treatment for a period of time. It was also learned that the mother had consulted a psychiatrist after the divorce, had been treated with sertraline 150 mg/day for three years with a diagnosis of anxiety disorder, and was currently not taking any medication.

Mental status examination revealed that speech rhythm was low and she did not initiate communication. Her affect and mood were depressive. It was noteworthy that there were many old and new transverse cuts on the left forearm. Intelligence level was clinically normal.

As a result of the evaluation, the patient was diagnosed with dysthymic disorder according to the diagnostic criteria of the Diagnostic and Statistical Manual of the American Psychiatric Association, 5th edition (10) In the psychometric evaluations, the Beck Depression Scale (11) was found to be 20 and the Anxiety Rating Scale for Children (12) was found to be 52. As there was a family history of bipolar disorder, treatment with aripiprazole 2.5 mg/day was started gradually. When questioned about comorbidity and drug use, it was learned that the patient had presented to the urology department about one year ago with complaints of urinary frequency and urinary incontinence, enuresis was diagnosed as a result of tests and imaging, and treatment with oxybutynin 5 mg/day was started. It was learned that she took two tablets by mistake, felt better that day and increased her dose because she realised that she felt better afterwards. It was stated that the patient had been taking an average of 5-6 tablets (30 mg/day) every day for the last one month and had tried up to a maximum of 7 tablets (35 mg/day). It was stated that she had been taking oxybutynin treatment for one year, but she continued to use it for relaxation for the last month, took 4-5 pieces every day in the same amounts and could relax. It was stated that she continued to take it for the relaxation and happiness she felt when she took it, and that she did not know what happened when she did not take the medicine because she took it regularly every day for the last one month. No side effects were described on the days she took the drug. It was learned that she had no alcohol and substance use except smoking.

The patient was followed up with weekly follow-up interviews. Psychoeducation about drug abuse, stress management was practised, and day structuring planning was made in the meetings. Aripiprazole treatment was started again upon the recognition of benefit from previous aripiprazole treatment. In the first control interview, she stated that she had not received oxybutynin treatment for a week, that she used aripiprazole treatment regularly, that she benefited from it, that she did not experience withdrawal after stopping oxybutynin use and that she did not have any withdrawal symptoms. The importance of regular follow-up was emphasised because the family had not been able to provide regular psychiatric follow-up of the adolescent before, and the family's participation in the treatment was ensured. The patient was followed up regularly in our outpatient clinic for three months. It was informed that the treatment was continued in an outpatient centre since they moved out of the city. Informed consent was obtained from the family and the patient for the publication of this report.

Discussion

Oxybutynin chloride has direct anesthetic and smooth muscle relaxant effects as well as anticholinergic effects (3). It is also used in patients with neurogenic bladder and enuresis, and the dose range is 5-20 mg/day. Overdosage of the drug has been reported to cause anorexia, insomnia, agitation, irritability, delusions, hallucinations, confusion and delirium (13).

The mesolimbic dopaminergic system, ventral tegmental area, nucleus accumbens and prefrontal cortex are thought to be the major neurological pathways involved in addiction, and the cholinergic system has also been reported to play a role in addiction (2). Activation of muscarinic receptors facilitates dopamine release and transmission to the nucleus accumbens. Blockade of muscarinic receptors may also block the reuptake and storage of

dopamine, leading to euphoria and deliriogenic-hallucinogenic effects with addictive potential (14).

Adolescents are one of the most vulnerable groups in terms of substance abuse. In addition to substances known to be addictive, drugs with sedative, anticholinergic-antimuscarinic and stimulant properties, prescribed for all types of treatment, may be abused by adolescents (15). In a review of the literature, three cases of abuse of oxybutynin in adolescents have been reported in our country, and in two cases a history of substance abuse was defined before the abuse of oxybutynin (6,7). When the common characteristics of the cases are evaluated, it is noteworthy that they were in late adolescence in terms of age, had depressive symptoms, started with the suggestion of friends, and continued because they felt good.

During adolescence there are many psychological as well as physical changes. Gaining an identity is one of the most important achievements of this period and this situation can lead to identity confusion in some individuals. Adolescents in the process of identity formation are at high risk of substance abuse. Negative early life events and chaotic family relationships can lead to increased confusion and inappropriate coping during adolescence (16). Given the fragmented family structure, chaotic domestic relationships, current adolescence and depressive complaints of our case, it can be considered risky in terms of substance use. Many studies have shown that the prevalence of smoking, alcohol and drug abuse is higher in adolescents with inadequate family functioning and a problematic family structure (17). The fact that our patient did not have a regular psychiatric referral, her current mental health difficulties and her realisation that she was being relieved by oxybutynin may have led to an increase in substance abuse. Early recognition of oxybutynin abuse during the patient's outpatient follow-up prevented the risk of potential addiction.

Overactive bladder is a condition seen in paediatric practice in children and adolescents, although not as frequently as in adults, especially when associated with urinary incontinence, which can have a negative psychosocial impact on children and their families, and it is known that the most commonly prescribed agent for its treatment is oxybutynin (8). It has been available on prescription in Turkey since March 2017 and can be easy to prescribe. Considering all these, other medications used for organic diseases and their usage patterns should be questioned in groups at risk of substance abuse and in adolescents, attention should be paid to the use of anticholinergic drugs and they should be prescribed with caution. Considering the euphorising effect of low dose oxybutynin in our case, it is important to be careful in clinical applications.

Conclusions

With this case report, it was aimed to raise awareness of physicians working in branches where oxybutynin treatment is used, to question the dose used, and to consider the evaluation of abuse in case the drugs run out earlier than they should.

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There is no conflict of interest

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Unveiling Gastroduodenal Fistula: A Comprehensive Exploration

Nadir Bir Gastroduodenal Fistül: Literatürün Gözden Geçirilmesi

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Öz

Gastroduodenal fistül (GDF), genellikle peptik ülserlere bağlı olarak ortaya çıkan ve çoğunlukla elektif olarak teşhis edilen nadir bir durumdur. Bu makale, 79 yaşında, tipik olarak belirti göstermeyen bir erkekte, GDF'nin perforasyonla kendini gösterdiği sıra dışı bir vakayı sunmaktadır. Bu vakanın özelliği, akut karın belirtileriyle gelen alışılmadık bir tablo sergilemesidir. Yapılan cerrahi inceleme, prepilorik bölgeden duodenumun dördüncü kısmına kadar uzanan yeni bir fistül yolunu ortaya çıkarmıştır. Bu beklenmedik bulgu, GDF'nin farklı şekillerde ortaya çıkabileceğini göstererek, bu nadir durumun tanı ve tedavisinde daha gelişmiş yöntemlere ihtiyaç olduğunu vurgulamaktadır.

Anahtar Kelimeler: Akut Karın, Çift Pilor, Gastroduodenal Fistül

Abstract

Gastroduodenal fistula (GDF) is a rare pathology, often associated with peptic ulcers and typically diagnosed electively. This article presents an unprecedented case of GDF perforation in a 79-year-old male, deviating from the asymptomatic norm. The unique feature lies in the acute abdominal presentation, challenging conventional understanding. The intricate surgical exploration revealed a novel fistulous tract extending from the prepyloric region to the fourth part of the duodenum. This atypical manifestation underscores the need for deeper research into GDF's diverse presentations, contributing to enhanced diagnostic and treatment strategies for this rare medical phenomenon.

Keywords: Acute Abdomen, Double Pylor, Gastroduodenal Fistula

Introduction

Gastroduodenal fistula, also known as double pylor (1, 2), is an exceedingly rare pathology that can manifest congenitally or acquired (3). Typically diagnosed during gastroscope examinations, this anomaly exhibits a prevalence ranging from 0.001% to 0.04%, with a higher incidence observed in males (4). Its acquired pathogenesis is commonly recognized as a complication of peptic ulcers (5). The majority of reported cases are identified as elective endoscopic findings, often treated with anti-ulcer therapies (5, 6). However, instances requiring urgent surgical intervention are exceptionally rare.

Gastroduodenal fistula is a significant pathology within the digestive system that can lead to severe health complications. The occurrence of this condition in children, particularly in association with the ingestion of multiple foreign bodies, presents a novel and noteworthy clinical feature that has not been previously explored (7).

The acquired pathogenesis, primarily acknowledged as a complication of peptic ulcers, renders Gastroduodenal fistula a complex condition requiring further investigation into its origin and progression. A deeper understanding of this condition could contribute to the development of effective treatment modalities and the formulation of preventive strategies (6).

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In conclusion, Gastroduodenal fistula, though rare, is a condition with the potential for serious consequences, such as peritonitis, sepsis, and multiorgan failure, especially when presenting acutely. Highlighting these risks underscores the clinical importance of timely diagnosis and intervention in managing this condition. The information presented in this article aims to enhance the general understanding of this pathology and stimulate further research in the field.

Herein, we present a case of Gastroduodenal Fistula perforation, an unprecedented occurrence in the medical literature.

Case

A 79-year-old male patient with a conventional-open surgery history in 1999 due to peptic ulcer perforation presented with intermittent epigastric pain complaints for the past 3-4 months. Approximately 12 hours prior to admission, he experienced sudden-onset, diffuse abdominal pain. Physical examination revealed generalized rigidity and a board-like abdomen. Laboratory findings indicated an elevated white blood cell count (WBC: 27.000/µL), high C-reactive protein (CRP: 220 mg/L), partial urea and creatinine elevation, and a fivefold increase in amylase. Radiological assessments, including a plain abdominal (PA) X-ray showing subdiaphragmatic free air, and a contrast-free abdominal computed tomography (CT) scan revealing diffuse fluid and air densities within the abdomen, did not identify any additional pathologies. Due to acute abdominal symptoms, the patient underwent emergency surgery.

During the operation, a thorough examination of the stomach, duodenum, small bowel loops, and colon revealed no pathology. Subsequently, the gastrocolic ligament was dissected, entering the

omental bursa, where widespread intestinal content was observed and aspirated. Exploration unveiled a fistulous tract between the posterior surface of the gastric antrum and the fourth part of the duodenum, along with a perforation in the middle of the fistulous tract, an occurrence not previously encountered (Figure 1-A). After mobilizing the duodenum by partially disrupting the Treitz ligament, the perforated fistula was sutured and approximated with polyglactin, followed by closure and division using staplers (Figure 1-B, 1-C respectively). Subsequently, supportive serosal stitches buried the roots of the fistulous tracts in the antrum and duodenum separately (Figure 1-D). The uncomplicated patient tolerated oral intake opened on the 4th postoperative day, and on the 8th postoperative day, he was discharged without complications. A follow-up upper gastrointestinal endoscopy at the 5th postoperative week revealed no pathology.

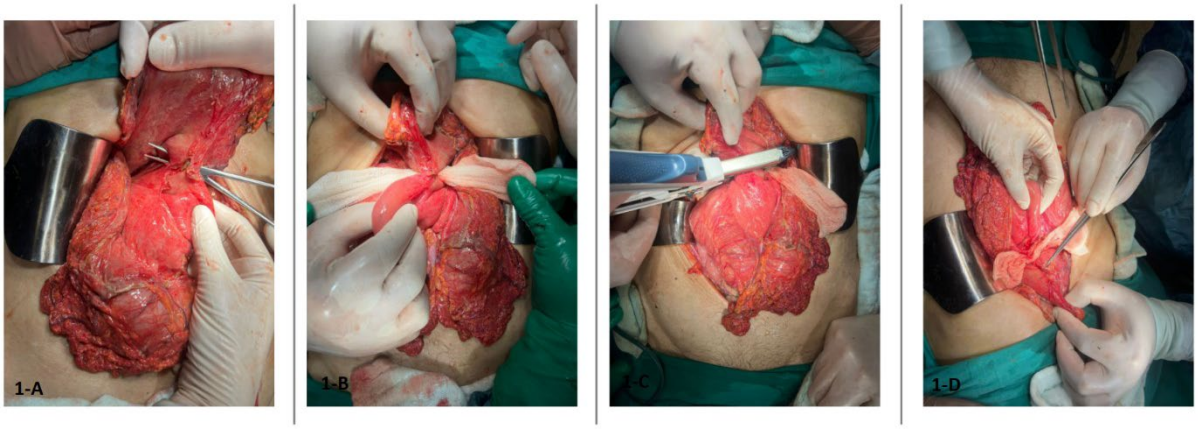

Figure 1. Gastroduodenal fistula perforation operation. 1-A: Fistula between the posterior antrum and the 4th continent of the duodenum and the perforated area above it. 1-B: Release and suspension of the fistula tract and primary repair of fistula perforation. 1-C: Transecting the gastrodudenal fistula with a linear cutting occluder. 1-D: Placing support sutures

Discussion

Gastroduodenal Fistula (GDF) has a well-documented historical background, beginning with Dittrich's first description in 1847, followed by significant advancements by Ludin and Hanganutz in 1924 and 1930, respectively (8). Notkin's work later established GDF's relevance in modern medical literature, highlighting its clinical implications and providing a basis for its recognition in practice.

Traditionally, GDF has been associated with benign gastrointestinal conditions, often linked to chronic peptic ulcers and rarely presenting with acute symptoms (9). However, recent literature suggests that GDF can manifest through various types, including gastrocolic, gastroduodenal, and gastrojejunocolic fistulas, each with unique clinical presentations and implications. The acute nature and extent of complications associated with GDF are significant, with documented complications such as sepsis, peritonitis, hemorrhage, and multiorgan failure, especially when undiagnosed or untreated. The frequency of these complications remains low but is clinically impactful, necessitating early identification and timely surgical intervention.

In our case, the GDF's unique pathway from the prepyloric region to the fourth part of the duodenum, coupled with acute abdominal symptoms, sets it apart from typical presentations. The fistulous tract perforation leading to an acute abdomen adds a layer of complexity that is rarely seen in GDF cases. Unfortunately, the patient's acute state prevented preoperative upper gastrointestinal endoscopy, thus complicating the diagnostic process. Surgical exploration required meticulous dissection to preserve critical vascular structures and minimize further risk.

This case exemplifies the need for a heightened clinical awareness of atypical GDF presentations and suggests the importance of including GDF in differential diagnoses of acute abdomen in elderly patients, particularly when chronic gastrointestinal conditions are present. Expanding upon current literature, this case underlines that while GDF is often asymptomatic or presents mildly, the potential for severe and life-threatening complications underscores its clinical importance.

Conclusions

In conclusion, our article highlights a distinctive case of GDF, deviating from the conventional asymptomatic profile and emphasizing the need for deeper exploration into its diverse presentations. The surgical intricacies and rarity of acute presentations underscore the uniqueness of our findings, contributing to the evolving landscape of gastroenterological literature.

Conflict of interest statement

The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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