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DergiPark tarafından yürürlüğe konulan kurallar çerçevesinde yazarların
"Etik İlkeler ve Yayın Politikası" ile "Yazım Kuralları" na uyulması konusunda ilgili başlıkları
dikkatlice incelemesi tavsiye edilmektedir.

Dergi 2023 yılından itibaren sadece İngilizce yazı kabul etmeye başlayacaktır.

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Değerli Bilim İnsanları,

Biyoteknolojik ve Stratejik Sağlık Araştırmaları Dergisi (JOURNAL OF BIOTECHNOLOGY AND STRATEGIC HEALTH RESEARCH), Deneysel, Biyoteknolojik, Klinik ve Stratejik Sağlık Araştırmaları Derneği'nin uluslararası, bağımsız, önyargısız ve çift-kör hakemlik ilkeleri çerçevesinde yayın yapan açık erişimli, bilimsel yayın organıdır. Dergi, Nisan, Agustos ve Aralık aylarında olmak üzere yılda 3 sayı yayınlanır. Ancak bu sene Cumhuriyetimizin 100.yılı anısına EKİM'2023'de bir özel sayı çıkarılması planlanmıştır. Dergi ağırlıklı olarak İngilizce yayın kabul etmektedir.

Derginin amacı; etik kurallara uyumlu hazırlanmış biyoteknolojik, kritik, stratejik sağlık araştırmaları ile ilgili bilimsel makaleleri, klinik ve deneysel çalışmaları, derleme, olgu sunumu, editöre mektup ve editöryel yorum türündeki yazıları yayınlayarak literatüre ve sağlık alanındaki tüm disiplinlerde katkı sağlamaktır.

Derginin hedef kitlesi; sağlık alanındaki tüm disiplinlerde çalışan araştırmacılardır.

Dergimizin 7. Yılı, Ekim'2023 "100.yıl Özel sayısı" ile yine birbirinden ilginç derleme ve araştırma yazıları ile karşınızdayız. Makalelerini gönderen değerli yazar arkadaşlarımıza ve zaman ayıran hakemlerimize teşekkür eder, bilginin kullanılarak toplum sağlığına değerli katkılar sağlamasını temenni ederiz.

Editör
Prof. Dr. Mustafa ALTINDİŞ
Editor in Chief

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YAZARLARA BİLGİLER

MAKALE YAZIM KURALLARI

Derginin Kapsamı

JOURNAL OF BIOTECHNOLOGY AND STRATEGIC HEALTH RESEARCH, yılda üç kez Deneysel, Biyoteknolojik, Klinik ve Stratejik Sağlık Araştırmaları Derneği tarafından yayımlanmakta olup tıp alanında ve sağlık bilimlerinin ilgili konularında yazılmış İngilizce veya Türkçe makaleler kabul edilmektedir. Dergiye kabul edilecek yazı türleri deneysel araştırmaları, klinik ve laboratuvar çalışmalarının sunulması amaçlı özgün makaleler, vaka sunumları, derleme makaleleri ve editöre mektuplardır.

A. Genel Bilgiler

➢ Etik Kurallar

Dergiye gönderilen makalelerin daha önce başka bir dergide değerlendirme sürecinde olmaması, yayım için kabul edilmemiş ve de yayınlanmamış, olması, bilimsel ve etik kurallara uygun şekilde hazırlanması gereklidir. Yazarlar, makalelerin bilimsel ve etik kurallara uygunluğundan sorumludur. (http://www.icmje.org/about-icmje/faqs/conflict-of-interest-disclosure-forms/).

Klinik araştırmaların protokolü etik komitesi tarafından onaylanmış olmalıdır. İnsanlar üzerinde yapılan tüm çalışmalarda "Yöntem" bölümünde çalışmanın ilgili komite tarafından onaylandığı veya çalışmanın Helsinki İlkeler Deklarasyonuna (www.wma.net/e/policy/b3.htm) uyularak gerçekleştirildiğine dair bir cümle yer almalıdır. Çalışmaya dahil edilen tüm insanların bilgilendirilmiş onam formunu imzaladığı metin içinde belirtilmelidir. JOURNAL OF BIOTECHNOLOGY AND STRATEGIC HEALTH RESEARCH'ne gönderilen yazıların Helsinki Deklarasyonuna uygun olarak yapıldığını, kurumsal etik ve yasal izinlerin alındığını varsayacak ve bu konuda sorumluluk kabul etmeyecektir. Çalışmada "Hayvan" öğesi kullanılmış ise yazarlar, makalenin "Yöntem" bölümünde Guide for the Care and Use of Laboratory Animals (www.nap.edu/catalog/5140.html) prensipleri doğrultusunda çalışmalarında hayvan haklarını koruduklarını ve kurumlarının etik kurullarından onay aldıklarını belirtmek zorundadır. Sonuç olarak, etik kurul kararı gerektiren klinik ve deneysel insan ve hayvanlar üzerindeki çalışmalar için etik kurul onayı alınmış olmalı, bu onay makalede "Etik Kurul Onay Numarası" ile belirtilmelidir ve belgelendirilmelidir.

Dergide çıkan yazıların tüm hakkı dergiye aittir. Yazılar için yazarlara telif hakkı ödenmez. Makaleye ek olarak yukarıdaki şartları kaşif taramalarına dayalı yazılarda Anabilim Dalı (Bilim Dalı) Başkanlığı, Başhekimlik veya Servis Şefliği tarafından arşivde çalışılmasına izin verdiğine dair bir belgenin çalışmaya eklenmesi zorunludur. Prospektif klinik çalışmalar için resmi gazetenin 29.01.1993 tarih ve 21480 sayılı nüshasında yayımlanan yönetmeliğe uygun bir şekilde Etik Kurulu onayı alınmalıdır. Dergide yer alan makalelerin etik sorumluluğu yazarlarına aittir.

Dergiye gönderilen makalelerden hakeme gönderilmesi uygun görülen makaleler konunun uzmanı hakemlere gönderilir. Makalenin yayımlanabilmesi için iki hakemin de olumlu görüş bildirmesi gerekmektedir. Değişikliğe gerek görüldüğü takdirde, istenilen değişiklikler yazarlarca 15 gün içerisinde yapıldıktan sonra yayın tekrar incelemeye alınır, yazım ve dil bilgisi hataları makalenin içeriğine dokunulmaksızın yayın kurulu tarafından düzeltilir.

Derleme yazılarında, tüm yazarların derleme konusu ile ilgili en az bir SCI/SCI-expanded indekse giren yayınının bulunması gerekmektedir.

Sonucu desteklemek için istatistiksel analiz genellikle gereklidir. İstatistiksel analiz, tıbbi dergilerdeki istatistik verilerini bildirme kurallarına göre yapılmalıdır (Altman DG, Gore SM, Gardner MJ, Pocock SJ. Statistical guidelines for contributors to medical journals. Br Med J 1983: 7; 1489-93). İstatistiksel analiz ile ilgili bilgi, Yöntemler bölümü içinde ayrı bir alt başlık olarak yazılmalı ve kullanılan yazılım kesinlikle tanımlanmalıdır.

Dergi İntihal İlkesi

JOURNAL OF BIOTECHNOLOGY AND STRATEGIC HEALTH RESEARCH'de makale göndermeden önce uygun intihal yazılım programlarıyla (iThenticate, Turnitin: Tezler için vb.) makalenizdeki benzerlik durumunu belirlemeniz beklenir. Benzerlik oranlarının dergimiz için kaynaklar hariç % 20'un altında olması istenmektedir.

Simgeler, Birimler ve Kısaltmalar

Dergimiz, İngilizce makalelerde Scientific Style and Format, The CSE Manual for Authors, Editors, and Publishers, Council of Science Editors, Reston, VA, USA (7th ed.) uzlaşılarını; Türkçe makalelerde ise TDK Yazım Kılavuzu, Türkiye Bilim Terimleri ve TÜBA Türkçe Bilim Terimleri Sözlüğü'nü esas almaktadır. p, ×, μ, η, or v gibi karakterler, sözcük işlem uygulamasının simge menüsünden seçilerek kullanılmalıdır. Sayılarla birimler arasında bir boşluk bırakılmalı (örn. "3 kg"), sayılarla yüzde simgesi arasında boşluk bırakılmamalıdır (örn. "%45"). Tüm kısaltma ve kısa adlar, ilk kez kullanıldıklarında tanımlanmalıdır. Canlıların ve mikroorganizmaların jenerik isimleri, tür adını değiştirmeden, uygun şekilde kısaltılmalı ve yatık olarak yazılmalıdır.

Makale Hazırlama Şekli ve Biçimi & Gönderim

Makale gönderimi çevrimiçi olarak http://dergipark.gov.tr/bshr adresine Microsoft Word dosyası olarak eklenmelidir. "Öz", "Ana Metin ve Kaynaklar (Çizelgeler dahil)" Microsoft Word dosyası (.doc veya .docx uzantılı) olarak, 12 yazı tipi boyutunda, Times New Roman karakterleriyle, 1,5 satır aralığıyla ve paragraflar iki yana yaslanmış olarak yazılmalıdır. Makalelerin değerlendirilmeye alınabilmesi için, başvuru esnasında 'Telif Hakkı devir formu' doldurulmalıdır. Bu formu içermeyen yazılar değerlendirmeye alınmaz. Makaleler, Ana metnin sayfa numaraları, her sayfanın sağ alt köşesinde belirtilmelidir.

Makaleler, Türkçe veya İngilizce yazılabilir.

B. Yazım Kuralları

Metin içi ve metin sonu kaynak gösterimi için, AMA (Amerikan Tıp Birliği/American Medical Association) Stili kullanılmalıdır (http://library.nymc.edu/informatics/amastyle.cfm; https://drive.google.com/drive/folders/1hzvgxnau1lBPUBYfKN1vTBKbPE31LBXQ) .

Dergide kör hakemlik uygulaması söz konusu olduğundan makale ana metin üstünde yazarlara ilişkin herhangi bir bilgi bulunmamalıdır.

Tüm makale yazarlarının, ORCID iD (Open Researcher and Contributor ID) numaraları başlık sayfasına eklenmelidir.

B. 1. Başlık Sayfası

Yazılar başlık sayfasından başlanarak numaralandırılmalı, sayfa numaraları sağ alt köşeye yazılmalıdır.Başlık sayfasında; yazının başlığı (Türkçe ve İngilizce), başlık altında tüm yazarların ad ve soyadları, kurumları yer almalıdır. Sorumlu yazarın adı ve soyadı, telefon numarası, e-posta ve yazışma adresleri bulunmalıdır. Makale başlığı, 25 kelime ile sınırlı, Türkçe ve İngilizce dillerinde verilmelidir. Kısa başlık (running title, running head) 50 karakterle (boşluk dahil) sınırlı şekilde Türkçe ve İngilizce olmalıdır.

B. 2. Öz Sayfası

Öz (Abstract), Türkçe ve İngilizce olarak en fazla 250 sözcük olacak şekilde; 'Amaç (Objective)', 'Yöntem (Methods)', 'Bulgular (Results)' ve 'Sonuç (Conclusion)' bölümlerinden oluşmalıdır. Derleme ve olgu sunumunda öz sayfası bölümlere ayrılmadan yazılmalıdır.

Öz'ün altına "anahtar kelimeler" (en az 3, en fazla 6) verilmelidir. Anahtar kelimeler Türkçe ve İngilizce yazılmalıdır. İngilizce anahtar kelimeler Index Medicus'da "Medical Subjects Headings" listesine uygun olmalıdır (Bkz: www.nlm.nih.gov/mesh/MBrowser.html). Türkçe anahtar kelimeler Türkiye Bilim Terimleri, uygun olarak verilmelidir (Bkz: www.bilimterimleri.com). Bulunamaması durumunda bire bir Türkçe tercümesi verilmelidir.

B. 3. Ana Metin

B. 3. 1. Özgün Araştırma

Sırasıyla ve kesin sınırlarla ayrılmış "Giriş", "Yöntem", "Sonuç" ve "Tartışma" bölümlerinden oluşmalıdır. Sonuç kısmı, ayrı bir bölüm olarak veya Tartışma'nın son paragrafı olarak yazılabilir. Tartışma kısmının son paragrafında çalışmanın sonuçları ifade edilebilir, ek bir başlık açılmasına gerek yoktur.

En çok 15 sayfa (öz, teşekkür ve kaynaklar hariç) olmalıdır.

Sistematik derleme ve meta-analiz özgün araştırma makalesi kapsamındadır. Yazarlar, taslaklarını gönderirken sistematik derleme ve meta-analiz için, PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) beyanatı (http://www.prisma-statement.org/). yönergesine uyduklarını gösteren standart kontrol listelerini kullanmalı ve istendiğinde sunmalıdır.

Sözcük sayısı öz, teşekkür ve kaynaklar hariç en çok 5 000 olmalıdır. Kaynak sayısı, 50'yi geçmemelidir(derleme hariç). Metin boyunca bilimsel terimler yatık olarak yazılmalıdır.

B.3.2. Derleme

En çok 20 sayfa (öz ve kaynaklar hariç) olmalıdır. Derlemeler, standart yazı şeklinden farklıdırlar. Yazı yazmanın evrensel formatı IMRAD derleme yazılarında uygulanmamaktadır. Ana hatlarıyla "Giriş" bölümü daha geniş olmakta ve derlemenin amacını ve yazı gerekçesini açıklamaktadır.

"Yöntem" ve "Bulgular" kısmı bulunmamaktadır. Tartışma kısmı yine geniş tutulacak ve kişisel deneyimler doğrultusunda aynı konuda yapılmış çalışmalar ve onların sentezi yapılacaktır. Sonuç anlamında bir yorum ve değerlendirme paragrafı bulunmalıdır. Kaynaklar ise tüm yazılara göre daha fazla sayıda olacaktır. Ancak mutlaka yazarın kendi çalışmaları da bulunacaktır.

B.3.3. Olgu Sunumu

En çok 10 sayfa (öz, teşekkür ve kaynaklar hariç) olmalıdırr. Olgu sunumlarında ise sırasıyla giriş, olgu sunumu ve tartışma bölümlerini içermelidir.

B.3.4. Editöre Mektup

En çok 5 sayfa (öz ve kaynaklar hariç) olmalıdır. Çizim ve çizelge içermez. Bir makaleye ithaf olarak yazılmış ise sayı ve tarih verilerek belirtilmeli ve metnin sonunda yazarın ismi, kurumu ve adresi bulunmalıdır.

B.4. Çizim ve Çizelgeler

Metin içerisinde kullanılan fotoğraf, grafik, şekil, resim gibi görsel sunum araçları 'Çizim' olarak tanımlanır. 'Tablo' ise sınıflandırılmış verilerin yer aldığı görsel sunum araçlarıdır. Tablolar kaynaklardan sonra başlıklarıyla birlikte verilmelidir. Tablolar, başlığın alt ve üstünde, ayrıca alt satırın altında yatay kenarlık ve sol sütunun sağ dikey kenarlığı olacak şekilde düzenlenmelidir.

Figür ve Tablolar, numaraları ile metin içinde geçtiği yerlerde ilgili cümlenin sonunda ayıraç içinde belirtilmeli; sırayla numaralandırılmalıdır.

Örnek tablo:

Tablo 1. Araştırmaya katılanların ilk başvurularını birinci basamakta çalışan hekime yapmama nedenleri

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YAZARLARA BİLGİLER

Başvurmama Nedeni	*n	%
Sadece psikiyatri uzmanı ruh sağlığı hizmeti sunabilir		
Birinci basamakta çalışan hekimin bu hizmeti sunduğunu bilmemem		
Ebeveyn kararıydı		
Birinci basamakta çalışan hekime güveniyorum ancak tercih etmedim		
	47	53,4
	17	19,3
	12	13,6
	12	13,6

* Toplam hasta sayısı

Tablolar, metne dahil edilmemeli ve sistem üzerinden "Görseller" başlığı seçilerek yüklenmelidir. Görseller; JPG, GIFF, PNG veya TIFF formatında gönderilmelidir. Metine ek olarak sisteme yüklenen tüm çizim başlıkları, "Çizim Başlığı" altında, kaynaklardan sonra listelenmelidir. Kullanılan kısaltmalar çizim ve çizelgelerin altındaki açıklamada 10 yazı boyutunda belirtilmelidir. Ondalıklı sayıların belirtilmesinde Türkçe metinlerde virgül işareti, İngilizce metinlerde nokta işareti kullanılmalıdır. Yüzde ile belirtilen sayılarda Türkçe metinlerde sayı önünde, İngilizce metinlerde ise sayı arkasında % işareti kullanılmalıdır.

B. 5. Açıklamalar

Çalışmada teşekkür, daha önce sunulduğu kongre, çıkar çatışması olmadığı, maddi destek, bağış ya da teknik yardım gibi konular metnin sonunda kaynaklardan önce belirtilmelidir. Çalışmayı maddi olarak destekleyen kişi ve kuruluşlar ve varsa bu kuruluşların yazarlarla olan çıkar ilişkileri belirtilmelidir. (Olmaması durumu da "Çalışmayı maddi olarak destekleyen kişi/kuruluş yoktur ve yazarların herhangi bir çıkar dayalı ilişkisi yoktur" şeklinde yazı yazılmalıdır. Araştırma desteği (Üniversite Bilimsel Araştırma projeleri , TÜBİTAK projeleri ve benzeri kurumlardan) alınmışsa, proje numarası belirtilmelidir.

C. Kaynak Gösterimi

Dergimiz, kaynak gösteriminde AMA stilini kullanılmaktadır ve kaynak yazımında atıf düzenleme programlarının kullanımını tavsiye edilmektedir (EndNote, Mendeley, Zotero vb.).

C. 1. Metin İçinde;

Kaynaklar, metinde geçiş sırasına göre numaralandırılmalıdır ve kaynak numaraları üst simge olarak verilmelidir. Örneğin,"… belirtilmektedir8. , bildirilmiştir8,13,18. , şeklindedir8-10

C. 2. 'Kaynaklar' Başlığı Altında;

Kaynaklar ayrı bir liste olarak metin içindeki sıralamalarına göre numaralandırılarak verilmelidir. Kaynak sayısı özgün araştırmalarda en çok 50, olgu sunumlarında en çok 20, editöre mektuplarda ise en çok 5 olmalıdır.

Kaynaktaki yazar sayısı 3 veya daha az ise tüm yazarlar belirtilmeli; 3'den fazla ise, Türkçe kaynak gösteriminde sadece ilk 3 isim yazılmalı "ve ark." şeklinde, İngilizce kaynak gösteriminde ise ilk 3 isim yazılmalı ve "et al." şeklinde gösterilmelidir.

Dergi isimleri Index Medicus/Medline/PubMed'de yer alan dergi kısaltmaları ile uyumlu olarak kısaltılmalıdır. Index Medicus'ta indekslenmeyen bir dergi kısaltılmadan yazılmalıdır. Çevrimiçi yayınlar için DOİ (digital object identifier) numarası verilmelidir.

Örnek:

1. Gage BF, Fihn SD, White RH. Management and dosing of warfarin therapy. The American Journal of Medicine. 2000; 109(6): 481-488. doi:10.1016/S0002-9343(00)00545-3.

Örnekler:

1. Debes-Marun CS, Dewald GW, Bryant S, et al. Chromosome abnormalities clustering and its implications for pathogenesis and prognosis in myeloma. Leukemia. 2003; 17: 427–436.
2. Ozcelik F, Oztosun M, Gülsün M, ve ark. İdiopatik trombositopenik purpura ön tanılı bir olguda EDTA'ya bağlı psödotrombositopeni. Turk J Biochem. 2012; 37(3): 336–339.

Örnek:

1. Yoldas O, Bulut A, Altindis M. Hepatit A Enfeksiyonlarının Güncel Yaklaşımı. Viral Hepatit J 2012; 18: 81-86.
2. Bir derginin ek sayısı (Supplement) kaynak gösterileceği zaman; İngilizce makalelerde (Suppl.) ve Türkçe makalelerde ise (ES) şeklinde gösterilmelidir.

Çevrimiçi makale ise tam yayın tarihi kullanılır. Genellikle cilt ve dergi sayıları, sayfa numaraları yoktur. Makaleye doğrudan ulaşım adresi ve erişildiği tarih verilmelidir.

Örnek:

5. Frederickson BL (2000, Mart 7). Cultivating positive emotions to optimize health and well-being. Prevention & Treatment 3, Makale 0001a. http://journals.apa.org./prevention/volume3/pre003000-1a.html adresinden 20 Kasım 2000'de erişildi.

Kitabın kaynak gösterimi ise yazarların adı, kitabın adı, birden çok basımı varsa kaçıncı basım olduğu, basımevi, basım yeri, basım tarihi belirtilmelidir

Örnek:

2. Strunk W Jr., White EB. The Elements of Style (4. baskı). Longman, New York, 2000.

Kaynak çok yazarlı bir kitabın bölümü ya da bir makalesi ise bölümün ya da makalenin yazarı, bölümün ya da makalenin adı, kitabın adı, kaçıncı baskı olduğu, cildi, kitabın yayın yönetmenleri, basım yeri, sayfaları, tarih yazılmalıdır.

Örnek:

3. Meltzer HY, Lowy MT. Neuroendocrin function in psychiatric disorders. American Handbook of Psychiatry, 2. Baskı, cilt 8, PA Berger, HKH Brodie (Ed), New York. Basic Books Inc, 1986; s. 110-117.

Çeviri kitaplar aşağıdaki şekilde kaynak olarak gösterilmelidir.

Örnek:

4. Liberman RP. Yetiyitiminden İyileşmeye: Psikiyatrik İyileştirim Elkitabı. American Psychiatric Publishing Inc. Washington DC. 2008. Çev. Mustafa Yıldız, Türkiye Sosyal Psikiyatri Derneği, Ankara, 2011.

Kaynak çevrimiçi (internette yer alıyor) ise erişim tarihi ile birlikte yazılmalıdır.

MAKALE SÜREÇ YÖNETİMİ

A. Çift-Kör Hakemlik

JOURNAL OF BIOTECHNOLOGY AND STRATEGIC HEALTH RESEARCH (J of BSHRS), yılda 3 kez yayınlanan ve çift-kör hakemlik sürecinden geçen bilimsel makalelerin yayınlandığı ulusal/uluslararası ve hakemli bir akademik dergidir. Yayınların incelenmesi için çalışmaların içeriğine ve hakemlerin uzmanlık alanlarına göre en az iki hakem, makale alan editörü/leri tarafından atanır. Bu süreçte hakem değerlendirme raporları elektronik ortamda isimsiz olarak gönderilir. Değerlendirmeyi yapan hakemlerin isimleri çift-kör yöntemi gereği raporlarda ve dergide belirtilmemektedir. Talep edilmesi halinde, hakem olarak dergiye katkı sağladığına ilişkin yazılı bir belge hakemlere verilebilir. Yazarlar, hakemlerle doğrudan iletişime geçemez, değerlendirme ve hakem raporları dergi yönetim sistemi aracılığıyla iletilir. Bu süreçte değerlendirme formları ve hakem raporları editör aracılığıyla sorumlu yazara iletilir.

B. Karar Alma Süreçleri

Yayınlanmak üzere gönderilen tüm çalışmalar, değerlendirme için alanlarında uzman en az iki hakeme gönderilir. İnceleme sürecinin tamamlanmasının ardından editör, söz konusu çalışmanın doğruluğu, araştırmacı ve okuyucular için önemi, hakem raporları, telif hakkı ihlali ve intihal gibi yasal düzenlemeleri de göz önünde bulundurarak hangi çalışmaların yayınlanacağına karar verir. Editör, bu kararı verirken diğer editörlerden veya hakemlerden de tavsiyeler alabilir.

C. İvedilik

Hakem değerlendirmesi yapmak üzere davet alan bir hakem, ilgili çalışma için hakemlik yapıp yapamayacağını yedi gün içinde editöre bildirmelidir. Kabul edilen hakemlik değerlendirme süreci onbeş, sorumlu yazara bildirilen değişikliklerin tamamlanması için, yazarlara verilen süre ortalama onbeş gündür. Sorumlu yazara son okuma için gönderilen metnin değerlendirme süresi ise üç gündür. Değerlendirme için hakemlere gönderilen çalışmalar gizli belge olarak tutulmalıdır. Çalışmalar başkalarına gösterilmemeli, içerikleri tartışılmamalıdır. Gerekli durumlarda editörün izni dahilinde hakemler başka meslektaşlarından tavsiye isteyebilirler. Editör, bu izni ancak istisnai bir koşul olması durumda verebilir. Gizlilik kuralı, hakemlik yapmayı reddeden kişileri de kapsamaktadır.

E. Tarafsızlık İlkesi

Değerlendirme sürecinde yazarlara yönelik kişisel eleştiri yapılmamalıdır. Değerlendirmeler, nesnel ve çalışmaların geliştirilmesine katkı sağlayacak şekilde olmalıdır.

F. Kaynak Belirtme

Hakemler, çalışmada atıf olarak belirtilmeyen alıntılar varsa bunları yazarlara bildirmekle yükümlüdür. Hakemler, alanda atıfta bulunulmayan eserlere ya da benzer eserlerle çakışan alıntılara özellikle dikkat etmelidir. Hakemler, daha önce yayınlanmış herhangi bir çalışma ya da bilgiyle benzerliği olan yayınların farkedilmesi durumunda editörleri bilgilendirmelidir.

G. Bilgilendirme ve Çıkar Çatışması

Hakemler, çalışmasını değerlendirmekle görevlendirildikleri herhangi bir yazar, şirket ya da kurumla işbirliğine dayalı herhangi bir bağlantıları olması durumunda değerlendirme yapmayı kabul etmemeli ve durumdan editörü haberdar etmelidir.

Hakemler, değerlendirme için gönderilmiş, yayınlanmamış eserleri ya da eserlerin bölümlerini yazar(lar)ının yazılı onayı olmadan kendi çalışmalarında kullanamaz. Değerlendirme sırasında elde edilen bilgi ve fikirler hakemler tarafından gizli tutulmalı ve kendi çıkarları için kullanılmamalıdır. Bu kurallar, hakemlik görevini kabul etmeyen kişileri de kapsamaktadır.

YAZI GERİ ÇEKME TÜM YAZARLARIN ONAYI İLE OLMALIDIR.

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Faks: +90.264.295 6629

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Journal of Biotechnology and Strategic Health Research

HEALTH RESEARCH

INSTRUCTIONS FOR AUTHORS

Scope of the Journal

The JOURNAL OF BIOTECHNOLOGY AND STRATEGIC HEALTH RESEARCH is published electronically 3 times a year by the Experimental, Biotechnological, Clinical and Strategic Health Research Association and accepts English or Turkish-language manuscripts in all fields of medicine(Experimental, Biotechnological, Clinical and Strategic Health Research) and other related health sciences. Contribution is open to researchers of all nationalities. The following types of papers are welcome: original articles (for the presentation of clinical and laboratory studies), case reports, review articles, and letters to the editor.

Submission Procedures

All manuscripts must be submitted electronically via the internet to the JOURNAL OF BIOTECHNOLOGY AND STRATEGIC HEALTH RESEARCH through the online system for ULAKBIM dergipark http://dergipark.gov.tr/bshr You will be guided stepwise through the creation and uploading of the various files.

There are no page charges.

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All experimental or clinical researches done in humans or animals should follow the ethical rules. The ethical approval form must be sent and the number of approval must be given in the manuscript. The ethical problems belong only to the author(s).

All copyright of the published papers belong to Experimental, Biotechnological, Clinical and Strategic Health Research Association.

The copyright fee is not paid to all authors.

In manuscripts based on scanning of archieve records, a consent form is needed that shows the permission for retrospective work and signed by Head of the Department, hospital manager or clinic manager.

Preparation of Manuscript Style and format:

Manuscripts should be submitted to http://dergipark.gov.tr/bshr as Microsoft word file in Times New Roman font. All manuscripts including references should be typed in 12 font size,one and a half (1.5) line space and justified. Upon submission, the copyright release form should be filled and downloaded. The manuscript submissions without a copyright release form will not be evaluated.

Each page of main text of the manuscript should be numbered on the right hand side. Manuscripts should be written in Turkish or English. Contributors who are not native English speakers are strongly advised to ensure that a colleague fluent in the English language or a professional language editor has reviewed their manuscript. Repetitive use of long sentences and passive voice should be avoided. It is strongly recommended that the text be run through computer spelling and grammar programs.

Symbols, Units, And Abbreviations:

In general, the journal follows the conventions of Scientific Style and Format, The CSE Manual for Authors, Editors, and Publishers, Council of Science Editors, Reston, VA, USA (7th ed.). Spaces must be inserted between numbers and units (e.g., 3 kg), but not between numbers and mathematical symbols (+, –, ±, ×, =, <, >) and between numbers and percent symbols (e.g., 45%). Please use International System (SI) units. All abbreviations and acronyms should be defined at first mention. Thereafter, generic names should be abbreviated as appropriate without altering the species name.

Typs of Manuscripts Original Article

It should consists of "Introduction", "Methods", "Results" and "Discussion". Conclusion may be written as a last paragraph of discussion, there is no need to add a separate section for conclusion. The whole length of text should be maximum 5 000 words (except abstract, acknowledgements and references). The numbers of references should be maximum 50. Also, scientific names should be spelled italics throughout the text.

Review

It should be maximum 6 000 words (except abstract and references). The author(s) should have at least one published paper in a journal indexed in SCI/SCI-expanded related to the topics of the review. The abstract should be as one paragraph and should be written without a section. The numbers of references should be maximum 100.

Case Report

It should be maximum 1 500 words (except abstract, acknowledgement and references). Case reports should consist of abstract, keywords, introduction, case report and discussion sections. The numbers of references should be maximum 10. Figures or Tables should follow the main text in a separate pages.

Letter to Editor

It should be maximum 1 000 words (except abstract and references). No Tables or Figures are included. If it was written refering to another article, the number and the date should also be added. The name, affiliation(s) and address of author(s) should be written at the end of the text. The numbers of references should be maximum 5.

Manuscript Arrangement

Manuscripts should be arranged as follows: "Title page", "Abstract", "Keywords", "Main text", "Acknowledgements", "References", "Tables",and "Figures".

Title page

All submissions must include a title page, which is to be uploaded as a separate document. The title page should contain the full title in sentence case (e.g., Urothelial cancers: clinical and imaging evaluation). The title should be limited to 25 words or less and should not containabbreviations.The title should be a brief phrase describing the contents of the paper.Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible. It should be written in capital letters both in Turkish and in English. Title in English should be written using italic letters for Turkish manuscripts and vice versa. The first and the family names of the authors should be written in small letters as the first letter being the capital.

The full names and affiliations of all authors should be given clearly and briefly with theirinstitutions, address with zip code and name of country, and the contact details of corresponding author (E-mail address and telephone). In addition, ORCID (Open Researcher and Contributor ID) numbers of all authors should be included into the title page.

Abstract

The abstract should be brief, indicating the purpose/significance of the research, methodology, major findings and the most significant conclusion (s). The abstract shouldnot contain literature citations that refer to the main list of reference attached to the complete article. The abstract should be written as a single paragraph and should be in reported speech format (past tense); complete sentences, active verbs and the third person should be used.

The abstract should be structured to include the study's "Objective", "Methods", "Results", and "Conclusion" under 4 separate headings. Abstracts of review articles should be a brief overview of the main points from the review. In reviews and case reports, abstract should be written without any sections. The abstract (English and Turkish) should not be more than 300 words.

Keywords

The authors must provide 3-6 keywords for indexing purposes and to facilitate the retrieval of articles by search engines. Keywords should be different from the words that make up the title of the article. Keywords should be written below the abstracts both inTurkish andEnglish. Acronyms should be avoided. For English keywords, always try to use terms from the Medical Subjects Headings list from Index Medicus (www.nlm.nih.gov/mesh/MBrowser.html). For Turkish keywords, terms from Turkish Scientific Terms (www.bilimterimleri.com) should be used.

Main text

Introduction

The introduction should be clear and concise, with relevant references on the study subject and the proposed approach or solution. There should be no subheadings. Excessive citation of literature should be avoided. Only necessary and the latest citations of literature that are required to indicate the reason forthe research undertaken and the essential background should be given.

Methods

Explain clearly but concisely your clinical, technical, or experimental procedures. A precise description of the selection of your observational or experimental subjects (for example patients or laboratory animals including controls) must be presented. Experimental research involving human or animals should be approved by ethical committiee.All chemicals and drugs used must be identified correctly, including the generic names, the name of the manufacturer, city and country in parenthesis. The techniques or methodology adopted should be supported with standard references. Briefly describe methods that have been published but are not well known as well as new or substantially modified methods. Description of established procedures are unnecessary. Apparatus should be described only is it is non-standard; commercially available apparatus used should be stated (including manufacturers' name, address in parenthesis). Only SI units should be used for each measurements.

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Results

The result section should provide complete details of the experiment that are required to support the conclusion of the study. The results should be written in the past tense when describing findings in authors experiments. Previously published findings should be written in the present tense. Speculation and the detailed interpretation of the data should not be included in the results but should be put into the discussion section.

Discussion

Statements from the "Introduction" and "Results" sections should not be repeated here. The final paragraph shouldhighlight the main conclusions of the study.

Tables and Figures

The visual presentations like photographs, graphics, picturesetc. must be labelled "Figures". Whereas, the "Tables" shows the classifieddata.Tables should be added after the "References" section. Figure legends should be placed into the end of the main text. Figures should be uploaded as a separate file following the Dergipark System.

All tables and figures must have a caption and/or legend and be numbered (e.g., Table 1., Figure 2.), unless there is only one table or figure, in which case it should be labelled "Table" or "Figure" with no numbering. Captions mustbe written in sentence case (e.g., Figure 1. Macroscopic appearance of the samples.). The font used in the figures should beTimes New Roman. If symbols such as ×, μ, η, or ν are used, they should be added using the Symbols menu of Word.

All tables and figures must be numbered consecutively as they are referred in the text. Please refer to tablesand figures with capitalisation and unabbreviated (e.g., "As shown in Figure 2. …", and not "Fig. 2" or "figure 2").
The resolution of images should not be less than 118 pixels/cm when width is set to 16 cm. Images must bescanned at 300 dpi resolution and submitted in .jpeg, .png or .tif format.

Graphics and diagrams must be drawn with a line weight between 0.5 and 1 point. Scanned or photocopied graphs anddiagrams are not accepted.

Charts must be prepared in 2 dimensions unless required by the data used. Charts unnecessarily prepared in3 dimensions are not accepted.

Figures that are charts, diagrams, or drawings must be submitted in a modifiable format, i.e. our graphicspersonnel should be able to modify them. Therefore, if the program with which the figure is drawn has a "Save as"option, it must be saved as .pdf. If the "Save as" option does not include .pdf extension, the figure must becopied and pasted into a blank Microsoft Word document as an editable object. It must not be pasted as an imagefile (.tiff or.jpeg) unless it is a photograph.

Tables and figures, including caption, title, column heads, and footnotes, must not exceed 16 × 20 cm andshould be no smaller than 8 cm in width. For all tables, please use Word's "Create Table" feature, with no tabbedtext or tables created with spaces and drawn lines. Please do not duplicate information that is already presented inthe figures.
Tables must be clearly typed, each on a separate sheet, and single-spaced. Tables may be continued on anothersheet if necessary, but the dimensions stated above still apply.

Tables should be arranged as a horizontal borderline as well as below the last line. Moreover, there sould be vertical line on the right of first column on the left hand site. Abbreviations used in the tables such as (*) should be explained below the table in 10 font size.

In Tables written in Turkish, decimal numbers should be written with comma, however in English text, decimal numbers should be written with dots. Percentages (%) should be placed in front of the numbers without space and behind the numbers in Turkish and English text, respectively.

Example for a Table:

Table1. The reasons of not applying to general practioner for the first application.

The reasons	*n**	*%*
Only Psychiatrist can do it		
No information about general practioner Parents decision		
Not preferred	*47*	*53.4*
17	*19.3*	
12	*13.6*	
12	*13.6*	

**Total number of patients.*

Acknowledgement

All acknowledgements, poster/oral presentations, financial supports, grants, technical supports and the conflict of interest should be mentioned at the end of the text.

Funding

The type of Project or the financial support such as scientific projects of University, TUBITAK projects etc. should be added at the end of the text including the numbers and the year of the projects.

References

While talking about the source in the text, the first author's surname ın Er and his firends' study12"…… or ın Er et al.12. Both authors should be given the surnames of both authors (similar results were found in the study conducted by Öncü and Ilke13).

Citations in the text should be identified by numbers assuperscript, for example, "The results were as follows: 4
If there are more than one references, separate the numbers with comma, for example, "Several interventions have been successful at increasing compliance.11,14"

In following journals, first and the last numbers should be seperated by "-" , for example: Diabetes mellitus is associated with a high risk of foot ulcers1-3 or "As reported previously,1,3-6"

Do not include personal communications, unpublished data, or other unpublished materials as references, although such material may be inserted (in parentheses) in the text. In the case of publications in languages other than English, the published English title should be provided if one exists, with an annotation such as "(article inTurkish with an abstract in English)". If the publication was not published with an English title, provide the original title only; do not provide a self-translation. A short title for use as a running head (not to exceed 30 characters in length, including spaces between words) is needed. References should be formatted as follows (please note the punctuationand capitalisation):
The list of references at the end of the paper should be given in order of their first appearance in the text. All authors should be included in reference lists unless there are more than 6, in which case only the first 3 should be given, followed by "et al." in English and "ve ark." in Turkish references.

The number of references should not be more than 60 in original articles, not more than 100 in review articles, not more than 20 in case reports and not more than 5 in letter to editor. The journal requires DOI numbers, when available, to be included in all references. Personal experiences and researches without a DOI number should not be used.

In order to arrange the reference list easly, our journal suggest the use of reference arrangement programmes such as EndNote or Mendeley etc.).

For a reference in the reference list, the surname of author, the first letter of author's name, the title of the reference, the name of the journal, the year of the journal, the numbers of its volume, issue and pages should be written. The name of the journal should be abbreviated as in AMA (American Medical Association) ((http://library.nymc.edu/informatics/amastyle.cfm). If the abbreviation is not avalible, whole name of the journal should be written.

Published papers

Yoldas O, Bulut A, Altindis M. Current Approach to Hepatitis A Infections. Viral Hepatit J 2012; 18: 81-86..
Debes-Marun CS, Dewald GW, Bryant S, et al. Chromosome abnormalities clustering and its implications for pathogenesis and prognosis in myeloma. Leukemia. 2003;17:427–436.
Ozcelik F, Oztosun M, Gülsün M, ve ark. Pseudothrombocytopenia due to EDTA in a case with idiopathic thrombocytopenic purpura. Turk J Biochem. 2012;37(3):336–339.

Gage BF, Fihn SD, White RH. Management and dosing of warfarin therapy. Am J Med. 2000;109(6):481- 488. doi:10.1016/S0002-9343(00)00545-3.

If a supplement of a journal is referred, (suppl.) in English and (ES) in Turkish manuscripts should be used.

Electronic journal articles

If a journal from a website is used, the date of publishing is used. Usually, there is no numbers of volume, issue or pages. The web adress and date of download should be given.

Example:

Acetaminophen poisoning. In: DynaMed [database online]. EBSCO Information Services. http://0-search.ebscohost.com.topcat.switchinc.org/login.aspx?direct=true&site=DynaMed&id=113862.

Updated

March 09, 2010. Accessed March 23, 2010.

Book

Harmening D. Modern Blood Banking & Transfusion Practices. 6th ed. Philadelphia, PA: F.A. Davis Company; 2012.
Strunk W Jr., White EB. The Elements of Style. 4th ed. New York, NY: Longman; 2000.
Chapter in a book
Solensky R. Drug allergy: desensitization and treatment of reactions to antibiotics and aspirin. In: Lockey P, ed. Allergens and Allergen Immunotherapy. 3rd ed. New York, NY: Marcel Dekker; 2004:585-606.
McCall RE, Tankersley CM. Phlebotomy and specimen considerations. In: Bishop ML, Fody EP, Schoeff LE, editors. Clinical Chemistry: Techniques, Principles, Correlations. Philadelphia, PA, USA: Lippincott Williams & Williams; 2010:33-73.

Conference proceedings

Weber KJ, Lee J, Decresce R, Subjasis M, Prinz R. Intraoperative PTH monitoring in parathyroid hyperplasia requires stricter criteria for success. Paper presented at: 25th Annual American Association of Endocrine Surgeons Meeting; April 6, 2004; Charlottesville, VA.

Chu H, Rosenthal M. Search engines for the World Wide Web: a comparative study and evaluation met-

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hodology. Paper presented at: American Society for Information Science Annual Conference; October 19-24, 1996; Baltimore, MD. http://www.asis.org/annual-96/electronicproceedings/chu.html. Accessed February 26, 2004.

Theses

Fenster SD. Cloning and Characterization of Piccolo, a Novel Component of the Presynaptic Cytoskeletal Matrix [master's thesis]. Birmingham: University of Alabama; 2000.

Publication Policy and Manuscript Evaluation Process

A. Double-blinded peer-reviewed method

Biotechnology and Strategic Health Research (J BSHRS) is published 3 times a year (April, August, December) and it is double-blinded peer-reviewed system national journal.

Editorial and publication processes of the BSHRS Derg. are shaped in accordance with the guidelines of the international organizations such as the International Council of Medical Journal Editors (ICMJE), the World Association of Medical Editors (WAME), the Council of Science Editors (CSE), the Committee on Publication Ethics (COPE), the European Association of Science Editors (EASE). The journal is in conformity with Principles of Transparency and Best Practice in Scholarly Publishing (doaj.org/bestpractice). Processing and publication is free of charge with the Biyoteknolojik ve Stratejik Sağlık Araştırmaları Dergisi. Authors are not charged a fee at any point during the publication process. All manuscripts should be submitted through the journal's web page at http://dergipark.gov.tr/bshr.

For the evaluation of papers, at least two referees are determined considering the content of the manuscript or the professional scientific area of the referees. In this step, referee assessment form is sent via internet without names. The personal data of the referee is not shown since the double-blind peer-reviewed method is used. Upon request, a written document given to referee as the referee for that contribute to the journal. The authors cannot directly contact with the referees. The referee's evaluation report is sent by the journal management system. The evaluation forms and the referees' reports are sent to the corresponding author(s) by the editor.

B. Decision process

After the referees' evaluation process, the editor decides whether the manuscript will be accepted or not considering the accuracy and the importance of the work, referee's reports, copyright infringement and ethical problems such as plagiarism.

As the editor decides about the manuscript, he or she may require the suggestions of the other member of editorial board or referees.

C. Instancy

A referee invited to the journal for the evaluation of a manuscript should inform the editor about the acceptance in 7 days. The referee should complete the evaluation in 15 days and the corresponding author(s) should download the revised manuscript in 15 days. The requested reading time for the last version of the manuscript by the corresponding author is only 3 days.

D. Confidentiality (Privacy Statement)

Personal information such as names and electronic mail addresses are only used for the scientific purposes of the journal. Other than these purposes this information will not be used and will not be shared with the third parties. The manuscripts sent to referees for assessment are kept as confidential documents. The manuscripts are not shown to other people and the contents of them should not be discussed. If it is necessary, reviewers may need suggestions from their colleagues after editorial permission. The editor may give that permission only in the presence of exceptional condition. The confidentiality rules are also valid for the referees not accepting the assessment of the manuscript.

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In the evaluation process, no personal criticism of the authors should be done. The evaluations should contribute to the development of works and be objective.

F. Citation to reference

The referees should inform the authors if there are any citations that are not referred in the manuscript. The referees should pay particular attention to the citations that do not refer to the subject or to the citations that coincide with similar works. The referees should inform the editors if any publications that have similarity to any previously published work or information are recognized.

G. Information and Conflict of Interest

The referees should not agree to make any evaluation if they have any relation with any author, company or institution in which they are tasked to evaluate their work and inform the editor. The referees may not use the unpublished works or sections of the works submitted for evaluation in their own work without the written consent of the author(s). The information and ideas obtained during the assessment should be kept secret by the referees and should not be used for their own interests. These rules include those who refuse the manuscript assessment.

H. Prevention of Plagiarism

J of Biotechnology and Strategic Health Research(J of BSHR) reports the similarity rates of the articles through the iThenticate and Turnitin programs and shows the care and sensitivity required to prevent plagiarism.

THE WITHDRAW OF THE ARTICLE MUST BE WITH THE APPROVAL OF ALL AUTHORS.

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Review / Derleme

http://dergipark.org.tr/tr/pub/bshr

Mevcut ve Gelişmekte Olan Aşı Teknolojileri; Kısa Derleme

Current and Emerging Vaccine Technologies; A Short Review

Elmas Pınar Kahraman Kılbaş[1], Mustafa Altındiş[2]

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Öz

Aşı teknolojileri, daha etkili ve çok yönlü aşılama stratejilerine duyulan ihtiyaç nedeniyle önemli ölçüde gelişti. Geleneksel aşılar öncelikle bağışıklık sistemini uyarmak için zayıflatılmış veya etkisiz hale getirilmiş patojenleri kullanıyordu. Ancak moleküler biyoloji ve immünolojideki son gelişmeler yeni aşı platformlarının geliştirilmesine yol açtı. Dikkate değer ilerlemelerden biri, COVID-19 aşılarından biri olan mRNA aşılarının geliştirilmesidir. Bu aşılar, hücrelere patojenin zararsız bir kısmını üretme talimatı vermek için sentetik mRNA'yı kullanarak güçlü bir bağışıklık tepkisi ortaya çıkarır. Umut verici başka bir yaklaşım, patojenik antijenleri kodlayan genetik materyali konakçı hücrelere iletmek için değiştirilmiş bir virüs kullanan viral vektör aşılarını içerir. Bu teknoloji, Ebola ve COVID-19 gibi hastalıklara karşı güçlü bir bağışıklık tepkisi sağlayarak umut vaat etmektedir. Protein alt birim aşılarındaki yenilikler, bir bağışıklık tepkisini tetiklemek için patojenin proteinler veya peptidler gibi zararsız parçalarının kullanılmasını içerir. Bu aşılar, genellikle adjuvanlar veya nanopartikül dağıtım sistemleri yoluyla geliştirilmiş güvenlik ve etkinlik sunar. Ayrıca, DNA aşıları gibi nükleik asit bazlı aşılardaki ilerlemeler, aşılama için potansiyel olarak güçlü ve esnek bir platform sunmaktadır. Ek olarak, günümüzde nanoteknoloji sayesinde bağışıklık tepkileri güçlendirerek aşı gelişimine katkı sağlanmıştır. Nanopartiküller, antijenleri veya adjuvanları kapsülleyerek aşı etkinliğini optimize edebilmektedir. Sonuç olarak, mevcut aşı teknolojileri, mRNA ve viral vektör aşıları, protein alt birim aşıları, nükleik asit bazlı aşılar ve nanoteknoloji dahil olmak üzere yenilikçi ve çeşitli yaklaşımlara doğru bir geçiş sergilemektedir. Bu ilerlemeler, ortaya çıkan bulaşıcı hastalıkların ele alınması ve aşının erişilebilirliğinin, güvenliğinin ve etkinliğinin iyileştirilmesi konusunda umut vaat etmektedir.

Anahtar Kelimeler: Aşı Teknolojileri, enfeksiyonlar, ımmunizasyon, mRNA aşıları, nanoaşılar.

Abstract

Vaccine technologies have evolved significantly due to the need for more effective and versatile vaccination strategies. Traditional vaccines primarily used weakened or inactivated pathogens to stimulate the immune system. However, recent advances in molecular biology and immunology have led to the development of new vaccine platforms. One notable advance is the development of mRNA vaccines, one of the COVID-19 vaccines. These vaccines elicit a strong immune response by using synthetic mRNA to instruct cells to produce a harmless portion of the pathogen. Another promising approach involves viral vector vaccines, which use a modified virus to deliver genetic material encoding pathogenic antigens into host cells. This technology shows promise by providing a strong immune response against diseases such as Ebola and COVID-19. Innovations in protein subunit vaccines involve using harmless parts of the pathogen, such as proteins or peptides, to trigger an immune response. These vaccines often offer improved safety and efficacy through adjuvants or nanoparticle delivery systems. Additionally, advances in nucleic acid-based vaccines, such as DNA vaccines, offer a potentially powerful and flexible platform for vaccination. In addition, nanotechnology has contributed to vaccine development by strengthening immune responses. Nanoparticles can optimize vaccine efficacy by encapsulating antigens or adjuvants. As a result, current vaccine technologies are shifting towards innovative and diverse approaches, including mRNA and viral vector vaccines, protein subunit vaccines, nucleic acid-based vaccines, and nanotechnology. These advances hold promise for addressing emerging infectious diseases and improving vaccine availability, safety, and effectiveness. In this review, the epidemiology of Monkeypox and the general characteristics of the causative agent of Monkeypox is reviewed, with current information and data.

Keywords *Vaccine Technologies, ınfections, ımmunization, mRNA vaccines, nanovaccines.*

GİRİŞ

Aşılar, hayat boyu enfeksiyonlardan, hastalıklardan ve biyolojik terör ajanlarından korunmak için etkili bir önleyici müdahale yöntemidir.[1] 1796 yılında geliştirilen ilk aşıdan bu yana, potansiyel enfeksiyonları önlemek için binlerce aşı adayı geliştirildi. Başarılı aşılama programları sayesinde, çoğu ülkede geçmişte milyonlarca insanın hayatına mal olan tehlikeli salgın enfeksiyonların önüne geçilmiştir.[2] Aşılarla önlenebilen bulaşıcı hastalıkların çoğu, özellikle gelişmekte olan ve az gelişmiş ülkelerde hâlâ yaşamı tehdit etmeye devam etmektedir.[3]

Profilaktik aşılar, bir patojene karşı etkili tepki ve hafıza için bağışıklık sistemini uyaran bir veya daha fazla spesifik antijen içerir. Aşılama sonrası uzun süreli bağışıklamanın sağlanması zorlayıcı olmuştur.[4] Bu durum da mevcut aşılarda biyoteknolojik gelişmeleri gerektirir. Mevcut aşılardaki immünolojik gelişmelere ek olarak, COVID-19 gibi yeni ortaya çıkan bulaşıcı hastalıklara ve laboratuvarda tasarlanmış patojenlere veya biyoterörizm uygulamasına yönelik potansiyel enfeksiyonlara karşı aşıların güvenilir ve hızlı geliştirilmesi için yeni teknolojik gelişmelere ihtiyaç vardır.[5,6] Canlı zayıflatılmış ve alt birim aşıları içeren geleneksel aşı platformlarının aksine, üçüncü nesil aşı teknolojileri, acil uygulamalar için hızlı tasarlanıp büyük dozların üretilmesine olanak sağlamaktadır. Bu derlemede, çeşitli etkenlere karşı geliştirilen yeni aşı teknolojilerine yönelik bilgiler sunulacaktır.

Bağışıklama

Aşıların İndüklediği Antikorlar

Rutin kullanımdaki neredeyse tüm aşıların esas olarak antikorların indüksiyonu yoluyla koruma sağladığı düşünülmektedir. Aşı kaynaklı korumada çeşitli fonksiyonel antikor türlerinin önemli olduğuna dair destekleyici kanıtlar vardır. Bu kanıtlar, immün yetmezlik durumları, pasif koruma çalışmaları ve immünolojik veriler olarak sınıflandırılabilir.

Antikorlarda veya diğer bağışıklık hücrelerinde immünolojik kusurları olan kişiler, belirli patojenlerle enfeksiyona karşı özellikle hassastır. Örneğin, kompleman sisteminde eksiklikleri olan kişiler, Neisseria meningitidis enfeksiyonunun neden olduğu meningokok hastalığına özellikle duyarlıdır çünkü bu enfeksiyonun kontrolü, kompleman aracılı bağışıklığa bağlıdır.[7] Antikor ve kompleman ile opsonize edilen *Streptococcus pneumoniae* bakterileri normal olarak dalaktaki fagositler tarafından kandan uzaklaştırılır. Bu nedenle pnömokok hastalığı özellikle dalak fonksiyonu azalmış bireylerde yaygındır.[8]

Ekzojen antikorların intramüsküler veya intravenöz infüzyonunun bazı enfeksiyonlara karşı koruma sağlayabildiği pasif korumaya verilebilecek en iyi örneklerdendir. Örneğin; anneye ait antikorlar plasenta üzerinden bebeğe pasif olarak transfer olur. Bu da yeni doğan bebeklere, doğumdan sonraki birkaç ay boyunca çok çeşitli patojenlere karşı koruma sağlamaktadır. Annenin boğmaca, tetanoz ve grip aşılarıyla aşılanması sayesinde, bebek doğumdan sonra bu önemli koruyucu adaptasyondan yararlanır.[9,10] Kalıtsal antikor eksikliği olan kişiler ciddi viral ve bakteriyel enfeksiyonlara karşı savunmasızdır, ancak bağışıklık sistemi sağlıklı bir donörden serum antikorlarının düzenli olarak uygulanması, antikor eksikliği olan kişiler için de neredeyse tamamen sağlıklı bir bağışıklık koruması sağlayabilmektedir.[11]

İmmünoloji alanında artan bilgiler, aşıların aracılık ettiği diğer koruma mekanizmalarına dair ufkumuzun genişlemesini sağlamaya devam etmektedir. Meningokok ve pnömokok gibi istilacı bakterilerin yüzey polisakkaritlerinden yapılan polisakkarit aşılar, bu hastalıklara karşı önemli ölçüde koruma sağlamaktadır.[12,13] Polisakkaritler T hücresinden bağımsız antijenler olduğundan bu aşıların T hücresi yanıtlarını tetiklemediği ve bu nedenle bunların korunmasına antikora bağımlı mekanizmalar aracılığıyla aracılık ettiği bilinmektedir. Protein-polisakarit konjuge aşılartarafından uyarılan T hücreleri, protein taşıyıcıyı tanır ve bu T hücreleri, polisakkariti tanıyan B hücrelerine yardım eder. Ancak polisakkariti tanıyan hiçbir T hücresi

uyarılmaz ve dolayısıyla bu aşıların sağladığı mükemmel korumada yalnızca antikorlar rol oynar.[14]

T Hücre Aracılı Bağışıklama

T hücrelerinin aşı ile bağışıklamadaki rolleri, lenf düğümlerinde B hücresi gelişimi ve antikor üretimine yardım sağlama rolleri dışında yeterince tanımlanmamıştır. Kalıtsal veya edinilmiş immün yetmezliği olan bireylerde, T hücresi eksikliğinin enfeksiyondan sonra patojenlerin üremesi kontrol edilememektedir. T hücresi eksikliği, kontrolsüz ve ölümcül varicella zoster virüsü enfeksiyonuyla sonuçlanabilmektedir.[15] T hücrelerinin aşı kaynaklı korumada rol oynadığına dair kanıtların sınırlı olmasının nedeni muhtemelen, birçok T hücresinin lenf düğümleri gibi dokularda yerleşik olması nedeniyle T hücrelerine erişimdeki zorluklardan kaynaklanmaktadır. Ayrıca hangi tip T hücresinin ölçülmesi gerektiği henüz tam olarak anlaşılmamıştır. T yardımcı 1 (TH1) hücreleri ve TH2 hücreleri, sırasıyla hücresel bağışıklık ve humoral bağışıklık oluşturmak için önemlidir, ancak TH1 hücreleri aynı zamanda IgG antikor alt sınıfları IgG1 ve IgG3'ün üretilmesiyle de ilişkilidir. Diğer TH hücre alt tipleri arasında TH17 hücreleri (bağırsak ve akciğer gibi mukozal yüzeylerde) ve T foliküler yardımcı hücreler (yüksek afiniteli antikorların üretimi için önemli olan ikincil lenfoid organlarda) bulunmaktadır.14 Araştırmalar, farelerde *S. pneumoniae*'nin taşıyıcılığına karşı bağışıklığın, *S. pneumoniae*'ye maruz bırakılan donör farelerden T hücrelerinin transferi yoluyla elde edilebileceğini göstermektedir. Bu da T hücresi aracılı bağışıklığın doğasını daha iyi anlamak için daha fazla araştırma yapılmasının gerekli olduğunu göstermektedir. Bu kanıtlar, enfeksiyonun önlenmesinde antikorların ana role sahip olduğunu, sitotoksik T hücrelerinin ise yerleşik enfeksiyonları kontrol etmek ve temizlemek için etkili olduğunu göstermektedir.[16]

Aşıların Tarihçesi

Aşıların varoluşuna dair ilk bulgular yedinci yüzyılda Hintli Budistlerin bağışıklık kazanmak için yılan zehrini yemeleriyle ortaya çıkmıştır. Aşıyla ilgili ilk yazılar, 11. yüzyıldan kalma olup, Çin edebiyatındaki "Çiçek hastalığının doğru tedavisi" adlı metinlerdir. Bu kitap, Jen Tsung'un hükümdarlığı sırasında (1022-1063) yaşayan ve çiçek hastalığından muzdarip hastalara çiçek aşısı yapma sanatını uygulayan Budist bir rahibeye atfedilmiştir. Başka bir Çin tıp kitabı olan "Tıbbın Altın Aynası" çiçek hastalığı aşısının dört formunutanımlamıştır.[17] Çiçek hastalığı aşısı ilk olarak Çin ve Hindistan'da uygulanmıştır. Bu yöntem, Küçük Asya ve Yakın Doğu'ya yayılmış ve 18. yüzyılın başında buradan Batı dünyasına geçmiş, böylece hastalığa karşı korumada yeni bir tedavi aracı olmuştur.[17]

17. yüzyılda Çinliler, hasta kişilerden alınan cerahatle ıslatılmış bir parça pamuğu burun deliklerine uygulayarak aşılama yapmışlardır. Diğer bir yöntem, bir yıl önce topladıkları yara kabuklarını kurutup küçük bambu kamışlarla burun deliklerine sürerek uygulama şeklindeydi. Üçüncü bir yöntem ise sağlıklı bir çocuğa çiçek hastalığı geçiren hastanın kullanılmış kıyafetlerini giydirmekti.[18]

1798 yılının Haziran ayında Edward Jenner (1749-1823) tarafından yazılan ve o zamana kadar sonuçları insanlık açısından korkunç olan çiçek hastalığına karşı mücadelede devrim yaratacak bir aşı çalışması yayımlandı. Bu keşfin başlangıcı, 14 Mayıs 1796'da Jenner'ın, inek sağan bir kadındaki inek çiçeği yarasından alınan materyali James Phipps adlı sekiz yaşındaki bir çocuğun kolundaki iki yüzeysel kesikten enjekte etmesiyle gerçekleşmişti.[19] Jennerian yöntemine "aşı" adı verilmiştir. 1885 yılında Louis Pasteur, zayıflatılmış mikroplar kullanılarak hastalıkların önlenebileceğini göstermiştir. Bunu, kuduz bir köpek tarafından ısırılan Joseph Meister adlı bir çocukta kuduzu başarılı bir şekilde önlemek için bir aşı kullanarak yapmıştır.[20] Aynı yıl İspanyol bakteriyolog Jaime Ferrán, Alicante salgınında test edilen bir anti-kolera aşısı keşfetmiştir.[21] 1890 yılında, immünoloji alanında öncü, tetanoz ve difteriye karşı aşıların kaşifi olan Emil Von Behring, ilk Nobel Fizyoloji ve Tıp Ödülü'ne layık görülmüş ve "askerlerin ve çocukların kurtarıcısı" olarak adlandırılmıştır.[22]

Aşılamadaki bir diğer ilerleme, 1922 yılında adını Albert Calmette ve Camile Guerin'den alan tüberküloz aşısı Bacillus Calmette Guerin (BCG)'in keşfi olmuştur.[23] Bu aşıyla birlikte, aşı güvenliği tarihindeki en büyük felaketlerden biri yaşanmıştır. 1930 yılında Almanya'nın Lübeck şehrinde Mycobacterium tuberculosis'in bir türünü içeren BCG ile aşılanan 75 bebek ölmüştür.[24] İlk tam hücre aşısı, 1926 yılında Danimarka'da Thorvald Madsen tarafından Bordetella pertussis süspansiyonundan üretilmiştir.[25]

1993 yılında çiçek, BCG, difteri-boğmaca-tetanoz (DPT), çocuk felci ve kızamık aşılarına ek olarak, hastalığın endemik olduğu ülkelerde hepatit B ve sarı hummaya karşı aşılama da bu programa dahil edilmiştir. 1998'de Haemophilus influenza tip b'ye (Hib) karşı aşı tanıtılmıştır[26]. Kitlesel aşı kampanyaları sayesinde yılda 5 milyon çiçek, 2,7 milyon kızamık, 2 milyon yenidoğan tetanoz ve 1 milyon boğmacadan ölümün önlendiğinin tahmin edildiği bildirilmiştir[26]. National Institutes of Health (NIH), 2006 yılında, İnsan Papilloma Virüsü (HPV) enfeksiyonuna karşı koruma sağlayan aşının ortaya çıktığını bildirmiştir.[27]

Geleneksel Aşılar

Genel olarak bağışıklama, bağışıklık sisteminin bir antijenle karşılaşması veya eksojen humoral bağışıklık sonucu oluşan bağışıklıktır. Bağışıklama, doğal enfeksiyonlarla veya bulaşıcı hastalıkların önlenmesi veya maligniteler gibi bazı hastalıkların tedavisi için aşılar kullanılarak sağlanabilir.[28] Birincil bir enfeksiyon için tam adaptif bağışıklık, B lenfositler tarafından humoral bağışıklığın ve T lenfositler tarafından hücresel bağışıklığın indüklenmesi ile meydana gelmektedir. Bu bağlamda, eş zamanlı humoral ve hücresel bağışıklık, gelecekteki olası antijenlerle maruziyet durumunda aynı enfeksiyon için daha yüksek koruyuculuk sağlayacaktır.[29] Bağışıklama için farklı aşı platformları bulunmakta olup, bu yöntemler başarıyla test edilmiştir. Günümüzde kullanılan aşı türleri; canlı zayıflatılmış aşılar (oral polio, suçiçeği ve kızamık aşıları vb.), inaktive aşılar (polio, boğmaca, kuduz, tifo aşıları vb.), toksoidler (difteri ve tetanoz vb.), alt ünite aşılar (Haemophilus influenza tip b, Salmonella Typhi ve hepatit B), viral vektör ve mRNA aşılarını içeren yeni nesil aşılardır.[30-33]

Genel aşı uygulamaları; subkutan, intradermal, intramüsküler, oral ve nazal yollarla gerçekleştirilmektedir. Ancak bu uygulama yollarının tümünde aşının etkliğini azaltan bazı bariyerler bulunmaktadır. Bağışıklamanın etkinliğini artırmaya yönelik yeni yaklaşımlar arasında, aşı enjeksiyonunun şekli ve rotası için yeni teknolojiler ve dermal, oral ve mukozal olarak geliştirilmektedir. Bu amaçla lipozomlar veya nanokapsüller gibi partikül taşıma sistemlerini kullanan nazal ve pulmoner immünizasyon, hidrojeller gibi modern sistemleri kullanan oral bağışıklama, mikropartikül sistemleri kullanılarak oral bağışıklama, polimer nanopartiküller kullanılarak tek dozlu aşılama, emülsiyon ve mikroakışkan sistemler kullanılarak aşı kapsülleme gibi yöntemler geliştirilmiştir.[34,35] (Tablo 1).

Tablo 1. Aşı etkinliğinin önündeki bariyerleri aşmaya yönelik geliştirilen yeni aşı teknolojileri.

Aşı uygulama yolları	Bariyerler	Kullanılan partiküller	Aşı teknolojileri
Nazal ve pulmoner immünizasyon	Mukus, enzimler, hücreler arası sıkı bağlantılar	Polimerik NP'ler, lipozomlari gümüş NO'ler, miseller, nanosfer, karbon nanotüpler Hücre iskeleleri, mikroiğneler, hidrojeller, partiküller	Mikropartiküller, polimerik NP'ler, mikroakışkan sistemler yoluyla tek doz immünizasyon, aşı enkapsülasyonu ve mikroakışkan sistemlerin üretimi
Kütanöz immünizasyon	pH, enzimler, deri katmanları, keratinositler, korneum	Hücre iskeleleri, mikroiğneler, hidrojeller, partiküller	
Oral immünizasyon	pH, mukus, enzimler, epitel tabaka, peristaltizm, mikrobiyota	Hücre iskeleleri, partiküller, hidrojeller	

Yeni Aşı Teknolojileri

Dünya çapında morbidite ve mortaliteyi azaltmak için yeni aşılara ihtiyaç duyulan birçok önemli hastalık bulunmaktadır. Grup B Streptokok aşıları, plasentayı geçen ve yenidoğanı pasif olarak koruyan anne antikorlarını tetiklemek amacıyla şu anda geliştirilme aşamasındadır.[36] Solunum Sinsityal Virüsü (RSV), gelişmiş ülkelerde bebeklik döneminde alt solunum yolu enfeksiyonlarına bağlı hastaneye yatışının en yaygın nedenlerindendir ve küresel olarak 12 aydan küçük çocuklarda önde gelen ölüm nedenlerinden biridir. RSV'ye özgü monoklonal antikorlarla aşılamayı içeren 60 kadar yeni RSV aşı adayı geliştirilme aşamasındadır. Lisanslı bir RSV aşısının bebek sağlığı ve pediatrik hastaneye yatışlar üzerinde büyük etkisi olacaktır.[11]

Yeni aşıların geliştirilmesindeki diğer bir ana hat, özellikle yara enfeksiyonları ve intravenöz kateterlerle ilişkili antibiyotiğe dirençli Gram pozitif bakteriler (Staphylococcus aureus vb.) ve çeşitli Gram negatif mikroorganizmalar (Klebsiella spp. ve Pseudomonas aeruginosa) olmak üzere hastane kaynaklı enfeksiyonlarla mücadele etmektir. Bu alanda ilerleme yavaş olmuştur. Aşı geliştirmede en büyük gelişmeler yaşlı yetişkinlere yöneliktir. Yaşlı yetişkin popülasyonunun önemli ölçüde artacağı düşünüldüğünde, bu popülasyonda enfeksiyonların önlenmesi bir halk sağlığı önceliğidir.[11]

Virüs Benzeri Partiküller (VLP)

Yeni aşı teknolojilerinden olan VLP'ler, aşılarda yalnızca nükleik asitlerin bulunması ve patojenin tamamından ziyade sentetik peptitveya polisakkarit antijenlerle geliştirilen konjuge aşılar, zayıflatılmış veya inaktif aşılara seçenek olabilecek aşı tasarımlarıdır. Ek olarak, kanser tedavisinde kullanılmakta olan hücresel aşılar da kullanımdadır.[37]

VLP'ler, virüsün nükleik asidi bulunmadan, viral kapsid proteinlerinin kendiliğinden birleşmesi ile meydana getirilen çoklu proteinlerden oluşmaktadır. Viral genom içermediklerinden replikasyon yeteneği yoktur.[38] İçerdiği yüksek dansiteli kapsid proteinleri sayesinde, bağışıklık yanıtın yüksek olmasını sağlayan bol miktarda viral epitop bulundurmaktadırlar.[39] Günümüzde HPV, HBV ve Plasmodium için lisanslı VLP aşılar yer almaktadır.[40]

Nükleik asit aşıları

HIV, HPV, Influenza, Ebola, Batı Nil virüsü, HBV, HCV, Sitomegalovirüs için geliştirilen DNA aşıları klinik çalışma aşamasındadır.[41] DNA aşılarının kısıtlılıkları, araştırmaların RNA bazlı aşılara yönelmesine neden olmuştur. Hücre çekirdeğine ulaşmaya gerek duyulmaması, hücreye girişinin yeterli olması RNA aşılarının avantajlarından bazılarıdır.[42] Ancak, RNA'nın molekül kararsızlığı ve in-vivo ortamda düşük verimde çalışması bu aşıların dezavantajlarını oluşturmaktadır. RNA bazlı aşı çalışmaları, genellikle kanserler üzerinde yoğunlaşmıştır.[43] Mikroorganizmalar için replike olmayan ve kendiliğinden çoğalan RNA aşıları olarak iki tür RNA aşısı üretilmiştir.[44] HIV, Rabies ve Zika virüs gibi virüsler için RNA bazlı aşıların çeşitli klinik çalışmaları yer almaktadır.[45]

Nükleik asit aşıları, genomun plazmid içerisinde aktarımı ya da mRNA'nın doğrudan uygulaması temeline dayanmaktadır. Endojen protein senteziyle doğal enfeksiyon sürecini taklit etmektedirler. Bu sayede T hücre yanıtına ek olarak antikor yanıtı da oluşmaktadır.[46] Veterinerlikte lisanslı DNA aşıları kullanılmaktadır.[47] COVID-19 pandemisi döneminde insanlar üzerinde yeni geliştirilen mRNA aşıları hücre kültüründe çoğaltma ihtiyacı olmadan sentetik biyolojik metotlarla hızlı bir biçimde üretilebilmektedir. Alman (Biontech-Pfizer) ve Amerikan (Moderna) menşeli COVID-19 aşıları bu aşıların örneğidir.[48]

Nanoaşılar

Nanoaşılar hem hızlı hem de uzun süreli humoral ve hücresel bağışıklığı indükleme potansiyeline sahiptir. Çoğunlukla oral, intravenöz, intranazal ve transdermal olmak üzere birkaç yöntem kullanılarak uygulanmaktadır.[49-51] Nanoaşıların temel çalışma prensibi, bağışıklık hücresi tarafından tanınmayı kolaylaştırmak için nanopartikülleri virüslerin boyutuna ve şekline benzetmektir.[49] Nanoaşı-

lar, bağışıklık sistemini uyarmak için bileşenlere bağlanan veya bu bileşenlerle özel olarak hazırlanan nano ölçekli parçacıklardan oluşmaktadır. Bu aşılar, hastalıklarla mücadele etmek için vücudun bağışıklık tepkisini uyarma kapasitesini kanıtlamıştır. Grip, salmonelloz, leishmaniasis, kuduz ve bruselloz gibi çeşitli zoonotik hastalıkları tedavi ve tespit etmek için nano bazlı formülasyonların oluşturulmasında büyük ilerleme kaydedilmiştir. Bu formülasyonlar arasında adjuvanlanmış pDNA hidrojeli, arginin bazlı nanotaşıyıcılar, kuantum noktaları ve poli-konjuge nanomiseller, mannosillenmiş tiyolatlı kitosan yer almaktadır.[52] Lipozom, VLP, polimerik nanopartikül, nanojel, lipid nanopartikül, eksozom ve emülsiyonlar enfeksiyon hastalıklarının profilaksisi için kullanılan nanoaşı türleridir.[53]

SARS-CoV-2 mRNA bazlı nanopartikül aşıları, Moderna tarafından Ulusal Alerji ve Bulaşıcı Hastalıklar Enstitüsü (NIAID) ile iş birliği içinde geliştirilmiştir.[54] Lipid nanopartikülleri içerisine kapsüllenen mRNA, spike proteinini kodlamaktadır.[55,56] Pfizer ve BioNTech tarafından geliştirilen BNT162 (b1, b2) aşısı, reseptör bağlama alanının bir trimerini kodlayan mRNA içermektedir.[53]

İnorganik partiküller

İnorganik partiküller, immün cevabın güçlendirilmesi için adjuvan ve antijen taşıyıcı bir araç olarak kullanılmaktadır. Altın, aluminyum, kalsiyum fosfat ve silika aşı geliştirme yöntemlerinde en fazla kullanılan inorganik partiküllerdir.[57] Altın parçacıkları kararlı bir yapıya sahiptir, farklı biçim ve büyüklüklerle rahatlıkla sentezlenebilmektedirler.[58] Yüzeyleri kolaylıkla değiştirilebildiğinden, uygulamada antijen konjugasyonunu kolaylaştırmaktadır.[59] Melanom, Influenza ve HBV gibi hastalıklar için farklı aşı çalışmalarında taşıyıcı olarak kullanılmıştır.[60,61] Altına ek olarak, aluminyum parçacıklarının da immün sistemi indüklemek için taşıyıcı ve adjuvan görevi gördüğü bildirilmiştir.[62,63]

İmmün cevabı uyaran kompleksler

Fosfolipidler, kolesterol, saponin ve protein antjenlerin kombine edilmesiyle kendi kendine meydana gelen kafes benzeri parçacıklardan oluşmaktadır.[64] İmmün cevabı uyaran kompleksin meydana gelmesi için amfipatik proteinler kullanılması gerekmektedir. Bu kompleksin alternatif bir formu olan "ISCOMATRIX" antijenler bulunmadan formülasyonu yapılan bir adjuvandır.[65] Hemen hemenbütün antijenler potansiyel olarak ISCOMATRIX adjuvanıyla birleşebileceğinden, bu metot kolaylık sağlamaktadır. Bu kompleks profilaktik ve terapotik aşıların klinik çalışmaları için kullanılmaktadır.[66]

Bitki benzeri parçacıklar

Kullanımı kolay, hızlı ve bol miktarlarda üretilebilmesi, maliyetinin düşük olması, kitle aşılamalarında kolaylıkla kullanılabilmesi gibi avantajlarından dolayı oral yolla uygulama için kullanışlı bir aşı yöntemidir.[67] Bitkilerdeki hücre duvarının, hücre içerisindeki maddeleri midenin asitli ortamından koruyabilmesi bu parçacıkların avantajlarından bir tanesidir. Aşılarda kullanılmak üzere antijen parçacıkları üretmek için transgenik bitki hücrelerinin üremesi sağlanmaktadır.[68] Patates, domates, muz, marul, havuç, tütün vb. bitkilerle Influenza, HBV ve Bacillus anthracis gibi farklı mikroorganizmalar için bitki hücresi bazlı aşı yöntemleri keşfedilmiştir.[67] Ayrıca alg bazlı aşılar da içerdiği hücre duvarı nedeniyle bu yöntemlerin içerisinde yer almaktadır. Bu aşıların bitki bazlı aşılara göre, genetik açıdan kolay değiştirilebilmeleri, biyoreaktörler aracılığıyla yetiştirilebilmeleri ve büyümeleri için geniş alanlar, iklim şartlarıya da uzun bir zaman gerektirmemeleri gibi bazı avantajları bulunmaktadır.69 HPV, HBV vb. etkenlerin, el ayak ağız hastalığı ve diabetes mellitus gibi farklı hastalıkların profilaksisi için alg bazlı aşı araştırmaları yer almaktadır.[67]

Gelecek Çalışmalar

Son yıllarda aşı geliştirme çalışmalarında kayda değer ilerlemeler olmasına rağmen, tüm aşılar konakçıda bir bağışıklık tepkisi oluşturamamakta ve ömür boyu koruma sağlayamamaktadır. Günümüzde kullanımda olan mevcut aşıları geliştirmek için, özellikle antijenik değişkenliğe sahip veya *S. pneumoniae* gibi çoklu serotiplere sahip pato-

jenler ve patojenik ekzotoksinler için aşı ihtiyaçlarını ele alacak yeni stratejilere ihtiyaç bulunmaktadır.[70] Rekombinant DNA teknolojilerindeki ilerlemeler, konak patogenezinde yer alan alt birimleri veya toksin reseptörlerini hedef alarak çok sayıda yeni aşı stratejisinin geliştirilmesini sağlayabilir. Ancak geliştirilen aşıların başarısı, aşı güvenliği, aşı geliştirmedeki zorluklar, maliyet, fayda ve riskler konusundaki dengenin sağlanmasıyla mümkün olmaktadır. Geliştirilecek olan aşıların başarısı, her patojen veya toksin için hangi teknolojinin tek başına veya kombinasyon halinde optimal olduğunun belirlenmesine ve konak yanıtına bağlıdır.[71]

Botulinum ve tetanoz toksini aşıları için yapılan son araştırmalarda, toksin proteininin tamamının en güçlü bağışıklık tepkisi sağladığını, genel yapı ve antijenik epitopları korurken toksinin biyolojik aktivitesini sıfırlamak için az sayıda amino asit ikamesinin uygulanmasının mümkün olduğu ifade edilmiştir. Ayrıca, biyolojik olarak boş bir rekombinant proteinden oluşan bir aşı oluşturmak için tek amino asit kalıntılarını seçici olarak mutasyona uğratmaya yönelik bir fikir, rekombinant alt birim aşıların güvenlik profilini geliştirmek için de gelecekte kullanılabilir. Bu yaklaşım, mRNA kodunun biyolojik olarak aktif olmayan ancak oldukça immünojenik bir protein üretecek şekilde kolayca değiştirilen mRNA aşılarına da kolaylıkla uygulanabilir. Gelecekteki umut veren fikirlerden bir tanesi de rekombinant toksinleri ve alt birim proteinlerinin kullanıldığı toksisite araştırmaları ile mRNA teknolojisinin birleşimidir. Sonuç olarak, aşı teknolojisindeki son gelişmeler ve patogenezde yer alan moleküler mekanizmalara ilişkin anlayışlar, yeni aşı çalışmaları için umut verici bir gelecek sunmaktadır.

SONUÇ

Yeni aşı teknolojileri geliştirilirken, mikroorganizmaların önceden var olan varyasyonlarının yanı sıra mutasyona uğrama, bağışıklık sisteminin etkisinden ve aşıların sağladığı korumadan kaçma gibi yetenekleri de dikkate alınmalıdır. Konağın bağışıklık sistemi ile patojenler arasındaki etkileşimin daha iyi anlaşılması aşıların geliştirilebilmesine büyük katkılar sağlayacaktır. Günümüzde, aşısı olmayan çok sayıda enfeksiyon hastalığı bulunmakta olup, muhtemel pandemiler göz önünde bulundurulduğunda yeni aşı teknolojilerine olan ihtiyaç giderek artmaktadır. Patojen ve konak arasındaki ilişkilerin yeterince anlaşılması, bir aşının etkili ve evrensel olabilmesini sağlayacak ve aşıların daha iyi tasarlanmasına yardımcı olacaktır.[72]

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Identification of Anaerobic Bacteria Isolated from Clinical Samples and Determination of Antibiotic Resistance Profiles

Klinik Örneklerden İzole Edilen Anaerop Bakterilerin Tiplendirilmesi ve Antibiyotik Direnç Profillerinin Belirlenmesi

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Abstract

Aim In this study, it was aimed to identify anaerobic bacteria isolated from various clinical samples, and to determine their antibiotic resistance by gradient method (E-test).

Material and Method The study was carried out between January 15 and November 1, 2021. The 213 of 863 samples were included in the study. Anaerobic strains were isolated by conventional methods and identified by an automated system. Antimicrobial susceptibility was determined by the gradient method according to the Clinical and Laboratory Standards Institute (CLSI) criteria.

Results Anaerobic bacteria were detected in 10.3% of the samples (n=22), aerobic/facultative anaerobic bacteria were detected in 34.8% (n=74), while growth was not observed in 54.9% (n=117) of the samples. The 76.9% of the samples (n=164) were abscess. The 72.7% (n=16) of anaerobic bacteria were Gram positive bacteria, and 27.3% (n=6) were Gram negative bacteria. The most common species were; Cutibacterium (22.7%, n=5), Actinomyces (18.3%, n=4), Prevotella (13.7%, n=3), Bacteroides (9.1%, n=2), Anaerococcus (9.1%, n=2), Clostridium species (9.1%, n=2). The antibiotic susceptibilities of all anaerobic bacteria were as following; moxifloxacin (95.5%, n=21), piperacillin-tazobactam (95.5%, n=21), amoxicillin-clavulanic acid (95.5%, n=21), cefoxitin (90.9%, n=20), meropenem (90.9%, n=20), clindamycin (77.3%, n=16), ampicillin (59.1%, n=13), and metronidazole (22.7%, n=5), respectively. The susceptibility rates of Gram positive bacilli were 91.7% (n=11) for ampicillin, amoxicillin-clavulanic acid, piperacillin-tazobactam, cefoxitin, moxifloxacin, meropenem, and 75% (n=9) for clindamycin. In Gram positive cocci, susceptibility to ampicillin was 50% (n=2), susceptibility to amoxicillin-clavulanic acid, piperacillin-tazobactam, cefoxitin, clindamycin, moxifloxacin were 100% (n=4), and to meropenem was 75% (n=3). The susceptibility rates for Gram-negative bacilli were 0.0% (n=0) for ampicillin, 100% (n=6) for amoxicillin-clavulanic acid, piperacillin-tazobactam, moxifloxacin, meropenem, 83.3% (n=5) for metronidazole, 66.7% (n=4) for cefoxitin, and 50% (n=3) for clindamycin.

Conclusion In our study, it was observed that the sensitivity rates for especially, metronidazole and ampicillin were low among anaerobic bacteria. The resistance profile of many anaerobic bacteria has changed significantly over the past decade, making the antimicrobial susceptibility of anaerobic bacteria unpredictable. For this reason, revealing and documenting local data on this subject at regular intervals will constitute an important reference for both empirical treatment, public health, and surveillance studies.

Keywords Anaerobe, *Cutibacterium spp.*, antibiotic concentration gradient method, E-test, metronidazole.

Özet

Amaç *Bu çalışmada, çeşitli klinik örneklerden izole edilen anaerop bakterilerin tanımlanması ve gradiyent yöntemi (E-test) ile antibiyotik dirençlerinin belirlenmesi amaçlandı.*

Gereç ve Yöntem *Çalışma, 15 Ocak - 1 Kasım 2021 tarihlerinde gerçekleştirildi. 863 örneğin 213'ü çalışmaya dahil edildi. Anaerop suşlar konvansiyonel yöntemlerle izole edildi ve otomatize sistemle tanımlandı. Antibiyotik duyarlılıkları Clinical and Laboratory Standards Institute (CLSI) kriterlerine göre gradiyent yöntemi ile belirlendi.*

Bulgular *Örneklerin %10,3'ünde (n=22) anaerop bakteri, %34,8'inde (n=74) aerop/fakültatif anaerop bakteri tespit edilirken, %54,9'unda (n=117) üreme görülmedi. Örneklerin %76,9'u (n=164) apse materyaliydi. Anaerop bakterilerin %72,7'si (n=16) Gram pozitif bakteri, %27,3'ü (n=6) Gram negatif basildi. En yaygın türler; Cutibacterium (%22,7; n=5), Actinomyces (%18,3; n=4), Prevotella (%13,7; n=3), Bacteroides (%9,1; n=2), Anaerococcus (%9,1; n=2), Clostridium türleri (%9,1; n=2) idi. Sırasıyla, tüm anaerop bakterilerin antibiyotik duyarlılıkları; moksifloksasin (%95,5; n=21), piperasilin-tazobaktam (%95,5, n=21), amoksisilin-klavulonik asit (%95,5; n=21), sefoksitin (%90,9; n=20), meropenem (%90,9; n=20) klindamisin (%77,3; n=16), ampisilin (%59,1; n=13) ve metronidazol (%22,7; n=5) idi. Gram pozitif basillerin duyarlılık oranları ampisilin, amoksisillin-klavulanik asit, piperasilin-tazobaktam, sefoksitine, moksifloksasin, meropenem için %91,7 (n=11), klindamisin için %75'di (n=9). Gram pozitif koklarda ampisiline duyarlılık %50 (n=2), amoksisillin-klavulanik asit, piperasilin-tazobaktam, sefoksitin, klindamisin, moksifloksasine duyarlılık %100 (n=4) iken, meropeneme duyarlılık %75'di (n=3). Gram negatif basillerde duyarlılık oranları ampisilin %0,0 (n=0), amoksisillin-klavulanik asit, piperasilin-tazobaktam, moksifloksasin, meropenem için %100 (n=6), metronidazol %83,3 (n=5), sefoksitin için %66,7 (n=4) ve klindamisin için %50 (n=3) idi.*

Sonuç *Çalışmamızda anaerop bakterilerde özellikle metronidazol ve ampisilin için duyarlılık oranlarının düşük olduğu gözlemlendi. Birçok anaerop bakterinin direnç profilinin, son on yılda önemli ölçüde değişmesi, anaerop bakterilerin antimikrobiyal duyarlılıklarını fazla tahmin edilemez hale getirmiştir. Bu sebeple bu konudaki lokal verilerin belli aralıklarla ortaya çıkarılması ve dökümante edilmesi hem ampirik tedavinin şekillenmesi, hem halk sağlığı, hem de surveyans çalışmaları için önemli bir referans oluşturacaktır.*

Anahtar Kelimeler *Anaerop, Cutibacterium spp., antibiyotik konsantrasyon gradiyent yöntemi, E-test, metronidazol.*

INTRODUCTION

Anaerobic bacteria are commensal in the microbiota of different parts of the human body such as the gastrointestinal tract, genital tract and mouth. These microorganisms, which do not cause infection under normal conditions, can become pathogenic as a result of translocation of bacteria due to disruption of tissue integrity or overgrowth due to impaired blood circulation and decreased oxygenation. Following the identification of pathogenic anaerobic bacteria in the mid-19th century, anaerobes could be overlooked because it was technically difficult to obtain pure cultures of microorganisms. The use of inadequate anaerobic incubation techniques often only allowed the isolation of the most common anaerobic pathogens, members of the Bacteroides fragilis group or Clostridium perfringens, known as 'moderate' anaerobes, which survive at oxygen levels of 2-8%.[1-2] This may allow microbiologists to consider anaerobic culture techniques to be completely adequate because they can regularly isolate 'anaerobes'. Accurate and practical identification of anaerobic bacteria at the species level is challenging because it requires speed and precision at every stage, from the selection of the appropriate sample type, to sample collection, transfer to the laboratory and diagnostic procedures. Nowadays, there are a variety of commercial kits, specialized media and instruments for the isolation, typing and antimicrobial susceptibility testing of many anaerobic bacteria in microbiology laboratories.[1-4]

The resistance profile of anaerobic bacteria has changed significantly in the last decade, both within and between countries. This makes it difficult to predict the antimicrobial susceptibility of anaerobic bacteria.[5] Therefore, periodically collecting and documenting local data on this subject is an important reference for shaping empirical treatment, public health and surveillance studies. According to the international guidelines, antimicrobial susceptibility testing (AST) of anaerobic bacteria is very expensive, time-consuming and requires experienced laboratory personnel, and therefore cannot be performed for every isolate in routine laboratories. According to the international guidelines, the use of the disk diffusion method for AST of anaerobic bacteria is not recommended. The agar dilution method is currently the gold standard for AST of anaerobic bacteria. Standard broth microdilution method is difficult to standardize as there is no homogeneous growth of anaerobic bacteria except Bacteroides spp..[6] Antimicrobial concentration gradient method (E-test) is the most commonly used test for anaerobic AST in routine laboratories. Minimum inhibition concentration (MIC) values obtained by the E-test are considered reliable and correlate well with the reference method.[3,7]

In this study, we aimed to identify anaerobic bacteria isolated from various clinical specimens of patients with suspected anaerobic infection at species level, and to determine their antibiotic resistance profiles by antimicrobial concentration gradient method E-test (BioMerieux Inc, Marcy L'Etoile, France).

MATERIALS and METHODS

This is a prospective descriptive study conducted between January 15 and November 1, 2021 with the approval of the Cukurova University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (TTU-2020-13333).

The study included clinical specimens such as tissues, aspirates, blood and other body fluids taken from the relevant foci of patients with suspected anaerobic infections such as diabetic foot ulcers, head and neck infections, breast abscess, brain abscess, psoas abscess, osteomyelitis, bacteremia, peritonitis in various clinics of Cukurova University Faculty of Medicine Balcalı Hospital. A total of the 863 clinical specimens sent to the microbiology laboratory, specimens that were not taken under sterile conditions, specimens that were not sent to the laboratory immediately after collection, specimens with missing patient information, specimens that were not suitable for anaerobic culture such as sputum, tracheal aspirate, bronchoalveolar

lavage, stool, midstream urine, skin swab specimens taken from body parts in contact with air were excluded (n=650) from the study. Abscess and body fluid samples were aspirated with a syringe, and tissue samples were taken in sterile saline in sterile small containers, and transported to the Microbiology Laboratory within 20 minutes without any delay. The samples were examined macroscopically for the presence of purulent, bloody mucus, foul odor and sulfur granules, and microscopically for the presence of polymorphonuclear leukocytes, pleomorphic staining, and spore formation by Gram staining and Giemsa staining.

For simultaneous anaerobic and aerobic cultures of the samples, 5% sheep blood Columbia Agar, Chocolate Agar PolyVitex, 5% sheep blood Schaedler agar, 5% sheep blood Schaedler Kanamycin Vancomycin Agar were used, and thioglycolate broth containing resazurin was used as enrichment media in the absence of growth (all media by BioMerieux Inc, Marcy L'Etoile, France). The seeded plates were placed in a 2.5 L anaerobic jar with Gas-Pak (GENboxanaer, BioMerieux Inc, Marcy L'Etoile, France) kit and an anaerobic medium indicator (Merck KGaA, Germany), and incubated at 35-37 °C for 48-72 hours. When the color of the indicator strip changed from blue to white, anaerobic environment was considered to be achieved. If no color change was observed on the strip within 1-2 hours, the procedures were repeated. After 48-72 hours of incubation, aerobic and anaerobic growths were examined and compared macroscopically, and evaluated microscopically by Gram staining. If colonies with the same morphologic structure grew in both media, facultative anaerobic bacteria were determined. When growth occurred only in anaerobic media, colonies were subjected to aerotolerance test.2 When anaerobic growth was observed in the test, the bacterium was considered obligate anaerobe. For identification, the automated diagnostic system VITEK 2 (BioMerieux Inc, Marcy L'Etoile, France) was used together with staining characteristics, morphology, susceptibility to colistin (10 µg), kanamycin (1000 µg) and vancomycin (5 µg) discs (Bioanalyse Inc., Ankara, Turkey).

Susceptibility tests of anaerobic bacteria identified at species level against ampicillin, amoxicillin clavulonic acid, piperacillin tazobactam, cefoxitin, meropenem, clindamycin, metronidazole, and moxifloxacin were performed using the antimicrobial concentration gradient method E-test (BioMerieux Inc, Marcy L'Etoile, France) with Brucella Blood Agar (BioMerieux Inc, Marcy L'Etoile, France) as media, and incubated at 35°C for 48 hours in an anaerobic atmosphere. All isolated and identified anaerobic bacteria were tested for beta-lactamase production by chromogenic Nitrocefin disk (Bioanalyse Inc., Ankara, Turkey). Bacteroides fragilis ATCC 25285 and Clostridium difficile ATCC 700057 standard strains were used as quality control strains. Antimicrobial susceptibility testing was interpreted according to the clinical breakpoints recommended by the Clinical and Laboratory Standards Institute (CLSI, 2012).[8]

RESULTS

Anaerobic bacteria were isolated in 10.3% (n=22) of 213 clinical samples cultured (Table 1). Of these, 17 (7.98%) grew as pure anaerobic bacteria and 5 (2.3%) as mixed with aerobic bacteria. Aerobic/facultative anaerobic bacteria growth was detected in 34.8% (n=74) of the cultures, including Staphylococcus spp. in 32, Escherichia coli in 19, Klebsiella spp. in 11, Streptococcus spp. in five, Pseudomonas spp. in three, Enterobacter spp. in three and Enterococcus spp. in one. No growth was observed in 54.9% (n=117) of the cultures.

The clinical samples from which anaerobic bacteria were isolated were abscess (n=11, 50%), blood (n=7, 31.8%), pleural fluid (n=2, 9.2%), cornea (n=1, 4.5%) and cerebrospinal fluid (n=1, 4.5%). Of the 5 samples with mixed growth, 4 were abscess samples and the associated aerobic bacteria were Escherichia coli (n=2), Proteus mirabilis in one sample, Staphylococcus epidermidis in one sample and Enterococcus avium in one sample.

Among the patients with anaerobic bacterial infection,

54.5% (n=12) were male and 45.5% (n=10) were female. Of these patients, 9% (n=2) were in the age group of 18 years and younger, 77.2% (n=17) were between 18-60 years, and 13.6% (n=3) were 60 years and older.

Table 1. Type of the clinical specimens and distribution of the isolated bacteria.

Sample type	Number of samples (n*, %)	Number of samples which only anaerobic bacteria were isolated (n, %)	Number of samples which aerobic/ facultative anaerobic bacteria were isolated (n, %)	Number of samples with mixed growth (n, %)
Abscess	164 (76.9)	7 (4.27)	58 (35.36)	4 (2.44)
Blood	10 (4.7)	7 (70)	2 (20)	None
Pleural fluid	12 (5.6)	2 (16.67)	8 (66.67)	None
Peritoneal fluid	7 (3.3)	None	5 (71.43)	None
Cornea	16 (7.6)	1 (6.25)	1 (6.25)	None
CSF**	1 (0.5)	None	None	1 (100)
Pericardial fluid	3 (1.4)	None	None	None
Total	213 (100)	17 (7.98)	74 (34.74)	5 (2.35)

* Number of patients
**CSF; Cerebrospinal fluid

Of the anaerobic bacterial isolates, 6 were Gram negative bacilli (27.3%) and 16 were Gram positive (72.7%), of which 12 were bacillus (54.54%) and 4 were cocci (18.18%). The most common anaerobes isolated were Propionibacterium/Cutibacterium species (22.7%, n=5), followed by Actinomyces species (18.3%, n=4), Prevotella species (13.7%, n=3), Bacteroides species (9.1%, n=2), Anaerococcus species (9.1%, n=2), Clostridium species (9.1%, n=2), Fusobacterium species (4.5%, n=1), Lactobacillus species (4.5%, n=1), Parvimonas micra (4.5%, n=1) and Peptoniphilus assacharolyticus (4.5%, n=1).

The β-lactamase positivity was detected in 4 of the anaerobic bacteria isolated (4/22, 18.2%). Two of the β-lactamase positive bacteria were Prevotella spp., one was Anaerococcus spp. and the other was Bacteroides spp (Table 2). Antimicrobial susceptibility profiles of the isolated anaerobic bacteria are summarized in Table 2. The most active antimicrobials were moxifloxacin (95.5%, n=21), piperacillin-tazobactam (95.5%, n=21) and amoxicillin-clavulonic acid (95.5%, n=21), followed by cefoxitin (90.9%, n=20), meropenem (90.9%, n=20), clindamycin (77.3%, n=16), ampicillin (59.1%, n=13) and metronidazole (22.7%, n=5). The susceptibilities of anaerobes to ampicillin were 91.7% (n=11) for Gram positive bacilli and 50% (n=2) for Gram positive cocci, while resistance was observed in all anaerobic Gram negative bacilli. For amoxicillin-clavulanic acid and piperacillin-tazobactam, anaerobic Gram positive bacilli showed 91.7% (n=11), Gram positive cocci 100% (n=4) and Gram negative bacteria 100% (n=6) susceptibility. The 91.7% (n=11) of anaerobic Gram positive bacilli, 100% (n=4) of Gram positive cocci and 66.7% (n=4) of Gram negative bacteria were susceptible to cefoxitin, while 33.3% (n=2) of Gram negative bacteria were moderately susceptible. The 75% (n=9) of anaerobic Gram positive bacilli, 100% (n=4) of Gram positive cocci and 50% (n=3) of anaerobic Gram negative bacilli were sensitive to clindamycin. While 83.3% (n=5) of anaerobic Gram negative bacilli were susceptible to metronidazole, 100% (n=16) resistance was observed in anaerobic Gram positive bacteria. Susceptibility to moxifloxacin was 91.7% (n=11) in anaerobic Gram positive bacilli and 100% in Gram positive cocci and Gram negative bacteria (n=10). Susceptibility to meropenem was 91.7% in anaerobic Gram positive bacilli, 75% (n=11) in Gram positive cocci and 100% (n=6) in Gram negative bacilli.

Table 2. Antimicrobial susceptibility profiles and β-lactamase test results of isolated anaerobic bacteria.

Isolated bacteria (Isolation number)	Ampicillin (µg/mL)	Amoxicillin-clavulanic acid (µg/mL)	Piperacillin-tazobactam (µg/mL)	Meropenem (µg/mL)	Metronidazole (µg/mL)	Clindamycin (µg/mL)	Moxifloxacin (µg/mL)	Cefoxitin (µg/mL)	β- lactamase
	MIC 0.016-256 µg/mL	MIC 0.016-256 µg/mL	MIC 0.016-256 µg/mL	MIC 0.002-32 µg/mL	MIC 0.016-256 µg/mL	MIC 0.016-256 µg/mL	MIC 0.002-32 µg/mL	MIC 0.016-256 µg/mL	
Gram positive anaerobes									
Anaerococcus prevotii* (5)	8 (R)	1.5 (S)	1.5 (S)	>32 (R)	>256 (R)	0.25 (S)	1.5 (S)	16 (S)	-
Anaerococcus prevotii* (43)	3 (R)	0.75 (S)	0.75 (S)	1.5 (S)	>256 (R)	0.125 (S)	0.125 (S)	12 (S)	+
Peptoniphilus asaccarolyticus (17)	0.023 (S)	0.032 (S)	0.032 (S)	0.012 (S)	>256 (R)	0.047 (S)	0.015 (S)	0.094 (S)	-
Parvimonas micra (208)	0.064 (S)	0.125 (S)	0.047 (S)	0.008 (S)	>256 (R)	0.064 (S)	0.125 (S)	4 (S)	-
Actinomyces naeslundii* (149)	0.032 (S)	0.023 (S)	0.123 (S)	0.004 (S)	>256 (R)	0.25 (S)	0.38 (S)	0.125 (S)	-
Actinomyces naeslundii* (190)	0.064 (S)	0.064 (S)	0.094 (S)	0.023 (S)	>256 (R)	>256 (R)	0.25 (S)	0.125 (S)	-
Actinomyces naeslundii* (27)	0.016 (S)	0.023 (S)	0.064 (S)	0.004 (S)	>256 (R)	0.047 (S)	0.125 (S)	0.032 (S)	-
Actinomyces naeslundii* (171)	0.032 (S)	0.023 (S)	0.064 (S)	0.032 (S)	>256 (R)	0.75 (S)	0.38 (S)	2 (S)	-
Cutibacterium acnes* (195)	0.032 (S)	0.023 (S)	0.125 (S)	0.016 (S)	>256 (R)	0.032 (S)	0.094 (S)	0.19 (S)	-
Cutibacterium acnes* (73)	0.047 (S)	0.047 (S)	0.19 (S)	0.47 (S)	>256 (R)	0.064 (S)	0.125 (S)	0.19 (S)	-
Cutibacterium acnes* (91)	0.047 (S)	0.032 (S)	0.125 (S)	0.008 (S)	>256 (R)	0.19 (S)	0.125 (S)	0.125 (S)	-
Cutibacterium gronulosum* (11)	0.25 (S)	0.025 (S)	1 (S)	0.125 (S)	>256 (R)	>256 (R)	0.047 (S)	0.75 (S)	-
Cutibacterium gronulosum* (84)	0.064 (S)	0.094 (S)	0.047 (S)	0.064 (S)	>256 (R)	0.032 (S)	0.064 (S)	0.19 (S)	-
Lactobacillus plantarum (103)	0.094 (S)	0.38 (S)	1 (S)	0.094 (S)	>256 (R)	0.016 (S)	1.5 (S)	>256 (R)	-
Clostridium group (142)	0.047 (S)	0.016 (S)	0.016 (S)	0.004 (S)	>256 (R)	0.023 (S)	0.094 (S)	0.047 (S)	-
Clostridium subterminale (211)	>256 (R)	>256 (R)	>256 (R)	>32 (R)	>256 (R)	>256 (R)	>32 (R)	>256 (R)	-
Gram negative anaerobes									
Bacteroides fragilis (32)	4 (R)	0.19 (S)	0.5 (S)	0.094 (S)	1 (S)	1 (S)	0.125 (S)	12 (S)	-
Bacteroides thetaiotamicron (61)	>256 (R)	4 (S)	16 (S)	0.38 (S)	0.38 (S)	2 (S)	1 (S)	24 (I)	+
Fusobacterium necrophorum (127)	>256 (R)	0.125 (S)	0.094 (S)	0.032 (S)	>256 (R)	>256 (R)	0.016 (S)	0.047 (S)	-
Prevotella bivia (205)	>256 (R)	4 (S)	8 (S)	0.125 (S)	0.25 (S)	>256 (R)	0.75 (S)	16 (S)	+
Prevotelle buccae (199)	>256 (R)	0.75 (S)	0.5 (S)	0.023 (S)	0.125 (S)	>256 (R)	0.019 (S)	0.5 (S)	+
Prevotelle oralis (74)	12 (R)	0.19 (S)	8 (S)	0.125 (S)	0.19 (S)	1.5 (S)	0.5 (S)	24 (I)	-

S: Sensitive, I: Moderately sensitive, R: Resistant, n: Number of patients, MIC: Minimum inhibitory concentration
*The same isolates were listed separately in the table, as their antibiotic susceptibilities were different.

DISCUSSION

Anaerobic bacteria constitute an important part of the human microbiome. They play an important role in various infections such as central nervous system, intraabdominal and foreign body infections, especially in polymicrobial infections.[9-11] Previous studies have shown that anaerobic bacteria isolated and their antimicrobial susceptibilities vary depending on the type of infection and hospital.[12-14] This situation shows the importance of local and national data in this regard.

Bacteroides, Prevotella, Propionibacterium/Cutibacterium species and Gram positive cocci are among the most frequently isolated anaerobic bacteria from clinical specimens.[11] In our study, pure anaerobic bacteria were isolated in 7.98% of 213 clinical specimens sent with the suspicion of anaerobic infection and cultured, of which 72.72% were Gram positive and 27.27% were Gram negative bacteria. The most frequently isolated anaerobic bacteria were Cutibacterium species (22.7%), Actinomyces species (18.1%) and Prevotella species (13.6%). In a few studies on anaerobic bacteria in our country, different rates of growth were found. In a study conducted in Afyon, a total of 4% anaerobic agents were reported in 5535 clinical samples sent for anaerobic culture in the three-year period between 2015-2017, of which 68% were Gram negative and 32% were Gram positive, and Propionibacterium/Cutibacterium acnes, Actinomyces spp and Clostridium spp. were the most common isolates.[15] In a study conducted in Diyarbakır, anaerobic bacteria were isolated from 73 (19.8%) of 368 clinical specimens; in 50 (13.6%) of these specimens, only anaerobic bacteria were isolated, and in 23 (6.3%) of these specimens, anaerobic bacteria as well as aerobic/facultative anaerobic agents were isolated.[16] In another study conducted in Konya, a total of 22 anaerobic bacteria were isolated from 14 of 100 clinical samples. In seven of these specimens, more than one anaerobic bacteria were found at the same time, while in eight samples anaerobic and facultative anaerobic bacteria were reported to grow together. The most frequently isolated bacteria were reported to be Bacteroides fragilis and Peptostreptococcus spp. And it was reorted that the two of six Bacteroides fragilis isolates were found to produce beta-lactamase enzyme, while the presence of beta-lactamase was not detected in other anaerobic strains.[17] In a study conducted in Sivas, no growth was observed in 409 (75.3%) of the samples, while various anaerobic bacteria were isolated in 134 samples (24.6%). And it was reported that Bacteroides spp. (29.9%), Peptopstreptococcus spp. (23.1%) and Propniobacterium spp. (20.2%) were the most common ones among the anaerobic bacteria isolated.[18] The reason why Bacteroides spp. were found to be the most common causative agent in the Konya and Sivas studies may be related to the higher number of intraabdominal samples. In a study conducted in Diyarbakır, Cutibacterium spp. were the most frequently isolated anaerobic bacteria in accordance with our study.[19] In a study conducted in Bulgaria, it was reported that Prevotella spp. were the most frequently isolated bacteria in abscess samples (22%).[20] These differences may be caused by the geographical location, age and other demographic characteristics of the patients, sample types and isolation methods.

In this study, anaerobic bacteria were mostly isolated from abscess materials. Of the cultured samples, 164 (77%) were abscesses, followed by blood (31.8%) and pleural fluid samples (9.1%). In other studies in which anaerobic bacteria were isolated, abscess specimens were reported most frequently, which is consistent with our study.[17-19,21]

It was previously reported that the antimicrobial resistance rates were increasing in anaerobic bacteria, which affected both treatment costs and mortality rates. And attention was drawn that regional susceptibility profiles were important in determining the empirical treatment of anaerobic infections.[11,22] In our study, 78% of the anaerobic bacteria for which AST was performed were resistant to metronidazole. And the resistance was mainly observed in Gram positive anaerobic bacteria (100%). This is indicative of intrinsic resistance found in most non-spore-forming

Gram positive anaerobic bacilli, especially Actinomyces, Propionibacterium/Cutibacterium and Lactobacillus species. Metronidazole showed good activity against most Gram negative bacilli (83.3%). In various countries of the world, metronidazole resistance rates of anaerobic bacteria have been reported in a wide and varied range, ranging between 1-58.3% in Gram positive and 1-50% in Gram negative bacilli.[11,21-22,23-26] Metronidazole resistance was reported as 96.2% for anaerobic Gram positive bacilli, 61.1% for Gram positive cocci and 33.3% for Gram negative bacilli in Diyarbakır, 0% for anaerobic Gram negative bacteria in Afyon and 94.9% for anaerobic Gram positive bacilli in Van.[15-16,27] In our study, the ampicillin resistance rate of anaerobic isolates that underwent AST was 40.9%, resistance was detected in 18.75% in Gram positive (8.3% in bacilli, 50% in cocci) and 100% in Gram negative isolates. In a study conducted in Malaysia, it was reported that the resistance rate of anaerobic Gram positive bacteria to ampicillin was 23.3% and that of Gram negative bacteria was 33.3%.[25] In various studies, penicillin resistance rates of anaerobic bacteria were reported as 30.8% in Gram positive bacilli, 19-50% in Gram positive cocci and 33.3-78.57% in Gram negative bacilli.[15-16,21,23,26] In almost all the studies, amoxicillin-clavulanic acid and piperacillin-tazobactam were reported to be the most susceptible antibiotics against anaerobic bacteria, consistent with our findings.[15,21,22,26,28] In this study, resistance to cefoxitin was found in 9.1%. The 12.5% of anaerobic Gram positive bacteria (bacilli 8.3%, cocci 0.0%) and 33.3% of Gram negative bacteria were resistant to cefoxitin. When domestic and foreign studies were analyzed, cefoxitin resistance was detected at quite different rates (3%-89%) in this bacterial group.[15-16,23,26,29-30] Variations in clindamycin susceptibility were also observed in the studies. In our study, clindamycin resistance was detected in 22.7%. The 18.7% of Gram positive bacteria (bacilli 25%, cocci 0.0%) and 50% of Gram negative bacilli were resistant to clindamycin. While these results were consistent with some studies,[23,26,28] our results were higher than the results of some other studies.[20,24] For example, in the study conducted in Bulgaria, which included mainly odontogenic abscess samples, Actiomyces spp. was most frequently isolated as Gram positive bacteria and Prevotella spp. as Gram negative bacteria, and resistance rates were tested by the agar dilution method. The resistance rates of isolates to clindamycin (2-3%) were significantly lower than this study.[20] The antimicrobial resistance profiles vary depending on geographical location, hospital centers, national antibiotic consumption, antimicrobials used for empirical therapy, diagnostic methods, bacterial species and sample types.[7,12]

In our study, susceptibility rates of anaerobic Gram positive bacteria were generally higher than those of Gram negative bacteria. The resistance to moxifloxacin was found at a rate of 4.5%. In addition, resistance was detected in 6.2% of Gram positives (8.3% of bacilli, 0.0% of cocci), while all Gram negatives were found to be susceptible to moxifloxacin. In contrast to this study, the studies conducted abroad, reported higher rates of resistance to moxifloxacin.[21,22-24,31] In a study conducted in Afyon, it was reported that no resistance to moxifloxacin was observed in Gram negative anaerobic bacteria in accordance with our results.[15] In this study, meropenem resistance of anaerobic bacteria was found to be 9.1%. It was found that 12.5% of Gram positive bacteria (bacilli 8.3%, cocci 25%) were resistant, and all Gram negative bacteria were susceptible. These results were compatible with the previous reports.[15,22-23,31]

Our study has some limitations. The molecular mechanism of resistance to antianaerobic drugs, risk factors that may cause resistance and their relationship with mortality have not been investigated. To understand the impact of antimicrobial resistance on patients and public health, it may be important to study the evolution and consequences of antimicrobial resistance in anaerobic bacteria together.

CONCLUSION

In our study, low susceptibility rates were observed especially for metronidazole and ampicillin in anaerobic bacteria. In particular, an alarming resistance rates of 77.3% to

metronidazole and 40.9% to ampicillin were detected. Due to the emergence of drug resistance in anaerobes, it would be useful to investigate newer and alternative options for patient management. It is time for susceptibility testing of anaerobic bacteria to become a routine service in microbiology laboratories. The data of this study can serve as a reference for monitoring resistance and determining empirical treatment, and can be used for periodic monitoring of resistance trends in surveillance studies.

Ethical Approval

The approval was obtained from the Cukurova University Faculty of Medicine Non-interventional Clinical Research Ethics Committee (No: 104, Date: 02.10.2020).

Peer-review

Externally and internally peer-reviewed.

Authorship Contributions

Consept: F.K., T.A., Design: F.K., T.A., H.H.G. Data Collection or Processing: F.K., T.A., H.H.G., Analysis and Interpretation: F.K., T.A., H.H.G., Literature Search: F.K., T.A., H.H.G., Writing: F.K., T.A., H.H.G.

Conflict of Interest

The authors declare no conflict of interest in relation to this article.

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The Effects of Dipeptidyl Peptidase-4 Inhibitors on Kidney Function in Advanced CKD

İleri Evre Kronik Böbrek Hastalarında Dipeptidil Peptidaz-4 İnhibitörlerinin Böbrek Fonksiyonu Üzerindeki Etkisi

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Abstract

Aim This study aimed to investigate the effects of DPP-4 inhibitors on kidney function in type 2 diabetes mellitus patients with stages 3-5 chronic kidney disease, focusing on estimated glomerular filtration rate and proteinuria.

Material and Method This is a retrospective case-control design, and data were collected from a single hospital's software and the Turkish Ministry of Health's National Data Tracking System. Diabetic patients with T2DM and CKD stages 3-5 were included, with dipeptidyl peptidase-4 inhibitor users (n=118) and non-users (n=48) forming the intervention and control groups, respectively. Baseline demographics, clinical characteristics, and outcomes were compared between groups.

Results At baseline, both groups demonstrated similar age, gender distribution, body mass index, and eGFR. Over a 12-month follow-up, while slight improvements in eGFR were observed in the intervention group and minor reductions in the control group, these changes did not reach statistical significance (p>0.05). Proteinuria showed a stable trend in the intervention group, whereas a significant increase was noted in the control group (p=0.035). Age significantly correlated with eGFR (p<0.001) but not proteinuria (p=0.156). The study found that DPP-4 inhibitor users experienced a statistically significant reduction in HbA1c levels (p=0.041) compared to minimal changes in the control group.

Conclusion The study suggests potential renoprotective effects of DPP-4 inhibitors in T2DM patients with advanced CKD, as evidenced by trends in eGFR and proteinuria stabilization.

Keywords Chronic kidney disease, DPP-4 inhibitors, eGFR, proteinuria, type 2 DM.

Özet

Amaç *Bu çalışmada, tahmini glomerüler filtrasyon hızı ve proteinüriye odaklanarak, evre 3-5 kronik böbrek hastalığı olan tip 2 diabetes mellitus hastalarında Dipeptidil Peptidaz-4 inhibitörlerinin böbrek fonksiyonu üzerindeki etkilerini araştırmayı amaçladık.*

Gereç ve Yöntem *Çalışma retrospektif vaka kontrol olarak tasarlandı. Veriler tek bir hastanenin yazılımından ve T.C. Sağlık Bakanlığı Ulusal Veri Takip Sisteminden toplanmıştır. Hastalar, vildaglatin kullanan (Müdahele; n=118) ve kullanmayanlar (kontrol; n=48) tip 2 diyabet ve aynı zamanda evre 3-5 kronik böbrek hstalarında oluşturuldu. Temel demografik özellikler, klinik özellikler ve sonuçlar gruplar arasında karşılaştırıldı.*

Bulgular *Başlangıçta, her iki grup da benzer yaş, cinsiyet dağılımı, vücut kitle indeksi ve tGFH gösterdi. 12 aylık takipte, müdahale grubunda tGFH'da hafif iyileşmeler ve kontrol grubunda hafif azalmalar gözlenirken, bu değişiklikler istatistiksel olarak anlamlı bulunmadı (p>0,05). Proteinüri müdahale grubunda istikrarlı bir eğilim gösterirken, kontrol grubunda anlamlı bir artış kaydedildi (p=0,035). Yaş, tGFH ile anlamlı korelasyon gösterirken (p<0.001) proteinüri ile korelasyon göstermedi (p=0.156). Çalışma da, vildagliptin kullanıcılarının, kontrol grubundaki minimum değişikliklerle karşılaştırıldığında HbA1c düzeylerinde istatistiksel olarak anlamlı bir düşüş (p=0,041) yaşadığını saptandı.*

Sonuç *Çalışma, tGFH ve proteinüri stabilizasyonundaki eğilimlerin kanıtladığı gibi, ilerlemiş kronik böbrek yetmezliği olan T2DM hastalarında DPP-4 inhibitörlerinin potansiyel renoprotektif etkilerini göstermektedir.*

Anahtar Kelimeler *DPP-4 inhibitörleri, kronik böbrek hastalığı, proteinüri, tahmini glomerüler filtasyon hızı, tip 2 DM.*

INTRODUCTION

Dipeptidyl peptidase 4 (DPP-4) inhibitors comprise a class of antihyperglycemic medications employed in the treatment of type 2 diabetes mellitus (T2DM), which is a notable predisposing factor for coronary disease, heart failure, stroke, and numerous cardiovascular events. Initially, it was thought that the enhancement of endocrine actions of glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP) solely accounted for the improvement in glycaemic control attributed to DPP-4 inhibitors. Nevertheless, current evidence indicates that this is unlikely to be the sole mechanism, as other pathways and mediators likely play a role.

Previous studies reported DPP-4 inhibitors are located on endothelial cells throughout the vascular system, including local capillaries of organs such as the kidney and heart.[1,2] The local blood concentrations of GIP and GLP-1 are higher than their systemic concentration.[3] The observed direct cardiac and renal effects in preclinical studies, along with findings from meta-analyses of clinical trials, indicate they may also have effects on non-glucose targets beyond their primary role of enhancing glycemic control.[2,4,5] In a narrative review, Daza-Arnedo et al.[6] discussed the renoprotective implications of DPP-4 inhibitors, highlighting their potential to mitigate inflammation, fibrosis, and oxidative damage.6 Fibrosis in diabetic kidney is thought to be due to microRNA29s suppression.[7] This was shown to be related to targeting both the TGF beta activation process and DPP-4 protein.[7] This study endeavors to examine the prospective advantages of DPP-4 inhibitors concerning estimated glomerular filtration rate (eGFR) and proteinuria among patients with CKD stage 3-5, irrespective of their influence on glucose regulation.

MATERIALS and METHODS

This retrospective case-control study was conducted between 2019-2020 years at our hospital. The data were recruited from our hospital's software and The Turkish Ministry of Health National Data Tracking System (E-Nabız). All procedures were conducted in accordance with ethical rules and the principles of the Declaration of Helsinki.

1. Case selection

Diabetic patients> 18 years with T2DM and CKD stage 3-5 were collected from the hospital software. The individuals who had at least three hospital visits with data of 12-month follow-up were enrolled. Hospitalization, death, prolonged infections, individuals who had a cause of a rapid deterioration in kidney functions other than T2DM, and the discontinuation or intermittent use of vildagliptin were considered for exclusion. We did not include patients on other oral antidiabetic agents. We also excluded patients with nephrotic syndrome (proteinuria over 3500 mg/day) at baseline (Figure 1.) Patients in stage 3 used vildagliptin 50 mg twice a day, while the dose for sage 4 and 5 was 50 mg once a day.

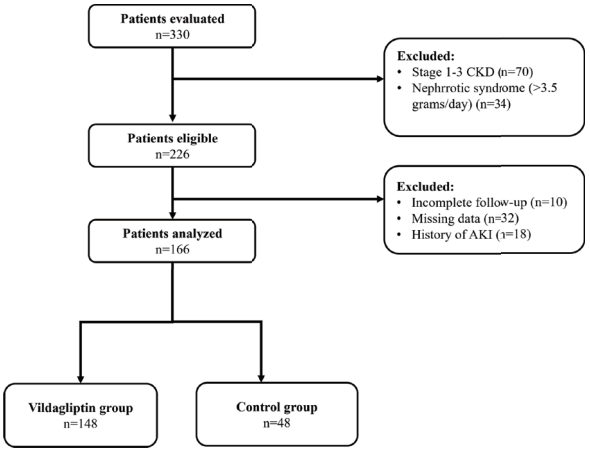

Figure 1. Flowchart of the study population

2. The comparison groups

The individuals with a diagnosis of T2DM were divided into vildagliptin users (intervention) and DPP-4 inhibitor-free (control) groups. In the intervention group, patients had received a DPP-4 inhibitor as an add-on to the previous antiglycemic regimen, while in the control group, the available treatment regimens were considered for adjustments. During the follow-up, antiglycemic regimens were modified according to clinical demands

3. Data collection

Duration of T2DM and DPP-4 inhibitor use, age, gender, body mass index (BMI), anti glycemic drugs, CKD stage, eGFR, proteinuria, and weight were noted at the onset of a DPP-4 inhibitor use. Comorbidities that potentially can damage the kidneys, such as heart disease, hypertension, glomerulonephritis, and polycystic kidney disease, were noted. The intervention and control groups were compared for eGFR and proteinuria at the end of the 12-month follow-up.

4. Measurements

BMI= weight (kg) / (height (m) X height (m), eGFR was calculated by using an online calculation program addressing Chronic Kidney Disease Epidemiology Collaboration 2009 Equation (CKD-EPI 2009), www.mdrd.com, and proteinuria was assessed by using spot urine creatinine-to-protein ratio (UPCR).

The study was carried out with the permission of the local Ethics Committee (Date: 13.12.2023, IRB no: E71522473/050.01.04/287).

5. Statistical Analysis

The variables were analyzed using SPSS Version 15.0 for Windows (IBM Corporation, Armonk, New York, United States). Continuous variables were presented as mean ± standard deviation and median (minimum/maximum), while categorical variables were expressed as n and (%). The distribution of the variables was assessed using the Kolmogorov-Smirnow test. The independent sample t-test and the Mann-Whitney U test were employed in the comparison of the parametric and nonparametric variables. Paired-sample t-Test was used to assess the variables before and after treatment. Categorical variables were compared using the Pearson Chi-Square test and the Fisher exact test Monte Carlo Simulation technique. Linear regression analysis was utilized to measure the effects of prognostic variables on eGFR and proteinuria. The analysis was performed at a 95% confidence level, and a p-value less than 0.05 was considered statistically significant.

RESULTS

A total of 166 participants were enrolled in the study (Figure 1, Table 1), with 118 participants in the intervention group and 48 in the control group (Table 2).

Table 1. Demographic and basal laboratory features of all participants

Characteristic	All patients No=166
Age, years	61.89±12.84
Gender, male/female, n	90/76
BMI, kg/m2	28.81±4.09
T2DM duration, years	13.27±6.75
Baseline eGFR, ml/min/1.73 m2	32.61±8.44
Proteinuria, mg/day (Basal)	213.12 (19-2854)
Baseline HgbA1c, %	8.03±1.15
Insulin users, yes/no, n, %	90(63.9) / 76(36.1)

Abbreviations: BMI: Body mass index, eGFR: estimated glomerular filtration rate, T2DM: type 2 diabetes mellitus

Table 2. Comparison of Basal demographic and laboratory features of all participants

Items	Vildagliptin Group (n=118)	Control Group (n= 48)	p
Age, years	61.89±12.23	62.96±14.38	0.862
Gender, male n, (%)	62 / (52.5)	28 (58.3)	0.497
DM duration, years	13.50±6.69	12.72±6.94	0.507
Basal BMI, kg/m2	28.86±4.17	28.64±3.94	0.803
eGFR, ml/min/1.73 m2	31.27±8.96	35.92±5.88	0.001
Proteinuria (mg/day)*	216.35 (16-3258)	199.05 (19-2740)	0.245
HgbA1c, mean ± SD	8.01±1.11	8.13±1.16	0.298
Basal Fasting glucose (mg/dl), mean ± SD	121.54±48.27	123±36.98	0.283
Uric acid (mg/dl), mean ± SD	6.72±1.78	6.62±1.75	0.726
Albumin (g/dl) mean ± SD	3.6±0.42	3.8±0.39	0.642
Sodium (mmol/L), mean ± SD	139.96±2.84	138±4.63	0.234
Potassium (mmol/L), mean ± SD	4.73±0.48	4.97±0.56	0.192
Calcium (mg/dl), mean ± SD	9.19±0.53	9.26±0.48	0.577

Abbreviations: BMI: Body mass index, eGFR: estimated glomerular filtration rate, T2DM: type 2 diabetes mellitus
*Presented as median (minimum-maximum).

The participants had a mean age of 61.89±12.84. The mean eGFR was 32.48±8.28 ml/min/1.73 m^2. Data regarding comorbidities and antihypertensive medicines were substantially lacking; thus, they were not considered for the assessment. The mean duration of T2DM and BMI were 13.27±6.75 years and 28.81±4.09 kg/m^2, respectively (Table 2). Oral antidiabetic drugs had been modified several times according to the clinical demands, so they were not considered for the assessment.

The two groups were similar in terms of age, gender distribution, BMI0 (at the onset of the study), BMI12 (at the end of the 12-month follow-up), and eGFR (p>0.05) (Table 3). eGFR showed a slight improvement in the intervention group and a slight reduction in the control group within the 12-month follow-up. However, the differences were not statistically significant (p>0.05). The duration of T2DM and BMI positively correlated with proteinuria (p=0.023 and r=0.268, p=0.010 and r=0.199, respectively). In the control group, proteinuria significantly increased at the 12th month (p=0.035), while in the intervention group, it remained stable. In the intervention group, HgbA1c reduced by 5.3%, and the change was statistically significant (p=0.041), while in the control group, HgbA1c reduced only 0.1% (p=0.975).

Age had an impact on eGFR (p<0.001 and r2=0.355) but not on proteinuria (p=0.156 and r2=0.09); however, BMI, T2DM duration, and gender did not have an impact on eGFR (p>0.05).

Table 3. Comparison of patient's outcomes before and after intervention in terms of primary endpoints

Items	Vildagliptin Group (n=118)	Control Group (n= 48)	p
BMI0, kg/m2, mean±SD	28.86±4.17	28.64±3.94	0.803
BMI12, kg/m2, mean±SD	28.37±4.09	28.39±4.10	0.901
eGFR0, ml/min/1.73 m2, mean±SD	31.27±8.96	35.92±5.88	0.001
eGFR12, ml/min/1.73 m2, mean±SD	31.83±10.83	34.59±6.99	0.163
Proteinuria0, mg/day*	216.35 (16-3258)	199.05 (19-2740)	0.245
Proteinuria12, mg/day*	222.15 (19-2854)	246.66 (21-2647)	0.082
HgbA1c0, %, mean±SD	8.01±1.11	8.13±1.16	0.298
HgbA1c12, %, mean±SD	7.58±1.66	7.99±1.57	0.059

Abbreviations: BMI0: Body mass index at the start of the study, BMI12: Body mass index after one year, DM: diabetes mellitus, eGFR0: Basal estimated glomerular filtration rate, eGFR12: estimated glomerular filtration rate after one year
*Presented as median (minimum-maximum)

DISCUSSION

The current study sought to investigate the potential effects of Dipeptidyl Peptidase-4 (DPP-4) inhibitors on kidney function in patients with Chronic Kidney Disease (CKD) stages 3-5, focusing on estimated glomerular filtration rate

(eGFR) and proteinuria, independent of their impact on glucose regulation. The discussion will delve into the findings of the study and their implications in the context of existing literature, highlighting the potential renoprotective effects of DPP-4 inhibitors.

The initial premise of DPP-4 inhibitors primarily targeting glycemic control through the enhancement of GLP-1 and GIP actions has evolved over time.[7,8] Emerging evidence suggests that these inhibitors may exert effects beyond glucose regulation.[9,10] Notably, DPP-4 inhibitors are found in various organs, including the kidneys and heart, where local concentrations of GLP-1 and GIP are higher than systemic levels.[7] Preclinical studies have revealed the direct cardiac and renal effects of DPP-4 inhibitors, prompting investigations into their potential impact on non-glucose targets. The inhibition of DPP-4 with saxagliptin was shown to have renoprotective in patients with comorbid diseases such as diabetes, obesity, and hypertension, in which activation of the renin-angiotensin system is expected.[11] DPP-4 inhibitors also reduced renal fibrosis, according to a preliminary investigation.[12]

Podocin is an important structure of the podocyte and plays a critical role in the integrity of the slit diaphragm.[13] Megalin is a multifunctional endocytic receptor protein found in many tissues, including the kidney, especially tubules, where it has an important role in renal-tubular reabsorption.[14] In the Acaris et al. study, they found that DPP-4 inhibition decreased proteinuria and prevented podocin and megalin reduction in CKD rats. This protection of podocin and megalin sheds light on the role of podocin and megalin in both glomerular and tubular protein filtration.[15] This expanding scope of action underscores the need for comprehensive evaluations of their effects on various organ systems, especially in patients with CKD.[1–5] In animal models of kidney disease, linagliptin, a DPP-4 inhibitor, elicited multiple renoprotective effects, including reducing albuminuria, glomerulosclerosis, periglomerular fibrosis, podocyte loss, and renal oxidative stress.[7,16] It is interesting to note that in patients with albuminuria (Urine albumin/creatinine ratio: 30-3000 mg/g) who were already receiving angiotensin-receptor blockers or angiotensin-converting enzyme inhibitors, linagliptin treatment was associated with a significant 32% reduction in urinary albumin-to-creatinine ratio.[17] In our study, the intervention group had stable proteinuria compared to the control group. Our study could not demonstrate that such a dramatic reduction may be due to the sample size. Despite our gold standard approach of giving either ACEI or ARB, we did not have complete information about medications. Another possible reason may be that our population includes late-stage CKD, in which the control of proteinuria can be difficult compared to early-stage kidney disease.

The findings of this retrospective case-control study contribute to this evolving understanding by examining the effects of DPP-4 inhibitors on kidney function in a real-world setting. The study population consisted of diabetic patients with CKD stages 3-5, a high-risk group vulnerable to kidney complications. The comparison of DPP-4 inhibitor users and non-users allowed for a comprehensive assessment of their potential renoprotective benefits. Notably, the study revealed comparable baseline characteristics between the intervention and control groups, including age, gender distribution, BMI, and eGFR.

The study's focus on eGFR and proteinuria as key indicators of kidney function is noteworthy. While the slight improvements in eGFR within the intervention group and the minor reduction in the control group did not reach statistical significance, they underscore the need for larger and longer-term studies to establish a conclusive effect on kidney function. The stabilization and improvement of GFR may be related to the antifibrotic effects of DPP-4 inhibition, which supports our finding.[12] This antifibrotic effect was studied in many medical situations.[12,18–20] Importantly, proteinuria, a recognized marker of renal damage, exhibited a favorable trend in the intervention group. DPP-4 inhibitors seemed to contribute to the stabiliza-

tion of proteinuria over the 12-month follow-up period, contrasting with the significant increase observed in the control group. These findings are consistent with the renoprotective implications discussed in a narrative review by Daza-Arnedo et al.[6], which highlighted the potential of DPP-4 inhibitors to mitigate inflammation, fibrosis, and oxidative damage in the kidneys.[6,12,21,22]

Age emerged as an influential factor on eGFR, aligning with the existing understanding that age is a significant determinant of kidney function decline.[23,24] The observed lack of impact on proteinuria is an interesting avenue for future research, suggesting that while age plays a role in overall renal function, it might not be a significant driver of proteinuria in this specific context.

It is worth acknowledging certain limitations of the study. The relatively small sample size and the retrospective design warrant a cautious interpretation of the results. Further, the absence of data regarding comorbidities and antihypertensive medications may limit a comprehensive assessment of confounding factors. However, the study's commitment to ethical guidelines, adherence to the principles of the Declaration of Helsinki, and the approval of the institutional ethics committee lend credibility to its findings.

CONCLUSION

This study contributes valuable insights into the potential renoprotective effects of DPP-4 inhibitors in patients with CKD stages.[3-5] The observed trends in eGFR and proteinuria, while not statistically significant, underscore the need for further investigation into the impact of DPP-4 inhibitors on kidney function in larger, well-designed prospective trials. As the landscape of diabetes management evolves, a comprehensive understanding of the multifaceted effects of antihyperglycemic agents on various organ systems, particularly in high-risk populations, holds immense clinical significance. This study serves as a stepping stone toward unraveling the complex interplay between DPP-4 inhibitors, kidney function, and long-term outcomes in diabetic patients with CKD.

Limitations of the Study

Retrospective Design: The study utilized a retrospective design, which inherently carries limitations in terms of data collection, potential biases, and the ability to establish causal relationships. Retrospective studies are more prone to selection bias, confounding variables, and incomplete or inaccurate data collection.

Small Sample Size

The study's sample size, particularly the number of participants in the control group (n=48), is relatively small. A small sample size reduces the statistical power and generalizability of the results, making it challenging to detect significant differences and limiting the ability to extrapolate findings to a broader population.

Data Availability and Completeness

The absence of comprehensive data regarding comorbidities, concomitant medications (including antihypertensive drugs), and relevant clinical parameters may introduce confounding variables that could influence the outcomes. Missing or incomplete data may affect the accuracy and reliability of the results.

Duration of Follow-up

The study's 12-month follow-up period may not be sufficient to capture long-term effects or changes in kidney function and proteinuria that could potentially develop over a more extended period. A longer follow-up is required to assess the durability of the observed effects and potential changes over time.

Baseline Differences

While the study aimed to match key baseline characteristics between the intervention and control groups, unmeasured or unknown confounding variables may still exist. These differences could impact the outcomes and poten-

tially lead to biased conclusions.

Treatment Regimen Changes

The study acknowledges that antiglycemic regimens were adjusted according to clinical demands during the follow-up period. Changes in treatment regimens, including the addition of other medications or modifications to insulin therapy, could influence kidney function and proteinuria independently of DPP-4 inhibitor use.

Lack of Randomization

The study did not utilize randomization to allocate participants to the intervention and control groups. This may introduce selection bias and limit the ability to establish a cause-and-effect relationship between DPP-4 inhibitor use and the observed outcomes.

Ethnic and Demographic Factors

The study's sample population may not be representative of diverse ethnic and demographic groups, limiting the generalizability of the findings to broader populations with different characteristics.

External Validity

The study was conducted at a single hospital, potentially limiting the external validity of the findings to other healthcare settings or regions with different healthcare practices and patient populations.

Potential Confounders

Although the study attempted to control for various confounders, there could be other unmeasured factors that contribute to the observed outcomes, such as diet, lifestyle, and socioeconomic factors.

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Ethical Approval

The study was carried out with the permission of Sakarya university faculty of medicine Clinical Research Ethics Committee (Decision No: 241662-287, Decision date: Date: 13.12.2018).

Peer-review

Externally and internally peer-reviewed.

Author Contributions

Concept: M.I., A.C.G, Design: M.I., Data Collection or Processing: A.C.G., M.I, Analysis or Interpretation: A.C.G, Literature Search: M.I., A.C.G, Writing: M.I., A.C.G.

Conflict of Interest

The authors have no conflicts of interest to declare.

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Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

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Evaluation of Toxoplasma gondii, CMV, and Rubella Seropositivity and Avidity Tests in the First Trimester of Pregnancy: Why to Test?

Gebeliğin İlk Üç Ayında Toxoplasma gondii, CMV ve Rubella Seropozitifliği ve Avidite Testlerinin Değerlendirilmesi: Neden Test Etmeli?

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Abstract

Aim The influence of intrauterine and perinatal infections on fetal and neonatal mortality rates and childhood morbidity is substantial. Toxoplasmosis gondii, CMV, and rubella are widely recognized as the major causative pathogens of in utero infections. The objective of this study is to investigate the seropositivity rates and avidity incidences of *T. gondii*, CMV, and rubella in pregnant women during the first trimester.

Material and Method The electrochemiluminescence immunoassay method (Elecsys, Roche, Germany) was used for the detection of the anti-toxo IgM, anti-toxo IgG, anti-CMV IgM, anti-CMV IgG, anti-rubella IgM, and anti-rubella IgG during the time period of January 1, 2021, to June 15, 2023, in pregnant women in their first trimester. The anti-toxo IgG, anti-CMV, and anti-rubella IgG avidity tests were performed with the enzyme-linked fluorescent assay method (VIDAS, bioMérieux, France). The data was retrospectively analyzed using the electronic archives.

Results Test results of a total of 15,356 pregnant women were evaluated. The seropositivity rates of *T. gondii* IgM and IgG were 2.1% and 22%, respectively. For *T. gondii*, a high avidity was observed in 75.8% of cases. We found the anti-CMV IgM and IgG seroprevalence as 1.6% and 96.9%, and the anti-rubella IgM and IgG seroprevalence as 0.8% and 98.7%, respectively. The IgG avidity rates with a high index for CMV and rubella were 99.4% and 99.1%, respectively.

Conclusion The present study revealed that the pregnant women exhibited an anti-toxo IgG seropositivity rate of 22% while demonstrating notably high IgG seropositivity rates for CMV and rubella. The seropositivity rates for *T. gondii*, CMV, and rubella IgM were found to be relatively low, but the rates of IgG avidity were shown to be high. A high IgG avidity index against these pathogens in first-trimester pregnant women with a positive IgM and IgG can rule out postconception infections. Treatment for *T. gondii* diagnosed during pregnancy, preventive behavioral measures for CMV and vaccination against rubella prior to pregnancy may help reduce congenital infections. Hence, it is imperative to prioritize the screening of pregnant women for *T. gondii*, CMV, and rubella, as it holds significant importance for the public health.

Keywords *Toxoplasma gondii*, CMV, rubella, serology, pregnancy, congenital infection

Özet

Amaç *İntrauterin ve perinatal enfeksiyonlar, fetal ve neonatal mortalite ve çocukluk çağı morbiditesi üzerinde oldukça büyük etkiye sahiptir. Toxoplasma gondii, sitomegalovirüs (CMV) ve rubella (kızamıkçık) fetüsün in utero enfeksiyonlarına neden olan başlıca patojen etkenlerdir. Bu çalışmanın amacı ilk trimesterdeki gebelerde T. gondii, CMV ve rubella seropozitiflik oranları ve avidite insidansını araştırmaktır.*

Gereç ve Yöntem *Hastanemize 1 Ocak 2021 - 15 Haziran 2023 tarihleri arasında ilk trimesterda başvuran gebelerde anti-toxo IgM, anti-toxo IgG, anti-CMV IgM, anti-CMV IgG, anti-rubella IgM ve anti-rubella IgG antikorlarının tespiti için elektrokemiluminesans immünolojik test yöntemi (Elecsys, Roche, Almanya) kullanıldı. Anti-toxo IgG, anti-CMV ve anti-rubella IgG avidite testleri, enzim bağlantılı floresan test yöntemi (VIDAS, bioMérieux, Fransa) ile gerçekleştirildi. Veriler elektronik arşiv üzerinden retrospektif olarak analiz edildi.*

Bulgular *Toplam 15,356 gebeye ait test sonuçları değerlendirildi. T. gondii IgM ve IgG seroprevalansı sırasıyla %2,1 ve %22 olarak belirlendi. T. gondii için %75,8'inde yüksek avidite gözlendi. Anti-CMV IgM ve IgG seroprevalansı sırasıyla %1,6 ve %96,9 tespit edildi. Anti-rubella IgM ve IgG seroprevalansı ise sırasıyla %0,8 ve %98,7 olarak belirlendi. CMV ve rubella için yüksek indeksli IgG avidite sonucu sırasıyla %99,4 ve %99,1 olarak bulundu.*

Sonuç *Bu çalışma, gebelerin %22 oranında bir anti-toxo IgG seropozitifliği sergilediğini, CMV ve rubella için ise oldukça yüksek IgG seropozitiflik oranları gösterdiğini ortaya koydu. T. gondii, CMV ve rubella için IgM seropozitiflik oranları nispeten düşük oranlarda bulunurken; IgG avidite oranlarının yüksek olduğu görüldü. IgM ve IgG pozitif ilk trimester gebelerinde bu etkenlere karşı belirlenen yüksek avidite test sonuçları gebelik sonrası enfeksiyonları dışlamak için kullanılabilir. Gebelik sırasında gelişen T. gondii tanı ve tedavisi, CMV bulaşını önleyici kişisel hijyen uygulamaları ve gebelik öncesi rubella aşılaması gibi önlemler konjenital enfeksiyonların azaltılmasına yardımcı olabilir. Bu nedenle, gebelerde T. gondii, CMV ve rubella taramasına öncelik verilmesi halk sağlığı açısından hayati önem taşımaktadır.*

Anahtar Kelimeler *Toxoplasma gondii, CMV, rubella, seroloji, gebelik, konjenital enfeksiyon*

INTRODUCTION

Intrauterine and perinatal infections exert a substantial impact on fetal and neonatal death rates and childhood morbidity.[1] The causative agents of in utero infection that are well-described include *Toxoplasmosis gondii*, CMV, and rubella.[1]

Toxoplasma gondii is an intracellular protozoan that is usually acquired early in life and whose definitive host is felines.[2] Humans are intermediate hosts. Transmission mostly occurs through raw or undercooked meat and meat products contaminated with parasitic bradyzoites; or by consuming raw vegetables and fruits contaminated with sporozoites.[3] In immunocompetent people, toxoplasma infection is typically asymptomatic or manifests as a mild illness with fever and malaise, lymphadenopathy being the main symptom. It causes significant morbidity and mortality in immunosuppressed patients.[4] Fetal infection occurs when there is transplacental transmission of tachyzoites prior to the production of maternal IgM antibodies during the primary maternal infection.[5] Although most newborns who are later found to have congenital toxoplasmosis do not present with any symptoms or obvious abnormalities at the time of delivery. The major clinical symptoms are chorioretinitis, hydrocephalus, and intracranial calcifications.[6] The risk of transmission is observed to be higher during later stages of gestation. However, it is important to note that the risk of severe complications such as neurologic and ocular abnormalities, is higher during first trimester infections.[6]

Cytomegalovirus (CMV) is classified as a member of the Herpesvirus family. It is the most common congenital viral infection and is widely prevalent among various age groups throughout the world, increasing with age.[7] Transmission occurs through direct contact with secretions such as saliva, urine or semen of infected patients or contaminated objects. The infection with CMV usually results in nonspecific symptoms such as headache, arthralgia, pharyngitis, rhinitis, myalgia, and fatigue with a mild fever. Immune-compromised individuals are susceptible to severe and disseminated illness.[8]

Primary maternal CMV infection during pregnancy is most commonly caused by close contact with young children.[9] The transplacental route is the major way through which mother-to-child transmission occurs.[10] The probability of vertical transmission rises as gestational age progresses.[10] The vast majority of infants infected by congenital CMV infection may be asymptomatic at delivery, yet are prone to late-onset neurologic impairments.[11] Isolated sensorineural hearing loss is the most common clinical presentation of congenital CMV infection.[11] In pregnants with a primary CMV infection, the risk of severe neonatal sequelae is 3%, and the risk of any adverse outcome is about 8%.[12] The observations made in symptomatic neonates encompass several clinical manifestations such as thrombocytopenia, jaundice, petechia, microcephaly, hepatosplenomegaly, ventriculomegaly, and chorioretinitis. Congenital CMV infection may have more severe sequela if primary maternal infection occurs before 20 weeks of gestation.[13] The congenital diseases can be caused by both primary and secondary infections.[13]

Rubella, often known as German measles, is an infectious and consequential human disease resulting from the rubella virus. Rubella is contracted through the inhalation of aerosols and direct contact with droplets containing nasopharyngeal secretions.[14] The manifestation of acquired rubella typically presents as a mild and self-limiting illness accompanied by a distinctive maculapapular rash called exanthem, occuring initially on the face.[14] Individuals who are affected may have prodromal symptoms characterized by a mild fever, malaise, conjunctivitis, sore throat, and headache.[15] Congenital infection is a result of the transmission of the virus crossing of the placenta hemotogenously.[14] Rubella induces cellular death, interferes with cellular division, and inflicts severe consequences on the growing fetus, resulting in spontaneous abortion, stillbirth, fetal infection, or intrauterine growth retardation.[14,15] The

incidence of congenital abnormalities following maternal infection is primarily confined to instances of maternal infection occurring within the initial 16 weeks of gestation.[16] Distinctive clinical presentation of congenital rubella syndrome include cardiac abnormalities such as patent ductus arteriosus, pulmonary stenosis, radiolucent bone lesions, and blueberry muffin skin lesions.[17,18]

The objective of this study is to investigate the seropositivity rates and avidity test results of *T. gondii*, CMV, and rubella in pregnant women who have undergone routine pregnancy visit during the first trimester.

MATERIALS and METHODS

The study was approved by the Ethics Board of the Faculty of Medicine of the University of Karatay (Decision no. 2023/024).

In the present study, a total of 15,847 pregnant women who consulted the Gynecology and Obstetrics outpatient clinic of Konya City Hospital from January 1, 2021, to June 15, 2023, for routine pregnancy monitoring were included in the study. Repeat tests within 3 days for 491 women were dismissed. The serum *T. gondii*, CMV, and rubella IgM and IgG antibodies, and IgG avidity tests were performed by the relevant techniques: The electrochemiluminescence immunoassay method (Elecsys, Roche, Germany) was employed for the detection of the anti-toxo IgM, anti-toxo IgG, anti-CMV IgM, anti-CMV IgG, anti-rubella IgM, and anti-rubella IgG. Anti-toxo IgM, anti-CMV IgM, and anti-rubella IgM tests with borderline results were tested twice. The anti-toxo IgG, anti-CMV, and anti-rubella IgG avidity tests were performed with the enzyme-linked fluorescent assay method (VIDAS, bioMérieux, France). The results were evaluated retrospectively by screening the electronic archive. Statistical analysis was conducted using the SPSS Statistics 22 software.

RESULTS

The serum *T. gondii* IgM positivity rate among 15,356 pregnant women was 2.1%; and *T. gondii* IgG positivity rate was determined as 22% in 8379 pregnant women. In the study, a total of 227 IgG avidity tests were conducted on patients who tested positive for both *T. gondii* IgM and IgG. For *T. gondii*, low avidity was observed in 13.6% (n = 31) of cases, while high avidity was detected in 75.8% (n = 172) of cases. Additionally, borderline avidity was found in 10.6% of the tests.

The anti-CMV IgM positivity rate among 3.500 pregnant women was 1.6%; and IgG positivity rate was determined as 96.9% in 2482. A total of 174 anti-CMV IgG avidity tests were conducted for patients who tested positive for both IgM and IgG against CMV. 99.4% (n = 173) of cases were detected as high avidity, while borderline avidity rate was 0.6% (n = 1). We did not detect low avidity for CMV infection.

The anti-rubella IgM positivity rate among 15.165 pregnant women was 0.8%; and IgG positivity rate was determined as 98.7% in 10.091 pregnant women. A total of 114 anti-rubella IgG avidity tests were conducted for patients who tested positive for both IgM and IgG against rubella. 99.1% (n = 113) of cases were detected as high avidity, while low avidity rate was 0.9% (n = 1) for rubella infection.

Table 1. presents the test numbers and the rates of seropositivity for IgM and IgG antibodies against *T. gondii*, CMV, and rubella in pregnant women, categorized by years. Table 2. shows the test numbers and the results of the avidity tests for *T. gondii*, CMV, and rubella according to years.

Table 1. IgG and IgM seropositivity rates against T. gondii, CMV, and Rubella by years.

		1 January-31 December 2021	1 January-31 December 2022	1 January-31 June 2023	Mean (Total n)
T. gondii	**High avidity % (n)**	%77.2 (71)	%80.5 (70)	%64.6 (31)	%75.8 (172)
	Borderline avidity % (n)	%9.8 (9)	%8 (7)	%16.7 (8)	%10.6 (24)
	Low avidity % (n)	%13 (12)	%11.5 (10)	% 18.5 (9)	%13.6 (31)
CMV	**High avidity % (n)**	99% (98)	100% (44)	100% (19)	99.4 (173)
	Borderline avidity % (n)	1% (1)	-	-	0.6% (1)
	Low avidity % (n)	-	-	-	-
Rubella	**High avidity % (n)**	97.8% (46)	100% (48)	100% (19)	99.1% (113)
	Borderline avidity % (n)	-	-	-	-
	Low avidity % (n)	2.2% (1)	-	-	0.9% (1)

Table 2. IgG Avidity indeces of T. gondii, CMV, and Rubella by years.

		1 January-31 December 2021	1 January-31 December 2022	1 January-31 June 2023	Mean (Total n)
T. gondii	**IgG positive % (n)**	23.2% (518)	21.7% (842)	21.2% (481)	22% (1841)
	IgG negative % (n)	76.8% (1710)	78.3% (3040)	78.8% (1788)	78% (6538)
	IgM positive % (n)	2% (129)	19.8% (122)	2.7% (77)	2.1% (328)
	IgM negative % (n)	97.3% (6149)	79.4% (6012)	95.9% (2735)	97% (14896)
	IgM borderline % (n)	0.7% (45)	0.8% (47)	1.4% (40)	0.9% (132)
CMV	**IgG positive % (n)**	100% (46)	99.6% (1470)	99.4% (954)	99.5% (2470)
	IgG negative % (n)	--	0.4% (6)	0.6% (6)	%0.5 (12)
	IgM positive % (n)	1.6% (13)	1.8% (32)	1.2% (12)	1.6% (57)
	IgM negative % (n)	96.5% (764)	96.6% (1687)	97.2% (937)	%96.9 (3388)
	IgM borderline % (n)	1.3% (11)	1.6% (29)	1.6% (15)	%1.5 (55)
Rubella	**IgG positive % (n)**	93.2% (3105)	92.5% (4205)	93.8% (2078)	93% (9388)
	IgG negative % (n)	6.8% (226)	7.5% (339)	6.2% (138)	7% (703)
	IgM positive % (n)	0.8% (52)	0.7% (43)	0.7% (20)	0.8% (115)
	IgM negative % (n)	98.7% (6084)	98.7% (6220)	98.7% (2663)	98.7% (14967)
	IgM borderline % (n)	0.5% (31)	0.6% (37)	0.6% (15)	0.5% (83)

DISCUSSION

According to the previous studies[19,20], it has been observed that timely treatment of maternal infection caused by *T. gondii* within the initial three weeks can effectively prevent fetal infection. The seroprevalence of toxoplasma in pregnant women exhibits significant variation, ranging from 45% to 80% in developing countries, while in Europe and the USA, it ranges from 7% to 34%.[21] Data on the incidence of acute toxoplasma infection during pregnancy are limited. The incidence of toxoplasma in the USA is estimated to be 0.2 per 1000 pregnant women.[22] It was reported that congenital toxoplasmosis infection was 1 in 10,000 live births in England between 1986 and 1992.[23]

The seroprevalence of anti-toxo IgG in Turkey ranges from 18% to 60%.[24-29] The variation in seropositivity across Turkey may be attributed to geographical variables and sociocultural differences. For example, in Şanlıurfa, a region known for its notably elevated seroprevalence of anti-toxo IgG antibodies, there exists a substantial consumption of raw meat as a result of its traditional culinary practices (çiğ köfte).[29] In multiple investigations conducted within Tur-

key, the seroprevalence of Anti-toxo IgM has been documented to range from 0.2% to 9%.[25,28,30] We found that the seroprevalence of anti-toxo IgG among pregnant women in their first trimester was 22%. Also, we found a 2.1% rate of anti-toxo IgM positivity, which aligns with existing literature on the subject within our region. In our study, a rate of 75.8% of high avidity, 10.6% of borderline avidity, and 13.6% of low avidity were detected in 227 patients with positive IgM and IgG antibodies against *T. gondii*. Several investigations conducted within Turkey have reported high avidity ranging from 70% to 96%. Additionally, a smaller percentage of individuals displayed borderline avidity levels, ranging from 3% to 24%, while a minority exhibited low avidity levels, ranging from 0% to 15%.[31-33]

In cases where a pregnant woman exhibits clinical suspicion, accompanied by fever and lymphadenopathy, and fetal ultrasound imaging revealing intracranial hyperechoic calcification areas or cerebral ventricular dilatation, it is recommended to conduct diagnostic tests for congenital toxoplasmosis.[22] It is not advisable to conduct routine pregnancy screening in countries with low toxoplasma prevalence and incidence, such as the United Kingdom, United States, and Canada.[34-36] In some European countries such as France, prenatal screening is performed at 1-2-3 month intervals.[37] It is conceivable that each country should decide on prenatal screening and its frequency by taking risk factors into consideration.[34] Hence, it is imperative to ascertain the immunological status of the mother prior to conception. There is no set policy for prenatal screening tests in Turkey, but pregnant women are routinely screened with serological tests in the first trimester.

Serological tests for the detection of IgM and IgG antibodies against toxoplasma are widely used routinely in diagnosing toxoplasma. The aforementioned tests are cost-effective, fast, and easy to perform. Conversely, the interpretation of positive toxoplasma diagnostic tests in asymptomatic pregnant women poses some challenges.[38] Firstly, it may not be possible to determine the timing of infection. Another disadvantage is that false positives are frequently encountered in these tests. When anti-toxo IgM is detected as positive or at the borderline level, it is necessary to confirm the result using a different method. Individuals who test positive for anti-toxo IgG and negative for IgM during the first trimester are considered immune and do not require more advanced testing.[39] If the pregnancy screening is conducted after the 20th week of gestation, further confirmatory tests may be necessary for pregnant women with positive IgG and negative IgM, who are clinically suspicious.[22]

The detection of serum anti-toxo IgM antibodies begins approximately two weeks after exposure to the parasite and can remain positive for several years. The anti-toxo IgG antibodies, on the other hand, become positive 6-8 weeks after infection and remain positive throughout the individual's lifetime.[38] The demonstration of IgM and IgG seroconversion is considered the most reliable diagnostic tests for acute toxoplasmosis. According to previous studies[38], a ≥ 2-fold increase in IgG titers obtained from blood samples taken three weeks apart and analyzed in the same laboratory simultaneously is indicative of an acute infection.

The likelihood of infection occurring after conception was shown to be low in pregnant women who tested positive for IgM and IgG antibodies for the first time towards the end of the first trimester.[38] A single positive IgM result in the first trimester is not sufficient for pregnancy termination or initiation of treatment.[25] In cases when both IgM and IgG are positive, avidity tests are necessary to differentiate between new and past infection or to exclude false positivity.[39] High avidity is an indication of an infection passed more than 4 months ago. On the other hand, low avidity is undiagnostic for new infection because it persists for years in some individuals.[40]

As in the case of *T. gondii*, age, geography, cultural and socioeconomic status affect CMV seroprevalence.[41] In de-

veloping countries, children are mostly infected by three years old, whereas in industrialized countries, infection occurs throughout childhood and adolescence.[42] Global seroprevalence of CMV in women of childbearing age is aproximately 83%.[7] During pregnancy, over 2% of seronegative pregnant women will acquire CMV infection.[7] Routine serologic maternal screening for CMV is discouraged due to various reasons such as, the lack of vaccine to prevent infection in individuals who lack antibodies against CMV, the challenge to differentiate between primary and non-primary infection, and the risk of fetal infection from reactivation or reinfection with a different viral strain as well as to establish the precise date of the infection.[35] However, the implementation of preventative behavioral interventions can help mitigate the transmission risk.[43]

Congenital CMV infection is the leading cause of sensorineural hearing loss.[7] Prenatal testing is indicated in pregnants with mononucleosis-like symptoms and abnormal USG examination consistent with congenital CMV infection, Demonstrating seroconversion is the gold standard for diagnosing primary CMV infection in pregnant women with clinical suspicion.[44] If the immune status of pregnant is unknown, positive results of both anti-CMV IgG and anti-CMV IgM can not discriminate initial infection, reactivation, reinfection, or latent illness. The detection of high avidity suggests that the infection is likely to have occurred at a minimum of six months prior, whereas low avidity implies a more recent onset of the infection.[44] In regions characterized by a high seroprevalence (80% - 100%), the incidence of neonatal CMV infection varies between 1% and 5%.[45] Conversely, in regions with a comparatively lower prevalence (40% - 70%), the incidence of congenital CMV infection ranges from 0.4% to 2%.[45] The prevalence of seroconversion during pregnancy is documented at 1% - 7%.[46] In fact, the seroprevalence of IgM of populations was found to be comparable between developing and industrialized countries. The seroprevalence of CMV IgM among adults in industrialized regions was estimated to range from 2.3% to 4.5%, while in source-limited countries, it ranged from 1.0% to 6.7%.[47] On the other hand, the seroprevalence of anti-CMV IgM among women of reproductive age exhibited slight variability across several regions, including Europe (1.0-4.6%), North America (2.3 - 4.5%), and Japan (0.8%)[48]. In a meta-analysis,[48] anti-CMV IgG seropositivity rates in pregnant women range from 84.10% to 100% in Turkey.[49] On the other hand, the seropositivity rates for anti-CMV IgM range from 0.12% to 3.2%.[48,49] In Turkey, a small number of studies[50-52] have reported CMV avidity between 0% and 7.1%. In our study, we found an anti-CMV seropositivity of 99.5%, an IgM incidence of 1.6% and a high avidity rate of 99,4%, which are consistent with existing data.

Rubella was widespread before the rubella vaccine, with the highest incidence among pre-school and schoolchildren. Epidemics have caused widespread illness and death.[53] An estimated 12.5 million individuals were infected during the 1964-1965 pandemic in US.[53] Over 20,000 cases of congenital rubella syndrome and 11,000 fetal deaths occurred during this outbreak.[52] Since the introduction of routine childhood rubella vaccination, the incidence of rubella has significantly decreased in Turkey and across the world.[53] On the other hand, rubella infections and resultant congenital rubella syndrome cases persist in areas with inadequate vaccination regimens.[53,54] WHO estimates that approximately 100,000 cases of congenital rubella syndrome occur annually worldwide.[15] Immigrants originating from war-torn nations have been identified as the primary carriers of rubella infection.[55] Periodic outbreaks of measles, particularly in the United States, are attributable to the growing resistance towards vaccination. There is a prediction that a comparable scenario could potentially arise in the context of rubella.[56]

The sole dependable indication of prior rubella infection is the detection of serum rubella IgG antibodies.[56] The most effective method for diagnosing acute rubella syndrome includes a fourfold rise in IgG titer when comparing serum specimens obtained during the acute phase and the

convalescent phase, and the detection of IgM antibodies against rubella.[57] If rubella IgM is detected in an asymtomatic pregnant woman without any prior history of contact, a rubella specific avidity assay may be useful to exclude a false positive result.[58] Rubella IgG antibodies in pregnant women in Turkey have been reported as 82% - 96.2% and anti-rubella IgM positivity as 0% - 1.9%.[49,52,59] The observed rates of low avidity for rubella among pregnant women in Turkey range from 0% to 82.9%, varying according to population characteristics and laboratory methods used.[60] A high seroprevalence of anti-Rubella IgG (93%), a low incidence of IgM positivity (0.8%), and a low detection rate for low avidity (0.9%) were observed in our study, suggesting the absence or very low incidence of rubella infection among pregnant women during the period of study and as an indication of high immunity against rubella among women of reproductive age. The seroprevalence data in our region have consistently exhibited similar levels over the course of several years.

The primary objective of our study was to make a scholarly contribution to the existing literature by providing insights into the seroprevalence rates, avidity results, and public health strategies of most well known causative agents of congenital infections. As a result, our findings were consistent with the data of our region, whose population is mainly engaged in agriculture and to some extent animal husbandry. Given the restricted availability of data on avidity tests for *T. gondii*, CMV, and rubella, it is anticipated that this article will provide a contribution to the existing body of national data. A limitation of our retrospective study is that an alternative confirmatory assay other than avidity test was not performed to exclude *T. gondii*, CMV and rubella IgM false positivity in pregnant women. In order to achieve this objective, it is important to conduct prospective research.

CONCLUSION

It is of utmost importance for public health to prioritize the screening of *T. gondii* in high-risk populations, particularly women of reproductive age and pregnant women, as well as to emphasize the significance of early detection and treatment of the infection. Timely diagnosis is crucial for promptly initiating appropriate therapy. On the other hand, the implementation of preventive behavioral treatments and good personel hygien can help reduce transmission of CMV in pregnant women. The main approach to mitigating the risk of rubella during pregnancy is administering vaccination before pregnancy. Consequently, being vigilant about congenital infections and screening pregnant women in the first trimester for *T. gondii*, CMV, and rubella can help reduce congenital infections.

Ethics Approval

The study was approved by the Ethics Board of the Faculty of Medicine of the KTO Karatay University (project no. 2023/024, date: 18.07.2023).

Peer-review

Externally and internally peer-reviewed.

Authorship Contributions

Concept: A.R.U., Ü.E., O.G. Design: A.R.U., Ü.E., O.G. Data Collection or Processing: A.R.U., Ü.E., O.G., Analysis or Interpretation: A.R.U., Ü.E., O.G. Literature Search: A.R.U., Ü.E., O.G. Writing: A.R.U., Ü.E., O.G.

Conflict of Interest

No conflict of interest was declared by the authors.

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Informed Consent

Informed consent was not obtained since it was a retrospective archive scan.

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E-Complaining in Health Services: A Research on sikayetvar.com Shares of Patients Related to the Radiology Department in Turkey

Sağlık Hizmetlerinde E-Şikâyet: Türkiye'de Radyoloji Bölümü ile İlgili Hastaların sikayetvar.com Paylaşımları Üzerine Bir Araştırma

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Abstract

Aim The aim of this study is to examine the complaints made on the internet site complaint about radiology services in Turkey. The study also aims to determine a general profile of individuals who complain about their dissatisfaction with the radiology department to the competent authorities and to systematically examine the reasons for their complaints.

Material and Method In this retrospective study, complaints made to the sikayetvar.com website were examined using the content analysis method, which is a qualitative research method. The last 110 complaints about the radiology department in Turkey, made on the internet to sikayetvar.com between July 2023 and August 2023, were included in the evaluation. 34 of these complaints were excluded from the study because they were sent to the wrong department, were insurance-related, and were unclear. Our study was conducted through the analysis of the remaining 76 complaints. Complaints made; They are classified according to gender, type of radiological procedure, results of the radiological procedure, whether it is a public or private hospital, whether they are inpatients, outpatients or emergency patients, satisfaction levels of the patients, number of views of the complaint and the subject of the complaint.

Results The data obtained were obtained from Reader et al. (15) was analyzed by adopting a deductive approach with the text analysis method, which is one of the content analysis types, in line with the patient complaint taxonomy. It was determined that 51 (67.1%) of 76 complaints about radiology services were made by women and 25 (32.9%) by men. It was found that magnetic resonance (MRI) (27.1%), conventional radiology (24.3%) and computed tomography (20.0%) received significantly more complaints than all other radiological procedures. It was determined that the most complaints were about Management (97.37%) and Communication (98.68%) (Clinical 21.05%). Quality (97.37%), security (15.79%), timing and access (86.84%), and communication (98.68%) comprised almost all of the complaint categories. Delays (88.16%), communication breakdown (93.42%), quality of care (15.79%) and staff attitude (88.16%) were included in almost all complaint subcategories. No complaints were made regarding diagnostic errors or treatment.

Conclusion It is thought that the obtained results can guide healthcare business managers in effective complaint management and help improve patient satisfaction. Knowing the sources of patient dissatisfaction with radiology services can help reduce the number of patient complaints and improve patient care.

Keywords *E-complaint, health services, patient satisfaction, radiology*

Özet

Amaç *Bu çalışmanın amacı Türkiye'de radyoloji hizmetleri ile ilgili internette şikâyetvar sitesine yapılan şikâyetlerin incelenmesidir. Çalışmada ayrıca radyoloji bölümü ile ilgili memnuniyetsizliklerini yetkili mercilere şikâyet eden bireylerin genel bir profillerini belirlemek ve şikâyet nedenlerini sistematik olarak incelemektir.*

Gereç ve Yöntem *Bu retrospektif çalışmada, şikâyetvar.com sitesine yapılan şikâyetler nitel araştırma yöntemi olan içerik analiz yöntemi ile incelenmiştir. Değerlendirmeye, Temmuz 2023-Ağutos 2023 tarihleri arasında Türkiye'de radyoloji bölümü ile ilgili internette şikâyetvar.com sitesine yapılan son 110 şikâyet alınmıştır. Bu şikayetlerden 34'ü yanlış bölüme olması, sigorta ile ilgili olması, belirsiz olması nedeniyle çalışma dışı bırakılmıştır. Çalışmamız kalan 76 şikayetin analizi üzerinden yapıldı. Yapılan şikâyetler; cinsiyete, radyolojik işlemin türüne, radyolojik işlemin sonuçlarına, kamu ya da özel hastane olmasına, yatan, ayakta veya acil hasta olmalarına, hastaların memnuniyet düzeylerine, şikâyetin görüntülenme sayılarına ve şikâyet konularına göre sınıflandırılmıştır.*

Bulgular *Elde edilen veriler Reader vd. (15) tarafından geliştirilen hasta şikâyet taksonomisi doğrultusunda içerik analizi türlerinden metin çözümleme yöntemi ile tümden gelimsel bir yaklaşım benimsenerek analiz edilmiştir. Radyoloji hizmetleri ile ilgili 76 şikâyetin 51'i (%67,1) kadınlar ve 25'i (%32,9) erkekler tarafından yapıldığı saptanmıştır. Manyetik rezonans (MR) (%27,1), konvansiyonel radyoloji (%24,3) ve bilgisayarlı tomografinin (%20,0) diğer tüm radyolojik işlemlere göre anlamlı derecede daha fazla şikayet aldığı saptandı. En çok şikâyet konusunun Yönetim (%97,37) ve İletişim (%98,68) temasında olduğu belirlenmiştir (Klinik %21,05). Şikayet kategorilerinin neredeyse tamamını kalite (%97,37), güvenlik (%15,79), zamanlama ve erişim (%86,84) ve iletişim (%98,68) oluşturdu. Gecikmeler (%88,16), iletişim kesintisi (%93,42), bakım kalitesi (%15,79) ve personelin tutumu (%88,16) neredeyse tüm şikayet alt kategorilerinde yer almaktaydı. Tanı hataları ile tedavi ile ilgili herhangi bir şikayette bulunulmamıştır*

Sonuç *Erişilen sonuçların etkili bir şikâyet yönetimi hususunda sağlık işletmesi yöneticilerine rehberlik edebileceği ve hasta tatminini iyileştirmeye yardımcı olabileceği düşünülmektedir. Radyoloji hizmetlerinde hasta memnuniyetsizliğinin kaynaklarının bilinmesi, hasta şikayetlerinin sayısının azaltılmasına ve hasta bakımının iyileştirilmesine yardımcı olabilir.*

Anahtar Kelimeler *E-şikayet, sağlık hizmetleri, hasta memnuniyeti, radyoloji*

INTRODUCTION

Unlike other service sectors, the health services sector is one of the most highly competitive sectors, where intense competition is experienced, which requires a high level of orchestration of different professional groups with a high level of specialization, where full-time service is provided, which cannot be consumed/stored as soon as it is produced,[1] which has no substitutes and makes its severity felt more unless it is met. It has a very sophisticated and complicated structure where even a simple mistake can cost human life.[2]

With the advancement of technology, the internet has become a part of consumers' daily lives. According to a study, more than half of the world's population (over 4 billion) uses the internet. There are 54.3 million internet users in Turkey.[3] Online internet use in health services. is increasing day by day. In Turkey, 718 million people applied to healthcare providers in 2017.[4] It is understood that each person goes to hospital approximately three times a year. According to the Ministry of Health data, the number of busy healthcare providers is 1669.[5] The heterogeneity of healthcare, a labor-intensive sector, is extreme. In this respect, the probability of service disruptions is higher. Service disruptions are one of the leading determinants of customer dissatisfaction.[6] Complaints may arise due to service errors or consumers not being aware of their rights. According to patient rights, which is one of the fundamental human rights, patients; benefiting from health services, being informed and requesting information, choosing and changing the health institution and its personnel, protecting privacy, refusing or terminating the treatment received, ensuring security, fulfilling religious obligations, receiving the respect required by human values, being made comfortable, having visits and companions, making complaints. and has the right to sue.[7]

Complaints arise when the consumer's demands are not met due to mistakes made by institutions during service delivery. According to Lovelock and Wright (2002), a complaint is a formal expression of dissatisfaction with the experience or any aspect of the service.[8] Complaint is the written or verbal expression of dissatisfaction resulting from non-fulfillment of needs, wishes and expectations.[9] Complaints may be related to mental, physical and emotional state.[10] In Turkey, a telephone reporting line was opened by the Ministry of Health in 1997 to receive complaints from patients, and the Ministry of Health Communication Center (SABİM) started its investigations on January 1, 2004, with the aim of delivering the complaints systematically. The Ministry of Health Communication Center (SABİM) offered the opportunity for participation to the parties related to the sector and thus made "interactive management" possible.[11]

In the globalizing world, along with the technological developments, individuals' value judgments regarding the concept of health have also changed. Especially with the rapid development of new media tools, individuals whose level of consciousness and awareness has increased and who do not refrain from questioning, try to convey their complaints not only to the institution but also to the people in their online environment and share their bad experiences with other consumers. This process has also led to the emergence of complaint sites that serve as a means for people with complaints to convey their complaints to the relevant institution. The main functions of complaint-related e-forums are to help consumers who have complaints against institutions convey their complaints to institutions on the virtual platform, to attract the attention of institutions for the solution of problems and to announce their negative experiences to other individuals.[2] The complexity of health services causes many complaints. Issues such as informing patients, staff-patient interaction, staff behavior, sense of trust, nutrition services, service quality, physical and environmental conditions, bureaucracy, fees, length of hospital stay and waiting times are stated as the most frequently complained issues by patients and their relatives.
Patient satisfaction is an important indicator reflecting the quality of health care.[12] The importance of assessing

patient dissatisfaction has been recognized as an essential component of patient-centered radiology practice, a concept in which healthcare professionals in the radiology department collaborate with patients and their families to identify and meet patients' needs and preferences.[13] There are not enough studies in the literature on the frequency and causes of patients' complaints about the services of the radiology department. In their study by Salazar et al. (2013), they reported that the general incidence of unwanted written complaints per radiological procedure was 2.38 per 100,000 and that most of these complaints (60.1%) resulted from the failure to provide patient-centered care.[14] The aim of this study is to examine the complaints made on the internet site "sikayetvar.com" about radiology services in Turkey. The study also aims to determine a general profile of individuals who complain about their dissatisfaction with the radiology department to the competent authorities and to systematically examine the reasons for their complaints.

MATERIALS and METHODS

In this retrospective study, the complaints made to the website sikayetvar.com about radiology services were examined with the content analysis method, which is a qualitative research method. In the evaluation, the last 76 complaints made to the internet site sikayetvar.com about radiology services between July 2023 and August 2023 were included in the sampling. It was assumed that the individuals who made the complaint in the study were over the age of 18. Because when registering on the websites, it is required to confirm that you are 18 years old and over. Complaints made; gender of the patient, type of radiological procedure (ultrasonography, CT, MR or interventional radiology), results of the radiological procedure, whether it is a public or private hospital, hospital status of the patient (inpatient, outpatient or emergency room), satisfaction level of the patients, whether the complaint is directed only to the radiology department are classified according to the number of views of the complaint and the subject of the complaint.

The coding taxonomy for patient complaints uses these three areas: "clinic" (complaints about the safety and quality of clinical care), "management" (complaints about the management of the health institution), and "relationships" (complaints about health care personnel). The clinical domain was divided into "quality" and "safety" categories, the management domain into "institutional issues" and "timing/access" categories, and the relations domain into "communication", "humanity/interest" and "patient rights" categories.[15]

The study is limited to the complaints of 76 patients made to the sikayetvar.com website and it is assumed that the complaints are correct. Applications where the complaint was not related to a procedure performed in the radiology department, was related to insurance companies, or the complaint was unclear were not included for analysis in this study.

Statistical Evaluation

SPSS 24 statistical software package (Statistical Package for the Social Sciences – IBM®) was used in the analysis of the data collected in the study. In the study, descriptive statistics regarding the distribution of responses to independent variables were presented as numbers and percentages for categorical variables, and as mean, standard deviation and median for numerical variables. The compatibility of continuous variables with the assumption of normal distribution was evaluated with the Kolmogorov-Smirnow test. Chi-square test was used for categorical variables and One Way Anova test was used for quantitative variables in pairwise and multiple comparisons. Frequency of complaints by type of radiological procedure was compared using Chi-square test with Bonferroni correction. The results were evaluated with a 95% confidence interval, $p<0.05$ as significant.

RESULTS

In this part of the study, 76 complaints made to the sikayetvar.com website about radiology services between July

2023 and August 2023 were examined.

Table 1. Demographic and clinical characteristics of the individuals in the study	
Gender, N (%)	
Woman	51 (67.1%)
Male	25 (32.9%)
Hospital Type, N(%)	
Public	32 (42.1%)
Special	44 (57.9%)
Patient's hospital status, N(%)	
Inpatient	4 (2.6%)
Outpatient treatment	70 (92.1%)
emergency room	2 (2.6%)
Type of radiological procedure (n:70), N(%)	
CT	14(20.0%)
Interventional	4 (5.7%)
HSG	3 (4.3%)
Conventional	17 (24.3%)
MRI	19 (27.1%)
USG	13 (18.6%)
Radiological procedure only, N (%)	
Yes	49 (64.5%)
No	27 (35.5%)
Result, N(%)	
Yes	32 (42.1%)
No	44 (57.9%)
Answer, N(%)	
Yes	33 (43.3%)
No	43 (56.6%)
Other partition related (n:16), N(%)	
Emergency room	5 (31.3%)
Emergency/surgical	1 (6.3%)
Anesthesia	1 (6.3%)
Pediatry	2 (12.5%)
General surgery	1 (6.3%)
Cardiovascular surgery	1 (6.3%)
Neurology	2 (12.5%)
Orthopedics	3 (18.8%)
Satisfaction level, N(%)	
1	59 (77.6%)
2	2 (2.6%)
3	15 (19.7%)
Display, Mean ±Std	1802.7±1612.4 (Min-Max: 90-11077)
N:number, %: percent, Mean: Mean, Std: Standard Deviation	

Individuals in the study; While 51 (67.1%) were female, 25 (32.9%) were male. While 32 (42.1%) of the hospitals complained about were public hospitals, 44 (57.9%) were private hospitals. While 4 (2.6%) of the complaining patients were inpatients, 70 (92.1%) were outpatients and 2 (2.6%) were emergency room patients. It was found that magnetic resonance (MRI) (27.1%), conventional radiology (24.3%) and computed tomography (20.0%) received significantly more complaints than all other radiological procedures (Figure 1.) 49 (64.5%) of the complaints were related only to radiological procedures. It was observed that 32 (42.1%) of the complaints received results and 33 (43.3%) received answers. Apart from radiology services, 5 (31.3%) of the other complaints were related to the emergency department, 1 (6.3%) was emergency/surgical, 1 (6.3%) was anesthesia, 2 (12.5%) were complaints. pediatrics, 1 (6.3%) in general surgery, 1 (6.3%) in cardiovascular surgery, 2 (12.5%) in neurology and 3 (18.8%) in orthopedics. was related. It was determined that the satisfaction level of 59 (77.6%) of the complaining individuals was "1", 2 (2.6%) had a satisfaction level of "2" and 15 (19.7%) had a satisfaction level of "3" (Table 1).

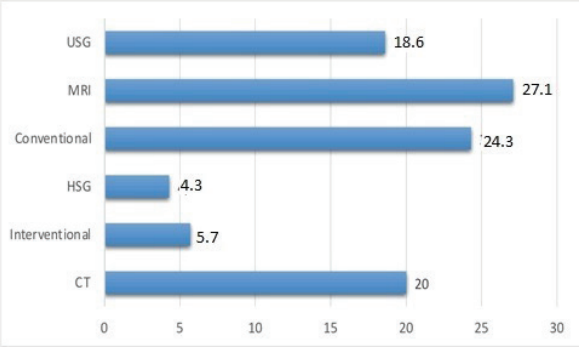

Figure 1. Types of radiological procedures complained about

Table 2. Pairwise comparison of complaint frequency between different radiological procedures						
	CT	**Interventional**	**HSG**	**Conventional**	**MRI**	**USG**
CT	-	p=0.023a	p=0.004a	p=0.724	p=0.586	p=0.852
Interventional			p=1.000	p=0.014 a	p=0.008a	p=0.022a
HSG				p=0.003a	P<0.001a	p=0.038a
Conventional					p=0.456a	p=0.327a
MRI						p=0.607a
aCalculated with z-test for proportions and post hoc Bonferroni correction						

All cross-sectional imaging modalities (CT, MR, and ultrasonography) also had significantly more complaints than conventional and infrared radiography (p<0.001). The frequency of other complaints did not differ significantly from each other (p>0.05) (Table 2).

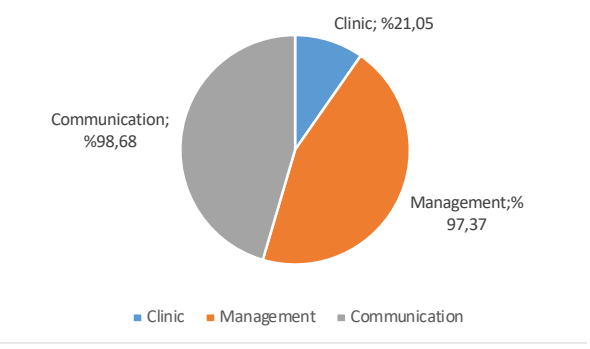

Figure 2. Distribution of complaints by domain names (%)

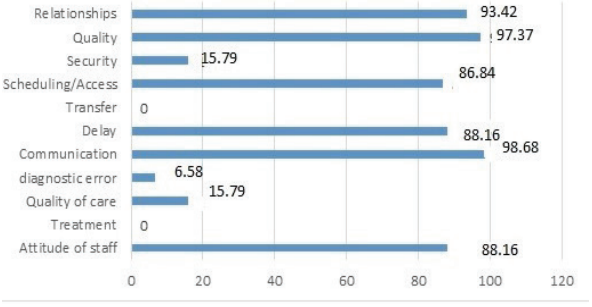

Figure 3. Distribution of complaints by categories (%)

It shows the distribution of complaints among different areas, categories and subcategories according to Reader et al.'s taxonomy of patient complaints (15). 16 (21.05%) of the complaints belonged to the clinic, 74 (97.37%) to the management, and 75 (98.68%) to the communication field (Figure 2). Quality (97.37%), security (15.79%), timing and access (86.84%), and communication (98.68%) comprised almost all of the complaint categories. Delays (88.16%), communication breakdown (93.42%), quality of care (15.79%) and staff attitude (88.16%) were included in almost all complaint subcategories. No complaints were made regarding diagnostic errors or treatment (Figure 3).

DISCUSSION

In the study, 76 complaints about radiology services made to the sikayetvar.com website were examined and a general profile of the individuals who complained about their dissatisfaction with the radiology department was determined and the reasons for the complaints were systematically evaluated. There are not enough studies in the literature on the frequency and causes of patients' complaints about the services of the radiology department. Therefore, our study is an original study.

As a result of our study, it was observed that the most complaints were made by female patients (67.1%). In a study by Alosaimi et al. (2018), which is similar to our study in the literature, in which they evaluated 672 complaints in the sample of Saudi Arabia, it was concluded that the highest number of complaints were made by women with 63% .16 Unlike our study, Hoşgör and Cengiz (2020) examined the general profile of individuals complaining about health services and the reasons for their complaints, and analyzed 15452 complainants and 16489 complaints in the study conducted by Hoşgör and Cengiz (2020). In their study,

they reported that the individuals who made the highest number of complaints were male patients aged 41 and over, who graduated from high school.[17] As a different perspective, Durduran et al. (2012); Based on the 2010 data of the Turkish Statistical Institute, they emphasize that although women in Turkey experience more health problems, it is generally men who apply to the patient rights unit because of their status as the person/head of the household, which is attributed to men in the traditional Turkish family structure.[18]

It was assumed that the individuals who made the complaint in our study were over the age of 18. Because when registering on the websites, it is required to confirm that you are 18 years old and over. In a study conducted by Webb (1995), it was determined that elderly patients applied for complaints twice as much as younger patients.[19] Similarly, in the study conducted by Lim et al. (1998) on complaints from the Family Medicine Services in Singapore, it was revealed that individuals in the 20-59 age group reported higher complaints than those in the 10-19 age group.[20] According to Önal and Civaner (2015), it has been interpreted that individuals' awareness of defending their rights increases with advancing age.[21] In addition, the fact that elderly patients feel less afraid that they will not be able to receive service due to their complaints from public health institutions can be seen as a reason for this situation.

As a result of our study, 75 (98.68%) of the applications that constitute the most common complaint of individuals related to radiology services are communication-related, followed by management (97.37%) problems, and the least complaint is clinical (21.05%) was found to originate. In our study, quality (97.37%), security (15.79%), timing and access (86.84%), and communication (98.68%) comprised almost all of the complaint categories. Delays (88.16%), communication breakdown (93.42%), quality of care (15.79%) and staff attitude (88.16%) were included in almost all complaint subcategories. No complaints were made regarding diagnostic errors or treatment. It can be said that the findings of our study and the results of Mattarozzi et al. (2017) are similar (Management: 68.1%; Relationships: 52.8%; Clinic: 36.8%).[22] Unlike the results of this study, in the study by Chaulk et al. (2019) in which 87 patient complaints were examined using the same taxonomy, the main theme of the most important complaint was; It was found to be Clinical (66%), Relationships (60%) and Management (31%).[23] Similarly, in Salazar et al.'s (2018) study, the most important reason for complaints was from the Clinic (52%), Management (24%) and the General Profile of Individuals Complaining about Health Services and Reasons for Complaints were Relationships (24%), while the complaints in the main theme were equally high,[24] In Harrison et al.'s (2016) study, it was determined that this order was Clinical (68%), Management (19%) and Relationships (13%).[25] The results obtained in this study show that the users of the service related to the radiology department in Turkey suffer from the problems related to the relations between the management of the health enterprises in the radiology department and the service provider, rather than the directly health-related issues, and that there is a more intensive improvement especially in these areas by the professional health administrators. and/or revisions need to be made.

In our study, it was also observed that patients complained about cross-sectional imaging methods (CT, MR, and ultrasonography) significantly more than in interventional radiology and conventional radiology ($p < 0.001$). The frequency of other complaints did not differ significantly from each other. Generally, while cross-sectional imaging methods are obtained, the planning, shooting and interpretation of the shots are more time-consuming and complex than traditional radiographic images. For this reason, disruptions experienced in standard care practices may be perceived negatively by patients. In a study by Ollivier et al.[26] they found that the majority of patients (73%) experienced distressing while undergoing MRI and CT scans, both because of the fear and anxiety felt due to

the scanning procedures themselves and the fear of what the results would be. These patient- and screening-related negative factors could potentially lower patients' threshold for reporting.

This study had some limitations. The study is limited to the complaints of 76 patients made to the sikayetvar.com website between July 2023 and August 2023 and it is assumed that the complaints are true. The fact that a specific complaint taxonomy is not used in most of the studies makes it difficult to reach a complete unity of definition regarding the reasons for complaints, and this may lead to subjectivity when classifying the reasons for complaints. Applications where the complaint was not related to a procedure performed in the radiology department or it was unclear whether this was the case were not included for analysis in this study.

CONCLUSION

This research was conducted by examining complaints regarding online radiology services offered in Turkey to www.sikayetvar.com.

Under the main theme of Management, the most complaint applications are for Institutional Problems and Timing and Access issues, respectively; Under the main theme of Relationships, Patient Rights, Humanity/Care and Communication issues; It was concluded that under the main theme of the clinic, it belongs to the issues of Quality and Patient Safety.

The most frequently reported complaint reasons in terms of subcategories are, in order: service problems, environment, access and patient admission, delays, privacy, respect, dignity and consideration, bureaucracy/red tape, incorrect/insufficient information, finance and billing, staff behavior, patient safety cases, treatment, communication defects, quality of care, It was concluded that there are discrimination, staff employment and other resources, skills and professional suitability, diagnostic errors, examination, informed consent, abuse, patient-staff dialogue, referrals, discharge, medication errors and patient guidance/monitoring.

Regarding the service problems related to the main theme of management; It may be suggested that professional health managers with undergraduate or postgraduate degrees in health management should be given more space in the management levels of hospitals. Regarding the environment/environment issue, it may be suggested that more emphasis should be given to hotel management services, especially in public and university hospitals, just like most private healthcare enterprises. For example, it may be possible to spend more effort on such points that are thought to positively affect the satisfaction levels of service recipients, such as the quality of the meals served, the comfort of the waiting and accommodation areas, the general cleanliness and hygiene of the institution, the optimization of the physical infrastructure capacity and the general atmosphere/ambiance of the hospital. Regarding access and patient admission issues; The development of national health policies or the revision of existing policies that will increase the physical and economic accessibility of services and improve registration-patient admission and appointment problems may be brought to the agenda. Regarding the solution of the problems of respect-dignity-caring, staff behavior, patient privacy and information related to the main theme of relationships; It may be beneficial to focus on cultural activities that can contribute to the improvement of the knowledge and awareness levels of all other health and non-health personnel, especially physicians and nurses, such as general communication, health communication, patient-oriented service provision, patient rights and values education. In this context, physicians; It is extremely critical that they do not ignore that they are trying to cure the patient, not the disease, and that they are sensitive individuals who expect to be respected, cared for, their privacy guaranteed, to be able to participate freely in treatment decisions, and to be adequately and accurately informed about their disease/general health con-

dition.

Regarding the solution of problems related to patient safety cases, treatment problems and quality of care related to the main theme of the clinic; Performing root-cause analysis, especially in determining what lies at the root of surgical complications, performing calibration and service maintenance of medical devices and equipment at certain and regular periods, recording undesirable situations that threaten patient safety and affecting the success/outcome of treatment, determining the factors affecting the success/outcome of treatment, institutions, health It may be recommended to clarify whether it is caused by staff or patients, to comply with standard clinical care plans in order to improve the quality of care, and finally, not to disrupt total quality management training.

It is thought that the results obtained in the study can guide healthcare business managers in effective complaint management and help improve patient satisfaction. Knowing the sources of patient dissatisfaction with radiology services can help reduce the number of patient complaints and improve patient care. More personalized contact between radiologists and patients may reduce the frequency of complaints, but this requires further research. Additionally, complaints regarding healthcare services associated with the radiology department in different countries can be compared. The results of this research can be shared and used in health education to gain insight into what patient complaints may be. The gap in the literature regarding malpractice in both radiology services and general health services can be filled. Additionally, studies can be carried out on methods on how complaints will be compensated.

Ethical Approval

Research involving information freely available in public domain- would not require ethics review.

Peer-review

Externally and internally peer-reviewed.

Authorship Contributions

Concept: F.M., A.Ş., Design: F.M., A.Ş., Data collection or Processing: F.M., A.Ş., Analysis or interpretation: F.M., A.Ş., Literature Search: F.M., A.Ş., Writing: F.M., A.Ş.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Myocardial Protection with Remote Ischemic Preconditioning in Congenital Heart Surgery: Does It Deliver What is Expected?

Konjenital Kalp Cerrahisinde Uzaktan Iskemik Önkoşullama ile Miyokardiyal Koruma: Bekleneni Sağlıyor mu?

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Abstract

Aim The discovery of the protective effects of antioxidant agents on organ functions enabled this system to be tested artificially. The impact of remote ischemic preconditioning on surgical clinical outcomes in patients with congenital heart defects is unclear. This study investigated the early consequences of ischemic preconditioning on cardiac protection.

Material and Method A prospective review of all patients who underwent complex congenital heart surgery procedures at a single center was performed. The antioxidant enzymatic analysis was performed on blood samples taken from randomly grouped patients.

Results The patients' surgical median age was 19.1 months (3.7- 57.7) in the ischemic preconditioning group (group 1) and 16.7 months (7.8- 35.9) in the control group (group 2). The patients' median follow-up period was 58.3 months (54.3- 62.1) in group 1 and 37.1 months (34.8- 41.7) in group 2. Early mortality was in 4 (4.4%) patients. There was no late mortality. There was a significant difference between the groups regarding Superoxide Dismutase, Malondialdehyde, and cardiac markers levels (p< 005).

Conclusion The effects of ischemic preconditioning on cardiac protection have not been proven yet. Especially in congenital cardiac patients, chronic stimuli such as hypoxia and cyanosis or drugs used before surgery may affect the study's results. Although there is no significant difference in mortality in these patients, a positive effect on the length of hospital stay is promising.

Keywords *Congenital heart defect, Ischemic preconditioning, myocardial protection*

Özet

Amaç *Antioksidan ajanların organ fonksiyonları üzerindeki koruyucu etkilerinin keşfedilmesi, bu sistemin yapay olarak test edilmesine olanak sağlamıştır. Bu ajanların endojen olarak vasküler yataktan salıverilmesini tetikleyecek geçici bir uzuv iskemisi oluşturmak suretiyle kalp, karaciğer gibi uzak dokuları iskemiye hazırlama prosedürü zamanla artarak denenmiştir. Ancak konjenital kalp defekti olan hastalarda uzaktan iskemik önkoşullamanın cerrahi klinik sonuçlar üzerindeki etkisi henüz belirsizdir. Bu çalışmada iskemik önkoşullamanın kardiyak koruma üzerindeki erken sonuçlarını araştırdık.*

Gereç ve Yöntem *Tek merkezde kompleks konjenital kalp cerrahisi uygulanan çalışma hastalarının prospektif incelemesi yapıldı. Rastgele gruplandırılmış hastalarda ameliyathanede anestezi hazırlığı aşamasında yapay olarak beşer dakikalık aralıklı bacak iskemisi oluşturulup sonrasında alınan kan örneklerinde antioksidan enzimatik analiz yapıldı.*

Bulgular *Çalışmaya dahil edilen toplam 67 hastanın ortanca cerrahi yaşı iskemik önkoşullama grubunda (grup 1; n=45) 19,1 ay (3,7-57,7), kontrol grubunda (grup 2; n=22) 16,7 ay (7,8-35,9) idi. Hastaların ortanca takip süresi grup 1'de 58,3 ay (54,3-62,1), grup 2'de 37,1 ay (34,8-41,7) idi. Erken mortalite 4 (%4,4) hastada görüldü. Geç mortalite görülmedi. Süperoksit Dismutaz, Malondialdehit ve kardiyak belirteçlerin düzeyleri açısından gruplar arasında anlamlı fark vardı (p< 005). Hastanede kalış süreleri grup 1'de 8 gün (6-14), grup 2'de 10 gün (8-22) idi (p=0,02).*

Sonuç *İskemik önkoşullamanın kardiyak korumaya etkisi henüz kanıtlanmamıştır. Özellikle doğuştan kalp hastalarında hipoksi, siyanoz gibi kronik uyaranlar ya da ameliyat öncesinde kullanılan ilaçlar çalışmanın sonuçlarını etkileyebilmektedir. Bu hastalarda mortalite açısından anlamlı bir fark olmasa da hastanede kalış süresine olumlu etki umut vericidir.*

Anahtar Kelimeler *Konjenital kalp defekti, iskemik önkoşullama, miyokardiyal koruma*

INTRODUCTION

Complex congenital heart diseases may predispose to forming many free radicals in the body due to their pathophysiology and exposure time. Free radical production may increase due to many factors, such as exposure to low saturation, the emergence of cyanosis, loss of pulmonary tissue compliance, and increased susceptibility to infection. During the operation, exposure to the patient's blood to extracorporeal circulation can trigger a similar process and create multi-organ involvement. Studies have reported low cardiac output syndrome (LCOS) incidence after open-heart surgery in newborn and infantile patients is around 25%- 40%.[1,2,3,4] This ratio's importance is the prolonged postoperative intensive care period and the additional complications it will cause. Other complex and high-mortality interventions may also be required, such as extracorporeal membrane oxygenation (ECMO) support.[5,6] In this study, we discuss the potential of remote ischemic preconditioning (RIPC) in protecting against the ischemic period during pediatric cardiac surgery.

Oxidative stress, characterized by an imbalance between oxidants and antioxidants in favor of oxidants, leads to impairment of physiological function.[7] Cell death and tissue damage are inevitable when tissue is exposed to ischemic conditions. However, this can be reversed depending on the recirculation time. Despite the recirculation here, there is a different concept called "reperfusion damage" paradoxically. The theory is that a mechanism that minimizes ischemia-reperfusion injury and is naturally found in the body in "remote ischemic preconditioning technique (RIPC)" could be pre-activated.[8,9] This natural mechanism is located in the vascular endothelium. Myocardial protection using the ischemic preconditioning method was first described in an animal experiment in 1986. According to this study, a few short ischemic periods created on the myocardium significantly reduce the infarction's severity after normal blood flow.[7] After coronary surgery, plasma isolated from the heart reperfused venous coronary sinus blood sample showed a 2-3-fold increase in protein carbonyls from ELISA measurement.[10]

The World Health Organization (WHO) defines a biomarker as any substance, structure, or process measured in the body or its products, affecting or predicting outcome/disease incidence.[11]

Phospholipid hydroperoxide glutathione peroxidase (PLGSH-PX) protects the membrane against peroxidation when vitamin E, an essential membrane-bound antioxidant, is insufficient. The body's low molecular weight thiols (cysteine, glutathione -GSH-) are very sensitive to oxidation.

Superoxide Dismutase (SOD) biological effects occur through different mechanisms. The best-known is its role in redox signaling, NO signalization, and its effects on mitochondria. The physiological function of SOD is to protect cells that metabolize oxygen against the harmful effects of superoxide free radicals (O2 −), such as lipid peroxidation. SOD also plays a role in the intracellular killing of phagocyted bacteria. SOD activity is higher in tissues with increased oxygen utilization and increases with tissue pO_2 increase. Inadequate removal of superoxide anion results in oxidative stress. Studies show that extracellular SOD3 (Cu- Zn SOD) is essential in oxidative stress-related pathophysiology, including hypertension, heart failure, ischemia-reperfusion, and lung injury.[12,13,14]

MATERIALS and METHODS

Ethical statement: This study was planned as a single-center pediatric cardiovascular surgery clinic in Istanbul Medipol University Hospital in 2015. University Ethics Committee approval was obtained (March 21, 2014 /Number: 10840098-53). Our study was designed in accordance with the Declaration of Helsinki. After prospective data collection, these data were confirmed with Data Processing Center data. Post-discharge mid-term follow-up data of the patients were obtained from the national data recording system and hospital database.

In the prospectively designed study, we based on the period from admission to the operating room to the postoperative follow-up period. Some cases were reoperations (staged surgery) with a history of palliative surgery. ECMO support for patients with respiratory or circulatory problems in the preoperative period, early postoperative bleeding, and reoperation due to pericardial tamponade were described as exclusion criteria from the study.

Technique and procedure application: The patients were divided into the RIPC group (group 1) and the control group (group 2). Patients were randomly distributed into groups. All patients have complex congenital cardiac anomalies. There were cyanotic and acyanotic patients in both groups. In the RPIC group, an age-appropriate size cuff was attached to one lower limb. In this application, the cuff was inflated with a pressure of 150 mmHg and kept for 5 minutes, and reperfusion waited for 5 minutes after the cuff was deflated. This cycle was repeated three times. After completing the last process, a blood sample was taken at the 5th minute (Figure 1).

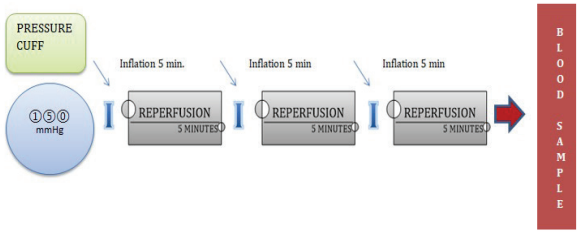

Figure 1. Remote ischemic preconditioning (RIPC) application method

The surgical procedure, anesthesia management, surgical team, and postoperative care were similar for both groups. Arterial blood pressure, central venous pressure, electrocardiographic monitoring, rectal temperature, and near-infrared spectroscopy (NIRS) (renal and cerebral) were followed during the operation. Non-pulsatile cardiopulmonary bypass was performed after standard aortic arterial and bicaval venous cannulation. Myocardial protection was provided with antegrade blood cardioplegia. Routine heparin administration and activated clotting time (ACT) follow-ups were performed. Protamine was used to neutralize the effect of heparin.

Patients with low cardiopulmonary performance data were taken to ECMO support immediately or within 24 hours after surgery. Patients who received ECMO support due to respiratory problems in the preoperative period were excluded from the study. Cardiac performance evaluation was performed by bedside echocardiography, vital sign monitoring, and arterial and mixed venous blood gas monitoring. The patients with low cardiopulmonary performance data were placed on ECMO support immediately after surgery or 24 hours later. Cardiac performance assessment was performed by bedside echocardiography, vital signs monitoring, and arterial and mixed venous blood gas monitoring.

Inotrope scoring (I.S.) and vasoactive inotrope scoring (V.I.S.) were calculated according to the data in the first 24 hours in patients taken to the intensive care unit after surgery.[15] These values were median and [inter quartile range; IQR]. The formulas used in this score calculation are shown in "information box 1".

Information Box 1. Inotrope score (I.S.) and vasoactive inotrope score calculate

I.S. = Dopamine (mcg/kg/min) + Dobutamine (mcg/kg/min) + 100 x Epinefrin (mcg/kg/min)

V.I.S. = I.S. + 10 x Milrinone (mcg/kg/min) + 10.000 x Vasopressin (units/kg/min) + 100 x Norepinefrin (mcg/kg/dose)

I.S., inotrope score; V.I.S., vasoactive inotrope score; mcg, microgram; kg, kilogram; min, minute.

Serum sample analysis method: Serum samples were collected from each patient, and the control was centrifuged for 20 minutes. Then, samples were stored at -82˚C until analysis. The study's samples were thawed the same day and taken to the Bezmialem University, Istanbul, research laboratory. One hundred thirty samples from 42 patients and 23 controls were divided into the clamping and second groups. We measured SOD (LOD: 0.5 U/mL, interassay CV 3.3%), Glutathione Peroxidase activities (GPX), and Malondialdehyde (MDA) (LOD: 31.2 ng/mL, CV< 10%) levels in patient and control sera using ELISA kits manufactured by ELABSCIENCE Biotechnology Co. LTD (USA, 14780 Memorial Drive, Suite 216, Houston, Texas 77079) in one day. MDA was measured using a competitive ELISA kit. The microtiter plate was pre-coated with an antigen specific to MDA. We added samples to the wells. MDA in the sample competes with a fixed amount of MDA in the solid phase to bind biotinylated detection antibodies specific to MDA. After washing, HRP (Horse Radish Peroxidase) conjugated Avidin is added to each microplate well and incubated. After washing, TMB substrate is added and terminated by a stop solution. The color change is measured photometrically at 450 nm. A standard curve was formed using data from the measurement. For Glutathione Peroxidase (GPX) activity, change in NADPH absorbance was measured kinetically at 340 nm. For Superoxide Dismutase (SOD) activity, the inhibition activity of SOD was determined by detecting the change in absorbance of WST-1 (Water-soluble tetrazolium) at 450nm. Later, creatine kinase muscle-brain (CK-MB) (mass immunoassay, CV:3.1%), Total creatine kinase (CK) (enzymatic, CV:0.5%), and Troponin I (TnI) (CMIA, CV:2%) levels were measured using Abbott reagents on Abbott Architect ci4100 (ABBOTT DIAGNOSTICS 5440 Patrick Henry Dr. Santa Clara, California 95054) in one run. QC procedures are run daily, and QC results were within ±2SD.

Statistical Analysis

IBM SPSS Statistics Software 21 (SPSS Inc., Chicago, Illinois, United States) was used for statistical analysis. Variables were examined using visual (histogram, probability plots) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk test) to determine whether they were typically dispersed. Descriptive analyses were presented using medians and IQR for non-normally distributed and ordered variables. Since the number of patients for enzymatic activity measurement did not show a normal distribution, nonparametric tests were performed to compare these parameters and sequential variables. Inter-group correlation analysis was performed using Spearman's correlation test. The Rho (ṕ) value indicated correlation severity. The Mann-Whitney U test was used for intragroup comparisons. A p-value of less than 0.05 was considered to show a statistically significant result.

RESULTS

Patients: Between April 2015 and December 2018, 67 pediatric patients with complex cardiac anomalies who underwent the first surgery and reoperation were included in the study (group 1, n=45; group 2, n=22). The demographic characteristics, cardiac diagnoses, surgical procedures, and intraoperative parameters of the patients are listed in Table 1.

Table 1. Demographic and operative data

	RIPC (n=45)	CTRL (n=22)	p-Value
Preoperative data			
Age (month) median (IQR)	19.1 (3.7-57.7)	16.7 (7.8- 35.9)	0.98
Gender (F/M)	20/25	8/14	0.53
STAT Category 1	23	10	
STAT Category 2	9	7	
STAT Category 3	7	3	
STAT Category 4	6	2	
STAT Category 5	-	-	
Cyanosis	30	13	0.54
Syndromic appearance	11	7	0.61
Postoperative data			
Re-do surgery	13	7	0.8
Postoperative ECMO	3	4	0.35
ICU stay (day) median (IQR)	2 (2-5)	3 (2-5)	0.33
Hospital stays (day) median (IQR)	8 (6-14)	10 (8-22)	0.02
Early mortality	1	3	0.26
Follow-up after discharge (month)	58.3 (54.3-62.1)	37.1 (34.8-41.7)	<0.01
From induction to CPB time (min.)	59 (53-69)	60.5 (48-69)	0.95
ACC time (min.)	59 (42-79)	65 (41.2-83.5)	0.54
CPB time (min.)	71 (55-99)	80 (66-102.2)	0.35
Reperfusion time (min.)	11 (10-20)	15 (10-18.7)	0.28
VIS	5.5 (1.7-8.2)	5.9 (4-8.4)	0.78
Arrhythmia after CPB	4	2	0.49

RIPC, remote ischemic preconditioning; CTRL, control; IQR, interquartile range; ECMO, extracorporeal membrane oxygenation; ICU, intensive care unit; ACC, aortic cross-clamp; CPB, cardiopulmonary bypass; VIS, vasoactive inotrope score.

The operative risk score of the patients was made according to the Society of Thoracic Surgery - European Society of Cardio-Thoracic Surgery (STS-EACTS [STAT]) classification system (Category 1 operations with the lowest risk of death and category 5, procedures with the highest risk of death).[16] This classification system is shown in Table 2.

Table 2. Common types of surgery within each STAT Category

STAT Category 1	Atrial septal defect repair, ventricular septal defect repair, coarctation repair, subaortic stenosis resection, pulmonary valve replacement, conduit replacement
STAT Category 2	Tetralogy of Fallot repair, Fontan operation, Ross operation
STAT Category 3	Hemi-Fontan operation, arterial switch operation, complete atrioventricular septal defect repair
STAT Category 4	Aortic arch repair, arterial switch operation with ventricular septal defect closure, heart transplant, aortopulmonary shunt, total anomalous pulmonary venous return repair, truncus arteriosus repair
STAT Category 5	Norwood (stage I) operation, hybrid stage 1 procedure, double switch operation, truncus arteriosus with interrupted aortic arch repair

The patients' surgical median age was 19.1 months (IQR= 3.7- 57.7 months) in Group 1 and 16.7 months (IQR= 7.8- 35.9 months) in Group 2. The median time between ischemic preconditioning and initiating cardiopulmonary bypass was 59 minutes (IQR= 53- 69 min) in group 1 and 60.5 minutes in group 2 (IQR= 48- 69 min) (p=0.95).

When the SOD analysis was compared according to the groups, the preoperative blood sample result was significantly higher in favor of group 1 (p= 0.015). No statistically significant difference was detected in the blood samples analyzed after surgery (p= 0.21).

MDA levels did not reveal a significant difference between groups in preoperative blood samples (p = 0.14). However, in the tests performed in the postoperative period, the difference was statistically significantly higher in favor of group 1 (p= 0.03).

A significant increase in GPX activity was detected in preoperative blood samples in favor of group 1 (p < 0.04). No statistically significant difference was detected in the blood samples analyzed after surgery (p= 0.24).

Our study used TnI, total CK, and CK-MB as postoperative cardiac protection markers. Troponin I level did not show a statistically significant difference between the groups in the preoperative period (p= 0.46). It was significantly lower in favor of group 1 in the postoperative period (p<0.01). CKMB values did not show a statistically significant difference between the groups in the preoperative period (p = 0.27). It was significantly lower in favor of group 1 in the postoperative period (p<0.01).

CK levels did not show a statistically significant difference between the groups in the preoperative period (p = 0.35). It was significantly lower in favor of group 1 in the postoperative period (p<0.01).

Enzymatic and biomarker levels are listed in Table 3.

No significant difference was found between the groups regarding postoperative arrhythmia incidence (p = 0.49). ECMO support was provided to 7 patients (group 1 n=3, group 2 n=4) due to LCOS in the early postoperative period (p=0.35). Among the postoperative cardiac performance indicators of the patients, the first 24-hour VIS values were also recorded. There was no significant difference between the groups (p=0.78). Here, since the inotropic support of patients receiving ECMO support will decrease, the VIS values of these patients were excluded.

The median length of stay in the intensive care unit (ICU) was two days (IQR= 2-5 days) in the RIPC group. In the control group, this value median was three days (2-5 days). There was no significant difference between the groups regarding the duration of stay in the ICU (p=0.33). The

Table 3. Statistical analysis of enzyme activities according to groups

	RIPC group		Control group		P-value
ENZYME	Median	IQR	Median	IQR	
Superoxide Dismutase (SOD)					
Preoperative	17.8	12.3-21	17.8	12.3-21	.05
Postoperative	16.9	11.3-19.7	16.9	11.3-19.7	.021
Glutathione Peroxidase activities (GPX)					
Preoperative	71	27-153	71	27-153	.08
Postoperative	70	21-139.4	70	21-139.4	.24
Malondialdehyde (MDA)					
Preoperative	84.9	69-97.4	84.9	69-97.4	.14
Postoperative	61.4	38.2-61	61.4	38.2-61	.03
Creatine Kinase (CK)					
Preoperative	45	36-63	45	36-63	.35
Postoperative	202	53-692	202	53-692	.01
Creatine Kinase, Muscle- Brain (CKMB)					
Preoperative	1.7	1.2-3.5	1.7	1.2-3.5	.27
Postoperative	4.9	1.4-59.9	4.9	1.4-59.9	<.01
Troponin I (TnI)					
Preoperative	9.1	3.2-107.6	9.1	3.2-107.6	.46
Postoperative	1222	9.2-13712	1222	9.2-13712	<.01

hospital's median length of stay was 8 days (IQR= 6-14 days) in the RIPC group. In the control group, this value median was 10 days (8-22 days). According to the groups, the patient's hospital stay duration showed a statistically significant difference (p= 0.02). This difference was in the direction of fewer hospital stays in Group 1.

The patients were followed up in 2 stages: the early post-operative period and after discharge. The early death rate was 4.4% (groups 1 n=1 and 2 n=2). Three of the EC-MO-dependent 7 patients died (42%). 1 of them was from group 1 patients. No mortality was observed in the mid-term follow-ups. The median follow-up period of group 1 patients was 58.3 months (IQR= 54.3- 62.1 months). The median follow-up period of group 2 patients was 37.1 months (IQR= 34.8- 41.7 months). Among these patients, there were three patients whose long-term records could not be reached. Five patients applied again for the second or third-stage operation post-discharge period. These patients were planned staged operations (e.g., Fontan completion) independently of RIPC. There was no significant difference in reoperation (p> 0.05).

DISCUSSION

Theoretical: The ischemic preconditioning theory was based on antioxidant enzymatic pathways present in the body, particularly endothelium-derived.

Purpose of application: In this study, our theoretical expectation was to activate the antioxidant activity before contributing to the body in combating operative stress. Our ultimate goal was to see this benefit in the cardiac tissue at the maximum level and minimize the damage. However, oxidative stress markers alone may not give specific results. Therefore, using more than one indicator is an advantage for accurate analysis.[18]

The relationship between SOD reported in studies and ischemia-reperfusion was also found in our research. It was at a statistically significant level (p< 0.05). There is a broad spectrum of congenital heart diseases, from genetic anomalies to acquired anomaly types. In other words, the balance change between free oxygen radicals and the antioxidant system may cause chromosomal abnormalities and accompanying cardiac effects. The effect of pharmacological agents may disrupt this balance. Or, there may be antioxidant dysfunction due to a genetic disorder. Increased SOD3 activity was shown in the erythrocytes of patients with Down syndrome; in our study, there were patients with intracardiac defects associated with Down syndrome. In light of these facts, in our research covering many types of congenital cardiac malformations, it may not be possible for the antioxidant system preconditioning to have an equal effect on all patients with RIPC application. In this case, this patient group's enzymatic activity is higher than others and will likely affect the results. The variety of metal cofactors (Cu, Zn, Mn) is critical in SOD activity and directly affects its biological role.19 The specific activity of Cu-Zn SOD is high in the erythrocytes of Down syndrome patients.[20,21]

Mezzetti A. et al.[22] examined enzymatic activity and capacity in human artery, vein, and heart tissue (right atrium) samples. As a result, they found a high rate in every tissue. In our study, the presence of enzymes that were found spontaneously and showed high cardiac tissue activity in both groups may have affected the statistical difference. In our study, GSHPX levels did not show a statistically significant difference between the groups. So, it can be concluded that preoperative activity was already high in both groups.

The other biomarker with significant elevation was MDA. Malondialdehyde (MDA) measurement is the most commonly used test to assess the degree of lipid peroxidation. MDA was also a widely used biomarker associated with cardiovascular events.[17] However, our study's correlation with other cardiac markers was weak (rho< 0.3). As Spiteller,[23] reviewed the role of lipid peroxidation in various chronic diseases, lipid oxidation end products emerged

as oxidative stress markers, with 4-HNE and MDA being among the most studied. However, since MDA is a product affected by heat, its plasma level may increase in its reactions.

Regarding cardiac biomarkers (TnI, CK, CKMB), the results were significantly lower in favor of the RIPC group. In other words, even under conditions where surgical modalities were similar, superiority was observed in terms of myocardial protection. Even though these results were not reflected in mortality at a statistically significant rate, they played an active role in reducing the length of hospital stay. Considering the secondary benefits of this situation, it will be of great importance in these high-risk patient groups.

Although there was no significant difference in the patients' duration in the ICU, the total hospital stay duration showed a substantial difference between the groups. This situation could be interpreted as a decrease in serous effusion or the reduction of the need for pharmacological support in RIPC patients because there was no difference between the groups regarding risk groups.

Studies have shown the role of free oxygen radicals in cardiovascular events. However, using antioxidant agents specifically as biomarkers has not been possible.

Limitation: One of the limiting factors in the study is the difficulty of obtaining a more significant number of enzymatic kits. It is essential in enzymatic processes that all samples taken are stored and analyzed under equal conditions. In addition, although the patients have a similar distribution in demographics, the possibility of not receiving reliable medication use and follow-up information from a sociocultural perspective may have affected the equality of conditions in the background. Again, considering the number of patients, efforts to create a more extensive series that can provide similar situations will also affect the study duration, design, and laboratory processes (separation, freezing, analysis, etc.).

CONCLUSION

The effects of pre/post-conditioning procedures by activating antioxidant systems on various organs have been published in the literature. However, no significant increase in survival was observed, especially in cardiac patients. More studies and different enzyme kits with an effective correlation rate could be investigated involving larger patient groups isolated from pharmacological agents or examining similar chromosomal anomalies as subgroups.

Ethics Approval

This study was planned as a single-center pediatric cardiovascular surgery clinic in Istanbul Medipol University Hospital in 2015. University Ethics Committee approval was obtained (March 21, 2014 /Number: 10840098-53).

Peer-review

Externally and internally peer-reviewed.

Authorship Contributions

Concept: A.A., E.C., Design: E.C., Data Collection or Processing: A.A., E.C., Analysis or Interpretation: T.A., E.C., Literature Search: A.A., E.C., Writing: A.A., T.A.

Conflict of Interest

The authors declared no conflict of interest.

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Informed Consent

The patients were sampled through a convenient sampling technique and enrolled after obtaining their written informed consent.

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Investigating False Positive Results in Urine Analysis Using the Immunoassay Method for Substance Metabolite Detection: A Retrospective Analysis

Doğrulama İstenen İdrarda Madde Analiz Sonuçlarının İncelenmesi

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Abstract

Aim This study aims to investigate the false positive outcomes in urine analysis via the immunoassay method within an addiction treatment center outpatient clinic. While widely utilized for substance detection, false positive results in the immunoassay method can cause misleading. This study aims to attract attention to false positivity and its implications.

Material and Method Conducted at an Alcohol and Drug Addiction Center outpatient clinic, the study retrospectively examines urine analyses from February to May 2023. Among 5109 immunoassay-based urine analyses, only 25 were subjected to confirmation through liquid chromatography-tandem mass spectrometry (LC-MS).

Results The findings demonstrate that LC-MS verified 40% of immunoassay-positive urine samples as true positives. Significantly, false positive results were notable, particularly in cases involving benzodiazepines. The study accentuates a noteworthy disparity between initial immunoassay outcomes and subsequent confirmatory tests, casting doubts on the reliability of the immunoassay method. A fundamental discovery is the consistent identification of pregabalin and gabapentin in urine samples yielding false positive benzodiazepine results during confirmation analysis. This revelation prompts inquiries into the potential cross-reactivity of these medications in immunoassay-based tests, suggesting the need for careful consideration in clinical and forensic contexts.

Conclusion The study underscores the importance of confirmatory testing for result accuracy and the multifaceted implications of false positives on patient-doctor relationships, treatment decisions, and patient safety. Acknowledging the study's limitations, such as its retrospective nature and limited participant pool, the research underscores the requirement for a comprehensive approach to substance detection, merging screening and confirmatory analyses to enhance diagnostic dependability.

Keywords *Benzodiazepine, false positive, immunoassay, LC-MS, opioid.*

Özet

Amaç *Bağımlılık yapan maddelerin taranması ve tespiti, gerek kişinin tedavi süreci, gerekse adli boyutları için önem arzetmektedir. Sıklıkla kullanılan bir yöntem olan idrarda immunoassay yöntemi ile madde tespiti, zaman zaman yanlış-pozitif sonuçlar verebilmektedir. Bu çalışmada, kliniğimizde immunoassay ile idrarında madde metaboliti saptanan sonuçların ne kadarının yanlış-pozitif olduğunun incelenmesi amaçlanmıştır.*

Gereç ve Yöntem *Bir AMATEM kliniğinde Şubat ve Mayıs 2023 tarihleri arasında yapılan idrar analizlerinin retrospektif olarak incelenmesi ile bu çalışma gerçekleştirilmiştir. 5109 hastanın verisi taranmış ve çalışma için uygun olan 25 hasta çalışmaya dahil edilmiştir; kişilerin yaş, cinsiyet, medeni durum ve idrar analiz sonuçlarına hastane kayıtlarından ulaşılmış, kendileriyle yüz yüze görüşülmemiştir. Çalışma için etik onam alınmıştır (no: E-23/1319).*

Bulgular *Çalışmanın sonuçlarına göre, LC-MS ile immünoassay yöntemiyle pozitif sonuç veren idrar örneklerinin sadece %40'ı pozitif olarak saptanmıştır. Opioid pozitifliğiyle doğrulamaya gönderilen idrarların sadece 3 (%23,1) tanesinin, benzodiazepin pozitifliğiyle doğrulamaya gönderilen idrarların ise 7 (%58,3) tanesinin pozitifliği konfirme edilmiştir. Benzodiazepin doğrulama sonucunda pozitif gelenlerin tamamında pregabalin ve gabapentin tespit edilmesi de çalışmamızın önemli bulgularından bir tanesidir.*

Sonuç *Çalışmamızın bulguları incelendiğinde, 5109 idrar analizinden sadece 25 tane doğrulama istenmiş olması ve sadece opioid ile benzodiazepin pozitifliğinde doğrulama istenmiş olması dikkate değerdir. İdrarda herhangi bir maddenin pozitifliği saptandığında, hastalar sık sık bunun yanlış pozitif olabileceğini iddia etmektedirler ve çalışmamızın sonuçlarına göre doğrulamaya gönderilen idrarların %60'ının yanlış-pozitif olduğu saptanmıştır. Son yıllarda kötüye kullanımı katlanarak artan pregabalin ve gabapentinin benzodiazepin yanlış-pozitifliğine neden olabileceğini ve immunoassay analizlerinin yanlış-pozitif sonuçlanabileceğini, bağımlılık alanında çalışan psikiyatristlerin göz önünde bulundurmalarında fayda vardır.*

Anahtar Kelimeler *Benzodiazepin, immunoassay, LC-MS, opioid, yanlış pozitif.*

INTRODUCTION

Detecting addictive substances in biological samples, particularly urine plays a significant role in clinical and legal contexts.[1] The immunoassay method has gained prominence as a reliable screening tool for detecting abused substances in urine samples due to its simplicity and availability.[2,3] However, despite its widespread use, the immunoassay method is not devoid of limitations, occasionally leading to false positive results.[4] The antibodies used in the immunoassay method can cause cross-reactivity and false-positivity.[5] Incorrect positive urine analysis results during the treatment processes of patients with substance use disorder will hinder the proper execution of this process.[6,7]

In the mass spectrometry technique, the relevant substance is directly detected, unlike the indirect measurement in the immunoassay method. Therefore, it is considered the best analytical technique for the most accurate substance screening analyses.[8] However, it is not widely used due to the lack of mass spectrometry equipment in every laboratory or the delay in obtaining analysis results for days. There are types, such as liquid chromatography-tandem mass spectrometry (LC-MS) or gas chromatography-tandem mass spectrometry (GC-MS).[9]

This study aims to explore the false positive results in urine analysis with the immunoassay method in an addiction treatment center outpatient. By conducting retrospective research, we intend to shed light on the prevalence, factors, and implications of false positive results in this diagnostic approach.

MATERIALS and METHODS

The study was conducted at the Alcohol and Drug Addiction Center outpatient clinics of Ankara Training and Research Hospital. Samples found positive for substance metabolites in urine analyses performed by the immunoassay method were analyzed by the LC-MS method for confirmation.

Records from February to May 2023 were retrospectively reviewed, revealing 5109 urine analyses conducted using the immunoassay method. Among these, it was observed that only 25 were sent for confirmation through LC-MS analysis. These 25 patients who had been referred for confirmation due to positive immunoassay-based urine analysis results were included in the study.

By reviewing the hospital's medical records, essential demographic information, including age, gender, marital status, and initial urine analysis results, were extracted. The study design did not entail direct interaction with the participants; the analysis was solely based on the available data. Ethical approval for the study was obtained from the Ankara Training and Research Hospital Clinical Studies Ethics Committee (decision no: E-23/1319).

The research data were analyzed using SPSS (Statistical Package for the Social Sciences for Windows v.22.0, SPSS Inc., Chicago, IL). Descriptive statistics were presented as mean (±) standard deviation, frequency distribution, and percentage.

RESULTS

The participants exhibited a gender distribution, with 88% male and 12% female. The demographic landscape encompassed various marital statuses, including 56% single, 20% married, and 24% divorced or widowed individuals. The average age of the participants was calculated to be 35.6±9.8 years.

Of the 25 urine samples, 13 were sent for confirmation due to opioid and 12 benzodiazepine positivity. Upon confirmation analysis, 40% of the urine samples were validated as positive, whereas the remaining 60% were negative (Table 1).

Table 1. Socio-demographic Characteristics of Participants		
	mean / n	SD / %
Age	35.6	9.8
Gender		
Male	22	88
Female	3	12
Marital status		
Single	14	56
Married	5	20
Divorced/widowed	6	24
Reason for confirmation		
Opioid+	13	52
Benzodiazepine+	12	48
Confirmation result		
Positive	10	40
Negative	15	60
Mean: mean; n: number; SD: standard deviation; %: percentage.		

Moreover, a closer examination of the samples revealed that only 23.1% of the urine samples sent for confirmation due to opioid positivity were eventually confirmed as positive, highlighting a noteworthy discordance between initial immunoassay results and subsequent confirmatory tests. Similarly, among the samples sent for benzodiazepine confirmation, a significantly higher proportion (58.3%) were confirmed as positive, indicating that many benzodiazepine-positive results from the immunoassay might be false positives.

One of the crucial results of this study was the consistent identification of pregabalin and gabapentin in all cases that yielded positive results for benzodiazepines during the confirmation analysis. This observation raises intriguing questions about the potential cross-reactivity of these medications in immunoassay-based tests, suggesting a nuanced consideration of their presence in clinical and forensic contexts (Table 2).

Table 2. Analysis of Groups Sent for Confirmation Due to Opioid or Benzodiazepine Positivity		
	Opioid+ (n=13)	Benzodiazepine+ (n=12)
Confirmation Result		
Positive	3 (23.1%)	7 (58.3%)
Negative	10 (76.9%)	5 (41.7%)
Substances Detected upon Confirmation		
Pregabalin	0	5
Gabapentin	0	2
Morphine	3	0
n: number; %: percentage.		

DISCUSSION

According to the findings of our study, only 25 confirmatory tests were sent out of 5109 urine analyses. Notably, more than half of the positive samples for the substance metabolites were found negative, according to the confirmation results. Gabapentin and pregabalin were found to cause benzodiazepine false positivity in the immunoassay method.

The implications of false positive results in urine analysis using the immunoassay method are multifaceted. A false positive result in urine analysis can have negative consequences for patients. Patients under treatment may be mistakenly categorized as having used heroin when they have not, which can undermine trust in the patient-doctor relationship.[9]

Another example is when someone who has used medication containing codeine for a cough is mistakenly categorized as having an opioid overdose due to a false positive urine result when an underlying issue affects their clinical condition. This misclassification might lead to overlooking the actual underlying problem.[10]

False positive benzodiazepine results can disrupt the treatment process for patients under buprenorphine therapy, as the co-administration of benzodiazepines and opioids can lead to respiratory depression.[11] Due to this potential risk,

clinicians might refrain from prescribing buprenorphine treatment to such patients.[12]

The present study not only emphasizes the need for confirmatory testing to ensure accurate results but also emphasizes the significance of understanding the factors that can contribute to false positive outcomes. The prevalence of false positive results in the studied cases, particularly in benzodiazepine-related instances, serves as a reminder that caution is required in interpreting immunoassay-based findings, especially in the context of substances that might share structural similarities.

As the abuse of substances like pregabalin and gabapentin gains momentum, their potential to trigger false positive benzodiazepine results warrants thorough consideration. Professionals in addiction psychiatry should be vigilant about the limitations and potential pitfalls of immunoassay methods, acknowledging the need for a comprehensive approach to substance detection that combines screening and confirmatory analyses.[13]

Our study also has certain limitations that need to be acknowledged. Firstly, its retrospective design and the derivation of data from health records restrict the generalizability of the results. Additionally, the limited number of participants constitutes another shortcoming of our study. Besides these limitations, this retrospective analysis sheds light on the intricate dynamics of false positive results in urine analysis using the immunoassay method. By providing insights into these results' prevalence, patterns, and implications, the study underlines the necessity of a holistic approach to substance detection, ensuring the reliability of diagnostic outcomes and the accuracy of conclusions drawn.

Acknowledgment

The authors declare they have no conflicts of interest to disclose.

Ethical Approval

University of Health Science, Ankara Education and Research Hospital ethics Commitee and following the Declaration of Helsinki (decision no: E-23/1319).

Peer-review

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Authorship Contributions

Concept: G.Z.İ., Ş.B.N., Design: G.Z.İ., Ş.B.N., Data collection or Processing: G.Z.İ., Analysis or interpretation: G.Z.İ., Ş.B.N., Literature Search: G.Z.İ., Ş.B.N., Writing: G.Z.İ., Ş.B.N.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Detection of Human Bocavirus in Respiratory Tract Specimens

Solunum Yolu Örneklerinde İnsan Bocavirüs Tespiti

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Abstract

Aim The aim of this study was to retrospectively examine the patients who presented with the complaints of respiratory tract infection and were found to have Human bocavirus (HBoV) in the samples studied with the respiratory tract pathogens panel.

Material and Method We retrospectively analyzed patients of all age groups who were diagnosed with HBoV by PCR in the respiratory tract pathogens panel between January 2021 and November 2022.

Results Between January 2021 and November 2022, 36 patients with HBoV DNA detected by PCR in nasopharyngeal swab samples taken from a total of 989 patients were examined. Of 989 patients, 557 were male and 432 were female (male/female 1.28). The median age of HBoV positive patients was 2.3. According to age groups, 1-2 age years-old showed the highest prevalence. In patients with positive HBoV DNA, the most common symptom was cough (77.7%) and catarrh (69.4%). HBoV was detected alone in 15 (41.7%) patients and together with other viruses in 21 (58.3%) patients in total. Rhinovirus/Enterovirus was found to be the most common co-pathogen.

Conclusion Patients positive for HBoV exhibited few respiratory symptoms as a result of single or co-pathogenicity, confirming its role in respiratory diseases. However, it is difficult to say that HBoV is the primary responsible pathogen in respiratory tract infections.

Keywords *Acute respiratory infection, children, human bocavirus, respiratory tract pathogens.*

Özet

Amaç *Solunum yolu enfeksiyonu şikâyetiyle gelen ve solunum yolu patojenleri paneli ile çalışılan örneklerde Human bocavirus (HBoV) saptanan hastaların retrospektif olarak incelenmesi*

Gereç ve Yöntem *Ocak 2021-Kasım 2022 tarihleri arasında solunum yolu patojenleri panelinde PCR yöntemi ile HBoV saptanan tüm yaş grubundaki hastaları geriye dönük olarak inceledik.*

Bulgular *Ocak 2021-Kasım 2022 tarihleri arasında toplam 989 hastadan alınan nazofaringeal sürüntü örneklerinde PCR ile HBoV DNA saptanan 36 hasta incelendi. Toplamda 989 hastanın 557'si erkek, 432'si kadındı (erkek/kadın1,28). HBoV pozitif hastaların medyan yaşı 2,3 idi. Yaş gruplarına göre 1-2 yaş en yüksek prevalansı göstermiştir. HBoV DNA'sı pozitif olan hastalarda en yüksek semptom öksürük (%77,7) ve nezle (%69,4) idi. HBoV 15 (%41,7) hastada tek başına, 21 (%58,3) hastada diğer virüslerle birlikte saptandı. Rhinovirus/enterovirus en yaygın ko-patojen olarak bulundu.*

Sonuç *HBoV için pozitif olan hastalar, tek veya ko-patojenitenin bir sonucu olarak, solunum yolu hastalıklarındaki rolünü doğrulayan birkaç solunum semptomu sergiledi. Bununla birlikte solunum yolu enfeksiyonlarında HBoV 'nin birincil sorumlu patojen olduğunu söylemek güçtür.*

Anahtar Kelimeler *Akut solunum yolu enfeksiyonu, çocuklar, human bocavirus, solunum yolu patojenleri.*

INTRODUCTION

Acute respiratory tract infections are among the most important causes of childhood mortality and morbidity. Although influenza viruses, parainfluenza viruses, respiratory syncytial virus (RSV), picornaviruses (rhinovirus or enteroviruses), adenoviruses and coronoviruses are the most common viruses causing respiratory tract infections, pathogenic microorganisms cannot be identified in some of these infections.[1,2] With the development of molecular methods, new viruses such as Human bocavirus (HBoV), Human metapneumovirus (HMPV), Human coronaviruses (HCoV-NL63, HCoV-HKU1, HCoV-OC43, HCoV-229E) have also been detected in respiratory tract specimens. The worldwide estimate of the total prevalence of HBoV in respiratory tract infections is 6.3%. The presence of co-pathogen rate in people with respiratory tract infection and HBoV positivity is between 8.3-100%.[3,4]

HBoV belongs to the Parvoviridae family, the Parvovirinae subfamily, and the Bocavirus genus. HBoV is a non-enveloped DNA virus with an icosahedral capsid, a 5.5 kb linear and single-stranded genome. In addition, HBoV subdivided into 4 genotypes. HBoV1 is predominantly found in the respiratory tract and often in association with another pathogenic viruses.[5,6] HBoV1 has been associated with upper respiratory tract infections and lower respiratory tract infections, wheezing, bronchiolitis, and pneumonia. HBoV2-4 is mainly found in stool samples from patients with gastroenteritis.[6-8]

In this study, our aim is to determine the frequency of HBoV in patients of all age groups admitted to the hospital with respiratory tract infection complaints and to describe the clinical features of infected patients.

MATERIALS and METHODS

Nasopharyngeal swab samples were taken from 989 patients who applied to Ondokuz Mayıs University Hospital with complaints of respiratory tract infections such as fever, cough, wheezing, dyspnea and nasal congestion between January 2021 and November 2022 and developed one or more of these symptoms. Swab samples were taken throughout the year, but especially in November, December, January, February, due to more severe symptoms. These swab samples were studied using Multiplex Real Time PCR to determine the causative pathogen. Qiastat-Dx (Qiagen, Germany) Respiratory SARS-CoV-2 Panel, which can detect 22 pathogens (SARS-CoV-2, influenza A, influenza A subtype H1N1/2009, influenza A subtype H1, influenza A subtype H3, influenza B, coronavirus 229E, coronavirus HKU1, coronavirus NL63, coronavirus OC43, parainfluenza virus 1, parainfluenza virus 2, parainfluenza virus 3, parainfluenza virus 4, RSV A/B, HMPV A/B, adenovirus, HBoV, rhinovirus/ enterovirus, Mycoplasma pneumoniae, Legionella pneumophilia and Bordetella pertussis), was used for PCR. The Qiastat-Dx Respiratory SARS-CoV-2 Panel cannot differentiate between rhinovirus/enterovirus. The swab samples taken were added to the transport medium (Universal Transport Medium, UTM), delivered to the laboratory within one hour, and most of the samples were studied within two hours. For the test, 300 µl of was taken from the transport medium and placed in the main port of the Qiastat-Dx Respiratory SARS-CoV-2 Panel Cartridge. No different buffer solution was used during the transfer of the sample to the device. The test was started by placing the Qiastat-Dx Respiratory SARS-CoV-2 Panel Cartridge into the QIAstat-Dx Analyzer 1.0. Extraction, amplification and analysis of nucleic acids in the sample detection was performed automatically by the QIAstat-Dx Analyzer 1.0.

The Qiastat-Dx Respiratory SARS-CoV-2 Panel detects HBoV DNA and however a universal primer was used for HBoV1-4, it was not possible to distinguish between different subtypes of HBoV, which is a limitation of our study.

Statistical Analysis

Comparative statistical analyzes were used in HBoV positive and negative patient groups. Categorical variables were expressed as age and percentage of numbers, and

continuous variables as median and range. All data analyzes were performed using SPSS+ statistics calculation program version 21.

RESULTS

The study included 986 patients whose nasopharyngeal swab samples were sent to the microbiology laboratory to be studied with a respiratory panel between January 2021 and November 2022. The age distrution of the patienst was 0-87 years. Of 989 patients, 557 were male and 432 were female (male/female 1.28). In total, 36 (3.6%) of 989 patients were found to be positive for HBoV DNA positive, and 26 (72.2%) of them were male. The presence of complaints such as cough, wheezing, dyspnea, fever, nasal congestion, catarrh and their diagnosis were bronchiolitis, and bronchopneumonia were investigatig through hospital information system. Table 1. showed details of the patients. Considering the age groups, one-two years -olds showed the highest prevalence.

Table 1. Characteristics of the patients

Variable	Category	Frequency	Male/ Positive	Female/ Positive	Total positive HBoV
Age	0-1 year	211	121/4	90/1	5
	1-2 years	92	52/7	40/4	11
	2-3 years	71	40/6	31/2	8
	3-4 years	60	35/2	25/1	3
	4-5 years	54	32/2	22/0	2
	5-18 years	350	204/5	146/1	6
	>18 years	151	73/0	78/1	1
Total		989	557/26	432/10	36

The median age of HBoV positive patients was 2.3 years-old. Cough was the most common symptom and followed by catarrh in patients who was positive for HBoV (Table 2).

Table 2. Frequency of symptoms among HBoV-positive children

Symptom	Frequency
Cough	28
Catarrh	25
Dyspnea	16
Nasal congestion	15
Wheeze	15
Fever	14
Vomiting	2

In addition, according to clinical data, 11 (30.5%) of 36 patients were diagnosed with pneumonia and nine (25.0%) were diagnosed with bronchiolitis. While only HBoV was detected in 15 of 36 (41.7%) patients, other factors were detected together with HBoV in 21 (58.3%) patients. The most common co-pathogens were with rhinovirus/enterovirus, SARS-CoV-2 and RSV (Figure 1.).

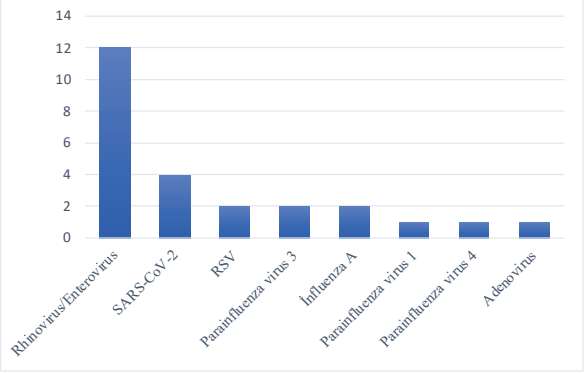

Figure 1. Viruses determined with HBoV.

Five rhinovirus/enterovirus, four SARS-CoV-2, one parainfluenza virus 3, one parainfluenza virus 4, one influenza A were found in eight pneumonıa cases in which HBoV was detected as a co-pathogen. Three rhinovirus/enterovirus and one influenza A were detected in four bronchiolitis cases with HBoV as co-pathogen. No co-pathogenicity of HBoV with bacteria or fungi was seen. When the distribution of HBoV positivity was examined by months, the highest positivity was seen in October, and the least in May and Jun (Figure 2.).

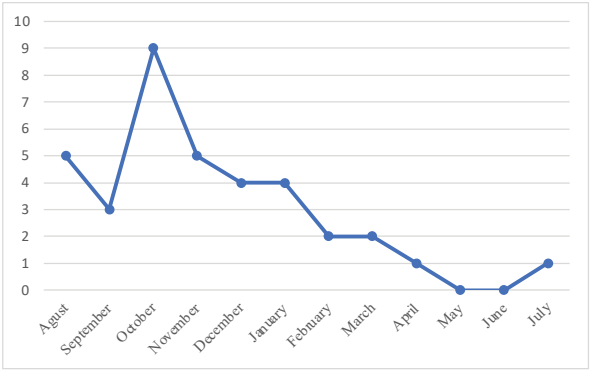

Figure 2. Distribution of HBoV positivity by months

DISCUSSION

HBoV, first identified in respiratory samples of Swedish children with lower respiratory tract infections, is increasingly associated with acute respiratory tract infection of unknown etiology, especially in young children. HBoV is detected more frequently in young children (<2 years) compared to older children and adults.[7,9,10]

Respiratory diseases such as colds, asthma, wheezing, bronchiolitis, pneumonia have been reported in many studies in connection with HBoV. It is not possible to clinically distinguish respiratory tract infections caused by different viruses or even bacteria such as rhinovirus, RSV, influenza virus and HBoV. In a recent study, respiratory tract infection symptoms seen in HBoV positive children in nasopharyngeal swap were most commonly cough (79%) followed by fever (67%) runny nose (66%).11-13 In a study by Joseph et al.[14] in Nigeria, they reported that the most common symptoms in children with HBoV were cough (100%), catarrh (100%) and nasal congestion (59.2%). In our study, cough (77.7%), catarrh (69.4%) and dyspnea (44.4%) were observed most frequently. In a study by Petrarca et al.[15], 34 (56.6%) of 60 HBoV positive patients had bronchiolitis and three (5%) had pneumonia; reported that HBoV alone was detected in 13 (38.2%) patients with bronchiolitis and in all patients with pneumonia. In our study, we found that 14 of 36 HBoV positive patients (38.8%) had pneumonia, nine (25%) had bronchiolitis, and five of nine patients with bronchiolitis and six of 14 patients with pneumonia had HBoV as a single pathogen. For HBoV positive patients, a more detailed anamnesis and examination will be useful to define clinical symptoms of HBoV and to better recognize HBoV.

In the study conducted by Ljubin-Sternak et al.16 in two different hospitals in Croatia, 957 respiratory tract samples taken from children aged 0-18 years who applied with the complaint of respiratory tract infection between May 2017 and March 2021 were examined. They reported that HBoV was detected in 73 (7.6%) of 957 children, 13 (17.8%) of them were found to be a single pathogen, and 60 (82.2%) were associated with one or more respiratory tract viruses. It was also stated that the most common accompanying virus was rhinovirus (35.8%). They also reported that the male: female ratio of HBoV positive patients was 41:32 (1.28:1) and the median age of HBoV positive patients was 1.36. They found that the highest rate (61.6%) according to age groups belonged to the 1-2.99 age group. In the study conducted by Madi et al.[17] in respiratory samples of 5941 patients with respiratory tract infection symptoms, HBoV was detected in 111/5941 (1.9%) samples. They stated that 59 (53.2%) of HBoV positive patients were male, 52 (46.8%) were female, and the median age was 1 year. While HBoV alone was detected in 48 (43.%) of 111 HBoV positive patients, it was found together with another virus in the remaining 63 (56.8%); reported that the most common association was with RSV (10.8%) and rhinovirus (9.9%). In the study conducted by Uyar et al.[18] with 95 patients, they detected HBoV in three (3.1%) people and it was reported that one of these three people was a single pathogen. Similar to these studies, in our study, the copatogenicity rate was found to be higher than the single detection of HBoV; rhinovirus/enterovirus (57.1%) was foundto be the most common virus accompanying HBoV. The male: female ratio of HBoV positive patients was 2.6. Similar to most studies, we observed more positivity in males. In our study, the HBoV positivity rate was found 3.6% for the whole age group and 4.17% for those under the age of[18]. In

addition, the median age ratio (2.3) was found to be higher in our study than these studies. The differences in HBoV positivity can be explained by the different study patterns and the age of the study group. While these studies covered the younger age group, this study was carried out on patients of all age groups.

Any seasonal distribution for HBoV is controversial as it varies by geographic region. Some studies reported that HBoV infections occurred with a high prevalence in winter and spring, some studies showed a higher prevalence in late spring and early summer, and some studies reported that no significant seasonal activity was observed.[15,19,20] In our study, it was seen that the distribution of HBoV intensifies in autumn. The differences with the seasonal distribution of HBoV are likely due to the different populations involved in the studies and different geographic regions.

It is difficult to prove the clinical significance and pathogenicity of HBoV due to its high co-pathogen ratio and to say that HBoV is the primary factor in infected patients. It can be said that HBoV is a factor that exacerbates respiratory diseases.[6,19] Although many studies have confirmed the severity of infection with HBoV positivity, some studies have not found a clear association between HBoV infection and different clinical manifestations.9 However, the frequency of HBoV detection in symptomatic patients is higher than in healthy controls.[21]

Studies have shown that the presence of HBoV continues for up to six months in nasopharyngeal samples taken from healthy asymptomatic children.[22] Therefore, newly acquired infection is not the only cause of HBoV DNA detection in the respiratory tract. It should also be considered that HBoV may remain latent in the respiratory tract. A positive PCR result for HBoV should be interpreted together with clinical symptoms.[9,22]

The first infection of HBoV occurs very early in life, as seen in epidemiological studies. There are few systematic studies involving adults, but studies show a very low prevalence of viruses in the respiratory tract of adults by PCR. More research is needed in adults and immunosuppressed individuals.[23,24]

Our retrospective study had some limitations; there were no healthy controls in the study and viral load could not be determined in the nasalopharyngeal specimens. Patients positive for HBoV exhibited few respiratory symptoms as a result of single or co-pathogenicity, confirming its role in respiratory diseases. However, it is difficult to say that HBoV is the primary responsible pathogen in respiratory tract infections. Although there is increasing evidence for the role of HBoV in respiratory infections, more studies are needed to fully understand the relationship between its pathogenicity and infection severity.

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None to declare.

Ethical Approval

Ethics Committee Approval: The study was approved by the Medical Ethics Committee of Ondokuz Mayıs University. (B.30.2.ODM.0.20.08/776-169)

Declaration of Helsinki

The study was conducted in accordance with the Declaration of Helsinki and followed the ethical standards of the country of origin. (B.30.2.ODM.0.20.08/776-169)

Peer-review

Externally and internally peer-reviewed.

Authorship Contributions

Concept: Y.T.C., Design: Y.T.C., Data Collection: Y.T.C., E.A. Analysis or Interpretation: Y.T.C., E.A., Literature Search: Y.T.C., E.A., Writing: Y.T.C., E.A.

Conflicts of Interest

The authors have none to declare

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Changes in Antimicrobial Susceptibility Patterns of Microorganisms Isolated from Urine Cultures in the Last Decade

İdrar Kültürlerinden İzole Edilen Mikroorganizmaların Antimikrobiyal Duyarlılık Paternlerinin Son On Yıldaki Değişimi

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Abstract

Aim Urinary tract infections are the infections for which antibiotics are most frequently prescribed to outpatients. The purpose of this study is to evaluate the ten-years change in antibiotic resistance profiles of microorganisms grown in urine cultures.

Material and Method The results of urine cultures that were sent to the Microbiology Laboratory between 01.01.2013 and 31.12.2022 were evaluated retrospectively. Identification and antimicrobial susceptibility of microorganisms were performed with the BD Phoenix 100 (Becton Dickinson, Maryland, USA) device between 2015-2016, and with the VITEK-2 compact system (BioMérieux, France) between 2013-2014, 2017-2022. While the antibiotic susceptibility results of the isolates were interpreted using the clinical breakpoints defined by the Clinical Laboratory and Standards Institute (CLSI) between 2013 and 2015, they were evaluated according to the European Committee for Antimicrobial Susceptibility Testing (EUCAST) criteria after 2015.

Results The most common microorganism is *Eschericha coli* with 49%(n=4.898), while the second most common agent is Klebsiella spp. with 13.8% (n=1.380) were gram-negative microorganisms. While the most sensitive antibiotics were carbapenem and aminoglycoside groups in *Escherichia coli* and *Klebsiella spp.*, the resistance to ampicillin, ciprofloxacin and cephalosporins in *Escherichia coli* was over 60%, while this rate was around 80% in Klebsiella spp. In our study, resistance rates of PIP-TZP, ceftazidime, carbapenems, aminoglycosides and quinolones were higher against *Acinetobacter spp*. than *Pseudomonas spp*. In general, resistance to *Acinetobacter spp. and Klebsiella spp*., and there was a tendency for the resistance rates of all microorganisms to increase over the years.

Conclusion Creating more detailed cumulative antibiogram reports, sharing these reports with relevant clinics within specified periods, establishing antimicrobial management teams that will work in harmony with infection control committees in hospitals, structuring a nationwide surveillance program; It will contribute to the determination of empirical treatment, prevention of the development of antimicrobial resistance and control of hospital-acquired infections.

Keywords Antimicrobial resistance, *Escherichia coli,* Urine culture.

Özet

Amaç *İdrar yolu enfeksiyonları, polikliniklere başvuran hastalara en sık antibiyotik reçete edilen enfeksiyonlardır. Bu çalışmanın amacı idrar kültürlerinde üreyen mikroorganizmaların antibiyotik direnç profillerindeki on yıllık değişimi değerlendirmektir.*

Gereç ve Yöntem *Mikrobiyoloji Laboratuvarı'na 01.01.2013 ile 31.12.2022 tarihleri arasında gönderilen idrar kültürü sonuçları retrospektif olarak değerlendirildi. Mikroorganizmaların tanımlanması ve antimikrobiyal duyarlılıkları iki ayrı sitemle, 2015-2016 yılları arasında BD Phoenix 100 (Becton Dickinson, Maryland, ABD) cihazıyla 2013-2014 ve 2017-2022 yılları arasında ise VITEK-2 kompakt sistemiyle (BioMérieux, Fransa) yapıldı. İzolatların antibiyotik duyarlılık sonuçları 2013-2015 yılları arasında Klinik Laboratuvar ve Standartlar Enstitüsü (CLSI) tarafından tanımlanan klinik sınır değerleri kullanılarak yorumlanırken 2015 sonrası Avrupa Antimikrobiyal Duyarlılık Testi Komitesi (EUCAST) kriterlerine göre değerlendirildi.*

Bulgular *En sık görülen mikroorganizma %49(n=4,898) ile Eschericha coli olurken ikinci en sık etken %13,8(n=1,380) ile Klebsiella spp. gibi gram negatif mikroorganizmalardı. Escherichia coli ve Klebsiella spp.'de en duyarlı antibiyotikler karbapenem ve aminoglikozid grubuyken Escherichia coli'de ampisilin, siprofloksasin ve sefalosporinlere direnç %60'ın üzerindeyken bu oran Klebsiella spp.'de %80 civarında tespit edildi. Çalışmamızda, piperasilin tazobaktam, seftazidim, karbapenemler, aminoglikozidler ve kinolonların Acinetobacter spp.'ye karşı direnç oranları Pseudomonas spp.'ye göre daha yüksekti. Genel olarak direnç Acinetobacter spp. ve Klebsiella spp.'da daha ciddiydi ve tüm mikroorganizmaların direnç oranlarında yıllar içinde artış eğilimi vardı.*

Sonuç *Daha detaylı kümülatif antibiyogram raporlarının oluşturulması, bu raporların belirlenen sürelerde ilgili kliniklerle paylaşılması, hastanelerde enfeksiyon kontrol komiteleri ile uyum içerisinde çalışacak antimikrobiyal yönetim ekiplerinin oluşturulması, ülke çapında sürveyans programının yapılandırılması; ampirik tedavinin belirlenmesine, antimikrobiyal direnç gelişiminin önlenmesine ve hastane kaynaklı enfeksiyonların kontrolüne katkıda bulunacaktır.*

Anahtar Kelimeler *Antimikrobiyal direnç, Escherichia coli, İdrar kültürü*

INTRODUCTION

According to the World Health Organization's 2019 report, antimicrobial resistance is one of the biggest threats to public health.[1] Urinary tract infections (UTI) are the most common infections both in the community and in the healthcare settings and are caused by major uropathogens such as *Escherichia coli (E. coli)*, *Klebsiella pneumoniae (K. pneumonia), Proteus mirabilis (P. mirabilis), Enterococcus faecalis (E. faecalis)* and *Pseudomonas aeruginosa (P. aeruginosa)*.[2] When the results of the "Global Prevalence of Infections in Urology (GPIU)" study covering 70 countries were examined, it was seen that most of the uropathogens had high rates of antibiotic resistance and multidrug resistance was especially striking.[3] UTI is one of the most common indications for outpatient empirical antibiotic prescribing.[4]

International guidelines recommend fosfomycin and nitrofurantoin as the first-line treatment of acute uncomplicated cystitis. Trimethoprim-sulfamethoxazole (TMP-SXT) can only be considered as the drug of first choice if local resistance to *E. coli* does not exceed 20 percent. Due to high resistance to aminopenicillins and long-term side effects of fluoroquinolones, they are no longer recommended as first-line treatment for urinary tract infections. Second-line options include oral cephalosporins such as cephalexin or cefixime, fluoroquinolones, and beta-lactams such as amoxicillin-clavulanate.[5]

In antimicrobial stewardship, microbiology laboratories have an important role in both determining empiric treatment and monitoring resistance profiles over the years by reporting cumulative antibiogram results regularly.[6] The aim of this study is to evaluate the ten-year change in the antibiotic resistance profiles of microorganisms grown in urine cultures.

MATERIALS and METHODS

A total of 35.132 urine culture samples sent to Gaziantep Dr. Ersin Arslan Training and Research Hospital Microbiology Laboratory between 01.01.2013 and 31.12.2022 were retrospectively evaluated. Urine samples were inoculated on sheep blood agar and Eosin Methylene Blue (EMB) agar by quantitative method using 0.01 ml loops. Urine culture plates inoculated for routine purposes were evaluated after incubation at 35-37 ºC for 16-24 hours in accordance with the guide recommendations. Additional procedures were performed for yeasts.[7] Identification of microorganisms and their antimicrobial susceptibility were performed with two separate systems, between 2015-2016 with the BD Phoenix 100 (Becton Dickinson, Maryland, USA) device, and between 2013-2014 and 2017-2022 with the VITEK-2 compact system (BioMérieux, France). Antibiotic susceptibility results of the isolates were interpreted using the clinical breakpoints defined by the Clinical Laboratory and Standards Institute (CLSI) between 2013 and 2015, and were evaluated according to European Antimicrobial Susceptibility Testing Committee (EUCAST) criteria after 2015.[8-9]

Descriptive statistical analysis: Resistance rates of isolates in the study were determined numerically using Microsoft Excel 2013 (Microsoft Corp. Redmond, WA, USA) and calculated as percentages.

RESULTS

A total of 35.132 urine culture results, who were admitted to outpatient clinics with UTI symptoms and sent to the laboratory from intensive care units and wards with the preliminary diagnosis of UTI, were retrospectively examined. During the 10-year period of our study, growth was observed in 28.5%(n=10.000) of the samples sent to our laboratory, while 63.8% (n=22422) did not show growth, and 7.7%(n=2.710) was evaluated as contamination. A total of 19.365 (55.1%) samples were sent from outpatient clinics, while 15.767 (44.9%) samples were sent from inpatients. Of the samples in which microbial growth was detected, 64.4%(n=6440) were female and 35.6%(n=3560) were male. Growth was most frequently detected in samples sent from outpatient clinics with 56.5%(n=5652),

followed by samples sent from intensive care with 27.6%(n=2756) and samples from patients sent from services with 15.9%(n=1592), respectively. When the distribution according to clinics is examined, 27.6%(n=2756) of the samples in which growth was detected were from intensive care, 18.6%(n=1859) from urology, 13%(n=1297) from nephrology, 10.3%(n=1030) from internal medicine, 9%(n=909) were sent from infectious diseases, 8.2%(n=819) were sent from pediatrics, and 2.1%(n=211) were sent from gynecology and obstetrics clinics.

The most common isolated microorganism was *E. coli* with 49%(n=4898), while the second most common agent was gram-negative microorganisms such as Klebsiella spp. with 13.8%(n=1.380). The third most common agent was *Enterecoccus spp*. with 7.8%(n=775) followed by coagulase negative staphylococci (CNS) with 6.6%(n=662), *Pseudomonas spp* with 4.7%(n=474), Candida spp. with 4.4%(n=440), *Proteus spp*. with 3.8%(n=379) and *Acinetobacter spp*. with 2.5%(254). The distribution percentage of causative microorganisms is shown in Figure 1.

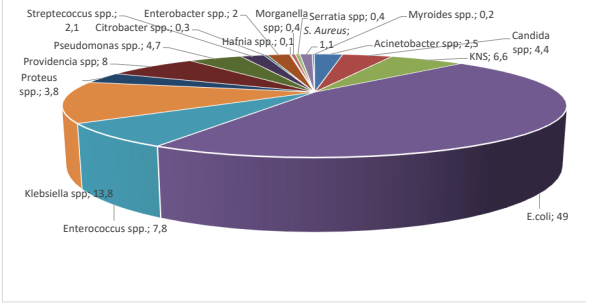

Figure 1. Distribution percentage of causative microorganisms

While the most sensitive antibiotics in *E. coli* and *Klebsiella spp*. were carbapenem and aminoglycoside groups, the resistance to ampicillin, ciprofloxacin and cephalosporins in *E. coli* was over 60%, while this rate was found to be around 80% in *Klebsiella spp*. (Figure 2-3).

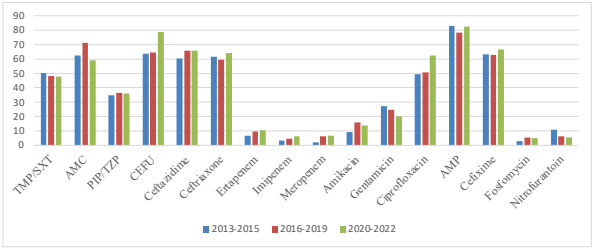

Figure 2. Antimicrobial resistance rates of E. coli

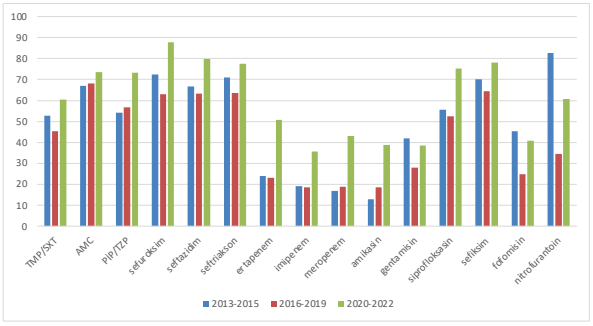

Figure 3. Antimicrobial resistance rates of Klebsiella spp.

It was observed that methicillin resistance increased from 50% in the 2013-2015 period for *S. aureus* to 60% in the 2020-2022 period, and from 70% to 80% for CNS (Figure 4).

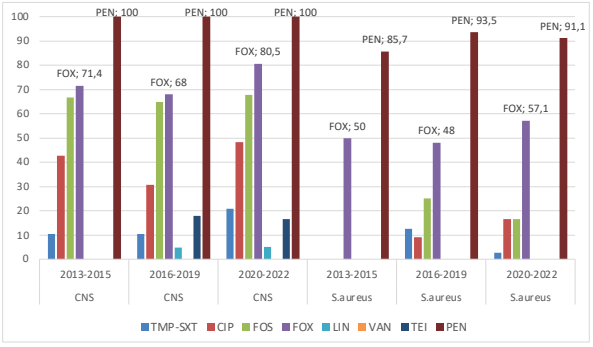

Figure 4. Antimicrobial resistance rates of Staphlococcus spp.

Vancomycin and teicoplanin resistance in *Enterecoccus spp*. increased from 5.8%, 4.3%, respectively, in the 2011-2013 period to 29.3% and 14.1% in the 2020-2022 period. The most sensitive antibiotic in *Acinetobacter spp*. was found to be TMP-SXT, while in *Pseudomonas spp*. it was

observed to be aminoglycosides, meropenem and ceftazidime (Figure 5).

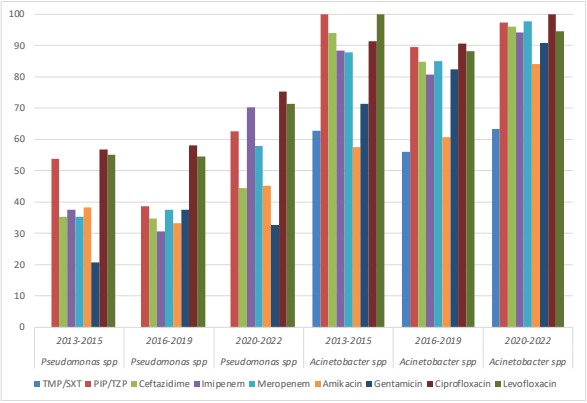

Figure 5. Antimicrobial resistance rates of Pseudomonas spp. ve Acinetobacter spp.

DISCUSSION

UTIs are among the most common bacterial infections occurring in both the community and health care settings worldwide, and most UTIs are usually treated empirically.[5]

In both community and healthcare settings, the Enterobacterales family predominates in UTIs, and the main pathogen isolated, *E. coli*, is the most common causative agent for both complicated and uncomplicated UTIs and this is followed by other pathogenic microorganisms such as *K. pneumoniae, P. mirabilis, Enterococcus spp*. and *Staphylococcus spp., P. mirabilis, P. aeruginosa, S. aureus*, group B *Streptococcus*, and *Candida species*.[10] In our study, *E. coli* was the most frequently detected agent with 49%, while Enterobacterales species ranked first with 69.6%.

Men and women of all ages can experience UTI, but due to female anatomy, the incidence of UTI was higher than in men.[11] The fact that 64.4%(n=6440) of the samples in which growth was detected in our study were female and 35.6%(n=3560) were male supports this information. When the National Healthcare Associated Infections Surveillance Network (USHIESA) agent distribution and antibiotic resistance report 2022 data is examined, amikacin (15%), amoxicillin-clavulanate (AMC) (59.2%), ampicillin (75.9%), gentamicin (24.8%), PIP-TZP (34.7%), ceftazidime (63.6%), ceftriaxone (60%), and ciprofloxacin (57.9%) resistance rates in *E. coli* were compatible with the resistance rates in our study.[12] Etiological agents such as *E. coli, K. pneumoniae* common cause of UTIs and poses the risk of nosocomial outbreaks.[13] The resistance rate of *Klebsiella spp*. was found to be more serious compared to E. coli. When the antibiogram data of the USHIESA 2022 report (for healthcare-associated UTI diagnoses caused by K. pneumoniae) were examined, amikacin (54.7%), AMC (82.7%), ceftazidime (85.2%), ceftriaxone (83%)) and ciprofloxacin (78.1%) resistance rates were similar to the resistance rates in our study.[12] When the antibiotic resistance of the isolates in Avcıoğlu et al.'s study was examined, resistance to ampicillin was found in 81%, gentamicin in 18%, TMP-SXT in 40%, nitrofurantoin in 4%, and fosfomycin in 4% and these rates were found to be compatible with our study. In the same study, AMC resistance was detected in 46% of the isolates, cefixime in 42%, ciprofloxacin in 41%, amikacin in 5%, and imipenem in 2%, and these rates were found to be lower than the resistance rates in our study.[14]

In our study, resistance rates to carbapenems, fosfomycin, and nitrofurantoin were generally <5% in *E. coli*; therefore, these drugs can be used for empiric treatment of UTI.
P. aeruginosa is a common cause of infection in hospitalized patients, especially those with compromised immune system. It is inherently resistant to many antimicrobial agents and has been difficult to control in the presence of healthcare-associated infections.[13] In our study, the resistance rates of PIP-TZP, ceftazidime, carbapenems, aminoglycosides and quinolones against *Acinetobacter spp*. were higher than against *Pseudomonas spp*. When the USHIESA 2022 report's antibiogram result for healthcare-associated UTI caused by *P. aeruginosa* and *Acineotobacter spp*. was examined, amikacin (24.4%, 81.8%), gentamicin (44.3%, 86.5%), meropenem (47.7%, 94.8%), PIP-TZP (72.7%, -), cefepime (70.3%, -) and ciprofloxacin (72.6%,

98.6%) resistance rates, were consistent with our study (between 2020-2022 period).[12] Tanriverdi et al. examined the urine culture results of a total of 4257 pediatric patients between 2015-2020. In their studies, resistance rates to imipenem, meropenem, amikacin, gentamicin and ciprofloxacin for *Pseudomonas spp*. and *Acinetobacter spp*. were found 15.9%-69.5%, 8.6%-69.5%, 7.44%-48.65%, 10.23%-66.72%, 7.3%-69.5%, respectively. These results were more sensitive compared to the results of our study.[15] In the study of Çuha et al., when the antibiotic resistance profiles were examined, the highest resistance rates were found in *Acinetobacter spp*., and over 60% resistance was observed in all antibiotics tested. Resistance to carbapenems was detected in 77.2%, PIP-TZP in 78.1% and ciprofloxacin in 78.6%. In *P. aeruginosa* isolates, resistance rates to aminoglycoside, carbapenem, PIP-TZP and antipseudomonal cephalosporins were determined to be below 20%, and ciprofloxacin resistance was detected to be 25.8%. These rates are much lower than the resistance rates of our study for both groups of microorganisms.[16]

In the study of Kalyoncu et al., AMC, nitrofurantoin, imipenem, levofloxacin, meropenem, cefepime, ceftazidime, ceftriaxone, ciprofloxacin and TMP-SXT antibiotic resistance rates for *E. coli, K. pneumoniae, P. aeruginosa* and *A. baumannii* isolates between 2017 and 2022, were found to be lower than the antibiotic resistance rates of our study covering the same years.[17]

Recently *Enterococcus spp*. has developed high levels of resistance to glycopeptides, including vancomycin, which is considered one of the last line of defense against multidrug-resistant organisms.[2] The USHIESA 2022 report's ampicillin, linezolid, teicoplanin and vancomycin resistance rates for *E. faecium* and *E. faecalis* are 96.4%-10%, 2.7%-1.7%, 26.7%-4.1% and 26%-2.9%, respectively. Since we gave total *Enterococcus spp*. resistance rates in our study, no comparison could be made.[12] Keskin et al.'s study, they did not detect vancomycin, teicoplanin and linezolid resistance in *Enterococcus spp*., while ampicillin (28%) and ciprofloxacin (42%) resistance rates were lower than our study.[18]

In this study, methicillin resistance rates in *S. aureus* and CNS ranged from 48% to 57.1% and 68% to 80.5%, respectively. In the study that included urine samples from a total of 2791 patients between November 2019 and November 2020 in our country, resistance to vancomycin, teicoplanin and linezolid was not detected in *S. aureus*, similar to our study, while resistance rates for TMP-SXT and ciprofloxacin were detected as 12% and 21%, respectively. Unlike our study, methicillin resistance (44%) was found to be lower.[18]

In general, in our study, resistance was more serious in *Acinetobacter spp*. and *Klebsiella spp*., and there was a tendency for the resistance rates of all microorganisms to increase over the years.

Our study has certain limitations. First of all, due to its single-center and retrospective nature, detailed clinical features such as underlying disease and detailed clinical diagnosis were not available. Secondly, although a total of 35.132 urine culture samples were examined in a 10-year period, a distinction between community-acquired UTI and hospital-acquired UTI could not be made and prospective studies are needed. Finally, since the isolates could not be stored, more detailed studies to determine antibiotic resistance genes could not be carried out.

Implementing good antimicrobial stewardship is critical to preventing the development of resistance and improving patient outcomes. The goal of antimicrobial stewardship is threefold and involves the implementation of specific strategies. The primary goal is to prevent treatment of asymptomatic bacteriuria; The second goal is to avoid the use of broad-spectrum fluoroquinolones; The third goal is to minimize the development of resistance by adhering to recommended drug courses and dosages.[6]

CONCLUSION

Creating more detailed cumulative antibiogram reports, sharing these reports with relevant clinics within specified periods, establishing antimicrobial management teams that will work in harmony with infection control committees in hospitals, structuring a nationwide surveillance program; It will contribute to the determination of empirical treatment, prevention of the development of antimicrobial resistance and control of hospital-acquired infections.

Ethical Approval

Gaziantep University Clinical Research Ethics Committee and following the Declaration of Helsinki (decision no: No 2023/267).

Peer-review

Externally and internally peer-reviewed.

Authorship Contributions

Concept: O.S.C., Design: O.S.C., Data collection or Processing: O.S.C., Analysis or interpretation: O.S.C., Literature Search: O.S.C., Writing: O.S.C.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Two Cases of Varicella Zoster Virus Meningitis without Fever and Rash: An Unexpected Clinical Presentation

Ateş ve Döküntü Olmayan İki Varicella Zoster Virüs Menenjit Olgusu: Beklenmeyen Klinik Sunum

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Abstract

Aseptic meningitis caused by varicella-zoster virus (VZV) is a rare phenomenon in the healthy population. Immunocompromised patients are predominantly affected by viral reactivation, characterized by rash and neurological symptoms, and meningitis as a rare complication. Herein, we report two cases of VZV meningitis in adult patients without rash and fever over a 4-month period. The first case was an immunocompetent 37-year-old male who presented with persistent headache, agitation and unclear speech. The second case was an immunocompromised 76-year-old male who was admitted to the emergency department with confusion, disorientation, dizziness, loss of consciousness, pain in the lower left extremities and difficulty in walking. Cerebrospinal fluid (CSF) analysis in both patients revealed a high leukocyte cell count with 97% lymphocytes. CSF gram staining and culture were negative. CSF polymerase chain reaction (PCR) analysis indicated VZV infection. Both patients were administered acyclovir for 14 days and were discharged without any neurological sequela. This case report series highlight the presentation of VZV as aseptic meningitis in both immunocompetent and immunocompromised patients without the typical clinical symptoms and should always be considered by the clinicians.

Keywords Cerebrospinal fluid, rapid diagnosis, PCR, varicella-zoster virus, meningitis

Özet

Varicella-zoster virüsünün (VZV) neden olduğu aseptik menenjit, sağlıklı popülasyonda nadir olarak görülmektedir. Bağışıklığı baskılanmış hastalar ağırlıklı olarak döküntü ve nörolojik semptomlar ile seyrederken, nadir bir komplikasyon olarak menenjit ile karakterize viral reaktivasyondan etkilenir. Bu çalışmada, 4 aylık bir süre içinde tanısı konulan, döküntü ve ateş olmayan iki VZV menenjit vakası sunuldu. İlk olgu baş ağrısı, ajitasyon ve konuşmada güçlük şikâyetleri ile hastanemize başvuran 37 yaşında immünkompetan erkek hasta idi. İkinci olgu ise bilinç bulanıklığı, oryantasyon bozukluğu, baş dönmesi, bilinç kaybı, sol alt ekstremitede ağrı ve yürümede güçlük şikayetleri ile acil servise başvuran 76 yaşındaki bağışıklığı baskılanmış erkek hasta idi. Her iki hastanın beyin omurilik sıvısı (BOS) analizinde, %97 lenfosit olmak üzere yüksek lökosit hücre sayısı görüldü. BOS gram boyama ve kültür negatif idi. BOS polimeraz zincir reaksiyonu (PZR) analizi, VZV açısından pozitif olarak sonuçlandı. Her iki hasta 14 gün süreyle asiklovir tedavisi aldı ve herhangi bir nörolojik sekel olmadan taburcu edildi. Bu iki olgu sunumu, VZV'nin tipik klinik semptomlar olmadan hem immünkompetan hem de bağışıklığı baskılanmış hastalarda aseptik menenjit olarak ortaya çıkabileceğini ve klinisyenler tarafından her zaman göz önünde bulundurulması gerektiğini vurgulamaktadır.

Anahtar Kelimeler *Beyin omurilik sıvısı, hızlı tanı, PZR, varicella-zoster virüs, menenjit*

INTRODUCTION

Neurotropic herpes viruses [herpes simplex type 1,2 (HSV-1, HSV-2), varicella-zoster virus (VZV)] are commonly seen in humans. A herpes virus family member varicella-zoster virus (VZV), also known as human herpes virus 3 (HHV-3), can spread through droplets, contact and airborne transmission, and causes varicella (chicken pox) in early childhood and herpes zoster (shingles).[1,2] Neurotropic viruses remain latent on dorsal roots, autonomic ganglia, and cranial nerves where they cause latent infections with the potential for reactivation.[3] When cellular immunity is compromised in the case of malignancy, trauma, co-morbidities, advanced age, and immunosuppression, the latent viral particles have the ability to reactivate. Similar to other herpesviruses, VZV can infect the central nervous system (CNS) and is responsible for CNS infections such as meningitis, angiitis, myelitis, or encephalitis due to its retrograde travel. Meningitis and other neurological complications due to VZV are commonly observed in the immunocompromised population with unfavourable outcomes. These complications are rarely seen in healthy immunocompetent people with primary features of rash and neurological symptoms.[4] VZV meningitis, which was originally identified in healthy patients in the 1980s, is currently regarded as the third most common type of viral meningitis following enterovirus and HSV.[5] Early diagnosis is critical for the prevention of morbidity and mortality. Diagnosis using the polymerase chain reaction (PCR) of cerebrospinal fluid (CSF) is a rapid and sensitive method for the detection VZV-related neurological complications.

Here we present two cases of unusual, atypical cases of adults presenting with aseptic meningitis with VZV without evidence of rash, which was primarily diagnosed with PCR using patients' CSF.

CASE REPORT

Case 1

A 37-year-old male was admitted to the emergency department by ambulance, after experiencing an unremitting headache for 6 days. He reported unresponsiveness, confusion and slurred speech on the morning of the admission day. He had a dermatomal varicella-zoster virus infection (chickenpox) history and he had received a varicella vaccination during his childhood.

On the day of admission, his blood tests were normal (Table 1.) and COVID-19 antigen test was negative. Patient's C-reactive protein (CRP) test result was 0.17 mg/dL (normal range 0.00-0.50). Hemogram results indicated white blood cell count ($6.4x10^3$/μL) and neutrophil count ($5.13\ x10^3$/μL) within the normal range. On examination, his vital signs were stable (temperature: 36^0C; blood pressure:100/60 mmHg; Glasgow coma scale:15; spO2:98; pulse:50). On neurological examination the patient did not present with signs of meningeal irritation. His chest X-ray was normal. No pathological findings were present in the cranial magnetic resonance imaging (MRI). Particularly, a stiff neck as a typical sign of meningitis was not found and he did not show any rash. He was admitted to the intensive care unit and a lumber puncture was performed. The patient CSF biochemical analysis indicated an elevated protein level of 98.4 mg/dL (normal range:15-45 mg/dL) (Table 2) and cellular analysis of the CSF revealed a high leukocyte cell count of 147.000 cells/μL with 97% lymphocytes and 3% polymorphonuclear neutrophils (PMNs), suggestive of a possible viral aetiology. The bacterial culture of CSF yielded no growth and no bacteria were detected in the CSF Gram staining. The molecular analysis of the CSF was performed using Qiastat-DX Meningitis/Encephalitis Panel (Qiagen, Hilden, Germany) (including bacterial, viral, and fungal pathogens) and was positive for VZV (Figure 1.).

The patient was immediately administered 750 mg of acyclovir intravenously three times daily for 14 days. On day two of hospital admission, his symptoms improved and he started his nutrition regimen. The patient's headache resolved gradually and he was discharged after 2 weeks. The patient was followed up after discharge and had complete

resolution of symptoms and no neurological sequalae.

Table 1. Biochemical analysis of blood samples for both patients upon admission

Test	Case 1	Case 2	Reference
Creatinine (mg/dL)	0.87	1.49	0.72-1.25
Urea (mg/dL)	28	93	19-44
Glucose (mg/dL)	114	n/a	<140
AST (SGOT) (U/L)	18	11	5-34
ALT (SGPT) (U/L)	17	8	0-55
Sodium (mmol/L)	137	131	136-145
Potassium (mmol/L)	3.5	5.8	3.5-5.1
CRP (sensitive) (mg/dL)	0.17	12.70	0.00-0.50

AST (SGOT): aspartate aminotransferase (serum glutamic-oxalo-acetic transaminase)
ALT (SGPT): alanine transaminase (serum glutamic-pyruvic transaminase)
CRP: C-reactive protein
n/a: Not available

Table 2. Biochemical analysis of CSF samples for both patients upon admission

Test	Case 1	Case 2	Reference
Glucose (mg/dL)	49	45	40-70
Total protein (mg/dL)	98.4	271.6	15-45
Sodium (mmol/L)	139	131	142-150
Potassium (mmol/L)	3.1	n/a	2.2-3.3
Chlorine (mmol/L)	120	98	118-132

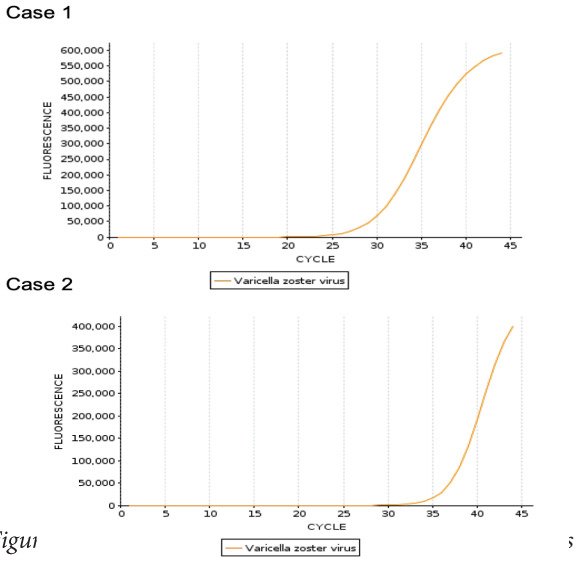
n/a: Not available

Case 2

A 76-year-old male was admitted to the emergency department with confusion, disorientation, dizziness, loss of consciousness, pain in the lower left extremities and difficulty in walking. His COVID-19 PCR test was negative. On admission, the patient was reported to have type 2 diabetes and interstitial lung disease as underlying conditions.

The patient's blood test results were analyzed. His serum creatinine and urea levels were found to be 1.49 mg/dL (normal range: 0.72-1.25 mg/dL) and 93 mg/dL (normal range: 18-55 mg/dL), respectively. His CRP levels were elevated at 12.70 mg/dL (normal range: 0.00-0.50 mg/dL) (Table 1). Hemogram results indicated high white blood cell count (12.4x10^3/μL) and high neutrophil count (9.61 x10^3/μL). Patient's tentative diagnosis was encephalomyelitis. A lumbar puncture was performed and patient's CSF sample was collected. CSF was analyzed using limbic encephalitis panel (LGI1, CASPR2, AMPA1, AMPA2 GABA B, NMDA antibodies) as well as paraneoplastic panel and concluded as negative for both tests. IgG index panel revealed elevated CSF albumin levels at 183 mg/dL (normal range: 15-40 mg/dL) as well as high CSF IgG at 52.40 mg/dL (normal range: 4.20-6.40 mg/dL). In addition, serum albumin levels were low at 29.20 g/L (normal range: 35.00-49.00). CSF oligoclonal electrophoresis result was positive for type 3, which may indicate systemic disease/multiple sclerosis. CSF culture result was negative with scarce polymorphonuclear neutrophilic leukocytes. CSF cell count was 293.000 cells/μL with 97% lymphocytes. The PCR analysis of the CSF was also performed using Qiastat-DX Meningitis/Encephalitis Panel and was found to be positive for VZV (Figure 1).

The patient was immediately administered 750 mg of acyclovir intravenously three times daily for 14 days and was discharged successfully.



Figu *s*

DNA in both patient's CSF sample

DISCUSSION

VZV, a member of Herpesviridae, is a common pathogen which spreads via airborne droplets or via direct contact with the virus.[2,6] It causes varicella (chicken pox) in childhood and zoster (shingles) in the adulthood. There are different clinical manifestations for VZV infections. VZV can cause vesicular rash by infecting the epidermis, may infect the neuronal tissues and reactivate after periods of time leading to various neurological complications. Meningoencephalitis, vasculopathy, cerebellitis vasculopathy, postherpetic neuralgia, zoster paresis, cranial nerve palsies, myelopathy and ocular disorders can be seen after zoster or shingle reactivation.[7] In rare conditions, the VZV can compromise the meninges nerves and cause aseptic meningitis, characterized with a negative CSF bacterial culture. Headache, high fever and vesicular rash are the predominant symptoms of VZV meningitis. Phonophobia, photophobia, neck pain and rigidity are less commonly seen with Kerning's and Brudzinski's sign. Absence of dermatomal rash in VZV meningitis is called Zoster Sine Herpete. More rarely, as reported in our case study, some patients develop VZV meningitis without any fever or rash. In our patient's case, the sole symptom was a persistent headache. VZV meningitis without rash was previously reported in literature.[6,8] Becerra et al. stated that in CNS cases, vesicular rash is absent in one third of the cases. The lack of rash can be defined by the pathophysiology. In this condition, low viral load and delayed neurological disease are observed in some cases.[5,6] Signs of meningitis, as described in the literature, were not observed in our patient, however he had elevated CSF protein levels (98.4 mg/dL (range:15-45 mg/dL)) and mononuclear cell predominance (97%), consistent with the literature.[7]

Suppressed cellular immunity have been associated with the disease. Immunosuppression such as advanced age, chemotherapy, autoimmune diseases and stress are risk factors VZV reactivation.[6] Apart from the immunosuppressed patients, meningitis due to VZV can be also observed in immunocompetent healthy individuals.[8,9] Here, we present two atypical cases of VZV meningitis in both immunocompetent and immunosuppressed patients without fever, rash or other neurological signs.

Molecular detection of the causative agent in CSF is considered as the gold standard for the diagnosis of VZV meningitis due to its high sensitivity and specificity. The third most common agent of CNS is VZV in adults after enterovirus and HSV. Therefore, it is imperative to identify the causative agent in a CNS infection. Additionally, meningitis without classical symptoms such as rash was previously confirmed by PCR and reported by Echevarria et al. These cases highlight the importance of molecular tests for VZV detection.[9,10] VZV PCR testing has enabled the detection of more cases, particularly when the skin manifestation is absent. Furthermore, the differentiation of HSV and VZV is crucial when a viral agent is suspected.

CONCLUSIONS

VZV is capable of causing CNS infections with varying complications both in immunocompetent and immunocompromised individuals. As highlighted by the two cases presented in this study, VZV meningitis can occur in both immunocompetent adults and individuals with underlying conditions and should always be considered by the clinicians even in the absence of rash, fever or neck stiffness.

Ethics Approval

Due to the nature of this retrospective study and the preserved anonymity of the patient, a waiver of ethics committee approval was obtained from Near East University. All methods were carried out in accordance with the guidelines and regulations of Declaration of Helsinki.

Peer-review

Externally and internally peer-reviewed.

Authorship Contributions

Concept: B.B, Design: B.B., A.B., Data Collection or Processing: B.B., A.B., K.S., Analysis or Interpretation: B.B,

K.S., Literature Search: A.B., Writing: B.B., A.B., K.S.

Conflict of Interest

No conflict of interest was declared by the authors.

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Informed Consent

The patient was sampled through convenient sampling technique and enrolled after obtaining written informed consent.

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