EISSN 1305-6441

İSTANBUL ÜNİVERSİTESİ YAYINEVİ

Indexed in Web of Science

jmed.istanbul.edu.tr

İstanbul Tıp Fakültesi Dergisi

Journal of Istanbul Faculty of Medicine

Cilt-Volume: 83 • Sayı-Issue: 4 • 2020

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AMAÇ KAPSAM VE YAYIN STANDARTLARI

İstanbul Tıp Fakültesi Dergisi (İst Tıp Fak Derg); bağımsız, önyargısız ve çift-kör hakemlik ilkeleri çerçevesinde yayın yapan İstanbul Üniversitesi, İstanbul Tıp Fakültesi'nin uluslararası ve açık erişimli bilimsel yayın organıdır. Dergi Ocak, Nisan, Temmuz ve Ekim aylarında olmak üzere üç ayda bir yayınlanır ve dört sayıda bir cildi tamamlanır. Yayın dili İngilizce ve Türkçe'dir.

İstanbul Tıp Fakültesi Dergisi (İst Tıp Fak Derg), tıbbın tüm alanlarında klinik ve deneysel özgün araştırmalar, ender görülebilecek olgu sunumları, özel ve aktüel konularda literatür derlemeleri ve editöre mektuplar yayınlamaktadır. Orijinal metot geliştirme, yeni bir girişim tekniği ve orijinal çalışmaların ön sonuçlarını içeren kısa raporlara da dergide yer verilmektedir.

Derginin hedef kitlesi; sağlık alanındaki tüm disiplinlerde çalışan hekimler ve akademisyenlerdir.

Derginin editörlük ve yayın süreçleri International Committee of Medical Journal Editors (ICMJE), World Association of Medical Editors (WAME), Council of Science Editors (CSE), Committee on Publication Ethics (COPE), European Association of Science Editors (EASE) ve National Information Standards Organization (NISO) organizasyonlarının kılavuzlarına uygun olarak biçimlendirilmiştir ve Principles of Transparency and Best Practice in Scholarly Publishing (doaj.org/bestpractice) ilkelerine uygun olarak yürütülmektedir.

İstanbul Tıp Fakültesi Dergisi (İst Tıp Fak Derg), Web of Science-Emerging Sources Citation Index ve TÜBİTAK ULAKBİM TR Dizin tarafından indekslenmektedir.

Makale değerlendirme ve yayın işlemleri için yazarlardan ücret talep edilmemektedir.

Derginin tüm masrafları İstanbul Üniversitesi tarafından karşılanmaktadır.

Dergide yayınlanan makalelerde ifade edilen görüşler ve fikirler İstanbul Tıp Fakültesi Dergisi Editör, Editör Yardımcıları, Yayın Kurulu ve Yayıncısının değil, yazar(lar)ın bakış açılarını yansıtır. Editör, Editör Yardımcıları, Yayın Kurulu ve Yayıncı bu gibi durumlar için hiç bir sorumluluk ya da yükümlülük kabul etmemektedir. Yayınlanan içerik ile ilgili tüm sorumluluk yazarlara aittir.

Yayınlanan tüm içeriğe ücretsiz olarak erişilebilir.

Editör: Birsen Karaman
Adres: İstanbul Üniversitesi, İstanbul Tıp Fakültesi Dekanlığı, Turgut Özal Cad. 34093 Çapa, Fatih, İstanbul, Türkiye
Tel.: +90 212 414 21 61
E-posta: itfdergisi@istanbul.edu.tr

Yayıncı: İstanbul Üniversitesi Yayınevi
Adres: İstanbul Üniversitesi Merkez Kampüsü, 34452 Beyazıt, Fatih, İstanbul, Türkiye
Tel.: +90 212 440 00 00
Faks: +90 212 217 22 92

İstanbul Tıp Fakültesi Dergisi

AIMS SCOPE AND PUBLICATION STANDARDS

Journal of Istanbul Faculty of Medicine (J Ist Faculty Med) an international, scientific, open access periodical published in accordance with independent, unbiased, and double-blinded peer-review principles. The journal is the official publication of Istanbul University, Istanbul Faculty of Medicine and it is published quarterly on January, April, July and October. The publication languages of the journal are English and Turkish.

Journal of Istanbul Faculty of Medicine (J Ist Faculty Med) aims to contribute to the literature by publishing manuscripts at the highest scientific level on all fields of medicine. The journal publishes original experimental and clinical research articles, reports of rare cases, reviews that contain sufficient amount of source data conveying the experiences of experts in a particular field, and letters to the editors as well as brief reports on a recently established method or technique or preliminary results of original studies related to all disciplines of medicine from all countries.

The journal's target audience includes researchers, physicians and healthcare professionals who are interested or working in all medical disciplines.

The editorial and publication processes of the journal are shaped in accordance with the guidelines of the International Committee of Medical Journal Editors (ICMJE), World Association of Medical Editors (WAME), Council of Science Editors (CSE), Committee on Publication Ethics (COPE), European Association of Science Editors (EASE), and National Information Standards Organization (NISO). The journal is in conformity with the Principles of Transparency and Best Practice in Scholarly Publishing (doaj.org/bestpractice).

Journal of Istanbul Faculty of Medicine is currently indexed in Web of Science-Emerging Sources Citation Index and TUBITAK ULAKBIM TR Index.

Processing and publication are free of charge with the journal. No fees are requested from the authors at any point throughout the evaluation and publication process.

All expenses of the journal are covered by the Istanbul University.

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Editor: Birsen Karaman
Address: Istanbul University, Istanbul Faculty of Medicine Deanery, Turgut Özal Cad. 34093, Çapa, Fatih, Istanbul, Turkey
Phone: +90 212 414 21 61
E-mail: itfdergisi@istanbul.edu.tr

Publisher: Istanbul University Press
Address: İstanbul Üniversitesi Merkez Kampüsü, 34452 Beyazıt, Fatih / Istanbul - Turkey
Phone: +90 212 440 00 00

YAZARLARA BİLGİ

İstanbul Tıp Fakültesi Dergisi (İst Tıp Fak Derg); bağımsız, önyargısız ve çift-kör hakemlik ilkeleri çerçevesinde yayın yapan İstanbul Üniversitesi, İstanbul Tıp Fakültesi'nin uluslararası ve açık erişimli bilimsel yayın organıdır. Dergi Ocak, Nisan, Temmuz ve Ekim aylarında olmak üzere üç ayda bir yayınlanır ve dört sayıda bir cildi tamamlanır. Yayın dili İngilizce ve Türkçe'dir.

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EDİTÖRYAL POLİTİKALAR VE HAKEM SÜRECİ

Derginin editörlük ve yayın süreçleri International Committee of Medical Journal Editors (ICMJE), World Association of Medical Editors (WAME), Council of Science Editors (CSE), Committee on Publication Ethics (COPE), European Association of Science Editors (EASE) ve National Information Standards Organization (NISO) organizasyonlarının kılavuzlarına uygun olarak biçimlendirilmiştir ve Principles of Transparency and Best Practice in Scholarly Publishing (doaj.org/bestpractice) ilkelerine uygun olarak yürütülmektedir.

Özgünlük, yüksek bilimsel kalite ve atıf potansiyeli bir makalenin yayına kabulü için en önemli kriterlerdir. Gönderilen yazıların daha önce başka bir elektronik ya da basılı dergide, kitapta veya farklı bir mecrada sunulmamış ya da yayınlanmamış olması gerekir. Toplantılarda sunulan çalışmalar için, sunum yapılan organizasyonun tam adı, tarihi, şehri ve ülkesi belirtilmelidir.

İstanbul Tıp Fakültesi Dergisi'ne gönderilen tüm makaleler çift-kör hakem değerlendirme sürecinden geçmektedir. Tarafsız değerlendirme sürecini sağlamak için her makale alanlarında uzman en az iki dış-bağımsız hakem tarafından değerlendirilir. Dergi Yayın Kurulu üyeleri tarafından gönderilecek makalelerin değerlendirme süreçleri, davet edilecek dış bağımsız editörler tarafından yönetilecektir. Bütün makalelerin karar verme süreçlerinde nihai karar yetkisi Editördedir.

Klinik ve deneysel çalışmalar, ilaç araştırmaları ve bazı olgu sunumları için World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects", (amended in October 2013, www.wma.net) çerçevesinde hazırlanmış Etik Komisyon raporu gerekmektedir. Gerekli görülmesi halinde Etik Komisyon raporu veya eşdeğeri olan resmi bir yazı yazarlardan talep edilebilir. İnsanlar üzerinde yapılmış deneysel çalışmaların sonuçlarını bildiren yazılarda, çalışmanın yapıldığı kişilere uygulanan prosedürlerin niteliği tümüyle açıklandıktan sonra, onaylarının alındığına ilişkin bir açıklamaya metin içinde yer verilmelidir. Hayvanlar üzerinde yapılan çalışmalarda ise ağrı, acı ve rahatsızlık verilmemesi için yapılmış olanlar açık olarak makalede belirtilmelidir. Hasta onamları, Etik Kurul raporunun alındığı kurumun adı, onay belgesinin numarası ve tarihi ana metin dosyasında yer alan Gereç ve Yöntem başlığı altında yazılmalıdır. Hastaların kimliklerinin gizliliğini korumak yazarların sorumluluğundadır. Hastaların kimliğini açığa çıkarabilecek fotoğraflar için hastadan ya da yasal temsilcilerinden alınan imzalı izinlerin de gönderilmesi gereklidir.

Bütün makalelerin benzerlik tespiti denetimi, iThenticate yazılımı aracılığıyla yapılmaktadır.

Yayın Kurulu, dergimize gönderilen çalışmalar hakkındaki intihal, atıf manipülasyonu ve veri sahteciliği iddia ve şüpheleri karşısında COPE kurallarına uygun olarak hareket edecektir.

Yazar olarak listelenen herkesin ICMJE (www.icmje.org) tarafından önerilen yazarlık koşullarını karşılaması gerekmektedir.

ICMJE, yazarların aşağıdaki 4 koşulu karşılamasını önermektedir:

1. Çalışmanın konseptine/tasarımına; ya da çalışma için verilerin toplanmasına, analiz edilmesine ve yorumlanmasına önemli katkı sağlamış olmak;
2. Yazı taslağını hazırlamış ya da önemli fikirsel içeriğin eleştirel incelemelerini yapmış olmak;
3. Yazının yayından önceki son halini gözden geçirmiş ve onaylamış olmak;
4. Çalışmanın herhangi bir bölümünün geçerliliği ve doğruluğuna ilişkin soruların uygun şekilde soruşturulduğunun ve çözümlendiğinin garantisini vermek amacıyla çalışmanın her yönünden sorumlu olmayı kabul etmek.

YAZARLARA BİLGİ

Bir yazar, çalışmada katkı sağladığı kısımların sorumluluğunu almasına ek olarak, diğer yazarların çalışmanın hangi kısımlarından sorumlu olduğunu da tanımlayabilmelidir. Ayrıca,her yazar diğerlerinin katkı bütünlüğüne güven duymalıdır.

Yazar olarak belirtilen her kişi yazarlığın dört koşulunu karşılamalıdır ve bu dört koşulu karşılayan her kişi yazar olarak tanımlanmalıdır. Dört kriterin hepsini karşılamayan kişilere makalenin başlık sayfasında teşekkür edilmelidir.

Yazarlık haklarına uygun hareket etmek ve hayalet ya da lütuf yazarlığın önlenmesini sağlamak amacıyla sorumlu yazarlar makale yükleme sürecinde http://jmed.istanbul.edu.tr/tr/content/makale-gonderme-kilavuzu/makale-gonderme-kilavuzu adresinden erişilebilen Yazar Katkı Formu'nu imzalamalı ve taranmış versiyonunu yazıyla birlikte göndermelidir. Yayın Kurulu'nun gönderilen bir makalede "lütuf yazarlık" olduğundan şüphelenmesi durumunda söz konusu makale değerlendirme yapılmaksızın reddedilecektir. Makale gönderimi kapsamında; sorumlu yazar makale gönderim ve değerlendirme süreçleri boyunca yazarlık ile ilgili tüm sorumluluğu kabul ettiğini bildiren kısa bir ön yazı göndermelidir.

İstanbul Tıp Fakültesi Dergisi, gönderilen makalelerin değerlendirme sürecine dahil olan yazarların ve bireylerin, potansiyel çıkar çatışmasına ya da önyargıya yol açabilecek finansal, kurumsal ve diğer ilişkiler dahil mevcut ya da potansiyel çıkar çatışmalarını beyan etmelerini talep ve teşvik eder.

Bir çalışma için bir birey ya da kurumdan alınan her türlü finansal destek ya da diğer destekler Yayın Kurulu'na beyan edilmeli ve potansiyel çıkar çatışmalarını beyan etmek amacıyla ICMJE Potansiyel Çıkar Çatışmaları Formu katkı sağlayan tüm yazarlar tarafından ayrı ayrı doldurulmalıdır. Editör, yazarlar ve hakemler ile ilgili potansiyel çıkar çatışması vakaları derginin Yayın Kurulu tarafından COPE ve ICMJE rehberleri kapsamında çözülmektedir.

Derginin Yayın Kurulu, itiraz ve şikayet vakalarını, COPE rehberleri kapsamında işleme almaktadır. Yazarlar, itiraz ve şikayetleri için doğrudan Yayıncılık Birimi ile temasa geçebilirler. İhtiyaç duyulduğunda Yayın Kurulu'nun kendi içinde çözemediği konular için tarafsız bir temsilci atanmaktadır. İtiraz ve şikayetler için karar verme süreçlerinde nihai kararı Baş Editör verecektir.

İstanbul Tıp Fakültesi Dergisi her makalenin http://jmed.istanbul.edu.tr/tr/content/makale-gonderme-kilavuzu/makale-gonderme-kilavuzu adresinden erişebileceğiniz Telif Hakkı Devir Formu ile beraber gönderilmesini talep eder. Yazarlar, basılı ya da elektronik formatta yer alan resimler, tablolar ya da diğer her türlü içerik dahil daha önce yayınlanmış içeriği kullanırken telif hakkı sahibinden izin almalıdırlar. Bu konudaki yasal, mali ve cezai sorumluluk yazarlara aittir.

Dergide yayınlanan makalelerde ifade edilen görüşler ve fikirler İstanbul Tıp Fakültesi Dergisi Editör, Editör Yardımcıları, Yayın Kurulu ve Yayıncısının değil, yazar(lar)ın bakış açılarını yansıtır. Editör, Editör Yardımcıları, Yayın Kurulu ve Yayıncı bu gibi durumlar için hiç bir sorumluluk ya da yükümlülük kabul etmemektedir. Yayınlanan içerik ile ilgili tüm sorumluluk yazarlara aittir.

MAKALE HAZIRLAMA

Makaleler, ICMJE-Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals (updated in December 2015 - http://www.icmje.org/icmje-recommendations.pdf) ile uyumlu olarak hazırlanmalıdır. Randomize çalışmalar CONSORT, gözlemsel çalışmalar STROBE, tanısal değerli çalışmalar STARD, sistematik derleme ve meta-analizler PRISMA, hayvan deneyli çalışmalar ARRIVE ve randomize olmayan davranış ve halk sağlığıyla ilgili çalışmalar TREND kılavuzlarına uyumlu olmalıdır.

Makaleler sadece http://jmed.istanbul.edu.tr/tr/content/makale-gonderme-kilavuzu/makale-gonderme-kilavuzu adresinde yer alan derginin online makale yükleme ve değerlendirme sistemi üzerinden gönderilebilir. Diğer mecralardan gönderilen makaleler değerlendirilmeye alınmayacaktır.

Gönderilen makalelerin dergi yazım kurallarına uygunluğu ilk olarak Yayıncılık Birimi tarafından kontrol edilecek, dergi yazım kurallarına uygun hazırlanmamış makaleler teknik düzeltme talepleri ile birlikte yazarlarına geri gönderilecektir.

YAZARLARA BİLGİ

Yazarların; Telif Hakkı Devir Formu, Yazar Formu ve ICMJE Potansiyel Çıkar Çatışmaları Formu'nu (bu form, tüm yazarlar tarafından doldurulmalıdır) ilk gönderim sırasında online makale sistemine yüklemeleri gerekmektedir. Bu formlara http://jmed.istanbul.edu.tr/tr/content/makale-gonderme-kilavuzu/makale-gonderme-kilavuzu adresinden erişilebilmektedir.

Kapak sayfası: Gönderilen tüm makalelerle birlikte ayrı bir kapak sayfası da gönderilmelidir. Bu sayfa;

- Makalenin Türkçe ve İngilizce başlığını ve 50 karakteri geçmeyen Türkçe ve İngilizce kısa başlığını,
- Yazarların isimlerini, kurumlarını, eğitim derecelerini ve ORCID numaralarını
- Finansal destek bilgisi ve diğer destek kaynakları hakkında detaylı bilgiyi,
- Sorumlu yazarın ismi, adresi, telefonu (cep telefonu dahil), faks numarası ve e-posta adresini,
- Makale hazırlama sürecine katkıda bulunan ama yazarlık kriterlerini karşılamayan bireylerle ilgili bilgileri içermelidir.

Özet: Editöre Mektup türündeki yazılar dışında kalan tüm makalelerin Türkçe ve İngilizce özetleri olmalıdır. Özgün Araştırma makalelerinin özetleri "Amaç", "Gereç ve Yöntem", "Bulgular"ve "Sonuç" alt başlıklarını içerecek biçimde hazırlanmalıdır. Olgu sunumu ve derleme türündeki yazıların Özet bölümleri alt başlık içermemelidir.

Anahtar Sözcükler: Tüm makaleler en az 3 en fazla 6 anahtar kelimeyle birlikte gönderilmeli, anahtar sözcükler özetin hemen altına yazılmalıdır. Kısaltmalar anahtar sözcük olarak kullanılmamalıdır. Anahtar sözcükler National Library of Medicine (NLM) tarafından hazırlanan Medical Subject Headings (MeSH) veritabanından seçilmelidir.

Makale Türleri

Özgün Araştırma: Ana metin "Giriş", "Gereç ve Yöntem", "Bulgular"ve "Tartışma"alt başlıklarını içermelidir. Özgün Araştırmalarla ilgili kısıtlamalar için lütfen Tablo 1'i inceleyiniz.

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). İstatiksel analiz ile ilgili bilgi, Yöntemler bölümü içinde ayrı bir alt başlık olarak yazılmalı ve kullanılan yazılım kesinlikle tanımlanmalıdır.

Birimler, uluslararası birim sistemi olan International System of Units (SI)'a uygun olarak hazırlanmadır.

Editöryel Yorum: Dergide yayınlanan bir araştırmanın, o konunun uzmanı olan veya üst düzeyde değerlendirme yapan bir hakemi tarafından kısaca yorumlanması amacını taşımaktadır. Yazarları, dergi tarafından seçilip davet edilir. Özet, anahtar sözcük, tablo, şekil, resim ve diğer görseller kullanılmaz.

Davetli Derleme: Yazının konusunda birikimi olan ve bu birikimleri uluslararası literatüre yayın ve atıf sayısı olarak yansımış uzmanlar tarafından hazırlanmış davetli yazılar değerlendirmeye alınır. Bir bilgi ya da konunun klinikte kullanılması için vardığı son düzeyi anlatan, tartışan, değerlendiren ve gelecekte yapılacak

Tablo 1. Makale türleri için kısıtlamalar

Makale türü	Sözcük limiti	Özet sözcük limiti	Kaynak limiti	Tablo limiti	Resim limiti
Özgün Araştırma	3500	250 (Alt başlıklı)	50	6	7 ya da toplamda 15 görsel
Davetli Derleme	5000	250	50	6	10 ya da toplamda 20 görsel
Olgu Sunumu	1000	200	15	Tablo yok	10 ya da toplamda 20 görsel
Editöre Mektup	500	Uygulanamaz	5	1	1

olan çalışmalara yön veren bir formatta hazırlanmalıdır. Ana metin "Giriş", "Klinik ve Araştırma Etkileri" ve "Sonuç" bölümlerini içermelidir. Davetli Derleme türündeki yazılarla ilgili kısıtlamalar için lütfen Tablo 1'i inceleyiniz.

Olgu Sunumu: Olgu sunumları için sınırlı sayıda yer ayrılmakta ve sadece ender görülen, tanı ve tedavisi güç olan hastalıklarla ilgili, yeni bir yöntem öneren, kitaplarda yer verilmeyen bilgileri yansıtan, ilgi çekici ve öğretici özelliği olan olgular yayına kabul edilmektedir. Ana metin; "Giriş", "Olgu Sunumu", "Tartışma" ve Sonuç" alt başlıklarını içermelidir. Olgu Sunumlarıyla ilgili kısıtlamalar için lütfen Tablo 1'i inceleyiniz.

Editöre Mektup: Dergide daha önce yayınlanan bir yazının önemini, gözden kaçan bir ayrıntısını ya da eksik kısımlarını tartışabilir. Ayrıca derginin kapsamına giren alanlarda okurların ilgisini çekebilecek konular ve özellikle eğitici olgular hakkında da Editöre Mektup formatında yazılar yayınlanabilir. Okuyucular da yayınlanan yazılar hakkında yorum içeren Editöre Mektup formatında yazılarını sunabilirler. Özet, anahtar sözcük, tablo, şekil, resim ve diğer görseller kullanılmaz. Ana metin alt başlıksız olmalıdır. Hakkında mektup yazılan yayına ait cilt, yıl, sayı, sayfa numaraları, yazı başlığı ve yazarların adları açık bir şekilde belirtilmeli, kaynak listesinde yazılmalı ve metin içinde atıfta bulunulmalıdır.

Tablolar
Tablolar ana dosyaya eklenmeli, kaynak listesi sonrasında sunulmalı, ana metin içerisindeki geçiş sıralarına uygun olarak numaralandırılmadır. Tabloların üzerinde tanımlayıcı bir başlık yer almalı ve tablo içerisinde geçen kısaltmaların açılımları tablo altına tanımlanmalıdır. Tablolar Microsoft Office Word dosyası içinde "Tablo Ekle" komutu kullanılarak hazırlanmalı ve kolay okunabilir şekilde düzenlenmelidir. Tablolarda sunulan veriler ana metinde sunulan verilerin tekrarı olmamalı; ana metindeki verileri destekleyici nitelikte olmalıdır.

Resim ve Resim Altyazıları
Resimler, grafikler ve fotoğraflar (TIFF ya da JPEG formatında) ayrı dosyalar halinde sisteme yüklenmelidir. Görseller bir Word dosyası dokümanı ya da ana doküman içerisinde sunulmamalıdır. Alt birimlere ayrılan görseller olduğunda, alt birimler tek bir görsel içerisinde verilmemelidir. Her bir alt birim sisteme ayrı bir dosya olarak yüklenmelidir. Resimler alt birimleri belli etme amacıyla etiketlenmemelidir (a, b, c vb.). Resimlerde altyazıları desteklemek için kalın ve ince oklar, ok başları, yıldızlar, asteriksler ve benzer işaretler kullanılabilir. Makalenin geri kalanında olduğu gibi resimler de kör olmalıdır. Bu sebeple, resimlerde yer alan kişi ve kurum bilgileri de körleştirilmelidir. Görsellerin minimum çözünürlüğü 300 DPI olmalıdır. Değerlendirme sürecindeki aksaklıkları önlemek için gönderilen bütün görsellerin çözünürlüğü net ve boyutu büyük (minimum boyutlar 100x100 mm) olmalıdır. Resim altyazıları ana metnin sonunda yer almalıdır.

Makale içerisinde geçen tüm kısaltmalar, ana metin ve özette ayrı ayrı olmak üzere ilk kez kullanıldıkları yerde tanımlanarak kısaltma tanımın ardından parantez içerisinde verilmelidir.

Ana metin içerisinde cihaz, yazılım, ilaç vb. ürünlerden bahsedildiğinde ürünün ismi, üreticisi, üretildiği şehir ve ülke bilgisini içeren ürün bilgisi parantez içinde verilmelidir; "Discovery St PET/CT scanner (General Electric, Milwaukee, WI, USA)".

Tüm kaynaklar, tablolar ve resimlere ana metin içinde uygun olan yerlerde sırayla numara verilerek atıf yapılmalıdır.

Özgün araştırmaların kısıtlamaları, engelleri ve yetersizliklerinden Sonuç paragrafı öncesi "Tartışma" bölümünde bahsedilmelidir.

REVİZYONLAR

Yazarlar makalelerinin revizyon dosyalarını gönderirken, ana metin üzerinde yaptıkları değişiklikleri işaretlemeli, ek olarak, hakemler tarafından öne sürülen önerilerle ilgili notlarını "Hakemlere Cevap" dosyasında göndermelidir. Hakemlere Cevap dosyasında her hakemin yorumunun ardından yazarın cevabı gelmeli ve değişikliklerin yapıldığı satır numaraları da ayrıca belirtilmelidir. Revize makaleler karar mektubunu takip eden 30 gün içerisinde dergiye gönderilmelidir. Makalenin revize versiyonu belirtilen süre içerisinde yüklenmezse, revizyon seçeneği iptal olabilir. Yazarların revizyon için ek süreye ihtiyaç duymaları duru-

munda uzatma taleplerini ilk 30 gün sona ermeden dergiye iletmeleri gerekmektedir.

Yayına kabul edilen makaleler dil bilgisi, noktalama ve biçim açısından kontrol edilir. Yayın süreci tamamlanan makaleler, yayın planına dahil edildikleri sayıyla birlikte yayınlanmadan önce erken çevrimiçi formatında dergi web sitesinde yayına alınır. Kabul edilen makalelerin baskıya hazır PDF dosyaları sorumlu yazarlara iletilir ve yayın onaylarının 2 gün içerisinde dergiye iletilmesi istenir.

KAYNAKLAR

Atıf yapılırken en son ve en güncel yayınlar tercih edilmelidir. Atıf yapılan erken çevrimiçi makalelerin DOI numaraları mutlaka sağlanmalıdır. Kaynakların doğruluğundan yazarlar sorumludur. Dergi isimleri Index Medicus/Medline/PubMed'de yer alan dergi kısaltmaları ile uyumlu olarak kısaltılmalıdır. Altı ya da daha az yazar olduğunda tüm yazar isimleri listelenmelidir. Eğer 7 ya da daha fazla yazar varsa ilk 6 yazar yazıldıktan sonra "et al." konulmalıdır. Ana metinde kaynaklara atıf yapılırken parantez içinde Arap rakamları kullanılmalıdır. Farklı yayın türleri için kaynak stilleri aşağıdaki örneklerde sunulmuştur:

Dergi makalesi: Blasco V, Colavolpe JC, Antonini F, Zieleskiewicz L, Nafati C, Albanèse J, et al. Long-term outcome in kidney recipients from donors treated with hydroxyethylstarch 130/0.4 and hydroxyethylstarch 200/0.6. Br J Anaesth 2015;115(5):797-8.

Kitap bölümü: Sherry S. Detection of thrombi. In: Strauss HE, Pitt B, James AE, editors. Cardiovascular Medicine. St Louis: Mosby; 1974.p.273-85.

Tek yazarlı kitap: Cohn PF. Silent myocardial ischemia and infarction. 3rd ed. New York: Marcel Dekker; 1993.

Yazar olarak editör(ler): Norman IJ, Redfern SJ, editors. Mental health care for elderly people. New York: Churchill Livingstone; 1996.

Toplantıda sunulan yazı: Bengisson S. Sothemin BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Rienhoff O, editors. MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sept 6-10; Geneva, Switzerland. Amsterdam: North-Holland; 1992.p.1561-5.

Bilimsel veya teknik rapor: Smith P. Golladay K. Payment for durable medical equipment billed during skilled nursing facility stays. Final report. Dallas (TX) Dept. of Health and Human Services (US). Office of Evaluation and Inspections: 1994 Oct. Report No: HHSIGOE 169200860.

Tez: Kaplan SI. Post-hospital home health care: the elderly access and utilization (dissertation). St. Louis (MO): Washington Univ. 1995.

Yayına kabul edilmiş ancak henüz basılmamış yazılar: Leshner AI. Molecular mechanisms of cocaine addiction. N Engl J Med In press 1997.

Erken Çevrimiçi Yayın: Aksu HU, Ertürk M, Gül M, Uslu N. Successful treatment of a patient with pulmonary embolism and biatrial thrombus. Anadolu Kardiyol Derg 2012 Dec 26. doi: 10.5152/akd.2013.062. [Epub ahead of print]

Elektronik formatta yayınlanan yazı: Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis (serial online) 1995 Jan-Mar (cited 1996 June 5): 1(1): (24 screens). Available from: URL: http:/www.cdc.gov/ncidodlElD/cid.htm.

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YAZARLARA BİLGİ

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Editör: Birsen Karaman
Adres: İstanbul Üniversitesi, İstanbul Tıp Fakültesi Dekanlığı, Turgut Özal Cad. 34093 Çapa, Fatih, İstanbul, Türkiye
Telefon: +90 212 414 21 61
E-mail: itfdergisi@istanbul.edu.tr

Yayıncı: İstanbul Üniversitesi Yayınevi
Adres: İstanbul Üniversitesi Merkez Kampüsü, 34452 Beyazıt, Fatih, İstanbul, Türkiye
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Abstract: A Turkish and an English abstract should be submitted with all submissions except for Letters to the Editor. Submitting a Turkish abstract is not compulsory for international authors. The abstract of Original Articles should be structured with subheadings (Objective, Materials and Methods, Results, and Conclusion). Abstracts of Case Reports and Reviews should be unstructured. Please check Table 1 below for word count specifications.

Keywords: Each submission must be accompanied by a minimum of three to a maximum of six keywords for subject indexing at the end of the abstract. The keywords should be listed in full without abbreviations. The keywords should be selected from the National Library of Medicine, Medical Subject Headings database (http://www.nlm.nih.gov/mesh/MBrowser.html).

Manuscript Types

Original Articles: This is the most important type of article since it provides new information based on original research. The main text of original articles should be structured with Introduction, Material and Method, Results, Discussion, and Conclusion subheadings. Please check Table 1 for the limitations for Original Articles.

Statistical analysis to support conclusions is usually necessary. Statistical analyses must be conducted in accordance with international statistical reporting standards (Altman DG, Gore SM, Gardner MJ, Pocock SJ. Statistical guidelines for contributors to medical journals. Br Med J 1983: 7; 1489-93). Information on statistical analyses should be provided with a separate subheading under the Materials and Methods section and the statistical software that was used during the process must be specified.

INSTRUCTION TO AUTHORS

Units should be prepared in accordance with the International System of Units (SI).

Editorial Comments: Editorial comments aim to provide a brief critical commentary by reviewers with expertise or with high reputation in the topic of the research article published in the journal. Authors are selected and invited by the journal to provide such comments. Abstract, Keywords, and Tables, Figures, Images, and other media are not included.

Invited Review Articles: Invited reviews prepared by authors who have extensive knowledge on a particular field and whose scientific background has been translated into a high volume of publications with a high citation potential are welcomed. The invited reviews should describe, discuss, and evaluate the current level of knowledge of a topic in clinical practice and should guide future studies. The main text should contain Introduction, Clinical and Research Consequences, and Conclusion sections. Please check Table 1 for the limitations for Invited Review Articles.

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Letters to the Editor: This type of manuscript discusses important parts, overlooked aspects, or lacking parts of a previously published article. Articles on subjects within the scope of the journal that might attract the readers' attention, particularly educative cases, may also be submitted in the form of a "Letter to the Editor." Readers can also present their comments on the published manuscripts in the form of a "Letter to the Editor." Abstract, Keywords, and Tables, Figures, Images, and other media should not be included. The text should be unstructured. The manuscript that is being commented on must be properly cited within this manuscript.

Tables

Tables should be included in the main document, presented after the reference list, and they should be numbered consecutively in the order they are referred to within the main text. A descriptive title must be placed above the tables. Abbreviations used in the tables should be defined below the tables by footnotes (even if they are defined within the main text). Tables should be created using the "insert table" command of the word processing software and they should be arranged clearly to provide easy reading. Data presented in the tables should not be a repetition of the data presented within the main text but should be supporting the main text.

Figures and Figure Legends

Figures, graphics, and photographs should be submitted as separate files (in TIFF or JPEG format)

Table 1. Limitations for each manuscript type

Type of manuscript	Word limit	Abstract word limit	Reference limit	Table limit	Figure limit
Original Article	3500	250 (Structured)	50	6	7 or tatal of 15 images
Invited Review Article	5000	250	50	6	10 or total of 20 images
Case Report	1000	200	15	No tables	10 or total of 20 images
Technical Note	1500	No abstract	15	No tables	10 or total of 20 images
Letter to the Editor	500	No abstract	5	1	1

through the submission system. The files should not be embedded in a Word document or the main document. When there are figure subunits, the subunits should not be merged to form a single image. Each subunit should be submitted separately through the submission system. Images should not be labeled (a, b, c, etc.) to indicate figure subunits. Thick and thin arrows, arrowheads, stars, asterisks, and similar marks can be used on the images to support figure legends. Like the rest of the submission, the figures too should be blind. Any information within the images that may indicate an individual or institution should be blinded. The minimum resolution of each submitted figure should be 300 DPI. To prevent delays in the evaluation process, all submitted figures should be clear in resolution and large in size (minimum dimensions: 100 × 100 mm). Figure legends should be listed at the end of the main document.

All acronyms and abbreviations used in the manuscript should be defined at first use, both in the abstract and in the main text. The abbreviation should be provided in parentheses following the definition.

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

All references, tables, and figures should be referred to within the main text, and they should be numbered consecutively in the order they are referred to within the main text.

Limitations, drawbacks, and the shortcomings of original articles should be mentioned in the Discussion section before the conclusion paragraph.

REVISIONS

When submitting a revised version of a paper, the author must submit a detailed "Response to the reviewers" that states point by point how each issue raised by the reviewers has been covered and where it can be found (each reviewer's comment, followed by the author's reply and line numbers where the changes have been made) as well as an annotated copy of the main document. Revised manuscripts must be submitted within 30 days from the date of the decision letter. If the revised version of the manuscript is not submitted within the allocated time, the revision option may be canceled. If the submitting author(s) believe that additional time is required, they should request this extension before the initial 30-day period is over.

Accepted manuscripts are copy-edited for grammar, punctuation, and format. Once the publication process of a manuscript is completed, it is published online on the journal's webpage as an ahead-of-print publication before it is included in its scheduled issue. A PDF proof of the accepted manuscript is sent to the corresponding author and their publication approval is requested within 2 days of their receipt of the proof.

REFERENCES

While citing publications, preference should be given to the latest, most up-to-date publications. If an ahead-of-print publication is cited, the DOI number should be provided. Authors are responsible for the accuracy of references. Journal titles should be abbreviated in accordance with the journal abbreviations in Index Medicus/ MEDLINE/PubMed. When there are six or fewer authors, all authors should be listed. If there are seven or more authors, the first six authors should be listed followed by "et al." In the main text of the manuscript, references should be cited using Arabic numbers in parentheses. The reference styles for different types of publications are presented in the following examples.

Journal Article: Blasco V, Colavolpe JC, Antonini F, Zieleskiewicz L, Nafati C, Albanèse J, et al. Long-termoutcome in kidneyrecipientsfromdonorstreatedwithhydroxyethylstarch 130/0.4 andhydroxyethylstarch 200/0.6. Br J Anaesth 2015;115(5):797-8.

Book Section: Suh KN, Keystone JS. Malaria and babesiosis. Gorbach SL, Barlett JG, Blacklow NR,

editors. Infectious Diseases. Philadelphia: Lippincott Williams; 2004.p.2290-308.

Books with a Single Author: Sweetman SC. Martindale the Complete Drug Reference. 34th ed. London: Pharmaceutical Press; 2005.

Editor(s) as Author: Huizing EH, de Groot JAM, editors. Functional reconstructive nasal surgery. Stuttgart-New York: Thieme; 2003.

Conference Proceedings: Bengisson S. Sothemin BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Rienhoff O, editors. MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sept 6-10; Geneva, Switzerland. Amsterdam: North-Holland; 1992. pp.1561-5.

Scientific or Technical Report: Cusick M, Chew EY, Hoogwerf B, Agrón E, Wu L, Lindley A, et al. Early Treatment Diabetic Retinopathy Study Research Group. Risk factors for renal replacement therapy in the Early Treatment Diabetic Retinopathy Study (ETDRS), Early Treatment Diabetic Retinopathy Study KidneyInt: 2004. Report No: 26.

Thesis: Yılmaz B. Ankara Üniversitesindeki Öğrencilerin Beslenme Durumları, Fiziksel Aktivitelerive Beden Kitle İndeksleri Kan Lipidleri Arasındaki Ilişkiler. H.Ü. SağlıkBilimleriEnstitüsü, DoktoraTezi. 2007.

Manuscripts Accepted for Publication, Not Published Yet: Slots J. The microflora of black stain on human primary teeth. Scand J Dent Res. 1974.

Epub Ahead of Print Articles: Cai L, Yeh BM, Westphalen AC, Roberts JP, Wang ZJ. Adult living donor liver imaging. DiagnIntervRadiol. 2016 Feb 24. doi: 10.5152/dir.2016.15323. [Epub ahead of print].

Manuscripts Published in Electronic Format: Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis (serial online) 1995 Jan-Mar (cited 1996 June 5): 1(1): (24 screens). Available from: URL: http:/ www.cdc.gov/ncidodlElD/cid.htm.

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Editor: Birsen Karaman
Address: Istanbul University, Istanbul Faculty of Medicine Deanery, Turgut Özal Cad. 34093, Çapa, Fatih, Istanbul, Turkey
Phone: +90 212 414 21 61
E-mail: itfdergisi@istanbul.edu.tr

Publisher: Istanbul University Press
Address: İstanbul Üniversitesi Merkez Kampüsü, 34452 Beyazıt, Fatih / Istanbul - Turkey
Phone: +90 212 440 00 00

İstanbul Tıp Fakültesi Dergisi
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LETTER TO THE EDITOR / EDİTÖRE MEKTUP
DOI: 10.26650/IUITFD.2019.0052
İst Tıp Fak Derg 2020 / J Ist Faculty Med 2020

DISSEMINE INTRAVASCULAR COAGULATION MAY BE THE PRESENTING FEATURE FOR CHRONIC MYELOMONOCYTIC LEUKEMIA

YAYGIN DAMAR İÇİ PIHTILAŞMA İLE GELEN KRONİK MYELOMONOSİTİK LÖSEMİ

Itır ŞİRİNOĞLU DEMİRİZ[1], A. Gülsan TÜRKÖZ SUCAK[2]

[1]Health Sciences University, Bakirkoy Dr Sadi Konuk Training and Research Hospital, Hematology, Istanbul, Turkey
[2]Bahcelievler Medical Park Hospital, Hematology, Stem Cell Transplantation Clinic, Istanbul, Turkey

ORCID IDs of the authors: I.Ş.D. 0000-0001-7931-7104; A.G.T.S. 0000-0002-7707-0029

Cite this article as: Sirinoglu Demiriz I, Sucak Turkoz G. Dissemine intravascular coagulation may be the presenting feature for chronic myelomonocytic leukemia. J Ist Faculty Med 2020;83(4):307-8. doi: 10.26650/IUITFD.2019.0052

Chronic myelomonocytic leukemia (CMML) is a malignant myeloid stem cell disease accompanied by dysplasia in the context of myeloproliferative disease. Peripheral cytopenias (mainly anemia and thrombocytopenia) and hepatosplenomegaly are common findings. Dramatic leukocytosis can also be seen without transformation to acute myeloid leukemia (AML). In some cases, this is associated with leukostasis and end organ damage. Splenomegaly is present in up to 25% of patients and is often accompanied by hepatomegaly, lymphadenopathy, or nodular cutaneous leukemic infiltrates. The acquired cogulation defect may be due to factor X binding to atypical monocytes, resulting in acquired factor X deficiency.

We would like to highlight the challenging diagnosis and treatment of CMML.

Cases of CMML have a persistent peripheral blood monocyte count >1000/microL (>10 %). Despite a relative increase in monocytes, the total white blood cell count may not be increased. Myeloid dysplasia may be seen in all myeloid cells, and unique abnormal mononuclear cells exhibiting features of both myelocytes and monocytes, termed "paramyeloid cells," are often detected (1).

The World Health Organization (WHO) criteria for the diagnosis of CMML was revised in 2016 as shown in Table 1 (2).

Table 1: WHO criteria for diagnosis of CMML.

Persistent PB monocytosis >1×10e9/L, with monocytes accounting for ≥10% of the white blood cell (WBC) count;
Not meeting WHO criteria for BCR-ABL1 CML, PMF, PV, or ET;
No evidence of PDGFRA, PDGFRB, or FGFR1 rearrangement or PCM1-JAK2 (should be specifically excluded in cases with eosinophilia);
<20% blasts in the blood and BM;
Dysplasia in 1 or more myeloid lineages. If myelodysplasia is absent or minimal, the diagnosis of CMML may still be made if the other requirements are met and;
An acquired clonal cytogenetic or molecular genetic abnormality is present in hematopoietic cells or the monocytosis (as previously defined) has persisted for at least 3 months and; all other causes of monocytosis have been excluded

CMML is also stratified into different forms according to WBC count, peripheral and bone marrow blast, and promonocyte counts as summarized in Table 2.

Corresponding author/İletişim kurulacak yazar: dritir@hotmail.com

Submitted/Başvuru: 24.07.2019 • **Revision Requested/Revizyon Talebi:** 12.05.2020 •
Last Revision Received/Son Revizyon: 15.05.2020 • **Accepted/Kabul:** 02.06.2020 • **Published Online/Online Yayın:** 19.10.2020

Table 2: Subgroups of CMML.

WBC count	The percentage of blasts plus promonocytes in the PB and BM
Myelodysplastic, with WBC count <13×10e9/L	CMML-0, (with <2% blasts in PB and <5% blasts in BM)
Proliferative, with WBC count >13×10e9/L	CMML-1 (with 2–4% blasts in PB and/or 5–9% blasts in BM);
	CMML-2 (with 5–19% blasts in PB, 10–19% in BM, and/or when any Auer rods are present)

The diagnosis in most cases is usually based upon peripheral blood and bone marrow abnormalities with clinical non-specific features. However bleeding or coagulopathy is extremely rare in the diagnostic period. To date, there have been only a few cases of bleeding diathesis in CMML patients and these cases were under treatment for CMML. Rare cases may be referred to us for DIC and later diagnosed for CMML.

Monocytes express a small amount of procoagulant activity (PCA). However, they can be stimulated to produce tissue factor and other direct factor X activators. This activation can be triggered by T lymphocytes, various antigens, cytokines, some lipoproteins, immune complexes, endotoxins (3-5). Monocytes may also be activated by tumor-specific antigens and immune complexes or other cytokines containing them. For example, in lung cancer, pulmonary alveolar macrophages adjacent to a tumor increased tissue factor activity in vitro compared to cells from normal controls or macrophages from the contralateral side of the tumor (6). In CMML patients, similar malignancies monocytes may provocate hypercoagulation and procoagulation.

Due to the heterogeneity of the disease, the clinical course and outcomes of patients with CMML are variable. Previous reports have demonstrated that a high proportion of BM blasts, elevated lactate dehydrogenase, male sex and a low Hb level were independent prognostic factors. Most recently, cytogenetic status and specific gene mutations have been identified as important prognostic factors. The general prognosis of patients with CMML is poor, with an expected median survival of approximately 30 months. Patients with low risk disease by both the MDACC and Mayo scoring systems can delay transplant until the disease has progressed.

For those who are not candidates for allogeneic HCT and who decide not to participate in a clinical trial, we suggest symptom-directed therapy with either cytoreductive therapy (eg, hydroxyurea) or hypomethylating agents (eg, azacitidine, decitabine). Cytoreductive therapy is usually preferred for patients with dramatic proliferative symptoms, while hypomethylating agents are preferred for patients with cytopenias and those with myeloproliferative symptoms in whom hydroxyurea is ineffective (7-10).

We should be alert for elderly patients presenting with DIC regarding chronic myeloid neoplasias. Of particular concern is that CMML may imitate more frequent hypercoagulative diseases such as acute promyelocytic leukemia. However, it would be helpful to simply recall the possibility and analyse the peripheral smear for differential diagnosis in detail. Remembering and diagnosing CMML in this group of patients may lead to a remission status after hypometillating agent protocol.

REFERENCES

1. Bernat AL, Priola SM, Elsawy A, et al. Chronic subdural collection overlying an intra-axial hemorrhagic lesion in chronic myelomonocytic leukemia: special report and review of the literature. Expert Rev Neurother 2018;18(5):371-7. [CrossRef]
2. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood 2016;127(20):2391-405. [CrossRef]
3. Altieri DC, Edgington TS. The saturable high affinity association of factor X to ADP-stimulated monocytes defines a novel function of the Mac-1 receptor. J Biol Chem 1988;263(15):7007-15.
4. Edwards RL, Rickles FR, Bobrove AM. Mononuclear cell tissue factor: cell of origin and requirements for activation. Blood 1979;54(2):359-70. [CrossRef]
5. Gregory SA, Kornbluth RS, Helin H, et al. Monocyte procoagulant inducing factor: a lymphokine involved in the T cell-instructed monocyte procoagulant response to antigen. J Immunol 1986;137(10):3231-9.
6. Edwards RL, Rickles FR, Cronlund M. Abnormalities of blood coagulation in patients with cancer. Mononuclear cell tissue factor generation. J Lab Clin Med 1981;98(6):917-28.
7. Kantarjian H, Issa JP, Rosenfeld CS, et al. Decitabine improves patient outcomes in myelodysplastic syndromes: results of a phase III randomized study. Cancer 2006;106(8):1794-803. [CrossRef]
8. Kantarjian H, Oki Y, Garcia-Manero G, et al. Results of a randomized study of 3 schedules of low-dose decitabine in higher-risk myelodysplastic syndrome and chronic myelomonocytic leukemia. Blood 2007;109(1):52-7. [CrossRef]
9. Kantarjian HM, O'Brien S, Shan J, et al. Update of the decitabine experience in higher risk myelodysplastic syndrome and analysis of prognostic factors associated with outcome. Cancer 2007;109(2):265-73. [CrossRef]
10. Santini V, Allione B, Zini G, et al. A phase II, multicentre trial of decitabine in higher-risk chronic myelomonocytic leukemia. Leukemia 2018;32(2):413-8. [CrossRef]

İSTANBUL ÜNİVERSİTESİ YAYINEVİ

RESEARCH / ARAŞTIRMA
DOI: 10.26650/IUITFD.2019.0093
İst Tıp Fak Derg 2020 / J Ist Faculty Med 2020

IS AMNIOTIC FLUID ANALYSIS DURING THE SECOND TRIMESTER A PREDICTOR FOR THE DETECTION OF PRETERM LABOR?

İKİNCİ TRİMESTERDE BAKILAN AMNİYOTİK SIVI ANALİZİ PRETERM DOĞUM ÖNGÖRÜSÜNDE BİR PREDİKTÖR MÜDÜR?

Yücel KOÇYİĞİT[1], Hale GÖKSEVER ÇELİK[2], Mehmet ŞİMŞEK[3]

[1]Acibadem Mehmet Ali Aydinlar University, School of Medicine, Department of Obstetrics and Gynecology, Istanbul, Turkey
[2]University of Health Sciences, Istanbul Kanuni Sultan Suleyman Training and Research Hospital, Department of Obstetrics and Gynecology, Istanbul, Turkey
[3]Akdeniz University, School of Medicine, Department of Obstetrics and Gynecology, Antalya, Turkey

ORCID IDs of the authors: Y.K. 0000-0002-0687-330X; H.G.Ç. 0000-0002-5162-3262; M.Ş. 0000-0002-1319-4663

Cite this article as: Kocyigit Y, Goksever Celik H, Simsek M. Is amniotic fluid analysis during the second trimester a predictor for the detection of preterm labor? J Ist Faculty Med 2020;83(4):309-14. doi: 10.26650/IUITFD.2019.0093

ABSTRACT

Objective: Premature activation of the hypothalamic-pituitary-adrenal axis due to stress can initiate preterm labor. Many mechanisms have been proposed to explain etiopathogenesis. The most important one is clinical and subclinical chorioamnionitis. We aimed to assess the possibility of early detection and prevention of preterm labor based on second trimester amniotic fluid analysis.

Material and Method: One hundred and twenty-five pregnant women with singleton pregnancies who underwent amniocentesis were included. The first 2 cm^3 of the amniotic fluid obtained during the amniocentesis was used for glucose, interleukin-1, interleukin-6, high sensitivity C-reactive protein, electrolytes, progesterone/estrogen analysis, and cell counts.

Results: Sixteen women (13.8%) went into labor prior to 37 weeks of gestation. The mean age of the study population was 33.2±6.25 years. Ages were similar between the preterm and term groups (36.06±3.91 vs 32.77±6.43). Furthermore, the analysis of all parameters in the amniotic fluid did not show any statistical significant difference between the groups.

Conclusion: The possible effects of subclinical infection and steroid hormonal changes that are implied in the etiology of preterm labor were investigated in our study, and no evidence was found to support that these factors played a role in the etiology of preterm labor.

Keywords: Amniocentesis, preterm labor, glucose, interleukin, high sensitivity C-reactive protein, leukocyte count

ÖZET

Amaç: Strese bağlı hipotalamik-pitüiter-adrenal aksın prematür aktivasyonu preterm doğumu başlatır. Etiyopatogenezi açıklamak üzere pek çok mekanizma ileri sürülmüştür. En önemlisi klinik ve subklinik koryoamniyonittir. Preterm eylem riski taşıyan hastaların daha erken dönemde belirlenmesi, önlenmesi ve hastaların yanlış tanı nedeni ile gereksiz yere tokoliz tedavisi almasının önlenmesini amaçladık.

Gereç ve Yöntem: Çalışmamızda 16-26 gebelik haftalarında 125 tekiz gebe, onamı alınarak çalışmaya dahil edildi. Hastalardan 3'ünde karyotip anomalisi (Trizomi 21) saptandı, 1 hastanın gebeliği şiddetli preeklampsi nedeni ile 32. haftada sonlandırıldı, 5 hastaya da ulaşılamadı. Yüz on altı hasta ile çalışmaya devam edildi. Amniyosentez işlemi alınan ilk 2 cc'lik amniyon mayisi kullanılarak glukoz, IL-1, IL-6, HsCRP, hücre sayımı, elektrolit ve progesteron/estrojen oranı çalışıldı.

Bulgular: Gebelerin 16'sı 37. haftasını doldurmadan doğum yaptı (%13,8). Çalışma popülasyonun ortalama yaşı 33,2±6,25 idi. Çalışmamızda preterm doğum yapan grup ile miyadında doğum yapan grubun yaş ortalaması istatistiksel olarak benzer bulundu (36,06±3,91 vs 32,77±6,43). Ayrıca her iki grupta hastaların amniyon mayisindeki glukoz, IL-1, IL-6, HsCRP, hücre sayımı, elektrolit ve progesteron/estrojen oranı istatiksel olarak anlamlı bulunmadı.

Sonuç: Çalışmamızda preterm eylemin etiyolojisinde suçlanan subklinik enfeksiyon ve steroid hormon değişimleri incelenmiş ve bu hipotezleri destekleyen kanıt bulunamamıştır.

Anahtar Kelimeler: Amniyosentez, preterm doğum, glukoz, interlökin, yüksek sensitiviteli C-reaktif protein, lökosit sayımı

Corresponding author/İletişim kurulacak yazar: hgoksever@yahoo.com

Submitted/Başvuru: 02.12.2019 • **Revision Requested/Revizyon Talebi:** 11.02.2020 •
Last Revision Received/Son Revizyon: 13.02.2020 • **Accepted/Kabul:** 01.04.2020 • **Published Online/Online Yayın:** 22.09.2020

INTRODUCTION

Preterm birth is one of the leading causes of neonatal morbidity and mortality. When congenital fatal anomalies are set aside, 75% of neonatal deaths are due to preterm birth (1, 2). The preterm birth rates in developed countries are between 7% and 12% of all births with a higher prevalence in developing countries (1, 3, 4). Worldwide, the prevalence of preterm birth rate is calculated approximately as 11%, and approximately 15 million children are born prior to 37 weeks of gestation each year (5).

Among surviving neonates, preterm birth may cause long-term morbidities such as cerebral palsy of prematurity and neurologic dysfunction (6). One of the primary objectives of obstetrics is to prevent preterm labor in order to avoid morbidities related to prematurity, as well as perinatal and neonatal deaths. Therefore, it is of utmost importance to detect high-risk patients for preterm birth at the early stages of pregnancy, before premature uterine contractions begin. The most convenient solution for preterm labor is to detect high-risk patients and to make a diagnosis at the right point in time and prevent preterm labor.

Many methods have been proposed for the early diagnosis of preterm labor. A role for biochemical markers in the early diagnosis of preterm labor is being discussed with increasing frequency. With potentially effective markers, it may be possible to detect high-risk patients and to provide close monitoring and early intervention (7-9). Some studies even compared the level of these markers in amniotic fluid with serum in order to understand their efficacy (10-12). Moreover, for patients who are determined to be at a low risk with such tests, aggressive tocolysis, lengthy hospital stays, and close patient follow-up can be avoided. Also, asymptomatic patients with high risk of preterm labor may be determined with these biochemical markers and timely interventions can be made to accelerate fetal maturation (13).

In our study, we sought to investigate the infection markers and steroid hormone levels in amniotic fluid in order to determine the risk of preterm labor before the symptoms appeared.

MATERIAL AND METHOD

Data source

One hundred and twenty-five pregnant women aged above 18 years with singleton pregnancies who underwent amniocentesis between 16 and 26 weeks of pregnancy for various reasons between March 2011 and September 2011 were included in the study.

The gestational ages of the patients who fulfilled the criteria were calculated according to their last menstruation dates and confirmed with ultrasonographic measurements. All participating women routinely gave informed consent for the use of their data for research purposes. The Medical Faculty Ethics Committee approved this study (18.04.2011/116) and the ethics standards of the 1975 Declaration of Helsinki as revised in 2000 were complied with. Patients whose amniotic fluid analysis revealed a chromosomal anomaly, and patients whose pregnancy ended prematurely due to fetal or maternal reasons other than preterm labor were excluded from the study.

Women with multiple gestation, being outside of 16-26 weeks of gestation, known intrauterine or vaginal infection, complications during current pregnancy, detected congenital malformation in fetus or history of preterm labor, late abortus or late pregnancy loss were excluded.

Technique

Amniocentesis was performed transabdominally with a 22-gauge injector under ultrasonographic guidance with a free hand technique. The first 2 cm^3 of the fluid samples were drawn into a sterile injector, sent to the laboratory and analyzed for glucose, interleukin-1 (IL-1), interleukin-6 (IL-6), high sensitivity C-reactive protein (HsCRP), electrolytes, progesterone/estrogen analysis, and cell counts. The patients were followed up for a healthy pregnancy and for the determination of the week of birth.

Statistical analysis

Statistical analysis was performed with SPSS software (Statistics Package for Social Sciences) version 24 for Mac. Independent samples t test, chi-square test and Mann-Whitney U test were analyzed to detect difference in mean values and characteristics between groups. Standard deviation (SD) was used to present the mean values. $p<0.05$ was considered statistically significant.

RESULTS

During the seven-month research period, 125 patients who had planned amniocentesis for genetic counselling were included in our study. They had no ultrasonographic anomalies and no intrauterine infections. Trisomy 21 was detected in 3 cases; one pregnancy was terminated at the 32nd week due to severe pre-eclampsia. Five patients could not be contacted. 9 patients with missing data were excluded from the study and the study was completed with 116 patients. The mean age of the study population was 33.2±6.25 years.

Sixteen patients (13.8%) gave birth before completing the 37th week of gestation. The remaining 100 patients (86.2%) had term pregnancies. When the patients with preterm and term birth were compared, the mean age of the preterm birth group was found to be higher. However, the difference was not statistically significant (p=0.069).

Table 1: Comparison of the patients in the study and control groups

	Preterm group (n=16, mean±SD or number (%) or min-max values)	Term group (n=100, mean±SD or number (%) or min-max values)	*p value*[a]
Mean maternal age	36.06±3.91	32.77±6.43	0.069
HsCRP<0.02 mg/dL	13 (81.2)	90 (90)	0.386
HsCRP≥0.02 mg/dL	3 (18.8)	10 (10)	0.386
Glucose level (mg/dL)	42.37±12.01	43.25±10.32	0.654
Estrogen level (pg/mL)	219.66±75.92	219.30±99.62	0.743
Progesterone level (ng/mL)	50.17±26.22	47.62±25.00	0.994
Progesterone/Estrogen ratio	0.25±0.12	0.23±0.11	0.660
IL-1 level (pg/mL)	37.63±15.50	40.40±50.79	0.211
IL-6 level (pg/mL)	814.79±663.94	744.58±721.56	0.278
Na level (mEq/L)	127.18±11.95	129.59±10.24	0.580
K level (mEq/L)	3.55±0.29	3.64±0.29	0.270
Cl level (mEq/L)	95.37±11.11	97.83±9.53	0.613
Leukocyte count ($x10^6/mm^3$)	0.13 (0.06-1.25)	0.16 (0.03-0.66)	0.990

SD: Standard deviation, hsCRP: High sensitivity C-reactive protein, IL-1: Interleukin-1, IL-6: Interleukin-6, Na: Sodium, K: Potassium, Cl: Chlorine
[a]Independent samples t test and chi-square test were applied.

Investigation of HsCRP level in amniotic fluid revealed that 13 patients had a HsCRP level of 0.02 mg/dL or above. Three of these patients were in the preterm group and 10 were in the term group. Statistical analysis showed no significant difference regarding HsCRP levels of the preterm and the term groups (p=0.654). When estrogen and progesterone levels and progesterone/estrogen ratio were analyzed, no statistically significant difference was observed. When the IL-1 and IL-6 levels were examined, there was no significant increase in the mean IL-1 level in the preterm group and there was minimal increment in the mean IL-6 level in the preterm group; however, the difference was not statistically significant. When the electrolyte levels (sodium (Na), potassium (K), and chlorine (Cl)) and leukocyte count in the amniotic fluid of the preterm and term groups were compared, no statistically significant difference was detected (Table 1).

DISCUSSION

Preterm birth is the leading cause of infant morbidity and mortality all over the world. For this reason, the pathogenic processes leading to preterm birth, development of preventive interventions and markers for the early prediction are major targets of obstetric research. The possible effects of subclinical infection and steroid hormonal changes that are implied in the etiology of preterm labor were investigated in our study. No significant evidence was found to support that these factors played a role in the etiology of preterm labor.

The theory claiming that the fetus is connected by an intrauterine inflammatory process during the very early stages of pregnancy by increasing the subclinical inflammatory response has been developed. A study that investigated amniotic fluid for genetic counselling purposes (between 16-20 weeks) found that the median CRP level in amniotic fluid was 183 ng/mL in women who gave birth prior to 34 gestational weeks, 113 ng/mL in those who gave birth between 34 and 37 gestational weeks, and 57 ng/mL in women who gave birth after 37 gestational weeks. A correlation was found between amniotic fluid CRP levels and preterm birth, and when a CRP cut-off level was set to 110 ng/mL, the probability of birth before 34 weeks of gestation could be predicted with 80.8% sensitivity and 69.5% specificity (14). Similarly, it was reported that when the CRP cut-off level in amniotic fluid was set at 0.65 mg/L, the probability of birth before 37 weeks could be estimated with 92.9% sensitivity and 78.7% specificity (15).

Another study published in the same year investigated a larger sample of patients and found that the probability of preterm labor was 9.3%. They obtained amniotic fluid between 15 and 18 weeks of gestation, and measured CRP, glucose, and leukocyte levels, and determined that there was no statistically significant difference between the preterm and term groups regarding the CRP levels and leukocyte counts. The glucose level, however, was found to be significantly lower in the preterm group. When glucose cut-off level was set at 46 mg/dL, the test was 100% sensitive and had a 100% negative predictive

value (16). Another prospective cohort series including 39 patients also found that all amniotic fluid biomarkers such as CRP, glucose, and IL-6 did not differ significantly between the pre-term and the term groups (17).

In this study, even though the CRP levels in the preterm group were found to be higher, there was no statistically significant difference between groups. There are, however, several studies demonstrating statistically significant results. A recent meta-analysis evaluating 14 prospective, retrospective, cohorts, and case-controlled studies concluded that higher amniotic fluid IL-6 and MMP-8 levels, and lower glucose level could be used as predictors for preterm delivery due to significant results (18, 19). Although these markers have been found to be predictors of preterm delivery, more evidence is needed to determine whether they should be used as screening tests in clinical practice (20).

Nevertheless, it is currently unknown whether or not a certain CRP level can trigger preterm birth. This raises the issue and difficulty of achieving a strong negative predictive value based on CRP levels alone. In addition, since CRP is the first acute phase reactant protein to appear in blood, there might be other cytokines responsible for the cascade of events leading to preterm delivery. Another issue is that although amniocentesis procedures typically last less than one minute, the needle may cause a slight CRP increase, and influence the contents of the sampled fluid.

Our study showed that glucose levels of the preterm and term groups were found to be at similar levels. A study that performed amniocentesis in 40 patients who were diagnosed as having preterm labor between weeks 28 to 36 gestation measured glucose levels and made cultures of the fluid samples to detect aerobic and anaerobic bacteria and mycoplasma. In culture-positive patients, glucose levels were significantly lower. When the cut-off was set at 16 mg/dL, the amniotic fluid glucose level could predict the infection with 77% sensitivity and 87% specificity. This study showed that for the diagnosis of intrauterine infection, the measurement of glucose level might prove to be a cheaper and easier method, as an alternative to culture. This method, however, can only be more widely used if the correlation between preterm labor and subclinical intraamniotic infection is determined (21).

Regarding the investigation of the role of subclinical intrauterine infections in preterm birth, a previously conducted study measured IL-6 and tumor necrosis factor (TNF)-α levels in amniotic fluid and examined the predictability of preterm labor. They found that IL-6 had 89.6% sensitivity and 80.3% specificity, whereas TNF-α had 81.3% sensitivity and 79.2% specificity for the detection of preterm labor. In the analysis made for positive intraamniotic culture, levels of IL-6 had 91.9% sensitivity and 73.8% specificity, and TNF-α had 78.4% sensitivity and 70.1% specificity (22). Similarly, an inverse correlation was also detected between amniotic fluid IL-6 levels and the gestational week of birth in other studies (23, 24).

Alvarez de Rosa et al investigated the relationship of preterm delivery and IL-1, 2, 6, 8, and IL-2 receptor levels in 103 pregnant women. The preterm delivery group was found to have significantly elevated IL-2 receptor levels and IL-6 levels were found to be significantly lower in the preterm delivery group among those who responded to tocolytics compared with non-responders. This study suggested that maternal serum IL-6 and IL-2 receptor levels might be of benefit in determining preterm labor and a possible response to tocolytics (25).

No statistically significant difference was observed between the groups in terms of amniotic fluid levels of IL-1 and IL-6 in our study. This result may be due to the low number of cases, or it may also be caused by the variation in cytokine expression due to the influence of environmental and genetic factors, such as race, which was shown to have such an effect (26).

In many animals, a change in systemic or local levels of steroid hormones is the factor that starts the delivery process. A decrease of progesterone levels has been shown in many animals (e.g. sheep, monkeys) to be the principal effector that starts delivery (27, 28). In humans, the events concerning steroid changes are not identical to those in sheep and take more time. The steroid changes in sheep occur in mere days, whereas the changes in humans start between weeks 34 and 36 of pregnancy and take longer than 5 weeks. The beginning of the estrogen increase begins between weeks 34 and 36 in-utero; however, there is no late increase before delivery (29). It is probable that instead of a triggering an increase, there is a certain concentration build-up in humans, or the changes are local and are not reflected in the mother's systemic circulation (30). Although the exact mechanism of the relationship between the decrease in progesterone and the start of labor is unknown, it is known that delivery can be delayed by administration of progesterone or synthetic progestin (31-33).

Furthermore, Romeo et al. investigated the local changes in progesterone and estrogen levels in term human deliveries, comparing the levels of estradiol, estriol, and progesterone by performing amniocentesis in 40 women at term pregnancy (20 pregnant women in active delivery and 20 pregnant women not in active delivery) (34). The study showed that progesterone/estrogen ratios were significantly lower in women in active delivery at term. It was emphasized that there were local changes in term delivery and that the progesterone/estrogen ratio was more related to the subject than the individual concentrations of estrogen and progesterone. However, another study investigated the relationship between estrogen/

progesterone ratio and term delivery and found no significant difference in plasma estrogen or progesterone concentrations of patients at term and patients at 37 to 42 weeks of gestation in whom labor hadn't started (35). In our study, no changes in the estrogen and progesterone levels, and the progesterone/estrogen ratio were detected in relation with preterm delivery. Although the hypothesis that estrogen and progesterone level changes are important in the etiology of preterm delivery is tempting, strong clues indicating no such connection exists have been reported.

Recent studies have attempted to detect intraamniotic changes through non-invasive techniques. This is promising as it may mean that in the future, detection of preterm delivery can be done as a routine detection test in gestational patients (36).

There are conflicting results in the literature regarding the parameters compared in this study. Data on the subclinical inflammation hypothesis and the steroid hormones hypothesis in the etiology of preterm delivery is not consistent and various studies point to different conclusions; therefore, there is a need for more studies in this subject, preferably conducted with non-invasive methods.

The strict inclusion criteria, the prospective and monocentric design are the strengths of our study. The size of study population is also acceptable. Nowadays, the more widespread use of non-invasive methods, rather than amniocentesis, can be considered as a limitation of our study. However, the definitive diagnosis of several conditions is still only possible by the performance of amniocentesis.

As a conclusion, although this study has found no significant differences between the subclinical infection markers and steroid hormonal changes in the second trimester amniotic fluid of preterm and term patients, a better understanding of the exact mechanisms of preterm labor in the future may uncover other potential ways for early detection of preterm delivery.

Ethics Committee Approval: Acibadem Mehmet Ali Aydinlar University, Medical Faculty Ethics Committee approved this study (18.04.2011/116) and the ethics standards of the 1975 Declaration of Helsinki as revised in 2000 were complied with.

Informed Consent: Written consent was obtained from the participants.

Peer Review: Externally peer-reviewed.

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

Conflict of Interest: Authors declared no conflict of interest.

Financial Disclosure: Authors declared no financial support.

Etik Komite Onayı: Acıbadem Mehmet Ali Aydınlar Universitesi, Tıp Fakültesi Etik Kurulu bu çalışmayı onayladı (18.04.2011 / 116) ve 2000 yılında revize edilen 1975 Helsinki Bildirgesi'nin etik standartlarına uyuldu.

Bilgilendirilmiş Onam: Katılımcılardan bilgilendirilmiş onam alınmıştır.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Çalışma Konsepti/Tasarım- Y.K., M.Ş.; Veri Toplama- Y.K., M.Ş.; Veri Analizi/Yorumlama- Y.K., H.G.Ç.; Yazı Taslağı- Y.K., H.G.Ç.; İçeriğin Eleştirel İncelemesi- Y.K., H.G.Ç., M.Ş.; Son Onay ve Sorumluluk- Y.K., H.G.Ç., M.Ş.; Süpervizyon- M.Ş., H.G.Ç.

Çıkar Çatışması: Yazarlar çıkar çatışması beyan etmemişlerdir.

Finansal Destek: Yazarlar finansal destek beyan etmemişlerdir.

REFERENCES

1. Amon E. Preterm labor. In: Reece EA, Hobbins JC, Mahoney MJ, Petrie RH, editors. Medicine of the fetus and mothers. Philadelphia: Lippincott-Raven 1999. p.1529-79.
2. Rush RW, Davey DA, Segall ML. The effect of preterm delivery on perinatal mortality. Br J Obstet Gynaecol 1978;85(11):806-11. [CrossRef]
3. Lockwood CJ, Senyei AE, Dische MR, Casal D, Shah KD, Thung SN, et al. Fetal fibronectin in cervical and vaginal secretions as a predictor of preterm delivery. N Engl J Med 1991;325(10):669-74. [CrossRef]
4. Bittar RE, Yamasaki AA, Sasaki S, Zugaib M. Cervical fetal fibronectin in patients at increased risk for preterm delivery. Am J Obstet Gynecol 1996;175(1):178-81. [CrossRef]
5. Blencowe H, Cousens S, Oestergaard MZ, Chou D, Moller AB, Narwal R, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet 2012;379(9832):2162-72. [CrossRef]
6. Vincer MJ, Allen AC, Joseph KS, Stinson DA, Scott H, Wood E. Increasing prevalence of cerebral palsy among very preterm infants: a population-based study. Pediatrics 2006;118(6):e1621-6. [CrossRef]
7. Iavazzo C, Tassis K, Gourgiotis D, Boutsikou M, Baka S, Hassiakos D, et al. The role of human beta defensins 2 and 3 in the second trimester amniotic fluid in predicting preterm labor and premature rupture of membranes. Arch Gynecol Obstet 2010;281(5):793-9. [CrossRef]
8. Hong SN, Joo BS, Chun S, Kim A, Kim HY. Prediction of preterm delivery using levels of vascular endothelial growth factor and leptin in amniotic fluid from the second trimester. Arch Gynecol Obstet 2015;291(2):265-71. [CrossRef]
9. Melekoglu R, Yilmaz E, Ciftci O, Kafadar YT, Celik E. Associations between second-trimester amniotic fluid levels of ADAMTS4, ADAMTS5, IL-6, and TNF-α and spontaneous preterm delivery in singleton pregnancies. J Perinat Med 2019;47(3):304-10. [CrossRef]

10. Oh KJ, Park KH, Kim SN, Jeong EH, Lee SY, Yoon HY. Predictive value of intra-amniotic and serum markers for inflammatory lesions of preterm placenta. Placenta 2011;32(10):732-6. [CrossRef]
11. Park H, Park KH, Kim YM, Kook SY, Jeon SJ, Yoo HN. Plasma inflammatory and immune proteins as predictors of intra-amniotic infection and spontaneous preterm delivery in women with preterm labor: a retrospective study. BMC Pregnancy Childbirth 2018;18(1):146. [CrossRef]
12. Virgiliou C, Gika HG, Witting M, Bletsou AA, Athanasiadis A, Zafrakas M, et al. Amniotic Fluid and Maternal Serum Metabolic Signatures in the Second Trimester Associated with Preterm Delivery. J Proteome Res 2017;16(2):898-910. [CrossRef]
13. Erzincan SG, Varol FG, Inan C, Sayin NC. Relationship between second-trimester amniotic fluid levels of Prokineticin-1 and Matrix Metalloproteinase-2 with adverse pregnancy outcome. Placenta 2018;62:25-7. [CrossRef]
14. Ghezzi F, Franchi M, Raio L, Di Naro E, Bossi G, D'Eril GV, et al. Elevated amniotic fluid C-reactive protein at the time of genetic amniocentesis is a marker for preterm delivery. Am J Obstet Gynecol 2002;186(2):268-73. [CrossRef]
15. Ozer KT, Kavak ZN, Gökaslan H, Elter K, Pekin T. Predictive power of maternal serum and amniotic fluid CRP and PAPP-A concentrations at the time of genetic amniocentesis for the preterm delivery. Eur J Obstet Gynecol Reprod Biol 2005;122(2):187-90. [CrossRef]
16. Tarim E, Bağiş T, Kiliçdağ EB, Sezgin N, Yanik F. Are amniotic fluid C-reactive protein and glucose levels, and white blood cell counts at the time of genetic amniocentesis related with preterm delivery? J Perinat Med 2005;33(6):524-9. [CrossRef]
17. Kesrouani A, Chalhoub E, El Rassy E, Germanos M, Khazzaka A, Rizkallah J, et al. Prediction of preterm delivery by second trimester inflammatory biomarkers in the amniotic fluid. Cytokine 2016;85:67-70. [CrossRef]
18. Liu Y, Liu Y, Zhang R, Zhu L, Feng Z. Early- or mid-trimester amniocentesis biomarkers for predicting preterm delivery: a meta-analysis. Ann Med 2017;49(1):1-10. [CrossRef]
19. Kim A, Lee ES, Shin JC, Kim HY. Identification of biomarkers for preterm delivery in mid-trimester amniotic fluid. Placenta 2013;34(10):873-8. [CrossRef]
20. Ozgu-Erdinc AS, Cavkaytar S, Aktulay A, Buyukkagnici U, Erkaya S, Danisman N. Mid-trimester maternal serum and amniotic fluid biomarkers for the prediction of preterm delivery and intrauterine growth retardation. J Obstet Gynaecol Res. 2014;40(6):1540-6. [CrossRef]
21. Uludogan M, Kuyumcuoglu U, Gorgen H, Delikara MN. Amniotic fluid glucose levels in intraamniotic infections. J Perinatol 1995;3(1-2):59-63.
22. Thomakos N, Daskalakis G, Papapanagiotou A, Papantoniou N, Mesogitis S, Antsaklis A. Amniotic fluid interleukin-6 and tumor necrosis factor-alpha at mid-trimester genetic amniocentesis: relationship to intra-amniotic microbial invasion and preterm delivery. Eur J Obstet Gynecol Reprod Biol 2010;148(2):147-51. [CrossRef]
23. Ghidini A, Jenkins CB, Spong CY, Pezzullo JC, Salafia CM, Eglinton GS. Elevated amniotic fluid interleukin-6 levels during the early second trimester are associated with greater risk of subsequent preterm delivery. Am J Reprod Immunol 1997;37(3):227-31. [CrossRef]
24. Wenstrom KD, Andrews WW, Hauth JC, Goldenberg RL, DuBard MB, Cliver SP. Elevated second-trimester amniotic fluid interleukin-6 levels predict preterm delivery. Am J Obstet Gynecol 1998;178(3):546-50. [CrossRef]
25. Alvarez-de-la-Rosa M, Rebollo FJ, Codoceo R, Gonzalez Gonzalez A. Maternal serum interleukin 1, 2, 6, 8 and interleukin-2 receptor levels in preterm labor and delivery. Eur J Obstet Gynecol Reprod Biol 2000;88(1):57-60. [CrossRef]
26. Velez DR, Menon R, Thorsen P, Jiang L, Simhan H, Morgan N, et al. Ethnic differences in interleukin 6 (IL-6) and IL6 receptor genes in spontaneous preterm birth and effects on amniotic fluid protein levels. Ann Hum Genet 2007;71(Pt 5):586-600. [CrossRef]
27. Haluska GJ, Cook MJ, Novy MJ. Inhibition and augmentation of progesterone production during pregnancy: effects on parturition in rhesus monkeys. Am J Obstet Gynecol 1997;176(3):682-91. [CrossRef]
28. Liggins GC, Fairclough RJ, Grieves SA, Forster CS, Knox BS. Parturition in the sheep. Ciba Found Symp. 1977;(47):5-30. [CrossRef]
29. Kota SK, Gayatri K, Jammula S, Kota SK, Krishna SV, Meher LK, et al. Endocrinology of parturition. Indian J Endocrinol Metab 2013;17(1):50-9. [CrossRef]
30. Davidson BJ, Murray RD, Challis JR, Valenzuela GJ. Estrogen, progesterone, prolactin, prostaglandin E2, prostaglandin F2 alpha, 13,14-dihydro-15-keto-prostaglandin F2 alpha, and 6-keto-prostaglandin F1 alpha gradients across the uterus in women in labor and not in labor. Am J Obstet Gynecol 1987;157(1):54-8. [CrossRef]
31. Yemini M, Borenstein R, Dreazen E, Apelman Z, Mogilner BM, Kessler I, et al. Prevention of premature labor by 17 alpha-hydroxyprogesterone caproate. Am J Obstet Gynecol 1985;151(5):574-7. [CrossRef]
32. Erny R, Pigne A, Prouvost C, Gamerre M, Malet C, Serment H, et al. The effects of oral administration of progesterone for premature labor. Am J Obstet Gynecol 1986;154(3):525-9. [CrossRef]
33. Khan-Dawood FS. In vitro conversion of pregnenolone to progesterone in human term placenta and fetal membranes before and after onset of labor. Am J Obstet Gynecol 1987;157(6):1333-7. [CrossRef]
34. Romero R, Scoccia B, Mazor M, Wu YK, Benveniste R. Evidence for a local change in the progesterone/estrogen ratio in human parturition at term. Am J Obstet Gynecol 1988;159(3):657-60. [CrossRef]
35. Mazor M, Wiznitzer A, Levy J, Sharoni Y, Meril Z, Minster A, et al. The relationship between estrogen/progesterone ratio and term human parturition. Isr J Med Sci 1993;29(2-3):97-9.
36. Park KH, Kim SN, Oh KJ, Lee SY, Jeong EH, Ryu A. Noninvasive prediction of intra-amniotic infection and/or inflammation in preterm premature rupture of membranes. Reprod Sci 2012;19(6):658-65. [CrossRef]

İSTANBUL ÜNİVERSİTESİ YAYINEVİ

RESEARCH / ARAŞTIRMA
DOI: 10.26650/IUITFD.2020.0074
İst Tıp Fak Derg 2020 / J Ist Faculty Med 2020

CHANGES IN CLINICAL AND CYTOGENETIC FINDINGS OF INVASIVE PRENATAL DIAGNOSIS FROM 1989 TO 2011 IN ISTANBUL; IMPACT OF THE BIOCHEMICAL SCREENING TESTS AND FETAL ULTRASONOGRAPHY

İSTANBUL'DA 1989-2011 YILLARI ARASINDA İNVAZİF PRENATAL TANININ KLİNİK VE SİTOGENETİK BULGULARINDAKİ DEĞİŞİKLİKLER; BİYOKİMYASAL TARAMA TESTLERİNİN VE FETAL ULTRASONOGRAFİNİN ETKİSİ

Seher BAŞARAN[1,2], Birsen KARAMAN[1-3], Melike KIRGIZ[2], İbrahim Halil KALELİOĞLU[4], Recep HAS[4], Tahir DEHGAN[2], Alkan YILDIRIM[3], Güven TOKSOY[1], Atıl YÜKSEL[4]

Istanbul University, Istanbul Faculty of Medicine, [1]Department of Medical Genetics, [4]Department of Obstetrics and Gynecology, Istanbul, Turkey
[2]PREMED, Center for Genetic Diagnosis, Şişli, Istanbul, Turkey
[3]Istanbul University, Child Health Institute, Pediatric Basic Sciences, Istanbul, Turkey

ORCID IDs of the authors: S.B. 0000-0001-8668-4746; B.K. 0000-0001-8640-0176; M.K. 0000-0003-1760-9958; H.İ.K. 0000-0003-1349-2561; R.H. 0000-0002-1372-8506; T.D. 0000-0001-8101-4145; A.Y. 0000-0002-8570-4298; G.T. 0000-0002-8103-9980; A.Y. 0000-0002-6487-0860

Cite this article as: Basaran S, Karaman B, Kirgiz M, Kalelioglu IH, Has R, Dehgan T, et al. Changes in clinical and cytogenetic findings of invasive prenatal diagnosis from 1989 to 2011 in Istanbul; Impact of the biochemical screening tests and fetal ultrasonography. J Ist Faculty Med 2020;83(4):315-24. doi: 10.26650/IUITFD.2020.0074

ABSTRACT

Objective: To determine the impact of maternal serum screening tests (MS-STs) and ultrasonography (US) on clinical and cytogenetic findings in invasive prenatal diagnosis (IPD)

Material and Method: Results of 23469 amniocentesis (AC) and 2492 chorionic villus sampling (CVS) obtained over 23 years were compared with regard to two periods; before and after year 2000.

Results: Cases with advanced maternal age (AMA) decreased, while MS-STs and fetal US increased in the timeline. The rate of chromosome aberration increased from 10.1% to 17.6% in CVS and from 3.2% to 4.3% in AC. The common aneuploidies summed up to 69.6% of anomalies (n=385) in CVS and 65.1% of anomalies (n=892) in AC. When known parental carriers were excluded, the rate of chromosomal rearrangements was 1.4% in CVS and 1% in AC. Discrepant cytogenetic results between CVS and AC were observed in 1.7% of CVS. The rate of true/possible true mosaicism, false-positive confined placental mosaicism (CPM) and false-negative CPM was 1.02%, 0.57% and 0.49% in CVS, respectively.

Conclusion: MS-ST and US had a serious impact on the indications for invasive procedures and the rates of chromosome ab-

ÖZET

Amaç: İnvazif prenatal tanıda (IPT) maternal serum tarama testlerinin (MS-TT) ve fetal ultrasonografinin (USG) klinik ve sitogenetik bulgular üzerine olan etkisini belirlemek.

Gereç ve Yöntem: 23 yıllık süreçte invazif girişimle elde edilen 23469 amniyosentez (AS) ve 2492 koryon villus aspirasyonu uygulama (KVA) sonuçları, 2000 yılı öncesi ve sonrası iki döneme ayrılarak karşılaştırıldı.

Bulgular: İleri anne yaşı (İAY) ile başvuran olgu sayısı azalırken, MS-TT ve fetal USG endikasyonu ile başvuran olguların sayısında artma gözlendi. Kromozom anomali oranı KVA'da %10,1'den %17,6'ya ve AS'de %3,2'den %4,3'e yükseldi. Yaygın anöploidiler, KVA'da anomalilerin %69,6'sını (n=385) ve AS'de anomalilerin %65,1'ini (n=892) oluşturdu. Bilinen kromozom anomali taşıyıcıları hariç tutulduğunda, kromozomal yeniden düzenlemelerin oranı KVA'da %1,4 ve AS'de %1 idi. KVA'nun %1,7'sinde KVA ve AS arasında uyumsuz sitogenetik sonuçlar gözlendi. KVA'da gerçek/olası gerçek mozaiklik, yalancı pozitif plasentayla sınırlı mozaisizm (PSM) ve yalancı negatif PSM oranı sırasıyla %1,02, %0,57 ve %0,49 bulundu.

Corresponding author/İletişim kurulacak yazar: basarans@istanbul.edu.tr

Submitted/Başvuru: 23.06.2020 • **Revision Requested/Revizyon Talebi:** 05.07.2020 •
Last Revision Received/Son Revizyon: 06.07.2020 • **Accepted/Kabul:** 11.07.2020 • **Published Online/Online Yayın:** 07.10.2020

errations. The presented data will establish a baseline for better genetic counseling regarding IPD and noninvasive PD (NIPD) in different risk groups.

Keywords: Invasive prenatal diagnosis, maternal serum screening tests, fetal ultrasonography, chromosome aberrations

Sonuç: MS-TT ve USG'nin invazif girişim endikasyonları ve saptanan kromozom anomali oranları üzerinde ciddi etkilerinin olduğu görülmüştür. Elde edilen veriler, farklı risk gruplarında İPT ve noninvazif prenatal tarama ile ilgili daha doğru ve yararlı genetik danışmanlık için bir temel oluşturacaktır.

Anahtar Kelimeler: İnvazif prenatal tanı, Maternal serum tarama testleri, fetal ultrasonografi, kromozom anomalileri

INTRODUCTION

The risk of trisomies increases with maternal age, decreases with advancing gestation (1-3). Although half of the trisomy 21 (T21) conceptuses survive to term, it is the most common chromosomal abnormality seen at birth and AMA as a screening parameter allowed us to diagnose about 1/4 of the T21s. The developing of the MS-ST known as "triple test" (TT) by using the three biochemical markers (Alfa fetoprotein, free ß-human Chorionic Gonadotropin, unconjugated estriol) in the 1980s increased the detection rate of T21 to 60-70% (4). Another improvement in the risk estimation was the US screening to search the non-structural anomalies entitled "soft markers" associated with fetal aneuploidies like increased nuchal translucency (NT), hypoplastic nasal bone, short femur length, etc. (5). First trimester-ST using NT thickness and two MS-biochemical markers (free ß-hCG and pregnancy-associated plasma protein A), raised the detection rate to ~ 90% for T21 at a false positive rate of 5%, which exceeded 95% incorporating nasal bone and other special ultrasound markers (6-8). Recent improvements in NIPD use MS-cell free DNA (cf-DNA) technique, which influences the management of the high-risk pregnancies today and in the near future (9, 10).

Since the cf-DNA takes its source from cytotrophoblasts, it is expected that the technique comprises the specification of the cytotrophoblasts. Therefore it is necessary to determine its efficiency and consequences (11). For this aim, we reevaluated our cytogenetic data obtained from the largest CVS and AC series during the past 23 years from Turkey. Istanbul with more than 15.000.000 inhabitants is the most populated city in Turkey and the number of deliveries exceeds 212,000 yearly (12). Although some western countries established national screening policies (13, 14), there is no nationally approved prenatal screening program in Turkey.

MATERIAL AND METHOD

During the 23 year study period, 25961 invasive procedures (2492 CVS and 23469 AC) were performed in two centers situated in Istanbul conducted by similar principles, one faculty hospital setting from 1989 on, which was the first established center for PD in Turkey and private one from 1996 on. The clinical and cytogenetic data were evaluated for two periods; from 1989 to 1999 and 2000-2011, retrospectively.

Risk factors determined by genetic counseling for IPD were classified as follows; (1) risk for monogenic disorders (MD), (2) no increased risk for chromosome aberrations (NIR) (maternal anxiety, etc.), (3) low risk (LR) includes cases with ~ 1% risk for fetal chromosome aberrations (child with chromosome aberration, IVF/ICSI pregnancies, etc.), (4) advanced maternal age (AMA), (5) increased risk in MS-STs (MS-ST), (6) pathological US findings (P-US), (7) parental balanced chromosomal rearrangements (PBCR). The number of invasive procedures in different indications according to the period and applied technique were shown in Table 1.

Table 1: The number of invasive procedures according to the referring indications and to the periods in CVS and AC series.

Indications	CVS series			AC series		
	First period n=662	Second period n=1830	∑n=2492	First period n=7426	Second period n=16043	∑n=23469
MD	352	626	978	86	106	192
NIR	4	9	13	188	289	477
LR	43	14	57	578	379	957
AMA	88	140	228	4633	6518	11151
ST	6	276	282	1343	4697	6040
P-US	112	714	826	543	3915	4458
PBCR	54	48	102	44	93	137
Confirmation	3	3	6	11	46	57

Mean maternal age in AMA indication was 39.84 in CVS (range 35-48) and 37,97 in AC (range 35-49) in the first period; 39 (range 35-47) in CVS and 38 (range 35-51) in AC in the second period. The cut-off level of combined risk was 1:300 in ST indication and mean maternal age was 32 (range 20-45) in the second period of CVS; 31 (range 16-45) in the first and 33 (range 18-47) in the second period of the AC for this indication group. Cases without increased risk by combined first trimester-ST, but increased NT measurement were evaluated under P-US. P-US group includes all minor or major, single or multiple anomalies detected in US. Cases with accompanying risk factors for chromosome aberrations of the MD group were placed into the related indications (109 AMA in CVS; 46 AMA and 5 P-US in AC) and remainders into the NIR indication in Tables 2a-2b and 3a-3b. Accompanying risk factors (AMA in 146 CVS and in 1333 AC; ST in 65 CVS and 109 AC) in P-US were considered only in Figures 1a-1b. When more than one indication was assigned (7.5% in CVS and 19.4% in AC in the first period and 25% in CVS and 35.4% in AC in the second period), these cases were included into the group with the highest relative risk for chromosome anomaly. The cases re-evaluated due to ambiguous or abnormal results referred from external laboratories were excluded (6 CVS and 57 AC cases) from the series.

CVS was performed by transabdominal route by using 18 or 20 Gauge needles. Transcervical technique was applied in 208 cases until 1998. AC was performed by using a 20 Gauge needle. Mean gestational age was 13.6±3 (range 9-29) in CVS and 18 (range 11-36), ±1.8 in AC. Procedure-related abortion risk was given as 1-2% for CVS and as 0.5% in the genetic counseling.

Both direct preparation/short term incubation (DP) and *in-situ* long term cell culture (LTCC) techniques were performed concurrently on CV samples, when adequate material was obtained, otherwise only LTCC. LTCCs were set up in two or three TC-25 flasks. Routinely 20 Giemsa banded metaphases at 450–550 band level were evaluated. If necessary, other banding and fluorescence *in situ* hybridization (FISH) techniques were applied. When the same anomaly was found in at least three DP-metaphases or in two flasks of LTCC, it was recorded as "mosaic", if it was possible, fetal tissues were further investigated. Classical polymorphic variants were not included in the anomalies. Chi-square analyses were performed for statistical analysis.

Table 2a: The changes in the number and frequencies of the chromosome aberrations according to the referring indications from first to second periods of CVS series.

CVS Series	First period			Second period			total rate %
	∑n	anomalies n	anomalies %	∑n	anomalies n	anomalies %	
NIR	308	2	0.7	533	8	1.5	1.2
LR	42	4	9.5	13	0	0	7.3
AMA	112	4	3.6	223	11	4.9	4.5
ST	6	1	16.7	276	52	18.8	18.8
P-US	110	19	17.3	714	225	31.5	29.6
PBCR	53	34	64.2	48	25	52.1	58.4
Total	631	64	10.1	1822	321	17.6	15.7

Table 2b: The changes in the number and frequencies of the chromosome aberrations according to the referring indications from first to second periods of AC series.

AC Series	First period			Second period			total rate %
	∑n	anomalies n	anomalies %	∑n	anomalies n	anomalies %	
NIR	267	1	0.4	358	5	1.4	1
LR	577	8	1.4	379	4	1.1	1.3
AMA	3891	93	2.4	6539	140	2.1	2.2
ST	2056	52	2.5	4693	136	2.9	2.8
P-US	536	55	10.3	3906	322	8.2	8.5
PBCR	43	26	60.5	93	50	53.8	55.9
Total	7370	235	3.2	15443	657	4.3	3.9

Table 3a: The number and the rate of distinct chromosome aberrations detected in CVS series divided according to the referring indications.

Chromosome anomalies*		NIR		LR		AMA		ST		P-US		PBCR	
		n	%	n	%	n	%	n	%	n	%	n	%
45,X and variants	n=42	1	2.4	0	-	1	2.4	4	9.5	36	85.7	0	-
PolysomyX/Y	n=6	2	33.3	0	-	2	33.3	0	-	2	33.3	0	-
Trisomy 21	n=132	0	-	2	1.5	7	35.6	29	22	88	66.7	6[a]	4.5
Trisomy 18	n=61	0	-	0	-	1	1.6	4	66.7	55	90.2	1[a]	1.6
Trisomy 13	n=27	0	-	0	-	1	3.7	2	7.4	23	85.2	1	3.7
Uncommon trisomies	n=20	2	10	1	5	1	5	8	40	8	40	0	-
Poliploidies	n=20	0	-	0	-	0	-	2	10	17	85	1	5
Balanced structural	n=51	5	9.8	1	2	2	3.9	1	2	7	13.2	35	68.6
Unbalanced structural	n=26	0	-	0	-	0	-	3	11.5	8	30.8	15	57.7
Total	n=385	10	2.6	4	1	15	3.9	53	13.8	244	63.4	59	15.3

*including translocations and mosaics
[a]Trisomy was detected additional to parental inherited balanced reciprocal translocation

Table 3b: The number and the rate of distinct chromosome aberrations detected in AC series divided according to the referring indication.

Chromosome anomalies*		NIR		LR		AMA		ST		P-US		PBCR	
		n	%	n	%	n	%	n	%	n	%	n	%
45,X and variants	n=58	1	0.2	0	-	6[b]	0.3	18[b]	31	33	56.9	0	-
Polysomy X/Y	n=68	0	-	0	-	32	47.1	18	26.5	18	26.5	0	-
Trisomy 21	n=339	0	-	5	1.5	82	24.2	78	23	173	51	1[a]	0.3
Trisomy 18	n=87	0	-	0	-	13[a]	14.9	7	8.1	66	75.9	1[a]	1.2
Trisomy 13	n=23	0	-	1	4.3	5	21.7	2	8.7	15	65.2	0	-
Uncommon trisomies	n=20	0	-	0	-	5	25	4	20	11	55	0	-
Polyploidies	n=17	0	-	0	-	1	5.9	1	5.9	15	88.2	0	-
Balanced structural	n=218	5	2.3	6	2.8	74	33.9	47	21.6	22	10.9	64	29.4
Unbalanced structural	n=62	0		0	-	15	24.2	13	21	24	38.7	10	16.1
Total	n=892	6	0.7	12	1.4	233	26.1	188	21.2	377	42.3	76	8.5

*including translocations and mosaics
[a]Trisomy was detected additional to the parental inherited balanced reciprocal translocation
[b]mosaic45,X/X structural anomalies

Informed consent was obtained from each patient.

RESULTS

The annual changes in the percentage of the main indications were figured out for 23 years (Figure 1a-1b) in CVS and AC series. The cases with accompanying risk factors (AMA, ST) in P-US indication were shown independently in these figures to demonstrate the impact of US on AMA and ST groups.

Out of 2492 CVS, 2459 could be karyotyped (success rate 98.7%) and of 23469 AC, 23395 (success rate 99.7%). A total of 25854 karyotypes were evaluated in this study. The maternal cell contamination (MCC) caused misdiagnosis in two cases of the earliest 500 LTCC of CV samples (0.8%).

A total of 1277 (385 in CVS and 892 in AC) chromosome aberrations were detected in this study. The overall rate of chromosome aberrations increased from 10.1% to 17.6% in CVS and from 3.2% to 4.3% in AC ($p<0.001$ in

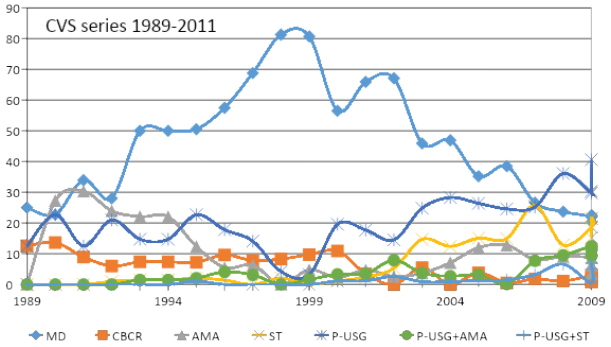

Figure 1a: Trends in CVS series during 23 years; annual proportion of the indications.

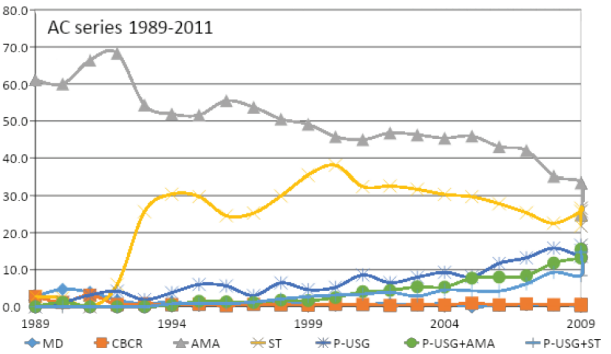

Figure 1b: Trends in AC series during 23 years; annual proportion of the indications.

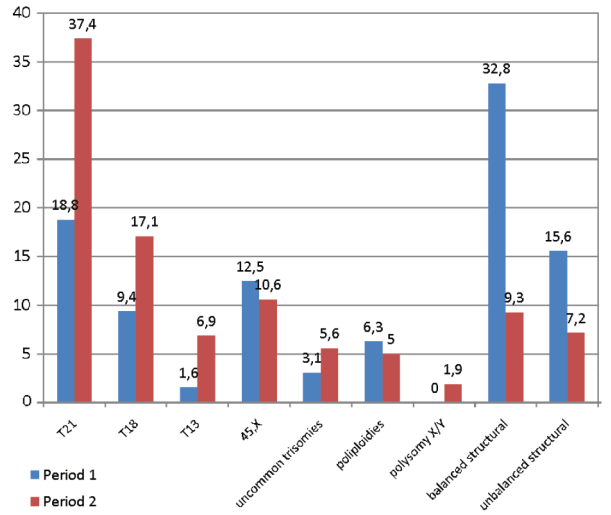

Figure 2a: The proportion of certain chromosome aberrations within the all aberrations compared between the periods of CVS series.

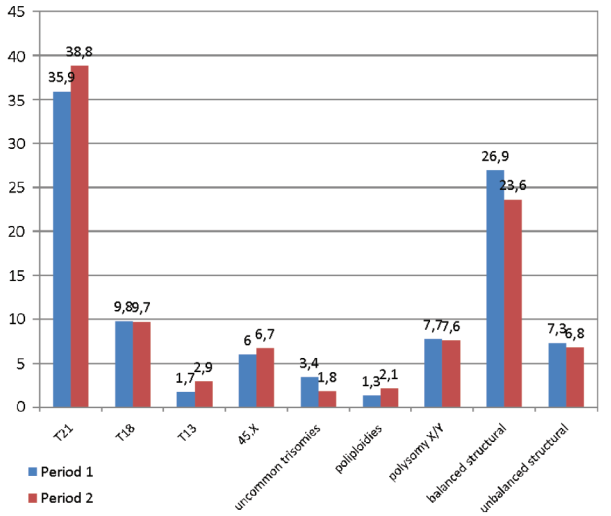

Figure 2b: The proportion of the certain chromosome aberrations within the all aberrations compared between the periods of AC series.

both series) from the first to the second period. This rate was higher in CVS versus AC in all indication groups. The highest abnormality rate except for the PBCR group was found in P-US, followed by ST and AMA in both series (Table 2a-2b).

When the proportion of certain aberrations within the all chromosome aberrations was compared between two periods, an increase of T21, T18, T13 and uncommon autosomal trisomies in CVS and of T21, T13 and 45,X in AC was observed in the second period (Figures 2a-2b). Most frequently detected aneuploidies were T21 and T18 in both series, followed by 45,X in CVS and by polysomy X/Y in AC (Figures 2a-2b).

Out of 51 balanced structural rearrangements detected in CVS series and of 218 detected in AC series, 68.6% and 29.4% of cases, respectively, were found in PBCR group (Table 3a-3b). Out of 26 unbalanced rearrangements in CVS and of 62 in AC, 57.7% and 16.1% of cases was diagnosed in PBCR group (Table 3a-3b). The remaining chromosomal rearrangements diagnosed due to the indications rather than PBCR indication provided the identification of 138 new families with balanced chromosomal rearrangements. The mode of inheritance has remained unknown in ten cases and *de novo* occurrence has been shown in 18 cases of CVS (0.7%) and 67 cases of AC (0.3%), which were mostly unbalanced rearrangements (15/18 in CVS and 39/67 in AC). Except for the PBCR group, the frequency of the balanced rearrangements were 0.6% in CVS and 0.7% in AC and of unbalanced rearrangements 0.5% in CVS and in 0.2% AC (overall frequencies were 1.1% in CVS and 0.9% in AC).

Discrepant karyotypes between DP and LTCC of CVS and AC were observed in 43 cases (1.7%). Four cases were

classified as "hidden true mosaicism" (0.16%), because the both techniques of CVS revealed a nonmosaic trisomy (three T16 and one T13), but AF-LTCC karyotypes were normal. Further I-FISH investigation in AF cells indicated mosaicism in two cases. Mosaicism was observed in 39 CVS (1.59%), of which 22 were "true mosaics" (0.9%) and three were "possible true mosaics" (0.12%). In 14 cases, DP revealed normal karyotype, while LTCC of CV a chromosome aberration, which were interpreted as "false negative DP-CPM" (0.57%). Involved anomalies were 45,X, T9, T16, T21, structural rearrangements (each twice), polyploidy, T7, T8, T10 (each once). In one case, DP and LTCC showed discrepant findings (false negative and false positive DP concurrently) and in another case presented with pathological US, the anomaly observed in DP (T22) could not be found in LTCC, which was interpreted as CPM with false negative LTCC. Three of the false positive-CPMs were additional to the nonmosaic trisomies (two T18 and one T21).

US findings (at least one minor or major anomaly) were present in about 67% of T21s, 90% of T18s, 85% of T13s, 86% of 45,X, 85% of polyploidies, 40% of uncommon trisomies in CVS, which were about 51%, 76%, 65%, 57%, 88%, 55% in AC series, respectively (derived from Tables 3a-3b).

Common aneuploidies including mosaics (T21 and T13 including translocations, T18, 45,X and polysomy X/Y)

Table 4a: The risk estimation for the common aneuploidies, for other unbalanced chromosomal aberrations associated with affected phenotype and for balanced rearrangements possibly associated with normal phenotype in CVS series in the main indications.

CVS Indication		Total chromosome aberrations		T21, 18, 13, 45,X and X/Y polysomies			Other chromosome aberrations								
							Uncommon trisomies, poliploidies, unbalanced rearrangements			Balanced rearrangements			Total risk		
	∑n	n	%	n	%	risk	n	%	risk	n	%	risk	∑n	%	risk
NIR	n=841	10	1.2	3	0.4	1:250	2	0.2	1:500	5	0.6	1:167	7	0.8	1:125
LR	n=55	4	7.3	2	3.6	1:28	1	1.8	1:56	1	1.8	1:56	2	3.6	1:28
AMA	n=335	15	4.5	12	3.6	1:28	1	0.3	1:333	2	0.6	1:167	3	0.9	1:111
ST	n=282	53	18.8	39	13.8	1:7	13	4.6	1:22	1	0.4	1:250	14	5	1:20
P-US	n=824	244	29.6	204	24.8	1:4	33	4	1:25	7	0.9	1:111	40	4.9	1:20
PBCR	n=101	59	58.4	8	7.9	1:13	16	15.8	1:6	35	34.7	1:3	51	50.5	1:2
Total	n=2453	385	15.7	260	10.6		50	2		16	0.7		66	2.7	

Table 4b: The risk estimation for the common aneuploidies, for other unbalanced chromosomal aberrations associated with affected phenotype and for balanced rearrangements possibly associated with normal phenotype in AC series in the main indications

AC Indication		Total chromosome aberrations		T21, 18, 13, 45,X and X/Y polysomies			Other chromosome aberrations								
							Uncommon trisomies, poliploidies, unbalanced rearrangements			Balanced rearrangements			Total risk		
	∑n	n	%	n	%	risk	n	%	risk	n	%	risk	∑n	%	risk
NIR	n=25	6	24	1	4	1:25	-	-	-	5	205	1:5	5	20	1:5
LR	n=86	12	14	6	7	1:14	-	-	-	6	7	1:14	6	7	1:14
AMA	n=10430	233	2.2	138	1.3	1:77	21	0.2	1:500	74	0.7	1:143	95	0.91	1:111
ST	n=6749	188	2.8	123	1.8	1:56	18	0.3	1:368	47	0.7	1:143	65	1	1:100
P-US	n=4442	377	8.5	305	6.9	1:15	50	1.1	1:91	22	0.5	1:188	72	1.6	1:63
PBCR	n=136	76	55.9	2	1.5	1:67	10	7.4	1:14	64	47.1	1:2	74	54.4	1:2
Total	n=23813	92	3.8	575	2.4		117	0.5		158	0.7		293	1.2	

summed up to 69.6% of all chromosome aberrations identified in CVS and 65.1% in AC series (derived from Figures 2a-2b). According to the indications, this percentage was about 30% in NIR, 50% in LR, 80% in AMA, 74% in ST and 84% in P-US in CVS, and 17%, 50%, 59%, 65% and 81% in AC, respectively (derived from Table 3a-3b). Remaining anomalies were grouped as; uncommon chromosome aberrations associated with abnormal phenotype and anomalies with possibly normal phenotype and the risks for these three groups of aberrations were calculated for the main indications (Table 4a-4b). The highest risk for common aneuploidies was in P-US (1:4 in CVS and 1:15 in AC) followed by ST and AMA indications in both series. The risk for uncommon chromosome aberrations associated with an abnormal phenotype, except PBCR indication was in ST indication of CVS (1:22) and in P-US (1:91) in AC series (Table 4a-4b).

DISCUSSION

Trends in indications and invasive procedures

The aim of the non-invasive STs is; 1) to assess more precisely risks of having a fetus with T21, T18 and T13, 2) to screen all pregnancies, including younger mothers, 3) to reduce the number of invasive procedures in normal pregnancies, 4) to diagnose more chromosome aberrations prenatally. Comprehensive studies from countries with well organized prenatal health care systems with national approved screening policies, demonstrated that the MS-ST and US screening changed the referral indications for IPD (14-16). All these reports pointed out to a clear decrease in AMA indication alone, but an increase in MS-ST and P-US indications and despite a decreased number of procedures, more chromosomal aberrations were diagnosed.

We retrospectively analyzed our data obtained from CVS and AC series, and compared the results according to two periods (see Material and Methods) to determine the impact of the MS-STs and fetal US examination on the IPD, and on the frequency and the type of the chromosome aberrations. Although there is no national screening policy in Turkey, our results demonstrate the tremendous impact of MS-STs and US on our cohort. Our policy was to offer CVS as an alternative to women having a risk of >5%. Since the high-risk families, like those at risk for MD and PBCR, were followed in our polyclinic and counseled about the facility of PD, they had the opportunity to choose CVS. The steady increase in the percentage of these indications in the 1990s could be explained by this policy. This percentage dropped back in the 2000s (Figure 1a), since relatively more CVS were performed due to the first trimester-ST and P-US indications. Parallel to the decrease of AMA, the increase of ST following the introduction of TT up until 1990 in AC (Figure 1b), and the introduction of first trimester-ST up until 2000 in CVS (Figure 1a) underlined the impact of STs on the families' decision on IPD. The increased percentage of cases having P-US findings (mostly soft markers) additional to AMA and/or ST indication demonstrated clearly the impact of US on IPD, even in PBCR indication (Figure 1a and 1b), since these families opted for AC in the 2000s especially, when US was normal. Pregnancies of the women with advanced age were monitored more closely and when first trimester-ST was positive, CVS; otherwise AC was a good choice for those women or IPD could be disapproved. Although ST became the most common indication for IPD in some countries, in our cohort, P-US in CVS and AMA in AC was the most common referral indication still in 2011 (14-16).

Cytogenetic results

As expected, the overall rate of chromosome aberrations in CVS was higher than in AC and also increased in the second period of both series (from 10.1% to 17.6% in CVS and from 3.2% to 4.3% in AC), which based on the facts that 1) first-trimester CVS can detect chromosome aberrations, which could not survive to the second-trimester of the pregnancy, 2) first-trimester US findings like cystic hygroma and other major malformations are closely related to severe/lethal chromosome aberrations, 3) the higher sensitivity of the first-trimester ST compared to TT, 4) through the wide usage of STs and US, pregnancies having higher risk than the others are selected for IPD, 5) FISH for the detection of microdeletions could be applied in presence of specific US findings, 6) presence of CPM, 7) CVS was offered and preferred by PBCR group having the highest risk for chromosome aberration. It is well known that some of the unbalanced products of the parental rearrangements can not survive to the second trimester of pregnancy. The ratio of balanced to unbalanced products in PBCR indication in CVS (35:21) versus in AC (64:11) observed in our study supported this explanation.

The unexpected high rates (1.2% in CVS and 1% in AC) of chromosome aberrations in the NIR group support the importance of fetal karyotyping in all prenatal samples, whatever the primary indication is for invasive procedure (Table 2a-2b). An interesting observation in the PBCR group was the high rate (1.69%) of trisomies (twice T18 and each once T21 and T22) of younger mothers (except one T18) and of paternal translocations. Three reports described the increased prevalence of T21 and T18 children, whose parents were translocation carriers, which was explained by the term of "interchromosomal effect" (ICE) (17, 18). Schinzel et al. (1992) showed in seven T21 cases of translocation carriers (two mat and five pat), that all extra chromosomes were maternally inherited, which was against the former hypothesis (19). Some reports in sperm cells of translocation carriers indicated the high frequency of disomy, however more studies are needed to clarify the effect of ICE on aneuploidies (20, 21).

Fetal US represents the most powerful and safe screening tool for chromosome aberrations. It is suggested that >95% of all major chromosomal anomalies can be diagnosed in the first trimester of pregnancy, when US screening and MS-STs are applied in combination (8). Aneuploidy prevalence ranges from 1% to over 80% with different US anomalies (22). As expected, P-US indication has the highest detection rate for chromosome aberration, except PBCR indication, which varies between 6-20% according to the selection criteria and used technique (overall frequency 29.6% in CVS and 8.5% in AC in this study). We observed that this frequency increased from the first to the second period (from 17.3% to 31.5%) in CVS, but slightly decreased in AC (from 10.3% to 8.2%). This result could be explained by the advanced experiences in US and our clinical strategy. Advanced experiences in US allowed us to detect more specific abnormalities (NT, nasal bone, etc.) associated with certain trisomies in the first trimester and also to offer CVS, which caused a selection of pregnancies having higher risk than the others for CVS in our cohort. US was more effective in the diagnosis of T18, T13 and 45,X, than for T21 and it is important to note that US can be normal in about 1/3 of the first and 1/2 of the second trimester of the pregnancies with T21 (derived from Table 3a-3b), which should be shared in the genetic counseling.

Higher rate of chromosome aberration in ST indication of CVS than AC and in the second period of both series than in the first period (Table 2a-2b) shows clearly, that the first trimester-ST is more sensitive than TT for chromosome aberrations, since CVS was most frequently performed procedure in pregnancies with positive first trimester-ST. STs are focused on T21 lesser, T18 and T13, but it seems to be sensitive for uncommon trisomies, 45,X and unbalanced rearrangements, too (Table 3a-3b).

The frequency of the chromosomal rearrangements increased from 0.26% to 0.58% by using a/the banding technique at a 400-500 band level. This frequency was 0.4% in the pioneer study and recent studies revealed a range between 0.53%-1.2% according to the patient selection criteria (including or excluding PBCR and P-US indication) (3, 23). The frequency of balanced rearrangements (0.6% in CVS; 0.7% in AC) was in agreement with the literature (0.35%; 0.82%), while the frequency of unbalanced rearrangements in CVS (0.5%) and in AC series (0.2%) was higher compared to the prevalence in newborns (0.2%) and previous prenatal reports (0.18%-0.38%) (23-25). The overall frequency of chromosomal rearrangements excluding PBCR group (1.1% in CVS and 0.9% in AC) and of *de novo* rearrangements in this study was also higher (0.7% in CVS and 0.3% in AC) than of the pioneer study (3), which could be explained by the including of the microdeletions diagnosed by FISH applied in the presence of specific US findings, CPMs and the lack of parental karyotyping in cases with poor obstetric history and IVF/ICSI pregnancies prior to PD in our cohort. During this study, 138 new families carrier of chromosomal rearrangements were disclosed following the detection of fetal chromosomal rearrangements, which allowed us to counsel more precisely for ongoing pregnancies and also to guide for the management of future pregnancies and to determine the consequences of other family members.

CV tissue is still embracing the problems of MCC and mosaicism. MCC poses a greater risk in LTCC than in DP. Removal of the decidual tissue from the CV sample is essential to overcome the MCC problem. Until sufficient experience has been gained, exclusion of the MCC by using chromosomal fluorescence polymorphisms is recommended (26). Chromosomal mosaicism is seen in 1-2% of CV samples (27, 28). Only about 10% of these mosaics are found in the fetus (29). Approximately 16 to 21% of pregnancies with CPM involving T2, T7, T8, T9, T16 and T22, is associated with IUGR, fetal loss, poor perinatal outcome, which has been explained by placental malfunction due to the high percentage of abnormal cells, hidden fetal mosaicism or uniparental disomy (UPD) (30-33). When a mosaic chromosomal anomaly is found in CVS, detailed US should be performed; when US is uneventful, AC should be offered to avoid the false positive diagnosis, especially in T16, T13, T22 cases and further cytogenetic investigations in fetal tissues using different techniques are strongly recommended before the pregnancy is terminated (34). Due to the non-adequate follow up, false positive CVS results can lead to termination of the normal fetuses, however false negatives can cause the birth of an affected child. The previous reports indicated that the false negative results can be reduced to 0.03-0.08%, when both DP and LTCC techniques are applied concurrently in CVS, otherwise it increases up to, when only DP is used (35). Our false negative findings (0.49%) were also limited to the DP. I-FISH application in uncultured AF cells was very effective to enlighten the low level mosaics in cases with normal karyotypes according to our experience. There are case reports describing discordant results with false negative QF-PCR in common trisomies. In a large study with 22 825 CVS, this discrepancy was 0.2% and explained by CPM (35). False negative and positive-CPM could be a serious potential source of error in NIPD using cf-DNA technique.

We reviewed our data, assuming that the hypothetical detection rate of cf-DNA test for common aneuploidies including mosaics 100%, to determine the risk for anomalies rather than common aneuploidies, which is important in pre-counselling for NIPD versus IPD. This risk was 1:111 in both series in AMA indication. With respect to the phenotypic effect of these anomalies; the risk for anomalies associated with abnormal phenotype was higher (1:333) in CVS than AC (1:500) (Table 5a-5b).

We agree with Collins and Impey emphasizing that "All screening and diagnostic methods have their own specific risks and benefits. It is vital that adequate time and importance is given to exploring the parents' understanding and expectations before any test is embarked on, no matter how 'safe' it is perceived to be" (36).

Our study, based on the results of the largest CVS and AC series in Turkey, shows how STs and US strongly influenced the clinical and cytogenetic results in IPD (37-39). We believe that abnormality rates detected in different indication groups in this study could be considered as the baseline risks in genetic counseling for certain indications in NIPD.

Acknowledgments: The authors are grateful to the cytogenetic team, who are working for more than 20 years together. The authors thank Prof. Dr. Peter Miny for critical scientific input on the manuscript.

Ethics Committee Approval: Ethics committee approval was not received due to the retrospective nature of the study.

Informed Consent: Written consent was obtained from the participants.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- S.B., B.K., M.K., İ.H.K., R.H., T.D., A.Y., G.T., A.Y.; Data Acquisition- S.B., B.K., M.K., İ.H.K., R.H., T.D., A.Y., G.T., A.Y.; Data Analysis/Interpretation- S.B., B.K., M.K., İ.H.K., R.H., T.D., A.Y., G.T., A.Y.; Drafting Manuscript- S.B., B.K., M.K., İ.H.K., R.H., T.D., A.Y., G.T., A.Y.; Critical Revision of Manuscript- S.B., B.K., M.K., İ.H.K., R.H., T.D., A.Y., G.T., A.Y.; Final Approval and Accountability- S.B., B.K., M.K., İ.H.K., R.H., T.D., A.Y., G.T., A.Y.; Technical or Material Support- S.B., B.K., M.K., İ.H.K., R.H., T.D., A.Y., G.T., A.Y.; Supervision- S.B., B.K., M.K., İ.H.K., R.H., T.D., A.Y., G.T., A.Y

Conflict of Interest: Authors declared no conflict of interest.

Financial Disclosure: Authors declared no financial support.

Teşekkür: Prof. Dr. Peter Miny'e makaleye bilimsel katkılarından dolayı ve 20 yıldan daha uzun bir süredir birlikte çalıştığımız sitogenetik ekibine teşekkür ederiz.

Etik Komite Onayı: Retrospektif çalışma olduğundan etik komite onayı alınmamıştır.

Bilgilendirilmiş Onam: Katılımcılardan bilgilendirilmiş onam alınmıştır.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Çalışma Konsepti/Tasarım-S.B., B.K., M.K., İ.H.K., R.H., T.D., A.Y., G.T., A.Y.; Veri Toplama- S.B., B.K., M.K., İ.H.K., R.H., T.D., A.Y., G.T., A.Y.; Veri Analizi/Yorumlama- S.B., B.K., M.K., İ.H.K., R.H., T.D., A.Y., G.T., A.Y.; Yazı Taslağı- S.B., B.K., M.K., İ.H.K., R.H., T.D., A.Y., G.T., A.Y.; İçeriğin Eleştirel İncelemesi- S.B., B.K., M.K., İ.H.K., R.H., T.D., A.Y., G.T., A.Y.; Son Onay ve Sorumluluk- S.B., B.K., M.K., İ.H.K., R.H., T.D., A.Y., G.T., A.Y.; Malzeme ve Teknik Destek- S.B., B.K., M.K., İ.H.K., R.H., T.D., A.Y., G.T., A.Y.; Süpervizyon- S.B., B.K., M.K., İ.H.K., R.H., T.D., A.Y., G.T., A.Y

Çıkar Çatışması: Yazarlar çıkar çatışması beyan etmemişlerdir.

Finansal Destek: Yazarlar finansal destek beyan etmemişlerdir.

REFERENCES

1. Adams MM, Erickson JD, Layde PM, Oakley GP. Down syndrome. Recent trends in the United States. JAMA 1981;246(7):758-760. [CrossRef]
2. Hook EB, Cross PK, Regal RR. The frequency of 47.+21, 47.+18, and 47,+13 at the uppermost extremes of maternal ages: results on 56,094 fetuses studied prenatally and comparisons with data on livebirths. Hum Genet 1984;28(3):211-20. [CrossRef]
3. Ferguson-Smith MA, Yates JRW. Maternal age specific rates for chromosomal aberrations factors influencing them: report of a collaborative European study on 52,965 amniocenteses. Prenat Diagn 1984;4(7):5-44. [CrossRef]
4. Cuckle HS, Wald NJ, Thompson SG. Estimating a woman's risk of having a pregnancy associated with Down's syndrome using her age and serum alpha-fetoprotein level. Br J Obstet Gynaecol 1987;94(5):387-402. [CrossRef]
5. Snijders RJM, Nicolaides KH. Sequential screening. In: Nicolaides KH, editor. Ultrasound markers for fetal chromosomal defects. Carnforth, UK: Parthenon; 1996.
6. Wald NJ, Hackshaw AK. Combining ultrasound and biochemistry in first-trimester screening for Down's syndrome. Prenat Diagn 1997;17(9):821-9. [CrossRef]
7. Spencer K, Macri JN, Aitken DA, Connor JM. Free beta hCG as first trimester marker for fetal trisomy. Lancet 1992;339(8807):1480. [CrossRef]
8. Nicolaides KH. Nuchal translucency and other first-trimester sonographic markers of chromosomal abnormalities. Am J Obstet Gynecol 2004;191(1):45-67. [CrossRef]
9. Lo DYM, Corbetta N, Chamberlain P, Rai V, Sargent IL, Redman CWG, Wainscoat JS. Presence of fetal DNA in maternal plasma and serum. Lancet 1997;350(9076):485-7. [CrossRef]
10. Wright CF, Chitty LS. Cell-free fetal DNA and RNA in maternal blood: implications for safer antenatal testing. BMJ 2009;339:b2451. [CrossRef]
11. Benn P, Cuckle H and Pergament E. Non-invasive prenatal diagnosis for Down syndrome: the paradigm will shift, but slowly. Ultrasound Obstet Gynecol2012;39(2):127-30. [CrossRef]
12. TUIK. www.turkstat.gov.tr Republic of Turkey Statistical Institute. Number:1325783/2015, Documents number: 7330775/7339623, Chapter 4.1, page 14.
13. Ekelund CK, Jørgensen FS, Petersen OB, et al. Danish Fetal Medicine Research Group. Impact of a new national screening policy for Down's sy ndrome in Denmark: population based cohort study. BMJ 2008;337:a2547. [CrossRef]
14. Lichtenbelt KD, Alizadeh BZ, Scheffer PG, et al. Trends in the utilization of invasive prenatal diagnosis in The Netherlands during 2000-2009. Prenat Diagn 2011;31(8):765-72. [CrossRef]

15. Mademont-Soler I, Morales C, Clusellas N, et al. Prenatal cytogenetic diagnosis in Spain: analysis and evaluation of the results obtained from amniotic fluid samples during the last decade. Eur J Obstet Gynecol Reprod Biol 2011;157(2):156-60. [CrossRef]
16. Morris JK, Waters JJ, de Souza E. The population impact of screening for Down syndrome: audit of 19 326 invasive diagnostic tests in England and Wales in 2008. Prenat Diagn 2012;32(6):596-601. [CrossRef]
17. Lindenbaum RH, Hulten M, McDermott A, Seabright M. The prevalence of translocations in parents of children with regular trisomy 21: A possible interchromosomal effect? J Med Genet 1985;22(1):24-8. [CrossRef]
18. Couzin DA, Watt JL, Stephen GS. Structural rearrangements in the parents of children with primary trisomy 21. J Med Genet 1987;24(5):280-2. [CrossRef]
19. Schinzel AA, Adelsberger PA, Binkert F, et al. No evidence for a paternal interchromosomal effect from analysis of the origin of nondisjunction in Down syndrome patients with concomitant familial chromosome rearrangements. Am J Hum Genet 1992;50(2):288-93.
20. Estop AM, Cieply K, Munne S, et al. Is there an interchromosomal effect in reciprocal translocation carriers? Sperm FISH studies. Hum Genet 2000;106(5):517-24. Erratum in: Hum Genet 2000;107(1):95. [CrossRef]
21. Anton E, Vidal F, Blanco J. Interchromosomal effect analyses by sperm FISH: incidence and distribution among reorganization carriers. Syst Biol Reprod Med 2011;57(6):268-78. [CrossRef]
22. Daniel A, Athayde N, Ogle R, George AM, et al. Prospective ranking of the sonographic markers for aneuploidy: Data of 2143 prenatal cytogenetic diagnoses referred for abnormalities on ultrasound. Aust N Z J Obstet Gynaecol 2003;43(1):16-26. [CrossRef]
23. Forabosco A, Percesepe A, Santucci S. Incidence of non-age-dependent chromosomal abnormalities: a population-based study on 88965 amniocenteses. Eur J Hum Genet 2009;17(7):897-903. [CrossRef]
24. Han SH, An JW, Jeong GY, Yoon HR, et al. Clinical and cytogenetic findings on 31,615 mid-trimester amniocenteses. Korean J Lab Med 2008;28(5):378-85. [CrossRef]
25. Jacobs PA, Browne C, Gregson N, Joyce C, White H. Estimates of the frequency of chromosome abnormalities detectable in unselected newborns using moderate levels of banding. J Med Genet 1992;29(2):103-8. [CrossRef]
26. Miny P, Holzgreve W, Başaran S, et al. Maternal cell contamination in chorionic villi cultures - exclusion by chromosomal fluorescence polymorphisms. Clin Genet 1985;28(3):262-3. [CrossRef]
27. Simoni G, Gimelli G, Cuoco C, et al. Discordance between prenatal cytogenetic diagnosis after chorionic vilus sampling and chromosomal constitution of the fetus. In: First trimester fetal diagnosis, Fraccaro M, Simoni G, Brambati B (ed.). Berlin: Springer Verlag. 1985;137. [CrossRef]
28. Hahnemann JM, Vejerslev LO. Accuracy of cytogenetic findings on chorion villus sampling (CVS)-Diagnostic consequences of CVS mosaicism and non-mosaic discrepancy in centres contributing to EUCROMIC 1986-1992. Prenat Diagn 1997;17(9):801-20. [CrossRef]
29. Phillips OP, Tharapel AT, Lerner JL, et al. Risk of fetal mosaicism when placental mosaicism is diagnosed by chorionic villus sampling. Am J Obstet Gynecol 1996;174(3):850-5. [CrossRef]
30. Johnson J, Wapner RJ, Davies GH, et al. Mosaicism in chorionic villus sampling: an association with poor perinatal outcome. Gynecology 1990;75(4):573.
31. Breed ASPM, Mantingh A, Vosters R, et al. Follow-up and pregnancy outcome after a diagnosis of mosaicism in CVS. Prenat Diagn 1991;11(8):577. [CrossRef]
32. Kalousek DK, Howard-Peebles PN, Olson SB, et al. Confirmation of CVS mosaicism in term placentae and high frequency of intrauterine growth retardation association with confined placental mosaicism. Prenat Diagn 1991;11(10):743-50. [CrossRef]
33. Wolstenholme J, Rooney DE, Davison EV. Confined placental mosaicism, IUGR, and adverse pregnancy outcome: a controlled retrospective UK collaborative survey. Prenat Diagn 1994;14(5):345-61. [CrossRef]
34. Miny P, Başaran S, Holzgreve W, Horst J, et al. False negative cytogenetic results in direct preparation after CVS. Prenat Diagn 1988;8(8):633. [CrossRef]
35. Holgado E, Liddle S, Ballard T, Levett L. Incidence of placental mosaicism leading to discrepant results between QF-PCR and karyotyping in 22,825 chorionic villus samples. Prenat Diagn 2011;31(11):1029-38. [CrossRef]
36. Collins SL, Impey L. Prenatal diagnosis: Types and techniques. Early Hum Dev 2012;88(1):3-8. [CrossRef]
37. Karaoguz MY, Bal F, Yakut T, Ercelen NO, Ergun MA, et al. Cytogenetic results of amniocentesis materials: incidence of abnormal karyotypes in the Turkish collaborative study. Genet Couns 2006;17(2):219-230.
38. Gunduz C, Cogulu O, Cankaya T, Bora E, et al. Trends in cytogenetic prenatal diagnosis in a reference hospital in Izmir/Turkey: a comparative study for four years. Genet Couns 2004;15(1):53-9.
39. Ilgin-Ruhi H, Yürür-Kutlay N, Tükün A, Bökesoy I. The role of genetic counseling on decisions of pregnant women aged 35 years or over regarding amniocentesis in Turkey. Eur J Med Genet 2005;48(1):13-19. [CrossRef]

İSTANBUL ÜNİVERSİTESİ YAYINEVİ

RESEARCH / ARAŞTIRMA
DOI: 10.26650/IUITFD.2019.0102
İst Tıp Fak Derg 2020 / J Ist Faculty Med 2020

COMPARISON OF MATERNAL B12 AND FOLATE STATUS IN PRENATALLY DIAGNOSED NEURAL TUBE DEFECTS: A CASE-CONTROL STUDY

PRENATAL FETAL NÖRAL TÜP DEFEKTİ TANISI KONULAN ANNELERDE SERUM B12 VE FOLAT DÜZEYLERİNİN KARŞILAŞTIRILMASI: VAKA-KONTROL ÇALIŞMASI

Gürcan TÜRKYILMAZ[1], Gökçe Naz KÜÇÜKBAŞ[2], Emircan ERTÜRK[1], Şebnem TÜRKYILMAZ[1], Onur KARAASLAN[2], Hanım Güler ŞAHİN[2]

[1]Van Education and Research Hospital, Department of Obstetrics and Gynecology, Van, Turkey
[2]Yüzüncü Yıl University, Department of Obstetrics and Gynecology, Van, Turkey

ORCID IDs of the authors: G.T. 0000-0002-5514-0233; G.N.K. 0000-0002-2755-3700; E.E. 0000-0003-0169-6903; Ş.T. 0000-0002-3984-5663; O.K. 0000-0002-4599-1173; H.G.Ş. 0000-0002-8596-0734

Cite this article as: Turkyilmaz G, Kucukbas GN, Erturk E, Turkyilmaz S, Karaaslan O, Sahin HG. Comparison of maternal B12 and folate status in prenatally diagnosed neural tube defects: A case-control study. J Ist Faculty Med 2020;83(4):325-9. doi: 10.26650/IUITFD.2019.0102

ABSTRACT

Objective: To evaluate folate and B12 levels in fetuses who had been diagnosed with neural tube defects (NTDs) and healthy fetuses in Van Yüzüncü Yıl University and Van State Education and Research hospitals between March-August 2019.

Material and Method: Thirty-eight pregnant women who had been diagnosed with fetuses with NTDs prenatally, and 40 healthy controls were recruited. The chi-square test and Mann-Whitney U test were employed to compare variables.

Results: None of the women had taken folic acid preconceptionally in the NTD group. However, 4 (10%) women had taken folic acid supplementation in the preconception period in the control group, and this was significantly different among the groups (p=0.04). The women who had taken folic acid in the first trimester of pregnancy were 9 (23.6%) and 32 (80%) in cases and controls, respectively, and it was significantly different (p=0.01). The mean B12 level was 248.7±65.4ng/ml in cases and 239.3±27.5 ng/ml in controls, and there was no significance between the groups (p=0.78). The mean folate level was 9.6±4.8 ng/ml in cases and 9.8±3.9 ng/ml in controls, and it was similar between the groups (p=0.62).

Conclusion: We did not show difference in folate and B12 levels. However, folic acid intake in preconception or in the first trimester of pregnancy was significantly higher in women who have healthy babies compared to the NTD group.

Keywords: Folate, neural tube defect, prenatal, supplementation, vitamin B12

ÖZET

Amaç: Van Yüzüncü Yıl Üniversitesi ve Van Eğitim ve Araştırma Hastanesi'nde NTD tanısı koyulan ve sağlıklı fetüsü olan gebelerin B12 ve folat düzeyini karşılaştırdık.

Gereç ve Yöntem: NTD tanısı koyulan 38 hasta ve 40 kontrol grubu çalışmaya dahil edildi. İstatistiksel analizde ki-kare testi ve Mann-Whitney U testi kullanıldı.

Bulgular: NTD grubunda hiçbir hastada prekonsepsiyonel dönemde folik asit kullanımı yoktu ancak kontrol grubunda 4 (%10) hastada prekonsepsiyonel folik asit kullanımı mevcuttu ve bu fark istatistiksel olarak anlamlıydı (p=0,04). NTD grubunda 9 (%23.6), kontrol grubunda 32 (%80) hastada ilk trimesterde folik asit desteği alınmıştı ve bu istatistiksel olarak anlamlıydı (p=0,01). Ortalama B12 seviyesi NTD grubunda 248.7±65.4ng/ml, kontrol grubunda 239.3±27.5 saptandı ve bu fark anlamlı değildi (p=0,78). Ortalama folat seviyesi NTD grubunda 9.6±4.8 ng/ml, kontrol grubunda 9.8±3.9 ng/ml saptandı ve bu fark anlamlı değildi (p=0,62).

Sonuç: Her iki grup arasında B12 ve folat seviyeleri farklı değildi ancak, sağlıklı bebeği olan grupta prekonsepsiyonel dönemde ve ilk trimesterde folik asit kullanımı NTD grubuna göre anlamlı olarak daha fazlaydı.

Anahtar Kelimeler: Folat, nöral tüp defekti, prenatal, vitamin B12

Corresponding author/İletişim kurulacak yazar: gurcanturkyilmaz@gmail.com

Submitted/Başvuru: 18.12.2019 • **Revision Requested/Revizyon Talebi:** 20.03.2020 •
Last Revision Received/Son Revizyon: 25.03.2020 • **Accepted/Kabul:** 09.05.2020 • **Published Online/Online Yayın:** 18.09.2020

INTRODUCTION

Neural tube defects (NTDs) are a spectrum of abnormalities that arise as a result of nonclosure of the neural tube until six weeks of gestation (1). Numerous types of NTDs are defined, including acrania, anencephaly, iniencephaly, craniorachischisis, encephalocele, and meningomyelocele. They are the most common central nervous system anomalies, and the incidence varies from 5/1000 to 0.5/1000 (2). Etiology of NTDs is considered multifactorial involving genetic predisposition and environmental factors such as nutritional deficiencies and drug exposure (3). Most NTDs occur sporadically, and in most affected pregnancies, there are no recognizable risk factors.

Epidemiologic studies more than 30 years ego showed a correlation between maternal folate status and NTDs (4). The administration of folic acid before and during the first weeks of gestation can reduce the incidence of NTDs by roughly 70% but can not prevent it completely; other factors must be involved (5). Furthermore, some studies suggest that nutrients other than folate, such as vitamin B12, may also be essential to neural tube closure and have a potential role in risk reduction (6, 7). Vitamin 12 is a fundamental cofactor for two enzymes in DNA synthesis, including folate-dependent methionine synthesis and folate independent methyl malonyl-CoA mutase (8). Similar to folic acid, vitamin B12 deficiency also predisposes to NTDs, and vitamin B12 intake with folic acid may reduce NTDs risk.

Although numerous studies have established the role of folic acid and vitamin B12 associated with NTDs risk, regarding the high prevalence of NTDs and the lack of reviews about the role of maternal folate and vitamin B12 levels in NTDs in the Eastern region of the country, we conducted this study to evaluate the association of maternal folate and vitamin B12 levels with NTDs.

MATERIAL AND METHOD

This prospective case-control study was conducted between March-August 2019 in Van Education and Research hospital and Van Yüzüncü Yıl University hospital. Those two hospitals are referral centers around the eastern region of Turkey. The NTD group consisted of 38 women who had been diagnosed with fetuses with NTDs prenatally. The control group consisted of 40 pregnant women who did not have a history of NTDs in previous pregnancies and who did not have fetuses with NTDs in the present pregnancy; also, maternal serum alfa fetoprotein (msAFP) levels were in the normal range between 16-20 weeks of gestations. Maternal clinic and demographic features such as gestational weeks at diagnosis, education status, folic acid intake in the preconception period, and folic acid intake in the first trimester were recorded.

Each woman was evaluated with regular ultrasound assessments, which were performed transabdominally using an abdominal 2-5 Mhz curvilinear transducer (Voluson, General Electric, Milwaukee, WI, USA). Fetal NTDs were diagnosed with a detailed ultrasonographic evaluation with high (msAFP) levels. MsAFP levels were measured after ultrasonographic examination in our clinics. Postnatal or postmortem confirmation of NTDs cases were verified.

Serum was separated from blood collected without anticoagulants for vitamin B12 and folate analysis. Radioimmunoassay using gamma counter (Genesis-USA) was used for serum folate and vitamin B12 measurements. Serum folate and vitamin B12 less than five ng/ml and 160 pg/ml were considered as the cut-off values, respectively.

Statistical analyses were performed using SPSS 16.0. The chi-square test and Mann-Whitney U test were employed to compare variables. Statistical significance was established at $p<0.05$.

RESULTS

The mean age of pregnant women was 24.4±4.8 and 25.6±4.7 in the NTDs group, and control group, respectively (p=0.86). BMI was 25.5±4.4 kg/m^2 in cases, and 24.6±3.9 kg/m^2 in control group and this difference was not significant. The mean gestational weeks at diagnosis

Table 1: The comparison of demographic and clinical features between NTD mothers and controls.

	Cases (n=38)	Controls (n=40)	p-value
Age	24.4±4.8	25.6±4.7	0.86
Gravity	3.2±1.5	2.5±1	0.69
BMI	25.5±4.4	24.6±3.9	0.73
Gestational weeks at diagnosis	18.4±4.2	21.2±3.3	0.61
Education status	Illiterate: 11 (28.9%) Primary school: 23 (60.5%) High school: 3 (7.8%) University: 1 (2.6%)	Illiterate: 10 (25%) Primary school: 24 (60%) High school: 3 (7.5%) University: 3 (7.5%)	
Folic acid intake in preconceptional period	0 (0%)	4 (10%)	0.04
Folic acid intake in first trimester	9 (23.6%)	32 (80%)	0.01

were 18.4±4.2 weeks, and the mean gestational weeks at routine ultrasonographic evaluation in controls was 21.2±3.3 (p=0.61) and there was no significant difference. Anencephaly in 10 (26.3%), encephalocele in 4 (10%), and meningomyelocele in 24 (63.1%) cases composed the NTDs group. 78.9% of cases and 75% of controls were illiterate or primary school graduated. None of the women had taken folic acid preconceptionally in cases. However, 4 (10%) women had taken folic acid supplementation in the preconception period in the control group, and this was significantly different among groups (p=0.04). Women who took folic acid in the first trimester of pregnancy were 9 (23.6%) and 32 (80%) in cases and controls, respectively, and it was significantly different (p=0.01). The mean B12 level was 248.7±65.4ng/ml in cases and 239.3±27.5 ng/ml in controls, and there was no significance between groups (p=0.78). The mean folate level was 9.6±4.8 ng/ml in cases and 9.8±3.9 ng/ml in controls, and it was similar between cases and controls (p=0.62). The comparison of demographic and clinical features between NTD mothers and controls is demonstrated in Table 1. The values of folate and vitamin B12 levels and p-values between NTDs mothers and controls are shown in Table 2.

Table 2: The comparison of demographic and clinical features between NTD mothers and controls.

	Case	Control	p
Folate (ng/ml)	9.6±4.8	9.8±3.9	0.62
B12 (pg/ml)	248.7±65.4	239.3±27.5	0.78

DISCUSSION

NTDs are multifactorial disorders with many genetic and environmental factors defined in the etiology. The development of the neural tube is a multi-step process which is controlled by numerous genes and modulated by environmental factors.

Folate cycles between molecules in specific biologic reactions carry one-carbon groups from other molecules to homocysteine to form methionine. This folate cycle is a vital biochemical reaction required for proper DNA synthesis, repair, and methylation. Thus, low folate levels can directly limit its availability to cells or indirectly disrupt methionine metabolism, thereby increasing homocysteine.

Correlation of serum folate concentrations and NTDs risk has been investigated widely in the literature, and results are controversial. Cech et al. showed that serum folate levels were significantly lower in the NTDs group than controls in their study, which involved 107 pregnant women with NTDs and 275 controls (9). Zhang et al. who compared 82 pregnancies with fetal NTDs and 110 controls, identified lower serum levels of folate and vitamin B12 in the NTDs group (10).

On the contrary, several other researchers showed no relationship. Mobasheri et al. reported that levels of folate and vitamin B12 did not significantly affect NTDs risk in their study (11). Aydın et al. evaluated serum folate and vitamin B12 levels in 35 pregnant women with NTDs fetuses and 38 controls. They revealed no relation between either vitamin B12 and folate levels and NTDs. (12). In our study, we did not show lower serum folate levels among the NTDs group.

The starting of folic acid intake before conception is fundamental to reduction of NTDs risk and folate given before and during the first four weeks of gestation can prevent more than half of NTDs. (13). Unfortunately, many pregnancies are unplanned, and women often do not know that they are pregnant until the crucial first 4 to 8 weeks of pregnancy. This is the time during which neural tube development occurs, hence the importance of ensuring adequate folic acid intake before pregnancy. Thus, in many countries such as the USA, food fortification programs have been launched to prevent neural NTDs without vitamin supplementation (14). In our county, there is no food fortification program, and folic acid supplementation in the appropriate period is crucial to reduce NTDs risk. In our study, folic acid intake was significantly higher in controls either in the preconception period or in the first trimester of pregnancy. This data shows that folic acid intake in the proper period of pregnancy may reduce NTDs risk; even serum folate levels were not significantly different among groups.

Since the etiology of NTDs is multifactorial, folic acid supplementation alone is not sufficient to entirely prevent NTDs. Vitamin B12 is a fundamental cofactor for two enzymes in DNA synthesis and may influence NTDs risk in pregnancy. Ray et al. showed significantly low maternal serum B12 levels in pregnancies with NTDs in their population-based study (2). Kirke et al. evaluated 81 pregnant women with NTDs and 247 controls and showed significantly lower vitamin B12 levels among NTDs cases compared with the controls (15). Molloy et al. compared vitamin B12 status in women who had NTDs in current pregnancy and who had NTDs affected child in a previous pregnancy with healthy controls. They found that inadequate vitamin B12 levels were associated with a significantly increased risk for NTDs. Furthermore, they suggested women keep optimum vitamin B12 levels above 300 ng/L (16).

Contrastingly, Ceyhan et al. assessed serum folate and vitamin B12 levels in 31 pregnant women with NTDs fetuses and 32 controls. They revealed no relation between either vitamin B12 and folate levels and NTDs (17). We did not show a significant difference in vitamin B12 levels between the two groups in our study.

Methionine synthase is a vitamin B12 dependent enzyme, and in the absence of vitamin B12 or folate, homocyste-

ine accumulates in the serum. Thus, increased serum homocysteine levels may be an indicator of folate or vitamin B12 deficiency. Mills et al. showed that pregnant women who had fetuses with NTDs have significantly higher homocysteine levels than controls in their case-control study (18). Yang et al. showed that mothers with NTDs offspring demonstrated dramatically higher mean plasma homocysteine levels than mothers with healthy offspring in their meta-analysis (19). Unfortunately, we did not measure serum homocysteine levels in our study due to technical paucity.

Recently, among folate-metabolism related genes, *MTHRF* has been the principal focus of attention. Previous studies have shown that the c.677C>T and c.1298A>C variations are associated with an increased risk of NTDs (20, 21). Also, Liu et al. revealed that MTHFR c.677C>T variation was significantly higher in the tissue or blood samples of NTDs fetuses compared to controls (22).

CONCLUSION

Our data indicate that either maternal folate and vitamin B12 levels are not significantly different among women who have NTDs fetuses compared to controls. Conversely, women who had taken folic acid in the preconception period or first trimester of pregnancy have a significantly lower risk of NTDs offspring. A dietary supplement of folate in the preconception and first weeks of gestation is crucial to prevent NTDs.

Ethics Committee Approval: This study was approved by Van Education and Research Hospital Ethics Committee (20011467).

Informed Consent: Written consent was obtained from the participants.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- G.T.; Data Acquisition- G.T., G.N.K., O.K., H.G.Ş.; Data Analysis/Interpretation- E.E., Ş.T.; Drafting Manuscript- G.T., E.E., Ş.T.; Critical Revision of Manuscript- H.G.Ş., O.K., G.N.K.; Final Approval and Accountability- G.T., G.N.K., E.E., Ş.T., O.K., H.G.Ş.; Technical or Material Support- E.E.; Supervision- H.G.Ş., G.T.

Conflict of Interest: Authors declared no conflict of interest.

Financial Disclosure: Authors declared no financial support.

Etik Komite Onayı: Bu çalışma için etik kurul onayı Van Eğitim ve Araştırma Hastanesi Etik Kurulu'ndan alınmıştır (20011467).

Bilgilendirilmiş Onam: Katılımcılardan bilgilendirilmiş onam alınmıştır.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Çalışma Konsepti/Tasarım- G.T.; Veri Toplama- G.T., G.N.K., O.K., H.G.Ş.; Veri Analizi/Yorumlama- E.E., Ş.T.; Yazı Taslağı- G.T., E.E., Ş.T.; İçeriğin Eleştirel İncelemesi- H.G.Ş., O.K., G.N.K.; Son Onay ve Sorumluluk- G.T., G.N.K., E.E., Ş.T., O.K., H.G.Ş.; Malzeme ve Teknik Destek- E.E.; Süpervizyon- H.G.Ş., G.T.

Çıkar Çatışması: Yazarlar çıkar çatışması beyan etmemişlerdir.

Finansal Destek: Yazarlar finansal destek beyan etmemişlerdir.

REFERENCES

1. Botto LD, Moore CA, Khoury MJ, Erickson JD. Neural-tube defects. N Engl J Med 1999;341(20):1509-19. [CrossRef]
2. Ray JG, Wyatt PR, Thompson MD, Vermeulen MJ, Meier C, Cole DE, et al. Vitamin B12 and the risk of neural tube defects in a folic acid fortfied population. Epidemiology 2007;18(3):362-6. [CrossRef]
3. Greene ND, Stainer P, Copp AJ. Genetics of human neural tube defects. Hum Mol Genet 2009;18 (R2):R113-R129. [CrossRef]
4. Czeizel AE, Dudas I. Prevention of the first occurance of neural-tube defects by periconceptional vitamin supplementation. N Engl J Med 1992;327(26):1832-5. [CrossRef]
5. Au KS, Ashley-Koch A, Northrup H. Epidemiologic and genetic aspects of spina bifida and other neural tube defects. Dev Disabil Res Rev 2010;16(1):6-15. [CrossRef]
6. Van Loon K, Besseghir K, Eshkol A. Neural tube defects after infertility treatment: a review. Fertil Steril 1992;58(5):875-84. [CrossRef]
7. Wald NJ, Hackshaw AD, Stone R. Blood folic acid and vitamin B12 in relation to neural tube defects: a case control study. Biol Neonate 1995;67(3):154-9. [CrossRef]
8. Weir DG, Scott JM. Cobalamins: Physiology, dietary sources and requirements. In: Sadler MJ, Strain JJ, Caballero B. eds. Encylopedia of Human Nutrition. San Diego, CA, Academic Press 1999:394-401.
9. Cech I, Burau KD. Serological differences in folate/vitamin B12 in pregnancies affected by neural tube defects. South Med J 2010;103(5):419-24. [CrossRef]
10. Zhang T, Xin R, Gu X, Wang F, Pei L, Zheng Z, et al. Maternal serum vitamin B12, folate and homocysteine and the risk of neural tube defects in the offspirng in a high risk area of China. Public Health Nutr 2009;12(5):680-6. [CrossRef]
11. Mobasheri E, Keshtkar A, Golalipour MJ. Maternal folate and vitamin B12 status and neural tube defects in northern Iran: a case control study. Iran J Pediatr 2010;20(2):167-73.
12. Aydın H, Arısoy R, Karaman A, Erdogdu E, Cetinkaya A, Demirci O, et al. Evaluation of maternal serum folate, vitamin B12, and homocysteine levels and factor V Leiden, factor 2 g.20212G>A, and MTHRF variations in prenatally diagnosed neural tube defects. Turk J Med Sci 2016;46(2):489-94. [CrossRef]
13. MRC Vitamin Study Research Group. Prevention of neural tube defects: results of the Medical Research Council vitamin study. Lancet 1991;338(8760):131-7. [CrossRef]
14. Tinker SC, Cogswell ME, Devine O, Berry RJ. Folic acid intake among U.S. women aged 15-44 years, National Health and Nutrition Examination Survey, 2003-2006. Am J Prev Med 2010;38(5):534-42. [CrossRef]

15. Kirke PN, Molloy AM, Daly LE, Burke H, Weir DG, Scott JM. Maternal plasma folate and vitamin B12 are independent risk factors for neural tube defects. QJM 1993;86(11):703-8.
16. Molloy AM, Kirke PN, Troandle JF, Burke H, Sutton M, Mills JL, et al. Maternal vitamin B12 status and risk of neural tube defects in a population with high neural tube defects prevalance and no folic acid fortification. Pediatrics 2009;123(3):917-23. [CrossRef]
17. Ceyhan ST, Beyan C, Atay V, Yaman H, Alanbay I, Başer I, et al. Serum vitamin B12 and homocysteine levels in pregnant women with neural tube defects. Gynecol Endocrinol 2010;26(8):578-81. [CrossRef]
18. Mills JL, Scott JM, Kirke PN, McPartlin JM, Conley MR, Weir DG, Molloy AM, Lee YJ. Homocysteine and neural tube defects. J Nutr 1996;126(3):756-60.
19. Yang M, Li W, Wan Z, Du Y. Elevated homocysteine levels in mothers with neural tube defects: a systematic review and meta-analysis. J Matern Fetal Neonatal Med 2017;30(17):2051-57. [CrossRef]
20. Van der Put NM, Steegers-eunissen RPM, Frosst P, Trijbels FJ, Eskes TK, et al. Mutated methylenetetrahydrofolate reductase as a risk factor for spina bifida. Lancet 1995;346(8982):1070-1. [CrossRef]
21. Shields DC, Kirke PN, Mills JL, Ramsbottom D, Molloy AM, Whitehead AS. The 'thermolabile' variant of methylenetetrahydrofolate reductase and neural tube defects: an evaluation of genetic risk and the relative importance of the genotypes of the embryo and the mother. Am J Hum Genet 1999;64(4):1045-55. [CrossRef]
22. Liu J, Qi J, Yu X, Zhu J, Zhang L, Luo X, et al. Investigations of single nucleotide polymorphisms in folate pathway genes in Chinese families with neural tube defect. J Neurol Sci 2014;337(1-2):61-6. [CrossRef]

İSTANBUL ÜNİVERSİTESİ YAYINEVİ

RESEARCH / ARAŞTIRMA
DOI: 10.26650/IUITFD.2020.0024
İst Tıp Fak Derg 2020 / J Ist Faculty Med 2020

DIAGNOSIS, MANAGEMENT AND CLINICOPATHOLOGICAL FEATURES OF ACUTE APPENDICITIS IN PREGNANT WOMEN AND ITS IMPACT ON FETAL OUTCOMES

GEBE KADINLARDA GÖRÜLEN AKUT APANDİSİTİN TANI, TEDAVİ VE KLİNİKOPATOLOJİK ÖZELLİKLERİ VE FETAL SONUÇLAR ÜZERİNE ETKİSİ

Rahman ŞENOCAK[1], Süleyman Utku ÇELİK[1], Şahin KAYMAK[1]

[1]University of Health Sciences, Gulhane Training and Research Hospital, Clinic of General Surgery, Ankara, Turkey

ORCID IDs of the authors: R.Ş. 0000-0002-9476-1817; S.U.Ç. 0000-0002-1570-6327; Ş.K. 0000-0003-4717-5791

Cite this article as: Senocak R, Celik SU, Kaymak S. Diagnosis, management and clinicopathological features of acute appendicitis in pregnant women and its impact on fetal outcomes. J Ist Faculty Med 2020;83(4):330-8. doi: 10.26650/IUITFD.2020.0024

ABSTRACT

Objective: To evaluate the clinicopathological features and feto-maternal outcomes of appendicitis during pregnancy.

Material and Method: This study involved comparisons of laboratory findings, preoperative ultrasonography (US), pathology and clinical outcomes of 17 pregnant and 59 age-matched non-pregnant women undergoing appendectomy.

Results: The total number of US scans, rates of non-visualized appendix on US, and length of hospital stay were higher in pregnant women than in non-pregnant subjects (p<0.001, p=0.035, and p=0.014; respectively). The rate of negative appendectomy was 1.5-times higher and the rate of complicated appendicitis was 7-times higher in pregnant compared with non-pregnant patients. The diagnostic accuracy of US was higher in the non-pregnant group (72.9% vs. 64.7%). In terms of *the hematological* parameters, no significant difference was found between the pregnant patients with and without appendicitis. There was one premature birth and one abortus in the second trimester, and one premature birth followed by a negative appendectomy in the third trimester.

Conclusion: The diagnosis of acute appendicitis in pregnancy may remain inconclusive despite comprehensive evaluation with clinical examination, laboratory studies, and US. We recommend that clinicians consider additional imaging scans when they suspect appendicitis during pregnancy to avoid unnecessary surgical interventions. Both complicated appendicitis and negative appendectomy can cause a non-negligible rate of fetal morbidity and mortality.

Keywords: Acute appendicitis, morbidity, pregnancy

ÖZET

Amaç: Çalışmanın amacı gebe kadınlarda görülen apandisitin tanı ve tedavi stratejileri ile klinikopatolojik özellikleri ve feto-maternal sonuçlarını değerlendirmektir.

Gereç ve Yöntem: Bu çalışmada apendektomi yapılan 17 gebe kadın ve yaşları eşleştirilmiş 59 gebe olmayan kadın laboratuvar bulguları, preoperatif ultrasonografi (USG), patoloji ve klinik sonuçlar açısından karşılaştırıldı.

Bulgular: Toplam USG tarama sayısı, görüntülenemeyen apandis oranları ve hastanede kalış süreleri gebe kadınlarda gebe olmayan kadınlara göre daha fazla idi (sırasıyla p<0,001, p=0,035 ve p=0,014). Gebe grupta negatif apendektomi oranı gebe olmayanlara göre 1,5 kat, komplike apandisit oranı ise 7 kat daha yüksekti. USG'nin tanısal doğruluğu ise gebe olmayan grupta daha yüksek bulundu (%72,9'a karşı %64,7). Hematolojik parametreler açısından apandisit olan ve olmayan gebe hastalar arasında anlamlı bir fark bulunmadı. İkinci trimesterde bir erken doğum ve bir abortus görülürken, üçüncü trimesterde bir hastada negatif apendektomiyi takiben bir erken doğum gerçekleşti.

Sonuç: Gebelik sırasında laboratuvar parametreleri ve USG ile konulan apandisit tanısı hatalı olabilmektedir. Bu yüzden, gereksiz cerrahi müdahalelerden kaçınmak için, klinisyenlerin hamilelik sırasında apandisitten şüphelendikleri durumlarda ek görüntüleme tetkikleri yapmayı düşünmelerini öneririz. Çünkü hem negatif apendektomi hem de komplike apandisit, ihmal edilemez bir fetal morbidite ve mortalite oranıyla sonuçlanabilir.

Anahtar Kelimeler: Akut apandisit, gebelik, morbidite

Corresponding author/İletişim kurulacak yazar: rahmansenocak@yahoo.com

Submitted/Başvuru: 20.03.2020 • **Revision Requested/Revizyon Talebi:** 31.03.2020 •
Last Revision Received/Son Revizyon: 17.04.2020 • **Accepted/Kabul:** 21.04.2020 • **Published Online/Online Yayın:** 02.10.2020

INTRODUCTION

During pregnancy, acute appendicitis is the most common condition occurring at a frequency of one in 500–1000 births, and it necessitates nonobstetric emergency surgery (1). It is usually difficult to diagnose acute appendicitis during pregnancy because non-specific symptoms such as nausea, vomiting, loss of appetite, and abdominal pain are often the case in appendicitis as well as pregnancy, and the classic signs of appendicitis can also be masked by physiologic leukocytosis, altered anatomical position of the appendix and increased abdominal wall laxity, especially during late pregnancy (2, 3). Also, due to the fact that diagnosing appendicitis in women is difficult, up to 50% of patients are misdiagnosed in the preoperative period (2, 4, 5).

Complicated appendicitis rates occurring during pregnancy may vary between 14.9% and 43% (6). Because non-complicated appendicitis may progress rapidly into perforation, and complicated appendicitis may be associated with premature birth, fetal loss, maternal and fetal morbidity, it is imperative to diagnose appendicitis in pregnant women correctly and quickly (7). Reduced efficiency of abdominal ultrasonography (US) due to anatomic reasons, avoidance of computed tomography (CT) typically due to sensitivity to radiation, and difficulties in obtaining magnetic resonance imaging (MRI) often contribute to delays in diagnosis (8-10). Traditionally, early surgery is performed to avoid complications such as potential perforations. However, it has been reported that this approach could result in a negative appendectomy in 11% to 50% of cases. While negative appendectomy is acceptable to a certain extent, it is also unclear whether negative appendectomy or complicated appendicitis may be associated with unfavorable fetal and pregnancy outcomes (11, 12).

It remains unclear what the optimal clinical and surgical approach to acute appendicitis during pregnancy is. Also unclear is whether or not negative appendectomy or complicated appendicitis may be associated with unfavorable fetal and pregnancy outcomes. In this study, the aim was to compare appendicitis in pregnant women to that in women of similar age, to determine its diagnostic, treatment and clinicopathological differences, to determine the accuracy of US, and to investigate the effect of appendicitis on maternal and fetal adverse outcomes.

MATERIAL AND METHOD

The Ethics Committee of Non-Interventional Clinical Research at the University of Health Sciences found no ethical issues in carrying out the present study as this study did not involve any prospective analysis of a new method but only research showing standard clinical practices or advancement of practices. This study was designed as a retrospective study. The participants were recruited from among 466 female patients who underwent appendectomy between July 2016 and December 2019. The demographic characteristics and preoperative and postoperative findings of the pregnant women undergoing appendectomy were compared with those of their non-pregnant counterparts. The patients were matched by age at a ratio of 1:3 to eliminate age bias.

Initially, pregnant women with acute abdominal pain were investigated in a gynecology and obstetrics unit. If the origin of the pain was thought to be due to a non-obstetrical reason, the patients were identified for further investigation and a complete transabdominal US with sectorial and linear probes was performed. Before surgery, an obstetrician assessed the women to determine gestational age and to monitor fetal vitality. US was repeated both after the surgical procedure and before the patient's discharge.

Patient demographics, preoperative laboratory parameters, imaging results, details of the surgical intervention, pathological findings, and length of hospital stay were analyzed. Final pathologic diagnoses of appendix were grouped into the following categories: normal appendix, non-complicated appendicitis, and complicated appendicitis. The final diagnosis was accepted as a negative appendectomy if the resected appendix showed no histologically proven inflammation. Maternal and fetal outcomes were finalized by examining patient records and then conducting phone interviews. Fetal loss was defined as any loss after 20 weeks of gestation. Preterm delivery was defined as a birth before 37-weeks completed gestation.

All statistical analyses were performed using Statistical Package for the Social Sciences version 16.0 for Windows (IBM®, Chicago, USA). Descriptive statistics were reported as means ± standard deviation in normally distributed numeric variables, as medians (minimum-maximum) in non-normally distributed data, and as frequency in categorical variables. The Shapiro-Wilk test was used to ensure the normality of the data. The Student's *t* test or Mann-Whitney *U* test were used to analyze continuous variables, where appropriate. Kruskal-Wallis test was used for comparisons of median values among more than two groups. Comparisons of categorical variables were compared using chi-square or Fisher's exact test. Propensity score analysis was used to make pregnant patients and non-pregnant patients homogeneous in terms of age and sex (1:3 ratio). The diagnostic performance of US for predicting acute appendicitis in the pregnant and non-pregnant women was also analyzed. All tests were two-sided, and a p value < 0.05 was considered statistically significant.

RESULTS

The baseline characteristics of 17 pregnant patients undergoing appendectomy were compared with 59 age-

matched non-pregnant controls also undergoing appendectomy between July 2016 and December 2019. A total of 4,511 pregnant women were monitored in the same hospital's obstetrics department during this time. Therefore, 0.38% of the pregnant women were calculated to have appendicitis.

The demographic and perioperative data of the patients are presented in Table 1 and Table 2. In the pregnant group, the median number of US scans done for diagnosis was significantly higher than in the non-pregnant group (p<0.001). There was a higher rate of non-visualized appendix vermiformis on US in the pregnant patients compared with that in the non-pregnant patients (41.2% vs. 16.9%, p=0.035). However, the median maximal diameter of the appendix was 8.5 mm (range, 6–14 mm) in the pregnant women and 9 mm (range, 5–17) in the non-pregnant women (p=0.976). While CT or MRI was not used as a secondary diagnostic imaging modality in the pregnant women, CT was performed on 28.8% of the non-pregnant patients.

No statistically significant difference was observed between the groups in terms of preoperative laboratory findings as well as the interval from admission to surgery. In 41.2% of the pregnant patients, the anesthesia method was spinal anesthesia, whereas this rate was 1.7% in the non-pregnant patients (p<0.001). There was no significant difference regarding surgical methods, surgical drain use, and the need for broad-spectrum antibiotics (p=0.103, p=0.388, and p=0.151, respectively). Preventive tocolytic therapy was administered in 35.3% of the pregnant patients. Length of hospital stay was statistically longer in pregnant patients (2 days vs. 1 day, p=0.014). In terms of pathological findings, the negative appendectomy rate was found to be 1.5 times higher (29.4% vs. 16.9%), and the complicated appendicitis rate was 7 times higher (23.5% vs. 3.4%) in the pregnant group than in the non-pregnant patients, respectively (p<0.001).

The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy of US for predicting appendicitis are presented in Table 3. When preoperative laboratory parameters and US findings were compared between the pregnant patients with and without appendicitis, no significant difference was found in laboratory findings (Table 4). However, median appendix diameter was significantly higher in the patients with acute appendicitis than those with negative appendectomy (p=0.044).

Table 5 shows the perioperative and fetomaternal out-

Table 1: Comparison of demographic characteristics of the pregnant and non-pregnant women.

	Pregnant women n=17	**Non-pregnant women n=59**	***p***
Age (year), median	27 (range, 19-40)	28 (range, 18-41)	0.713
Gestational age (week), median	17 (range, 6-36)		N/A
First trimester	7 (41.2)		
Second trimester	6 (35.3)		
Third trimester	4 (23.5)		
Preoperative ultrasound findings			
Total number of ultrasound scans, median	2 (range, 1-5)	1 (range, 0-2)	**<0.001**
Probable acute appendicitis, n (%)	10 (58.8)	49 (83.1)	**0.035**
Non-visualized/normal, n (%)	7 (41.2)	10 (16.9)	**0.035**
Appendix diameter, median	8.5 (range, 6-14)	9 (range, 5-17)	0.976
Definitive diagnosis with CT, n (%)	0	17 (28.8)	N/A
Preoperative laboratory parameters			
Leucocyte (x10^9/L), mean±SD	13.9±3.1	13.3±4.8	0.569
Neutrophil (x10^9/L), mean±SD	11.4±3.2	10.5±4.8	0.490
Lymphocyte (x10^9/L), mean±SD	1.9±0.7	1.9±0.8	0.587
NLR, mean±SD	7.2±4.1	8.5±9.4	0.572
PLR, mean±SD	153.5±86.5	180.6±104.5	0.331
C-reactive protein (mg/L), mean±SD	26.5±48.7	47.0±60.6	0.215

N/A; not applicable, CT; computed tomography, NLR; neutrophil-to-lymphocyte ratio, PLR; platelet-to-lymphocyte

Table 2: Comparison of perioperative findings and clinicopathological features of the pregnant and non-pregnant women.

	Pregnant women n=17	Non-pregnant women n=59	*p*
Interval between hospital admission and surgery (hour), median	10 (range, 2-45)	8 (range, 1.5-35)	0.428
Anesthesia procedure, n (%)			**<0.001**
Spinal	7 (41.2)	1 (1.7)	
General	10 (58.8)	58 (98.3)	
Surgical procedure, n (%)			0.103
Open	16 (94.1)	45 (76.3)	
Laparoscopic	1 (5.9)	14 (23.7)	
Surgical drain placement, n (%)	1 (5.9)	8 (13.6)	0.388
Broad spectrum antibiotic use, n (%)	4 (23.5)	6 (10.2)	0.151
Preventive tocolysis, n (%)	6 (35.3)		N/A
Hospital stay (day), median	2 (range, 1-18)	1 (range, 1-8)	**0.014**
Pathological findings, n (%)			**<0.001**
Normal	5 (29.4)	10 (16.9)	
Non-complicated appendicitis	8 (47.1)	47 (79.7)	
Complicated appendicitis	4 (23.5)	2 (3.4)	

N/A; not applicable

Table 3: Sensitivity, specificity, PPV, NPV, and diagnostic accuracy of sonography for predicting appendicitis in pregnant and non-pregnant women.

	Sensitivity (%95 CI)	Specificity (%95 CI)	PPV (%95 CI)	NPV (%95 CI)	Diagnostic accuracy (%95 CI)	Diagnostic odds ratio (%95 CI)
Pregnant	66.7 (34.9-80.0)	60.0 (14.7-94.7)	80.0 (56.0-92.6)	42.9 (20.4-68.7)	64.7 (38.3-85.8)	3.00 (0.23-45.23)
Non-pregnant	83.7 (70.3-92.7)	20.0 (2.5-55.6)	83.7 (78.6-87.8)	20.0 (5.85-50.15)	72.9 (59.8-83.6)	1.28 (0.15-8.72)

PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval

Table 4: The preoperative ultrasound findings and laboratory parameters of the pregnant women with acute appendicitis and those with a normal appendix.

	Pathological results		
	Normal n=5	Appendicitis n=12	*p*
Preoperative ultrasound findings			
Total number of ultrasound scans, median	3 (range, 1-5)	1.5 (range, 1-5)	0.130
Appendix diameter, median	6.3 (range, 5.5-7)	8.9 (range, 7.3-14)	**0.044**
Preoperative laboratory parameters			
Leucocyte (x10^9/L), mean±SD	15.4±1.1	13.3±3.4	0.264
Neutrophil (x10^9/L), mean±SD	12.8±2.1	10.8±3.4	0.237
Lymphocyte (x10^9/L), mean±SD	2.1±0.7	1.8±0.7	0.475
NLR, mean±SD	7.3±4.6	7.1±4.1	0.928
PLR, mean±SD	144.9±111.5	157.1±79.5	0.801
C-reactive protein (mg/L), mean±SD	11.1±11.3	32.5±56.9	0.478

NLR; neutrophil-to-lymphocyte ratio, PLR; platelet-to-lymphocyte

Table 5: Comparison of the preoperative and postoperative features of the pregnant women in terms of gestational age.

	First trimester n=7	Second trimester n=6	Third trimester n=4	*P*
Age (year), median	25 (range, 20-39)	25.5 (range, 19-40)	30 (27-35)	0.489
Interval between hospital admission and surgery (hour), median	12 (3-45)	7.5 (3-29)	5.5 (2-18)	0.186
Preoperative ultrasound findings				
Total number of ultrasound scans, median	2 (range, 1-5)	1.5 (range, 1-5)	1.5 (range, 1-3)	0.739
Probable acute appendicitis, n (%)	6 (85.7)	3 (50.0)	1 (25.0)	0.124
Non-visualized/normal, n (%)	1 (14.3)	3 (50.0)	3 (75.0)	0.124
Appendix diameter, median	7.9 (range, 5.5-11.5)	11 (range, 8.5-14)	8 (range, 8-8)	0.273
Preoperative laboratory parameters				
Leucocyte (x10^9/L), median	13.8 (range, 6.9-16.4)	13.2 (range, 9.7-19.9)	15.8 (range, 13.3-16.4)	0.555
Neutrophil (x10^9/L), median	9.6 (range, 4.7-15.1)	11.3 (range, 7.1-18.1)	12.7 (range, 11.2-14.1)	0.238
Lymphocyte (x10^9/L), median	1.8 (range, 1.0-3.2)	1.6 (range, 1.0-2.0)	2.4 (range, 0.9-2.6)	0.453
NLR, median	3.7 (range, 2.9-15.2)	7.8 (range, 3.6-14.6)	5.7 (range, 4.7-12.4)	0.472
PLR, median	111 (range, 70-339)	166 (range, 112-297)	110 (range, 61-315)	0.273
C-reactive protein (mg/L), median	5.9 (range, 4.5-12)	16.9 (range, 4.9-192)	17.1 (range, 5.3-32.5)	0.258
Surgical procedure, n (%)				
Open	6 (85.7)	6 (100)	4 (100)	0.468
Laparoscopic	1 (14.3)	0	0	
Hospital stay (day), median	2 (range, 1-18)	2 (range, 1-3)	2.5 (range, 1-5)	0.796
Preventive tocolysis, n (%)	3 (42.9)	1 (16.7)	2 (50.0)	0.481
Pathological findings, n (%)				**0.033**
Normal	3 (42.9)	0	2 (50.0)*	
Non-complicated appendicitis	4 (57.1)	2 (33.3)	2 (50.0)	
Complicated appendicitis	0	4 (66.7)†	0	
Pregnancy process				0.427
Planned birth	7 (100)	4 (66.6)	3 (75.0)	
Preterm birth	0	1 (16.7)	1 (25.0)	
Fetal loss	0	1 (16.7)	0	

NLR; neutrophil-to-lymphocyte ratio, PLR; platelet-to-lymphocyte, †One fetal loss and one preterm birth, *One preterm birth

comes according to gestational age. There was no significant difference between trimesters in terms of age, interval from admission to surgery, total US scans, appendix diameter, laboratory parameters, surgical procedures, hospital stays, and preventive tocolysis. While the rate of negative appendectomy and noncomplicated appendicitis was higher in the first and last trimesters, the rate of complicated appendicitis was higher in the second trimester (p=0.033). There was no premature birth in women on whom appendectomy had been performed in the first trimester; however, there was one premature birth and one abortus (both had complicated appendicitis) in the second trimester, and one premature birth followed by negative appendectomy in the third trimester (p=0.427).

DISCUSSION

Given the unreliability of the clinical signs of appendicitis in pregnancy, an aggressive surgical approach has typically been recommended to prevent perforation, which leads to increased risk of adverse outcomes (5, 10, 13). However, in recent literature, negative appendectomy

rates have been reported to have risen when this strategy was introduced, resulting in fetal loss and early delivery (5, 12, 14). The need for early surgical management in case of appendiceal perforation and the need for enhancing preoperative diagnostic accuracy with additional imaging modality to avoid negative appendectomy have to be balanced. Currently, advances in the accuracy of diagnostic imaging methods and the efficacy of antibiotic treatment have made the traditional approach controversial. In the present study, we analyzed all pregnant women, who underwent appendectomy for acute appendicitis to demonstrate clinicopathologically different features of appendicitis in pregnancy and any potential adverse fetomaternal outcomes.

Appendicitis is the most prevalent cause of non-traumatic emergencies of the digestive tract necessitating surgical treatment in pregnant women, with a prevalence of 0.1-0.2% (6, 15). In this study, the appendicitis incidence during pregnancy was 1 in 265 births (0.38%) during the period between 2016 and 2019. In a study regarding acute appendicitis depending on trimesters, Zingone et al. (16) found the rates of acute appendicitis to be nearly same in the first and second trimesters (7.4 and 7.3 per 10,000 person-years) and to be lower in the last trimester (4.6 per 10,000 person-years). Some recent studies have reported that the incidence of appendicitis was higher during the second trimester of pregnancy than during the first or third trimesters (14, 17). In our study, appendicitis cases were observed most frequently in the first trimester (41.2%) and least frequently in the third trimester (23.5%).

It has been reported that the preoperative diagnosis of appendicitis can be difficult during pregnancy. Anatomical and physiological changes in pregnancy may make the diagnosis more uncertain and delay the treatment of appendicitis (3, 5, 10, 14, 18). While nausea and vomiting occur frequently during pregnancy, rebound tenderness and guarding are not often seen in pregnant women with appendicitis due to the shift of the appendix upward and laterally as the uterus grows, which results in diminished response to peritoneal irritation (9, 19). It was reported in previous literature that only less than half of patients with pathologically proven appendicitis had a classic history of abdominal pain (6, 9). Thus, the clinical diagnosis of appendicitis may pose a challenge for the physician.

Recently, in a large cohort study of pregnant women treated for appendicitis, Segev et al. (20) reported no significant difference between pregnant and non-pregnant women in terms of the time interval between the first symptoms and the visit to the emergency room, and from the emergency admission to surgical intervention; however an earlier study by Hiersch et al. (13) showed a significantly shorter period in pregnant patients from hospital admission to surgery. In our study, the time interval between admission to emergency department and surgery was similar among the groups. However, in accordance with the previous studies (13, 20), it was observed that the median hospital stay was significantly longer for the pregnant women (2 days vs. 1 day). Additionally, in the pregnant group, the median number of US scans for diagnosis was higher than in the non-pregnant group. These findings suggest that the traditional aggressive approach was not preferred in appendicitis in pregnancy and that the patients were more closely monitored for potential complications in the postoperative period.

It has been shown that leukocytosis occurs due to an increased number of neutrophils in blood circulation during pregnancy and that neutrophils begin to increase in the second trimester, then remain constant with the total number ranging between 9 and 15000 (15, 17, 20). The differential diagnosis of appendicitis in pregnancy includes several inflammatory conditions, all of which are characterized by leukocytosis. It was suggested that the inflammatory markers seemed to have a less diagnostic value in pregnant women with suspected appendicitis compared to their healthy pregnant counterparts. Similarly, we did not find a significant difference between the pregnant women with appendicitis versus the non-pregnant women with appendicitis with regard to leucocyte and lymphocyte counts nor with regard to the neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte, and CRP values on admission (13, 15). Although not statistically significant, the mean CRP level was found to be 3 times higher in the pregnant women with appendicitis than in the negative appendectomy group (11.1 mg/L vs. 32.5 mg/L, p=0.478). As reported in another study, the contributions of inflammation markers alone are limited, and thus their use must only be to contribute to diagnosis together with physical examination and imaging tests (18).

US is the main imaging modality for the diagnosis of acute appendicitis in both pregnant and nonpregnant patients (6, 14). Since ionizing radiation during pregnancy has some potential negative impacts on the fetus, we avoid using CT in pregnant women and women of reproductive age. In the current study, US was used preoperatively in all women to be sure of an accurate diagnosis of appendicitis and to avoid unnecessary surgery. It has been reported that US yields a high rate of non-visualization of the appendix during pregnancy in up to 75% of cases (9, 15) and a low sensitivity in 36%–71% of cases (3, 6, 13). These results can be explained by the fact that the use of US is limited due to its high operator dependence. We observed that the non-visualization of appendix on US was higher in the pregnant than in non-pregnant patients (41.2% vs. 16.9%; p=0.035). Previous studies showed that the rate of negative appendectomy in pregnant women reduced from 54% to 36% when US was used and to 8% when US was followed by CT (20, 21). The different rates of negative appendecto-

my among the studies can be explained by the differences in the rates of imaging assessment before the surgical intervention. Our negative appendectomy rate was 29.4% for the pregnant women and 16.9% for the non-pregnant women according to the pathological results. These rates are consistent with the 13%–50% range reported for negative appendectomy during pregnancy (22, 23) and the 15%–35% range for non-pregnant patients with appendicitis (5). This difference was due to the use of CT in 28.8% of the non-pregnant patients. When initial US findings are indeterminate, advanced imaging methods with improved preoperative diagnostic accuracy are required to decrease the number of negative appendectomies.

Currently, the main surgical technique in the management of acute appendicitis during pregnancy is yet to be established. The preferred surgical method is based upon the trimester of pregnancy and the surgeon's own preference (3, 9, 13, 24). Our findings indicated that the laparoscopic management of appendicitis was more frequently used in the non-pregnant women than in the pregnant women. Whereas both open and laparoscopic procedures were performed in the first trimester, only open appendectomies were performed in the second and third trimesters, showing that a majority of the pregnant women were subject to open appendectomy. However, a number of studies have indicated that there might be an increased risk of preterm delivery or fetal loss in those undergoing laparoscopic appendicectomy compared with those undergoing open appendicectomy (24, 25). Whether the choice of incision should be transverse or oblique or at or above McBurney's point in pregnant women is also a controversial issue (18, 26). Our study showed that oblique incisions at McBurney's point could be performed successfully in all pregnant patients. Moreover, in our study, the anesthesia method was primarily regional anesthesia in 41.2% of the pregnant patients, whereas this was only 1.7% in the non-pregnant patients. There is no conclusive evidence demonstrating superior safety for regional anesthesia. General anesthesia is still frequently required. Not surprisingly, the length of hospital stay was statistically longer in pregnant women who underwent appendectomy than among the non-pregnant women, which may be associated with the examination of pregnant women with additional imaging techniques, and thus, lengthened hospital stays. We also did not find any differences in terms of perioperative variables such as surgical procedures, drain use, and use of broad-spectrum antibiotics between the pregnant women with appendicitis and the non-pregnant women.

Different results have been reported in terms of whether complicated appendicitis in pregnancy occurs at a greater or similar rate than in non-pregnant patients (5, 17, 20). In our study, the rate of complicated appendicitis was 7 times higher in the pregnant compared to the non-pregnant patients. By contrast, non-complicated appendicitis was approximately 1.5 times less common. There was no statistically significant difference in the interval from admission to surgery. However, this lack of difference may also be attributed to the fact that a third of the pregnant patients were operated on after more than 24 hours, which therefore, resulted in a delay in diagnosis. While negative appendectomy rates were higher in the first and third trimesters, the rate of complicated appendicitis was higher in the second trimester. Tocolytics are indicated to preserve pregnancy if premature labor is the case. Although its efficacy during non-obstetric surgeries is debatable, prophylactic tocolytics may be considered during the third trimester in lower abdominal or pelvic surgeries with inflammatory conditions. In this study, a preventive tocolytic agent was administered especially in the last trimester.

In a study by Mcgory et al. (5), fetal loss rate was reported to be 6% in patients undergoing appendectomy for complicated appendicitis, 2% in patients with noncomplicated appendicitis and 4% in patients with a normal appendix. Other studies have also reported the rate of preterm delivery in pregnant women with acute appendicitis up to 11.4% (21). In the present study, the majority of the pregnant women (82.3%) gave birth to a full-term baby. While there was no premature delivery in any of the appendectomies performed in the first trimester despite the high negative appendectomy rate (42.9%), two premature deliveries and one fetal loss occurred in the second and third trimesters, respectively. One of the premature deliveries occurred following an appendectomy on a 36-week pregnant patient, and the other at the 32nd gestational week after surgery at the 17th week of another pregnant patient. The fetus was lost during the 24th gestational week following an appendectomy on a 21-week pregnant patient. In conclusion, it was agreed that one patient had a preterm birth (during the third trimester) and another had a fetal loss (during the first trimester) due to appendectomy. Based on these results, it is hard to determine whether complicated appendicitis or negative appendectomy has a fetal impact. However, it can be assumed that any interventions during late pregnancy are risky.

This study is subject to a number of limitations. Firstly, there are limitations inherent in any retrospective analysis. For instance, the variations in the timing of antibiotic treatment might have changed pathological progression, and the sample size may undermine the statistical interpretation of outcomes with relation to trimesters. Another limitation is that different radiologists evaluated the findings of the patients. Although they were all experienced radiologists, there may be some variations in the evaluations. The major strength of this study was to stratify the pregnant women with acute appendicitis according to gestation age, which had an impact on clinical outcomes and patient management.

In conclusion, we found some statistically significant differences in length of hospital stay, total numbers of US scans, types of anesthesia procedures, and the rates of complicated appendicitis and negative appendectomy between the pregnant and non-pregnant women. Accurate diagnosis of acute appendicitis in pregnancy remains uncertain based upon laboratory parameters and US scans. We recommend that clinicians consider additional imaging modalities when they suspect appendicitis in pregnancy to avoid unnecessary surgical interventions. Both a complicated appendicitis and negative appendectomy during pregnancy can cause a non-negligible rate of fetal morbidity and mortality.

Ethics Committee Approval: The research was conducted according to the principles of the World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects"

Informed Consent: Informed consent was not received due to the retrospective nature of the study.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- R.Ş.; Data Acquisition- R.Ş., S.U.Ç., Ş.K.; Data Analysis/ Interpretation- R.Ş., S.U.Ç.; Drafting Manuscript- R.Ş., S.U.Ç., Ş.K.; Critical Revision of Manuscript- R.Ş., S.U.Ç.; Final Approval and Accountability- R.Ş., S.U.Ç., Ş.K.; Technical or Material Support- R.Ş.; Supervision- R.Ş.

Conflict of Interest: Authors declared no conflict of interest.

Financial Disclosure: Authors declared no financial support.

Etik Komite Onayı: Araştırma Dünya Tabipler Birliği Helsinki Bildirgesi'nin "insanlar üzerinde yapılan tıbbi araştırmalarla ilgili etik ilkeler" prensiplerine göre yürütülmüştür.

Bilgilendirilmiş Onam: Retrospektif bir çalışma olduğundan bilgilendirilmiş onam alınmamıştır.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar katkıları: Çalışma Konsepti/Tasarım- R.Ş.; Veri Toplama- R.Ş., S.U.Ç., Ş.K.; Veri Analizi/Yorumlama- R.Ş., S.U.Ç.; Yazı Taslağı- R.Ş., S.U.Ç., Ş.K.; İçeriğin Eleştirel İncelemesi- R.Ş., S.U.Ç.; Son Onay ve Sorumluluk- R.Ş., S.U.Ç., Ş.K.; Malzeme ve Teknik Destek- R.Ş.; Süpervizyon- R.Ş.

Çıkar Çatışması: Yazarlar çıkar çatışması beyan etmemişlerdir.

Finansal Destek: Yazarlar finansal destek beyan etmemişlerdir.

REFERENCES

1. Abbasi N, Patenaude V, Abenhaim HA. Management and outcomes of acute appendicitis in pregnancy-population-based study of over 7000 cases. BJOG 2014;121(12):1509-14. [CrossRef]
2. Andersen B, Nielsen TF. Appendicitis in pregnancy: diagnosis, management and complications. Acta Obstet Gynecol Scand 1999;78 (9):758-62. [CrossRef]
3. Aras A, Karaman E, Peksen C, Kiziltan R, Kotan MC. The diagnosis of acute appendicitis in pregnant versus non-pregnant women: A comparative study. Rev Assoc Med Bras 2016;62(7):622-7. [CrossRef]
4. Hee P, Viktrup L. The diagnosis of appendicitis during pregnancy and maternal and fetal outcome after appendectomy. Int J Gynaecol Obstet 1999;65(2):129-35. [CrossRef]
5. McGory ML, Zingmond DS, Tillou A, Hiatt JR, Ko CY, Cryer HM. Negative appendectomy in pregnant women is associated with a substantial risk of fetal loss. J Am Coll Surg 2007;205(4):534-40. [CrossRef]
6. Pedrosa I, Lafornara M, Pandharipande PV, Goldsmith JD, Rofsky NM. Pregnant patients suspected of having acute appendicitis: effect of MR imaging on negative laparotomy rate and appendiceal perforation rate. Radiology 2009;250(3):749-57. [CrossRef]
7. Ueberrueck T, Koch A, Meyer L, Hinkel M, Gastinger I. Ninety-four appendectomies for suspected acute appendicitis during pregnancy. World J Surg 2004;28(5):508-11. [CrossRef]
8. Woodfield CA, Lazarus E, Chen KC, Mayo-Smith WW. Abdominal pain in pregnancy: diagnoses and imaging unique to pregnancy--review. Am J Roentgenol 2010;194(6 Suppl):WS14-30. [CrossRef]
9. Aggenbach L, Zeeman GG, Cantineau AE, Gordijn SJ, Hofker HS. Impact of appendicitis during pregnancy: no delay in accurate diagnosis and treatment. Int J Surg 2015;15:84-9. [CrossRef]
10. Ibiebele I, Schnitzler M, Nippita T, Ford JB. Appendicectomy during pregnancy and the risk of preterm birth: A population data linkage study. Aust N Z J Obstet Gynaecol 2019;59(1):45-53. [CrossRef]
11. Agholor K, Omo-Aghoja L, Okonofua F. Rate of negative appendectomy in pregnant women in Benin City, Nigeria. J Obstet Gynaecol Res 2011;37(11):1540-8. [CrossRef]
12. Ito K, Ito H, Whang EE, Tavakkolizadeh A. Appendectomy in pregnancy: evaluation of the risks of a negative appendectomy. Am J Surg 2012;203(2):145-50. [CrossRef]
13. Hiersch L, Yogev Y, Ashwal E, From A, Ben-Haroush A, Peled Y. The impact of pregnancy on the accuracy and delay in diagnosis of acute appendicitis. J Matern Fetal Neonatal Med 2014;27(13):1357-60. [CrossRef]
14. Poletti PA, Botsikas D, Becker M, Picarra M, Rutschmann OT, Buchs NC, et al. Suspicion of appendicitis in pregnant women: emergency evaluation by sonography and low-dose CT with oral contrast. Eur Radiol 2019;29 (1):345-52. [CrossRef]
15. Bhandari TR, Shahi S, Acharya S. Acute Appendicitis in pregnancy and the developing world. Int Sch Res Notices 2017;2017:2636759. [CrossRef]
16. Zingone F, Sultan AA, Humes DJ, West J. Risk of acute appendicitis in and around pregnancy: a population-based cohort study from England. Ann Surg 2015;261(2):332-7. [CrossRef]
17. Lee SH, Lee JY, Choi YY, Lee JG. Laparoscopic appendectomy versus open appendectomy for suspected appendicitis during pregnancy: a systematic review and updated meta-analysis. BMC Surg 2019;19 (1):41. [CrossRef]

18. Cinar H, Aygun A, Derebey M, Tarim IA, Akalin C, Buyukakincak S, et al. Significance of hemogram on diagnosis of acute appendicitis during pregnancy. Ulus Travma Acil Cerrahi Derg 2018;24 (5):423-8. [CrossRef]
19. Maslovitz S, Gutman G, Lessing JB, Kupferminc MJ, Gamzu R. The significance of clinical signs and blood indices for the diagnosis of appendicitis during pregnancy. Gynecol Obstet Invest 2003;56(4):188-91. [CrossRef]
20. Segev L, Segev Y, Rayman S, Nissan A, Sadot E. Acute appendicitis during pregnancy: Different from the nonpregnant state? World J Surg 2017;41(1):75-81. [CrossRef]
21. Wallace CA, Petrov MS, Soybel DI, Ferzoco SJ, Ashley SW, Tavakkolizadeh A. Influence of imaging on the negative appendectomy rate in pregnancy. J Gastrointest Surg 2008;12(1):46-50. [CrossRef]
22. Al-Qudah MS, Amr M, Sroujieh A, Issa A. Appendectomy in pregnancy: the experience of a university hospital. J Obstet Gynaecol 1999;19(4):362-4. [CrossRef]
23. Terzi A, Yildiz F, Vural M, Coban S, Cece H, Kaya M. A case series of 46 appendectomies during pregnancy. Wien Klin Wochenschr 2010;122(23-24):686-90. [CrossRef]
24. Frountzas M, Nikolaou C, Stergios K, Kontzoglou K, Toutouzas K, Pergialiotis V. Is the laparoscopic approach a safe choice for the management of acute appendicitis in pregnant women? A meta-analysis of observational studies. Ann R Coll Surg Engl 2019;101(4):235-48. [CrossRef]
25. Wilasrusmee C, Sukrat B, McEvoy M, Attia J, Thakkinstian A. Systematic review and meta-analysis of safety of laparoscopic versus open appendicectomy for suspected appendicitis in pregnancy. Br J Surg 2012;99(11):1470-8. [CrossRef]
26. Popkin CA, Lopez PP, Cohn SM, Brown M, Lynn M. The incision of choice for pregnant women with appendicitis is through McBurney's point. Am J Surg 2002;183(1):20-2. [CrossRef]

İSTANBUL ÜNİVERSİTESİ YAYINEVİ

RESEARCH / ARAŞTIRMA
DOI: 10.26650/IUITFD.2019.0097
İst Tıp Fak Derg 2020 / J Ist Faculty Med 2020

AN ALTERNATIVE THERAPY OPTION IN METASTATIC THYROID CANCER: PEPTIDE RECEPTOR RADIONUCLIDE THERAPY

METASTATİK TİROİD KANSERİNDE ALTERNATİF TEDAVİ SEÇENEĞİ: PEPTİD RESEPTÖR RADYONÜKLİD TEDAVİ

Havva YEŞİL ÇINKIR[1], Umut ELBOĞA[2]

Gaziantep University, Faculty of Medicine, [1]Department of Medical Oncology, [2]Department of Nuclear Medicine, Gaziantep, Turkey

ORCID IDs of the authors: H.Y.Ç. 0000-0002-7870-8741; U.E. 0000-0002-3650-8258

Cite this article as: Yesil Cinkir, H, Elboga U. An alternative therapy option in metastatic thyroid cancer: peptide receptor radionuclide therapy. J Ist Faculty Med 2020;83(4):339-44. doi: 10.26650/IUITFD.2019.0097

ABSTRACT

Objective: In this study, we aimed to evaluate the results of peptide receptor radionuclide therapy (PRRT) in the treatment of metastatic thyroid cancer patients.

Material and Method: In total, 10 patients with metastatic thyroid cancer treated with PRRT were evaluated. There were 5 medullary thyroid cancer (MTC) patients and 5 patients had differentiated thyroid cancer (DTC).

Results: Median age at first PRRT was 61.5 (38-79) years and 5/10 (50%) were female. The mean overall survival (OS) was 19.2 months (95% CI; 4.1-34.3) after the first PRRT. The mean progression-free survival (PFS) was 4.5 months (95% CI; 2.8-6.3). According to pathologic subgroup analysis, the mean OS were 13.8 months (95% CI; 4.0-23.7) in DTC and 24.2 (95% CI; 0-48.8) in MTC after first PRRT (p:0.555). Five patients had stable disease and one patient had partial response. Minor hematological toxicity was observed in 4 patients.

Conclusion: PRRT appears to be an alternative treatment option for thyroid cancer. It is thought that the results will be more desirable as the patients take the treatment in the earlier stages.

Keywords: Radionuclide therapy, survival, thyroid cancer

ÖZET

Amaç: Bu çalışmada, metastatik tiroid kanserli hastaların tedavisinde peptid reseptörü radyonüklid tedavisinin (PRRT) sonuçlarını değerlendirmeyi amaçladık.

Gereç ve Yöntem: PRRT ile tedavi edilen metastatik tiroid kanseri olan 10 hasta değerlendirildi. Beş medüller tiroid karsinomu (MTC) ve 5 differansiye tiroid karsinomu (DTC) mevcuttu.

Bulgular: İlk PRRT uygulamasında ortalama yaş 61,5 (38-79) yıl ve hastaların 5/10 (%50)'u kadındı. Ortalama genel sağkalım (GSK), ilk PRRT' den sonra 19,2 ay (%95 CI; 4,1-34,3) idi. Ortalama progresyonsuz sağkalım 4,5 aydı (%95 CI; 2,8-6,3). Patolojik alt grup analizine göre ilk PRRT sonrası ortalama GSK; DTC'de 13,8 ay (%95 CI; 4,0-23,7) ve MTC'de 24,2 (%95 CI; 0-48,8) idi (p:0.555). Beş hastada stabil hastalık, bir hastada kısmi yanıt saptandı. Dört hastada minör hematolojik toksisite gözlendi.

Sonuç: PRRT tiroid kanseri için alternatif bir tedavi seçeneği olarak görünmektedir. Hastaların tedaviyi erken evrelerde almasıyla sonuçların daha iyi olacağı düşünülmektedir.

Anahtar Kelimeler: Radyonüklid tedavi, sağkalım, tiroid kanseri

INTRODUCTION

Thyroid cancer is a common malignancy of the head and neck region, whose incidence rate has been increasing in recent years (1). Differentiated type of thyroid cancer (DTC) (follicular and papillary) has an excellent 10-year survival rate of 85-99% and the postoperative recurrence rate of 23-30% (2, 3). Medullary thyroid cancer (MTC) is often associated with multiple endocrine neoplasia and represents approximately 3% of thyroid cancers (4). The 5-year relative survival of MTC is 93% for stage I to III, and 28% for stage IV (5). The conventional treatment options alter depending on the subtype and stage of the cancer. These options for thyroid cancer include surgery, radioactive iodine (^{131}I)

Corresponding author/İletişim kurulacak yazar: drhavva1982@gmail.com

Submitted/Başvuru: 02.12.2019 • **Revision Requested/Revizyon Talebi:** 30.01.2020 •
Last Revision Received/Son Revizyon: 30.03.2020 • **Accepted/Kabul:** 01.05.2020 • **Published Online/Online Yayın:** 22.09.2020

(RAI) therapy, radiotherapy (RT), and targeted therapy with several tyrosine kinase inhibitors (TKIs). The presence of somatostatin receptors in endocrin tumors has been demonstrated in DTC and MTC (6, 7). Therefore, the use of somatostatin analogs as ligands and targeting of the tumor with a radionuclide appears to be an attractive option. In this study, we aimed to analyse the results of peptide receptor radionuclide therapy (PRRT) in the treatment of patients with metastatic thyroid cancer.

MATERIAL AND METHOD

Study design and patients

In total, 10 patients (MTC: 5, papillary thyroid cancer (PTC): 4, follicular thyroid cancer (FTC): 1) treated with PRRT were evaluated at the Gaziantep University Faculty of Medicine between 2015-2019, retrospectively. The inclusion criteria for the study were; diagnosis of a histopathologically confirmed DTC or MTC, age ≥18 years, an available clinicopathological and follow-up data, occurrence of non-regional lymh nodes or distant metastases, non-RAI-avid or RAI refractory DTC, progression on previous treatments (chemotherapy, radiotherapy or TKIs), presence of visible somatostatin receptor expression at the ^{68}Ga labelled DOTATATE (synthetic somatostatin analogue peptide) positron emission tomography (PET). The exclusion criteria were; presence of cardiac, hepatic, hematological and renal dysfunctions, occurence of other malignant tumors.

The study was approved by the ethics committee of the Gaziantep University (Decision no: 2019/316, date: 28.08.2019). From all patients, written informed consent was obtained before the administration of radiolabeled substances.

Peptide receptor radionuclide therapy

Firstly, ^{68}Ga labelled DOTATATE (synthetic somatostatin analogue peptide) PET was performed and SSTR expression was detected. The existence of an SSTR led us to consider that ^{177}Lutetium (^{177}Lu) labelled DOTATATE could be used as an alternative treatment. The infused dose of ^{177}Lu labelled DOTATATE was 200 mCi. The treatment was applied at 6-10 weeks intervals.

Before initiating the ^{177}Lu labelled DOTATATE treatment, hematological and renal function tests were analyzed. The inclusion criteria for performing PRRT were hemoglobin ≥10 g/dL, white blood cell (leukocyte) count ≥4x10^3/μL, platelet count ≥100x10^3/μL, serum creatinine ≤1.2 mg/dL or creatinine clearance ≥60 mL/min, and Eastern Cooperative Oncology Group (ECOG) performans status (PS) ≤2.

For protecting renal function, the fluid protocol performed before and after ^{177}Lu labelled DOTATATE treatment was as follows: 750 miligram magnesium sulfate and 10 mg metoclopramide were injected into a 1000 cc ringer lactate solution, and applied over 60-75 minutes.

Response assesment was performed after treatment of ≥2 cycles based on standardized uptake value (SUV) max (8). The responses were: complete metabolic response, partial metabolic response (PR) (decrease with 15-25%), stable disease (SD) (no change or decrease with 15%) and progressive metabolic disease (P) (new lesions or increase of >25%).

Common Terminology Criteria for Adverse Events (CTCAE) was used for adverse events scoring.

Statistical analysis

The outcomes of treatment were progression-free survival (PFS), overall survival (OS), response rates and toxicities. PFS was described as the interval from treatment initiation to progression, last documented patient visit or death. OS was defined as the time between treatment initiation and death or until the last documented patient visit. The Kaplan-Meier method and Log-rank statistics were used for survival analysis. $P<0.05$ was defined as statistically significant. The Statistical Package for Social Sciences version 22.0 for Windows (SPSS, Inc. Chicago, IL, USA) was performed for all statistical analyzes.

RESULTS

Ten patients were treated with ^{177}Lu labelled DOTATATE. The clinical characteristics of patients are summarized in Table 1. The median age was 61.5 (38-79) years at first PRRT, and 5/10 (50%) were females. Nine out of the 10 (90%) patients had a baseline ECOG PS of 0-1. None of the patients had an endocrine paraneoplastic syndrome. A total thyroidectomy was performed in all patients. All the patients had metastasis to the lymph nodes, 8 patients had bone and 6 patients had lung metastases.

Of the 10 patients, 5 have (3 of PTC, 2 of MTC) died. Response assessment was performed in 7 patients who were treated with >2 cycles. The remaining 3 patients were excluded. Two patients died after first and second therapy, one patient was lost to follow up. 5 patients had SD and one patient had PR. Progression was observed in one patient.

The mean and the median OS were 19.2 months (95% CI; 4.1-34.3) and 9.1 months (95% CI; 0-25.2) after the first PRRT (Figure 1). The mean and the median OS were 90.5 months (95% CI; 67.1-113.8) and 94.7 months (95% CI; 56.7-132.6) after the diagnosis. The mean and the median PFS were 4.5 months (95% CI; 2.8-6.3) and 2.9 months (95% CI; 0.9-5.0) (Figure 2).

In total, ten patients received 31 PRRT courses. Minor hematological toxicity was detected in 4 patients. Leukopenia (CTCAE grade I) was observed in 3 patients. Anemia (CTCAE grade 2) was observed in one patient and grade

Table 1: Patient's characteristics, baseline data, and status after the last PRRT

	1	2	3	4	5	6	7	8	9	10
Gender	**M**	**F**	**M**	**M**	**M**	**M**	**F**	**F**	**F**	**F**
Tumor type	MTC	MTC	MTC	MTC	MTC	PTC	PTC	PTC	FTC	PTC
Previous treatments	T, ND, CT, RT, TKIs	T, ND	T, ND, CT, RT, TKIs	T, ND, CT, TKIs	T, ND, CT, TKIs	T, ND, RAI, TKIs	T, ND, RAI, TKIs	T, ND, RAI, TKIs	T, ND, RAI, TKIs	T, ND, RAI, TKIs
Stage	IV_C	IV_C	IV_C	IV_C	IV_C	IV_B	IV_B	IV_B	IV_B	II
Age at 1st PRRT	59	79	53	59	38	64	77	64	67	49
PRRT line	6	1	6	5	3	3	3	4	3	5
PRRT course	2	4	6	4	2	3	1	4	3	2
Metastases before PRRT	Lo, L, B, LAP	Lo, LAP	L, B, LAP	LAP	B, LAP	L, B, LAP	L, B, LAP	L, B, LAP	L, B, LAP	Lo, B, LAP
PRRT response	Unkn	Unkn	S	S	S	PR	Unkn	P	S	S
Status after last PRRT	E	A	E	A	A	E	E	A	A	E

A: Alive, B: Bone, CT: Chemotherapy, E: Exitus, F: Female, FTC: Follicular thyroid cancer, L: Lung, LAP: Lymph node involvement, Lo: Local, M: Male, MTC: Medullary thyroid cancer, ND: Neck dissection, P: Progression, PR: Partial response, PRRT: Peptide receptor radionuclide therapy, PTC: Papillary thyroid cancer, RAI: Radioactive iodine, RT: Radiotherapy, S: Stable, Unkn: Unknown, T: Thyroidectomy, TKIs: Tyrosine kinase inhibitors

I was in one patient. None of the patients experienced thrombocytopenia. None of the patients experienced hepatotoxicity or nephrotoxicity.

According to pathologic subgroup analysis;

DTC: The median age was 64 (49-77) years at first PRRT. They recevied radioiodine therapies and TKIs before

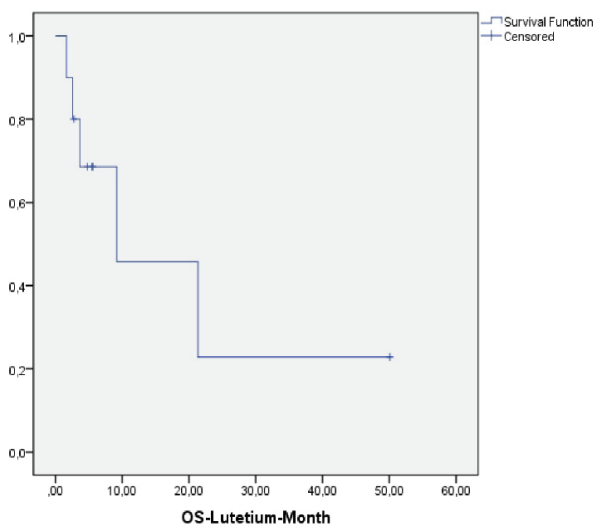

Figure 1: Kaplan-Meier analysis: mean overall survival after the first PRRT cycle was 19.2 months (95% CI; 4.1-34.3).

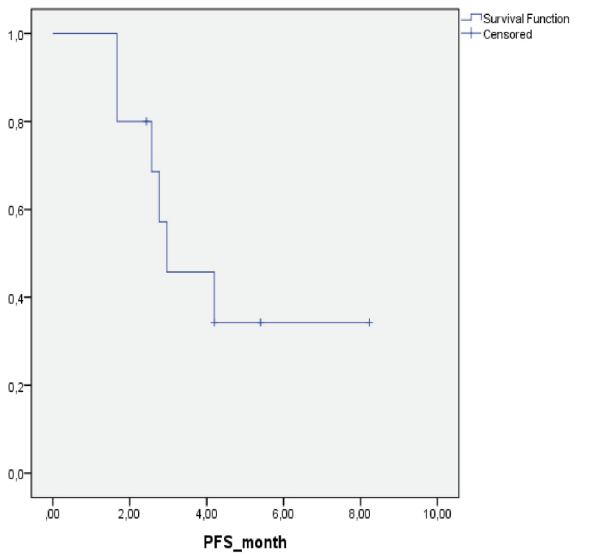

Figure 2: Kaplan-Meier analysis: mean progression free survival 4.5 months (95% CI; 2.8-6.3).

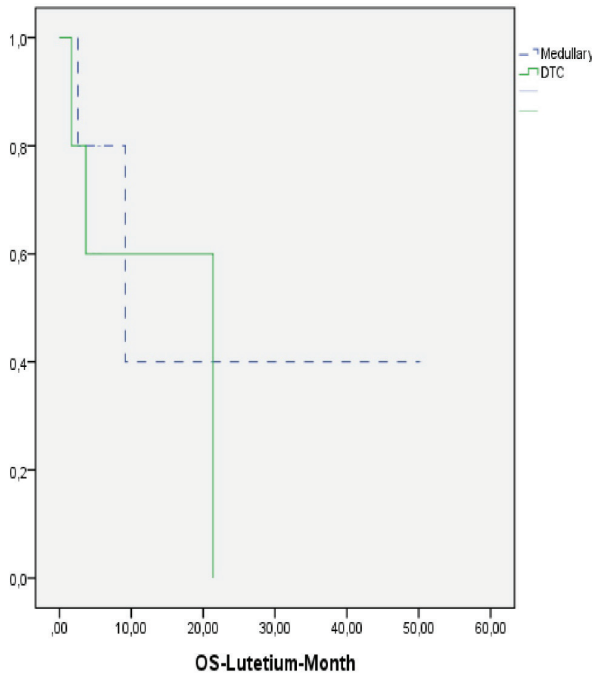

Figure 3: Mean overall survival (OS) were 13.8 months (95% CI; 4.0-23.7) in DTC and 24.2 (95% CI; 0-48.8) in MTC after first PRRT (p=0.555).

PPRT: The mean OS was 13.8 months (95% CI; 4.0-23.7) after first PRRT (Figure 3, p=0.555). The mean PFS was 5.5 months (95% CI; 1.7-9.4).
MTC: At first PRRT, the median age was 59 (38-79) years. The germline mutation of RET proto-oncogene was not determined. They recevied chemotherapy, radiotherapy (RT) and TKIs before PRRT. The mean OS was 24.2 (95% CI; 0-48.8) after first PRRT (Figure 3, p=0.555). The mean PFS was 37.3 months (95% CI; 17.6-56.9).

DISCUSSION

Thyroid cancers' prognosis and treatment depend on the type and stage of the tumor at diagnosis. Since thyroid cells have been shown to express SSTR, radionuclide applications have been the treatment of choice (6, 9). In the light of this information, we aimed to present the interim results of patients with thyroid cancer who underwent radionuclide therapy in our center.

An absence of SSTR2a expression in the tissue biopsy might be a poor prognostic sign in MTC (9). A low level of SSTR2a expression was associated with poor outcome and more aggressive grades of tumor in gastroenteropancreatic neuroendocrine tumors (10). The 10-year survival rates in stage IV MTC patients were 96% in SSTR2a positive patients and 43% in SSTR2a negative patients (11). ^{111}In-DTPA-octreotide scans were used to analyse SSTR2a uptake in 35 non-treated patients (10). Nowadays, ^{68}Ga-DOTATATE PET scan has been utilized more frequently. It has imaging advantages. These are excate staging of the patient, and presences of pharmacological (higher affinity to SSTR2a), physical (higher gamma energies) and technical (e.g. positron imaging, attenuation correction) differences (12). In our hospital, we used ^{68}Ga-DOTATATE PET for demonstrating the SSTR expression. After this procedure, patients with positive involvement were treated.

Survival is increased and recurrence rates are decreased with total thyroidectomy. Treatment with radioactive iodine (RAI) has been an integral adjuvant role in the DTC. The risk of recurrence in DTC is 30%, and 66% of these relapses are in the first decade after initial treatment (8). Also, RAI therapy is the backbone of treatment in recurrence. Iodine-avidity of metastases is a very significant prognostic factor in DTC. The OS rate of patients with non-RAI-avid is notably lower than that of patients with iodine-avid lesions (13-15). Unfortunately, treatment options are limited in patients non-RAI-avid or resistant to this treatment. Systemic treatment such as with TKIs is another alternative. However, TKIs cause severe side-effects, and these drugs can decrease quality of life and beginning with this as a treatment option should be carefully considered (16). Therefore, there is a demand for new treatment modalities for this group of patients. The fact that SSTR expression is defined in DTC has been an alternative treatment option in these patients (17-19). Our patients had received other standard treatments and PRRT was applied upon progression observation. The response rates reported in the literature were 30-80% in patients with DTC (17, 20). In our study, the rate of disease control was 75% (2=stable response, 1=partial response), which was similar to previous studies.

Like DTC, locally advanced tumor or distant metastases have limited systemic treatment options in MTC. Conventional chemotherapy has an inadequate efficacy and treatment-related toxicity rates are high (21). With advances in tumor biology, treatments such as vandetanib and cabozantinib, and a tyrosine kinase inhibitor that targets the RET proto-oncogene, have been used in clinical practice (22, 23). An increase in survival was reported with these treatments, but grade 3-4 side effects were observed in most patients (vandetanib=44%, cabozantinib=69%). Therefore, alternative treatment options with fewer side effects were needed. With the detection of SSTR expression in MTC, PPRT has become the new treatment option (9). According to previous studies, the response rates were 29-80%, in our study we found stable disease response in all 3 patients who underwent treatment response evaluation (18, 24).

In our study, the mean PFS and OS were in favor of MTC. According to the literature, the survival rate for MTC is not as good as for differentiated thyroid cancer (25). However, our results were the exact opposite of the literature. This demonstrates that our patient's population had more aggressive characteristics.

The median treatment line was 3.5 (range, 1-6) for all patients (DTC=3, range, 3-5; MTC=5, range, 1-6). Survival data were poor due to the fact that PRRT was applied in advanced stages for both DTC and MTC patients. Previous studies have suggested that PRRT should be used in the early stages (26, 27). It has been advocated that success rates may increase with the combined use of chemotherapy agents that increase radiation sensitivity. In our study, all patients were unresponsive to other treatments and PRRT was the last treatment option. Exceptionally, PRRT was administered as an initial treatment in an elderly patient (age, 79 years). PRRT, which is considered to be less toxic, was applied because the patient was of advanced age and did not accept other conventional treatments.

Treatment-induced toxicity both reduces treatment success and creates incompatibility. Radiolabelled somatostatin analogues are an important risk for nephrotoxicity. In previous studies, renal protection could be achieved by infusion of amino acid solutions and by monitoring the radiation dose to the kidney to a maximum of 23-27 Gy (28, 29). We also applied a protocol including fluid replacement and anti-emetic treatment to our patients for renal protection. Hematological toxicity is another important side effect. In our results, the patients tolerated the treatment well and we did not observe CTCAE grade >2 toxicity.

There were restrictive aspects of our study. The first factor was the small sample size of patients. Secondly, the patient group was heterogeneous. Thirdly, the follow-up time was quite short. The number of patients who started treatment has increased in the last 2 years. Therefore, the results of the patients were more negative than previous reports.

CONCLUSION

PRRT appears to be an alternative treatment option for thyroid cancer. It is thought that the results will be more desirable as the patients take the treatment in the earlier stages.

Ethics Committee Approval: This study was approved by the Ethical Committee of the Gaziantep University (Decision no: 2019/316).

Informed Consent: Written consent was obtained from the participants.

Peer Review: Externally peer-reviewed.

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

Conflict of Interest: Authors declared no conflict of interest.

Financial Disclosure: Authors declared no financial support.

Etik Komite Onayı: Bu çalışma için etik komite onayı Gaziantep Üniversitesi Etik Kurulu'ndan alınmıştır (Karar no: 2019/316).

Bilgilendirilmiş Onam: Katılımcılardan bilgilendirilmiş onam alınmıştır.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Çalışma Konsepti/Tasarım- H.Y.Ç., U.E.; Veri Toplama- H.Y.Ç., U.E.; Veri Analizi/Yorumlama- H.Y.Ç., U.E; Yazı Taslağı- H.Y.Ç.; İçeriğin Eleştirel İncelemesi- H.Y.Ç., U.E.; Son Onay ve Sorumluluk- H.Y.Ç., U.E.; Süpervizyon- H.Y.Ç., U.E.

Çıkar Çatışması: Yazarlar çıkar çatışması beyan etmemişlerdir.

Finansal Destek: Yazarlar finansal destek beyan etmemişlerdir.

REFERENCES

1. Mao Y, Xing M. Recent incidences and differential trends of thyroid cancer in the USA. Endocr Relat Cancer 2016;23:313-22. [CrossRef]
2. Eustatia-Rutten CF, Corssmit EP, Biermasz NR, Pereira AM, Romijn JA, Smit JW. Survival and death causes in differentiated thyroid carcinoma. J Clin Endocrinol Metab 2006;91(1):313-9. [CrossRef]
3. Yang X, Liang J, Li TJ, Yang K, Liang DQ, Yu Z, et al. Postoperative stimulated thyroglobulin level and recurrence risk stratification in differentiated thyroid cancer. Chin Med J 2015;128(8):1058-64. [CrossRef]
4. Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ, et al. Revised American Thyroid Association Management Guidelines for Patients with Thyroid Nodules and Differentiated Thyroid Cancer: the American Thyroid Association (ATA) Guidelines Taskforce on Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid 2009;19(11):1167-214. [CrossRef]
5. Hundahl SA, Fleming ID, Fremgen AM, Menck HR. A National Cancer Data Base report on 53,856 cases of thyroid carcinoma treated in the U.S., 1985-1995. Cancer 1998;83(12):2638-48. [CrossRef]
6. Klagge A, Krause K, Schierle K, Steinert F, Dralle H, Fuhrer D. Somatostatin receptor subtype expression in human thyroid tumours. Horm Metab Res 2010;42(4):237-40. [CrossRef]
7. Papotti M, Croce S, Bello M, Bongiovanni M, Allia E, Schindler M, et al. Expression of somatostatin receptor types 2, 3 and 5 in biopsies and surgical specimens of human lung tumours. Correlation with preoperative octreotide scintigraphy. Virchows Arch 2001;439(6):787-97. [CrossRef]

8. Mazzaferri EL, Jhiang SM. Long-term impact of initial surgical and medical therapy on papillary and follicular thyroid cancer. Am J Med 1994;97(5):418-28. [CrossRef]
9. Beukhof CM, Brabander T, Nederveen FH, Velthuysen ML, de Rijke YB, Hofland LJ, et al. Peptide receptor radionuclide therapy in patients with medullary thyroid carcinoma: predictors and pitfalls. BMC Cancer 2019;19(1):325. [CrossRef]
10. Wang Y, Wang W, Jin K, Fang C, Lin Y, Xue L, et al. Somatostatin receptor expression indicates improved prognosis in gastroenteropancreatic neuroendocrine neoplasm, and octreotide longacting release is effective and safe in Chinese patients with advanced gastroenteropancreatic neuroendocrine tumors. Oncol Lett 2017;13(3):1165-74. [CrossRef]
11. de Vries LH, Lodewijk L, Willems SM, Dreijerink KMA, de Keizer B, van Diest PJ, et al. SSTR2A expression in medullary thyroid carcinoma is correlated with longer survival. Endocrine 2018;62(3):639-47. [CrossRef]
12. Ruf J, Heuck F, Schiefer J, Denecke T, Elgeti F, Pascher A, et al. Impact of multiphase 68Ga-DOTATOC-PET/CT on therapy management in patients with neuroendocrine tumors. Neuroendocrinology 2010;91(1):101-9. [CrossRef]
13. Durante C, Haddy N, Baudin E, Leboulleux S, Hartl D, Travagli JP, et al. Long-term outcome of 444 patients with distant metastases from papillary and follicular thyroid carcinoma: benefits and limits of radioiodine therapy. J Clin Endocrinol Metab 2006;91(8):2892-99. [CrossRef]
14. Baudin E and Schlumberger M. New therapeutic approaches for metastatic thyroid carcinoma. Lancet Oncol 2007;8(2):148-56. [CrossRef]
15. Biermann K, Biersack HJ, Sabet A, Janzen V. Alternative therapeutic approaches in the treatment of primary and secondary dedifferentiated and medullary thyroid carcinoma. Semin Nucl Med 2011;41(2):139-48. [CrossRef]
16. Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, et al. 2015 American Thyroid Association management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: the American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid 2016;26(1):1-133. [CrossRef]
17. Budiawan H, Salavati A, Kulkarni HR, Baum RP. Peptide receptor radionuclide therapy of treatment-refractory metastatic thyroid cancer using (90)Yttrium and (177) Lutetium labeled somatostatin analogs: toxicity, response and survival analysis. Am J Nucl Med Mol Imaging 2013;4(1):39-52.
18. Salavati A, Puranik A, Kulkarni HR, Budiawan H, Baum RP. Peptide receptor radionuclide therapy (PRRT) of medullary and nonmedullary thyroid cancer using radiolabeled somatostatin analogues. Semin Nucl Med 2016;46(3):215-24. [CrossRef]
19. Ain KB, Taylor KD, Tofiq S, Venkataraman G. Somatostatin receptor subtype expression in human thyroid and thyroid carcinoma cell lines. J Clin Endocrinol Metab 1997;82(6):1857-62. [CrossRef]
20. Versari A, Sollini M, Frasoldati A, Fraternali A, Filice A, Froio A, et al.Differentiated thyroid cancer:a new perspective with radiolabeled somatostatin analogues for imaging and treatment of patients. Thyroid 2014;24(4):715-26. [CrossRef]
21. Martins RG, Rajendran JG, Capell P, Byrd DR, Mankoff DA. Medullary thyroid cancer: options for systemic therapy of metastatic disease? J Clin Oncol 2006;24(11):1653-5. [CrossRef]
22. Kurzrock R, Sherman SI, Ball DW, Forastiere AA, Cohen RB, Mehra R, Pfister DG, et al. Activity of XL184 (Cabozantinib), an Oral Tyrosine Kinase Inhibitor, in Patients With Medullary Thyroid Cancer. J Clin Oncol 2011;29(19):2660-6. [CrossRef]
23. Wells SA Jr, Robinson BG, Gagel RF, Dralle H, Fagin JA, Santoro M, et al.Vandetanib in patients with locally advanced or metastatic medullary thyroid cancer: a randomized, doubleblind phase III trial. J Clin Oncol 2012;30(2):134-41. [CrossRef]
24. Iten F, Müller B, Schindler C, Rochlitz C, Oertli D, Mäcke HR, et al. Response to [90Yttrium-DOTA]-TOC treatment is associated with long-term survival benefit in metastasized medullary thyroid cancer: a phase II clinical trial. Clin Cancer Res 2007;13(22 pt 1):6696-702. [CrossRef]
25. Sippel RS, Kunnimalaiyaan M, Chen H. Current management of medullary thyroid cancer. Oncologist 2008;13(5):539-47. [CrossRef]
26. Salavati A, Prasad V, Schneider CP, Herbst R, Baum RP. Peptide receptor radionuclide therapy of Merkel cell carcinoma using (177) lutetium-labeled somatostatin analogs in combination with radiosensitizing chemotherapy: a potential novel treatment based on molecular pathology. Ann Nucl Med 2012;26(4):365-9. [CrossRef]
27. Schmidt MC, Uhrhan K, Markiefka B, Hasselbring L, Schlaak M, Cremer B, et al. (68)Ga-DotaTATE PET-CT followed by Peptide Receptor Radiotherapy in combination with capecitabine in two patients with Merkel Cell Carcinoma. Int J Clin Exp Med 2012;5(4):363-6.
28. Rolleman EJ, Valkema R, de Jong M, Kooij PP, Krenning EP. Safe and effective inhibition of renal uptake of radiolabelled octreotide by a combination of lysine and arginine. Eur J Nucl Med Mol Imaging 2003;30(1):9-15. [CrossRef]
29. Krenning EP, Kwekkeboom DJ, Valkema R, Pauwels S, Kvols LK, Jong M. Peptide Receptor Radionuclide Therapy. Ann. N.Y. Acad. Sci. 2004;1014:234-45. [CrossRef]

İSTANBUL ÜNİVERSİTESİ YAYINEVİ

RESEARCH / ARAŞTIRMA
DOI: 10.26650/IUITFD.2020.0051
İst Tıp Fak Derg 2020 / J Ist Faculty Med 2020

CONTRIBUTION OF SARCOMERIC GENE VARIANTS TO THE PREDICTION OF SUDDEN CARDIAC DEATH RISK IN FAMILIAL HYPERTROPHIC CARDIOMYOPATHY

AİLESEL HİPERTROFİK KARDİYOMİYOPATİDE SARKOMERİK GEN VARYANTLARININ ANİ KARDİYAK ÖLÜM RİSKİNİN ÖNGÖRÜLMESİNE KATKISI

Evrim KÖMÜRCÜ BAYRAK[1,2], Filiz GÜÇLÜ GEYİK[2], Gökhan KAHVECİ[3], Fatih BAYRAK[4]

[1]Istanbul University, Istanbul Faculty of Medicine, Departments of Medical Genetics, Istanbul, Turkey
[2]Istanbul University, Aziz Sancar Institute of Experimental Medicine, Department of Genetics, Istanbul, Turkey
[3]Kartal Kosuyolu Training and Research Hospital, Clinic of Cardiology, Istanbul, Turkey
[4]Acibadem Mehmet Ali Aydinlar University, Department of Cardiology, Istanbul, Turkey

ORCID IDs of the authors: E.K.B. 0000-0003-1271-1208; F.G.G. 0000-0003-4257-9930; G.K. 0000-0001-8367-6505; F.B. 0000-0002-1574-1085

Cite this article as: Komurcu Bayrak E, Guclu Geyik F, Kahveci G, Bayrak F. Contribution of sarcomeric gene variants to the prediction of sudden cardiac death risk in familial hypertrophic cardiomyopathy. J Ist Faculty Med 2020;83(4):345-54. doi: 10.26650/IUITFD.2020.0051

ABSTRACT

Objective: Hypertrophic cardiomyopathy (HCM) is one of sudden cardiac death (SCD) causes. This study aimed to identify high-risk pathogenic variants for SCD in the three sarcomeric genes with the most frequent mutations in HCM.

Material and Method: The study included 12 adult HCM index cases with a family history of SCD and/or HCM, and 31 of their family members. All the participants were evaluated with detailed cardiac examinations. The exonic regions of the *MYH7*, *MYBPC3* and *TNNT2* genes were analysed using CorTAG HCM1 resequencing arrays.

Results: Six pathogenic variants causing amino acid substitutions were found in 8 of the index cases with HCM. Five of them were identified as previously defined missense variants of Val698Ala, Arg719Trp, Met822Leu and Arg663Cys (in three cases) in the *MYH7* gene, and Arg102Trp in the *TNNT2* gene. For the first time in an HCM family with a history of late-onset SCD, Tyr525Asn and c.*27-21G> A variants in the *MYBPC3* gene were identified as compound heterozygous. These variants were not present in control subjects (n=777) from the Turkish population.

Conclusion: In this study, novel variants in the *MYBPC3* gene were identified in an HCM family with SCD history. However, there was no clear association between pathogenic variants and the risk of SCD.

Keywords: Sarcomeric gene variant, familial hypertrophic cardiomyopathy, sudden cardiac death

ÖZET

Amaç: Hipertrofik kardiyomiyopati (HKM), ani kardiyak ölümün (AKÖ) nedenlerinden biridir. Bu çalışmada, HKM'de en sık mutasyon bulunan üç sarkomerik gende, AKÖ için yüksek riskli patojenik varyantların belirlenmesi amaçlandı.

Gereç ve Yöntem: Çalışmaya, AKÖ ve/veya HKM için aile öyküsü olan 12 yetişkin HKM'li indeks vaka ve 31 aile üyesi dahil edildi. Tüm katılımcılar, kardiyolojik olarak değerlendirildi. *MYH7*, *MYBPC3* ve *TNNT2* genlerinin ekzonik bölgeleri, CorTAG HCM1 dizileme sistemi kullanılarak analiz edildi.

Bulgular: HKM'li indeks vakaların 8'inde, amino asit değişimine neden olan 6 farklı patojenik varyant bulundu. Bunlardan beşinin, *MYH7* genindeki Val698Ala, Arg719Trp, Met822Leu ve Arg663Cys (üç vakada) ve *TNNT2* genindeki Arg102Trp değişimlerinin daha önce tanımlanmış yanlış anlamlı patojenik varyantlar olduğu belirlendi. İleri yaşta AKÖ öyküsü olan bir HKM ailesinde, *MYBPC3* geninde Tyr525Asn ve c.*27-21G>A varyantlar bileşik heterozigot olarak ilk defa tanımlandı. Bu varyantlar, Türk popülasyonu kontrol örneklerinde (n=777) saptanmadı.

Sonuç: Bu çalışmada, AKÖ öyküsü olan bir HKM ailesinde yeni varyantlar tanımlandı. Ancak, HKM ailelerinde saptanan patojenik varyantlar ile AKÖ riski arasında net bir ilişki bulunamadı.

Anahtar Kelimeler: Sarkomerik gen varyantı, ailesel hipertrofik kardiyomiyopati, ani kardiyak ölüm

Corresponding author/İletişim kurulacak yazar: ebayrak@istanbul.edu.tr

Submitted/Başvuru: 06.05.2020 • **Revision Requested/Revizyon Talebi:** 27.05.2020 •
Last Revision Received/Son Revizyon: 27.05.2020 • **Accepted/Kabul:** 01.06.2020 • **Published Online/Online Yayın:** 30.09.2020

INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is a cardiac disease characterized by left ventricular hypertrophy, causing sudden cardiac death (SCD) or progressive heart failure (1-3). HCM exhibits familial aggregation usually with an autosomal dominant inheritance, occurring in about 1 in 500 individuals (1). The first gene localization of familial HCM was mapped to the locus of ß-MyHC on chromosome 14q12 in a large French-Canadian family in 1989 (4). From this date, more than 1400 variants in the sarcomeric and non-sarcomeric genes in HCM have been identified using various techniques (5-9).

Although, in recent times, more than 40 genes have been identified in HCM patients by new genetic sequencing (NGS) (8, 10), HCM is mostly due to pathogenic single nucleotide variants (SNVs) (53-85%) in the *MYH7* gene encoding cardiac ß-myosin heavy chain (ß-MyHC), the *MYBPC3* gene encoding cardiac myosin binding protein-C (cMyBP-C) and the *TNNT2* gene encoding cardiac troponin T (cTnT) (2, 3, 11).

In this study, our aim was to identify novel high-risk pathogenic variants in the *MYH7, MYBPC3* and *TNNT2* genes in Turkish HCM families with SCD history, and also to perform retrospective clinical evaluations of variant carriers.

MATERIAL AND METHOD

The participants of study

The study included 12 index HCM cases (8 male/4 female; age range at diagnosis 16–67 years) with a family history of HCM and/or SCD, and their 31 family members. Study subjects were evaluated with a clinical history, physical examination, electrocardiography and echocardiography. This study was conducted in accordance with the ethics standards of the Ethics Committee of the University of Istanbul, Istanbul Faculty of Medicine and with the Helsinki Declaration (1964), and informed consent was obtained from the study subjects.

Variant detection

The genomic DNA samples were extracted from peripheral leukocytes. The coding regions and flanking intronic sequences of the *MYH7, MYBPC3* and *TNNT2* genes were screened in 12 index cases using array-based resequencing (CorTAG HCM1 Mutation Detection Assay based on Affymetrix CustomSeq Resequencing Arrays). This assay comprised primer sets for the long-range amplification of all coding regions and intron flanks. Pools of fragmented PCR-products were then hybridized and analysed with high-density oligonucleotide probe arrays. All novel variants and previously reported pathogenic variants were confirmed with Sanger sequencing after touchdown polymerase chain reaction (PCR) and control of the amplicons by 2% agarose gel electrophoresis. Primer sequences are shown in Table 1. The individual and possible pathogenic variants were researched in index cases and family members using the RFLP-PCR method or direct Sanger sequencing. The usage restriction enzymes in the RFLP-PCR method for genotyping of variants were listed in Table 2. The selection of the variant specific restriction enzyme and the lengths of the fragments were determined using the software RestrictionMapper (http://www.restrictionmapper.org.)

Database analysis

dbSNP, ClinVar, VarSome, American College of Medical Genetics and Genomics-ACMG Standards (12) for interpretation classification of variants, and HGMD Professional (Human Genome Mutation Database; BIOBASE) were used to describe the variants in the sarcomeric genes. The variants detected in our HCM patients were also screened in 777 distinct individuals from the in-house exome database of the Advanced Genomics and Bioinformatics Research Center (IGBAM) as a control group.

In silico analysis

The pathogenicity and conservation scores of variants were checked in VarSome using *in silico* tools such as MutationTaster, DANN, SIFT, PROVEAN and GERP. Moreover, Alamut (Interactive Biosoftware, trial version 2.6, Rouen, France) was used to predict whether the novel intronic variants change the characteristics of the splice signals and exonic splicing enhancers (ESE) binding sites on genes. ESEs predictive tools were used to identify the ESEs site for serine/arginine proteins such as SF2/ASF, SC35, SRp40, and SRp55. And also, in this software, the splice site variants were analysed using five prediction tools (SpliceSiteFinder-like, NNSPLICE, GeneSplicer, Human Splicing Finder and MaxEntScan). Default thresholds were used for all the analyses.

RESULTS

In this study, disease-causing variants of the *MYH7, MYBPC3* and *TNNT2* genes in 12 HCM families were searched, and pathogenic variants were identified in eight of them (Figure 1). Twelve index cases had a left ventricular maximal wall thickness >15 mm, and had a family history of SCD and/or HCM. In the genetic analysis of three sarcomeric genes of the index cases, a total of fifteen single nucleotide variations (SNV) were detected. Only six (five already defined and one novel) of these SNVs were pathogenic variants causing amino acid substitutions. Two novel uncertain significance intronic variants as well as seven non-pathogenic variants were also detected (Table 1). Minimal allele frequencies, gene localizations, primer sequences, restriction enzyme type for RFLP-PCR analysis of these variants are demonstrated in Table 1. The minimum allele frequencies of all SNVs in the Turkish population were checked in 777 controls of the in-house exome data-

Table 1: The general information of the identified SNVs in index cases with HCM

The variant position on reference transcript	SNV-ID	MAF	Amino acid substitution	Clinical significance (HGMD)	ACMG Classification	Localization	FP-5'>3'	RP-5'>3'	RE (Amplicon length)	Index cases
NM_000257.3 (*MYH7*)										
c.639+32G>T	novel intronic	0		vus	PM2, BP4	7. intron	cagttgtggattttgggtgt	ggaagagagtggtgatgagt	Bse MII (514 bp)	TK1
c.1062C>T	rs735712	51/1000	p.Gly354Gly	benign	PM1, BA1, BP6, BP7	12. exon	ggtatgggagtctcagaacc	agaagagagatgactgctga	Hae II (337 bp)	T1, A1, N1
c.1755C>A	rs201860580	1/1000	p.Ile585Ile	VUS, benign	PM1, BP6, BP7	16. exon	agcctactaccttaacaccc	aacctgacattgaagtggtg	SfaN I (526 bp)	TK1
c.1579-53C>T	rs45448096	1/1000		likely benign	BS1, BP4	16. intron	agcctactaccttaacaccc	aacctgacattgaagtggtg	ApoI (526 bp)	A1
c.1987C>T	rs397516127	0.0000165/2#	**p.Arg663Cys**	pathogenic (CM973126)	PM1, PM2, PM5, PP2, PP3	18. exon	tcatctctgtgacttctcga	atgtccatcagagtgcctta	Non* (424 bp)	K2, M3, N1
c.2093T>C	rs397516130	0	**p.Val698Ala**	likely pathogenic (CM054010)	PM1, PM2, PP2, PP3, PP5	19. exon	tcagaacccagaacttcagt	aggaaaagcatcagaggagt	Alf I (518 bp)	H1
c.2155C>T	rs121913637	0	**p.Arg719Trp**	pathogenic (CM941086)	PM1, PM2, PM5, PP2, PP3, PP5	19. exon	tcagaacccagaacttcagt	aggaaaagcatcagaggagt	Non* (518 bp)	D4
c.2464A>C	rs730880742	0	**p.Met822Leu**	pathogenic (CM138708)	PS1, PM1, PM2, PM5, PP2, PP5	22. exon	tatgaacacactgcagtcac	tcctctgactgaaggaacaa	EcoR II (536 bp)	KY1
NM_000256.3 (*MYBPC3*)										
c.472G>A	rs3729986	33/1000	p.Val158Met	benign	PM1, PP2, BA1, BP4, BP6	4. exon	tgggtgacagagcaagac	ctctccatgtcccctctct	BspH I (442 bp)	AO1
c.706A>G	rs3729989	0.100/12114#	p.Ser236Gly	benign (CM043536)	PM1, PP2, BA1, BP4, BP6	6. exon	ggggattacaggcctgag	ctaccctgcaagccttgg	Bbv I (406 bp)	M3, Y1**, N1
c.786C>T	rs11570058	61/1000	p.Thr262Thr	benign	BA1, BP6, BP7	7. exon	gtcatgaatgggcaagtctg	cagtgctgggatttggag	Gsu I (651 bp)	M3, Y1**, N1
c.1573T>A	novel exonic	0	**p.Tyr525Asn**	likely pathogenic	PM1, PM2, PP2, PP3	17. exon	tccctctctctctccttt	caagccctaaagcctcatg	Non* (803 bp)	T1
c.*27-21G>A	novel intronic	0		vus	PM2, BP4	34. intron	gttccatgtttgtttccagc	agacattgtttcttgaggcc	Non* (852 bp)	T1
NM_000364.3 (*TNNT2*)										
c.304C>T	rs397516456	0.00000824/1#	**p.Arg102Trp**	pathogenic (CM971501)	PM1, PM2, PM5, PP2, PP3, PP5	9. exon	gtagagcataggtgggatgg	gagacctcacacctagctg	Bsr I (458 bp)	Y1
c.601-296C>T	rs374006913	2/1000		benign	BS1, BS2, BP4	12. intron	catgtctctccttgcctttg	ggaaaatatgtgaggcagtcc	Hae II (699 bp)	AO1

The bolds are missense pathogenic variants, MAF: Minor allele frequency of in-house data (or public data, #ExAC), HGMD: Human Genome Mutation Database, RE: the restriction enzyme used in RFLP-PCR method, Non*; confirmed by PCR and Sanger sequencing, FP: forward primer, RP: reverse primer, ** homozygote for related variant, the abbreviations is according to ACMG guideline (Richards *et al.* 2015), PM1; location in a mutational hot spot and/or critical functional domain, PM2; absent from control databanks, PM5; novel missense variant, BA1; allele frequency is greater than 5%, PP2; the relation with mechanism of disease for missense variant, PP3; multiple lines of computational evidence support a deleterious effect, PP5; reputable source recently reports variant as pathogenic, PS1; same amino acid change as a previously established pathogenic variant regardless of nucleotide change, BP4; impact on gene in computational analysis, BS1; allele frequency is greater than expected for disorder, BP6; in recently reports variant as benign, BP7; a silent variant, BS2; the observation in a healthy adult individual for a disorder with full penetrance expected at an early age.

Table 2: The clinical characteristics of index cases and variant carriers in HCM families.

IDs of cases	Current age or age of death, years	Age of diagnosis, years	Gender	Diagnosis	Syncope/ presyncope	Shortness of breath (NYHA)	Left atrium size (cm)	LV end-diastolic diameter (cm)	LV end-systolic diameter (cm)	LV maximal wall thickness (cm)	LV ejection fraction (%)
Y2	46	35	M	1	0	I	3.74	3.90	2.29	2.7	73
Y1	43	32	M	1	0	I	3.21	3.65	2.05	2.20	76
T1	79	67	F	1	0	II	5.05	4.18	2.41	2.86	74
T4	47	47	F	0	0	I	3.10	4.60	2.80	1.00	69
T5	43	43	M	1	0	I	3.58	4.78	2.70	1.60	75
KY1	45	31	F	2	0	I	5.9	4.94	2.70	2.18	78
KY2	24	24	M	0	0	I	2.80	4.20	2.50	1.00	65
KY4	56	42	M	2	0	I	4.40	3.60	1.40	2.32	92
KY8	26	26	M	2	0	I	4.20	3.60	1.50	2.26	88
KY9	20	20	F	2	0	I	4.30	4.40	2.40	2.24	67
KY10	18	18	M	2	0	I	4.20	4.60	2.80	2.40	69
KY12	40	40	M	0	0	I	3.50	4.90	3.20	1.10	65
KY13	20	20	M	0	0	I	3.10	4.80	3.00	1.10	66
KY15	15	15	M	0	0	I	2.80	3.70	2.30	1.10	70
KY16	41	41	M	1	0	I	4.30	3.70	2.00	1.80	80
D4	32	22	M	1	0	I	4.10	5.04	3.43	2.81	68
D1	67	57	M	1	0	I	4.30	5.00	3.30	2.30	65
H1	59	39	F	1	0	II	4.80	4.70	2.60	2.39	75
H3	32	20	F	1	0	I	2.77	4.25	2.14	2.34	87
N1	55	45	M	1	1	II	4.60	4.70	3.40	2.50	55
M3	27	19	M	1	0	I	4.14	4.90	3.27	1.73	62
M1	44	39	F	1	0	I	4.35	4.47	2.41	2.37	77
K2	17	16	M	2	0	I	3.61	3.10	1.07	2.10	93
K1	42	42	M	1	0	I	3.70	4.05	2.45	2.40	71
HT1	54	54	M	3	1	I	4.60	4.14	1.70	2.20	89
A1	28	28	M	1	1	II	4.88	4.49	2.78	3.30	69
TK1	54	54	M	1	0	I	4.31	3.90	2.55	1.90	66
AO1	66	57	F	2	0	III	5.66	3.06	2.29	2.13	52

Diagnosis groups; 1, nonobstructive asymmetric septal hypertrophy; 2, obstructive asymmetric septal hypertrophy; 3, nonobstructive concentric hypertrophy, the bolds are index cases.

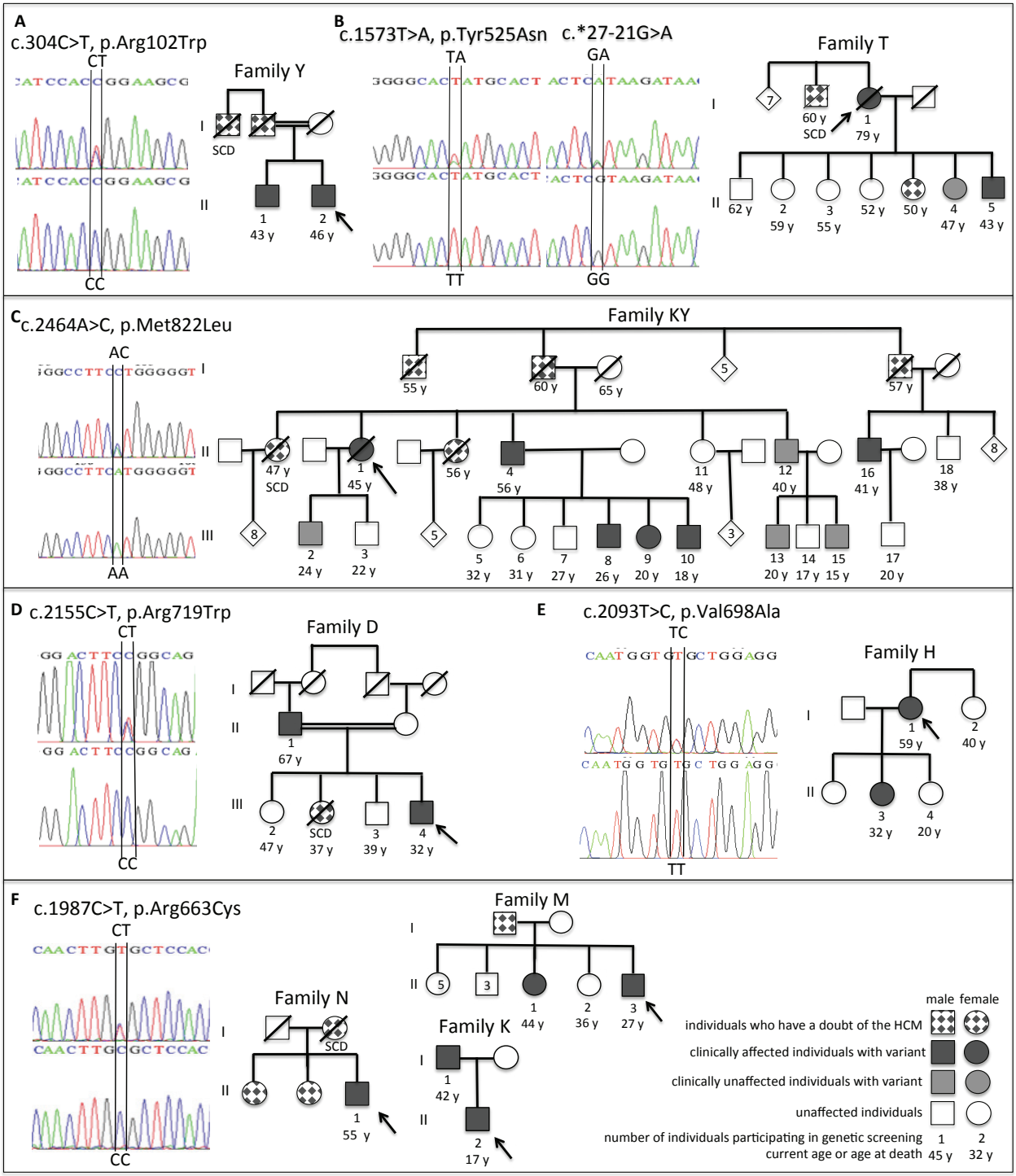

Figure 1: The family screening of the identified clinical significance variants in the *TNNT2* (A), *MYBPC3* (B) and *MYH7* (C-F) genes. Index cases are indicated with arrows.

base and also in public databases (ExAC) (Table 1). The pathogenic variants causing amino acid substitutions and two novel intronic variants (c.639+32G>T in *MYH7* and c.*27-21G>A in *MYBPC3*) had not been previously identified in this Turkish in-house database. The main clinical data of the 12 index cases and other clinically diagnosed family members with HCM is summarised in Table 2.

The pathogenic variants of *TNNT2* and *MYBPC3* genes

The previously described Arg102Trp missense variant (also called Arg92Trp) in the *TNNT2* gene was found in two brothers with HCM and a positive family history for SCD in Family Y with consanguineous marriage (Figure 1A). The uncle of the index case had SCD at a young age without clinical diagnosis.

Two novel p.Tyr525Asn and c.*27-21G>A compound heterozygous variants in the *MYBPC3* gene were identified in another Turkish family with HCM and a positive family history for SCD (Figure 1B). The c.*27-21G>A variant in *MYBPC3* was segregated with a novel Tyr525Asn missense variant in Family T. A genetic analysis could not be performed in a member of Family T with SCD at 60 years old. Index case (T1), with compound heterozygous variants, had mild clinical HCM and possible late onset hypertrophy. Cases T4 and T5 had these compound variants but only T5 had HCM, and T4 with a normal echocardiography (Table 2). Cases T2 and T3 had no variant with normal echocardiographic findings (Figure 1B).

The pathogenic variants of *MYH7* gene

The other four variants causing amino acid substitutions in the *MYH7* gene were previously reported missense pathogenic variants in patients with HCM according to variant databases. The Met822Leu variant in exon 22 of the *MYH7* gene was screened in 18 members of Family KY with a positive family history for SCD (Figure 1C). Ten family members were found to have this variant, out of which 6 had severe cardiac hypertrophy. Other 4 asymptomatic pathogenic variant carriers (called as KY2, KY12, KY13 and KY15) had normal echocardiographic findings. The unaffected family members without this variant had no cardiac disease. However, asymptomatic case KY12 and his two sons (called KY13 and KY15) had this variant. The Met822Leu variant site was conserved among the genomes of 35 mammals (GERP score; 4.6), and was determined as disease causing *in silico* tools (MutationTaster, FATHMM and SIFT).

The Arg719Trp variant in the *MYH7* gene was determined in an index case with cardiac defibrillator (implanted for documented ventricular tachycardia) in Family D (Figure 1D). Although, this pathogenic variant seems to be associated with a positive family history for SCD (sister with SCD at age 37 without documentation of HCM), the father (HCM documented) of the index case had a good prognosis without cardiac events.

The Val698Ala pathogenic variant was found to be related with severe cardiac hypertrophy in Family H (Figure 1E). However, there was no SCD history in this family.

The Arg663Cys pathogenic variant was identified in index cases of three unrelated families (Figure 1F). These three index cases (N1, K2 and M3) also have additional individual variants (Table 1). The HCM diagnosis age of this variant carriers (n=4) was less than 42 years old in Family K and Family M. This variant was also found in the index case (N1) of Family N (mother with SCD without HCM diagnosis). The cases of two other families (Family M and Family K) without a history of SCD had mild clinical forms of HCM. The clinical characteristics of the cases diagnosed HCM that were called N1, M1, M3, K1 and K2 in these families are shown in Table 2.

In silico analysis of novel intronic variants

Two novel clinically uncertain significance variants in the intron 7 of *MYH7* (c.639+32G>T) and in the intron 34 of *MYBPC3* (c.*27-21G>A) were identified in two HCM patients with a family history for SCD (TK1 and T1 called index cases, respectively). The conservation score among the genomes of 35 mammals were calculated as 3.51 for c.639+32G>T and 4.75 for c.*27-21G>A in the GERP *in silico* tool. It was observed that these variants were preserved evolutionarily. The splicing effect of these variants was investigated with *in silico* analysis using the Alamut software, which integrates several prediction tools (SpliceSiteFinder-like, NNSPLICE, GeneSplicer, Human Splicing Finder, MaxEntScan) and also SR proteins binding site prediction tools (ESEfinder and RESQUE-ESE). In exogenous splice enhancers (ESEs) site tools, these intronic variants have been found to alter motif scores for binding sites of SR proteins (Figure 2A and 2B). The splice site tools of Alamut predicted that the donor splice site scores of c.*27-21G>A variant had disappeared (Figure 2C). However, there was no difference in the splice site scores in the c.639+32G>T variant (data not shown). The association of the c.639+32G>T variant with HCM could not be determined precisely in TK1 because other family members were not available.

The polymorphic variants of *TNNT2, MYBPC3* and *MYH7* genes

The clinically benign variants with the exception of pathogenic exonic and two novel intronic variants were detected in index cases. The information of the gene localization, the effects on codon and allele frequencies of these SNVs are given in Table 1.

This polymorphic variants in index cases were defined according to ACMG (14) (Table 1).

The Ser236Gly missense variant in the *MYBPC3* gene, previously associated with HCM and also available in the Human Genome Mutation Database (HGMD) was identified in three index cases called M3, Y1 and N1.

DISCUSSION

In this study, disease-causing variants in three HCM-associated sarcomeric genes (*MYH7, MYBPC3* and *TNNT2*) were identified in 67% of the Turkish families with HCM

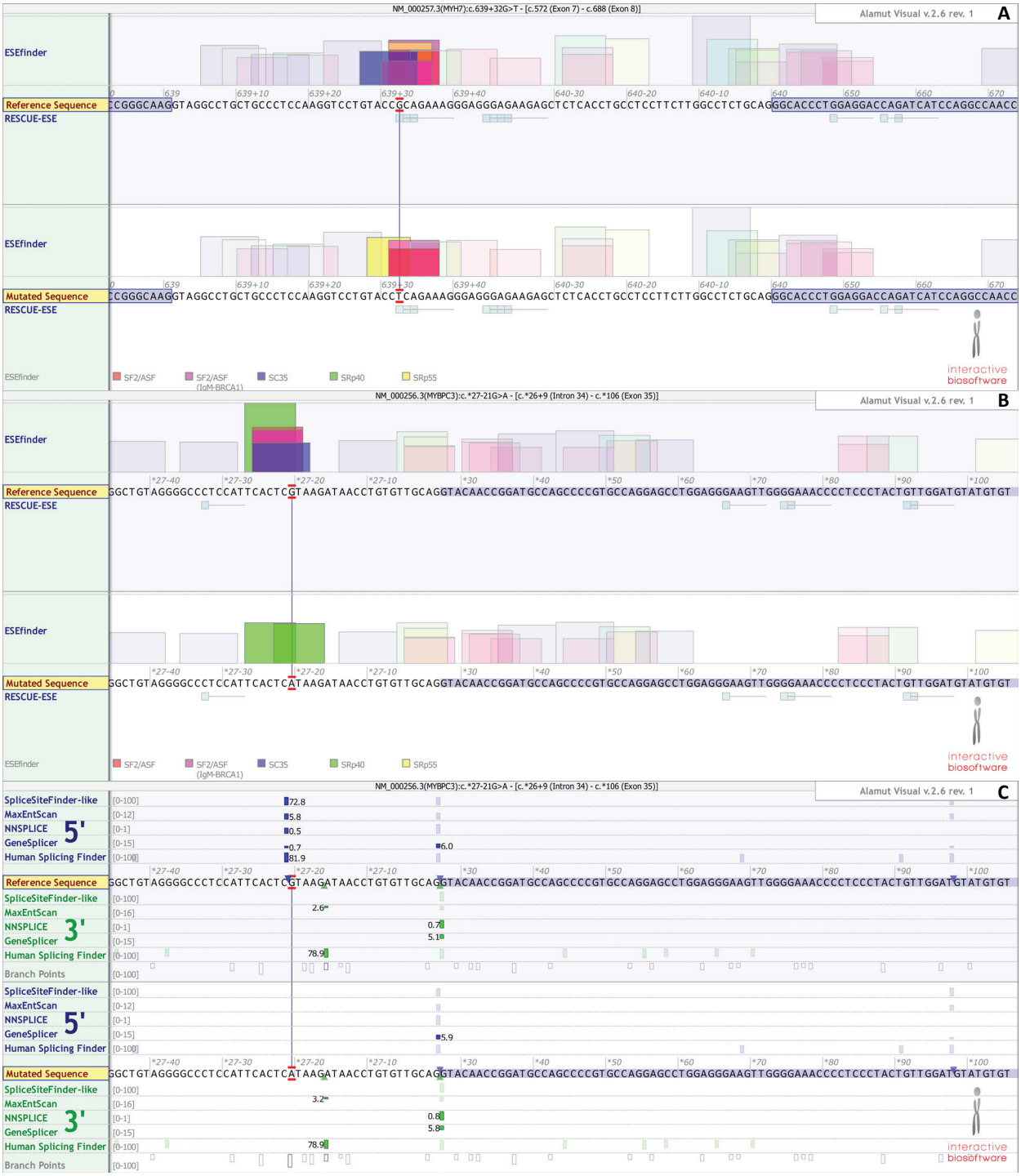

Figure 2: *MYH7* c.639+32G>T and *MYBPC3* c.*27-21G>A variants with potential effect on the exonic splicing enhancers (ESE) binding site (A and B) and the splicing site (C) as predicted using the Alamut software. The exonic sequences are shown as blue boxes. The splicing site scores from each mutation prediction tool are displayed in blue vertical bars for the 5' (donor) sites, and as green vertical bars for the 3' (acceptor) sites.

using sequence-based re-sequencing. The association between these pathogenic variants and SCD risk in familial HCM was also investigated with a retrospective clinical analysis. To date, there is no population-based study to demonstrate the prevalence of hypertrophic cardiomyopathy (HCM) and no large-scale mutation screening

studies for HCM in Turkey. In previous studies, reported mutations of the *MYH7* gene have been demonstrated in Turkish HCM patients (13, 14). In the study performed by Kucukates et al., the Arg403Gln missense mutation in *MYH7* was observed in 8 of 32 cases in 3 families (13). In another study, previously reported four mutations in *MYH7* were not found in 18 HCM patients (14). A novel insertion mutation in *MYBPC3* was detected in a Turkish family with HCM in the mutation screening of the *MYBPC3* and *MYH7* genes using PCR and Sanger sequencing (15). In addition to pathogenic variants, the identification of functional variants may be important for the modifier or founder effect (16) of variants in the disease phenotype. Therefore, in this study, all individual variants of the index cases were presented, but the population-specific effects of these variants in larger Turkish case-control studies could not be investigated.

The autosomal dominant inherited HCM is a complex disease that is characterized by an heterogenous clinical and genetic expression. It is caused primarily by missense mutations, although causative nonsense, frame-shift, and in-frame insertion/deletion mutations have also been observed, particularly in sarcomeric genes (3, 17). As a matter of fact, we have identified missense pathogenic variants in this study. According to the HGMD public database and Pubmed screening with a specific keyword, the Met822Leu missense variant in the *MYH7* gene has not been reported previously. However, we found one publication (18) that identified this pathogenic variant with the Alamut software and VarSome, and also another publication (19) that claims to identify the same variant for the first time in the HGMD Professional database. Since the clinical details of variant carriers were not mentioned in these studies, we could not compare them with the findings of our patients. Therefore, further experimental studies should be conducted to demonstrate the clinical significance and the association of this variant with SCD.

In our study, two novel SNVs in the intron 7 of *MYH7* (c.639+32G>T) and in the intron 34 of *MYBPC3* (c.*27-21G>A) were determined. These variants were analysed to determine possible-binding sites of ESEs for splice-site recognition and for serine/arginine proteins (20) using the Alamut software. *In silico* analysis predicted that c.*27-21G>A variant has a possible impact on mRNA splicing but no effect for the c.639+32G>T variant. These results indicate that *in vitro* studies are required to study the possible impacts of intronic variants on mRNA splicing and expression. However, in this study, the possible effects of these novel defined variants could not be supported by *in vitro* functional studies.

Furthermore, we identified one novel missense variant, in exon 17 of the *MYBPC3* gene (Tyr525Asn). So far, the different mutations related with HCM were reported in the same codon as the Tyr525His (21), the Tyr525Ser (22) and the Tyr525Term (23). The cases with a mild HCM form carrying both Tyr525Asn and c.*27-21G>A variants as compound heterozygote had late-observed SCD in Family T. This intronic variant in the 3'-UTR of the *MYBPC3* transcript suggests that it may affect the mutant allele expression.

In a previous study, similar to our results with family D, it was determined that the *MYH7* Arg719Trp mutation in four families increased the risk of death associated with HCM and caused the malignant phenotype (24). The Val698Ala mutation was firstly identified in Australian proband with hypertrophic cardiomyopathy but there is no clinical information for comparison to our patients (25). The Arg663Cys mutation was described as a "hot spot", and identified nine of the 58 HCM patients (26). This present finding was similar to our results. Indeed, in this study, we found this pathogenic variant in three of the 12 index patients who were not related to each other. In addition, we determined that these three cases had other individual variants besides the main pathogenic variant, and the risk of SCD and cardiac symptoms seems to be different among these three families. These additional polymorphic individual variants might be causing different functional effects on cardiomyocytes with a pathogenic sarcomeric protein.

In our study, the pathogenic variants were not detected in 4 of 12 patients with familial HCM, possibly because disease-causing variants can be found in other HCM-associated genes. In NGS studies applied in recent years, it has been determined that pathogenic variants in both the exonic and intronic regions of more than 40 candidate genes were found to cause the HCM phenotype (3, 8-10). The use of next generation sequencing for the diagnosis of multigenic diseases such as HCM appears to be advantageous to identify all variants. However, as the number of sequenced genes increases, variant analysis becomes difficult, so firstly, candidate genes with a high mutation detection rate can be searched. It is known that 53-85% of HCM is caused by pathogenic variants in the *MYH7, MYBPC3* and *TNNT2* genes (2, 3, 11). The molecular diagnosis rate of this study was 66%, as in the expected range. As supported by this study's results, a positive family history affects the genetic diagnosis rate of HCM.

In recent studies, as in this study, it has not been confirmed that there is a clear prognostic association between the pathogenic variants and the risk of SCD in HCM (3, 17). Prospective clinical follow-up of these patients with variants will clarify the prognostic importance of these findings. Future advances in genotype-based risk stratification are expected to shed light on the management of these patients, particularly in terms of sudden cardiac death (3).

Genetic testing in HCM patients is mainly used for the identification of family members at risk of the disease and for genetic counselling (27). Although the mutation-screening strategy in families with HCM is cost-effective (28), the issue still needs to be further evaluated for reproduction, competitive sports and professional career counselling. As a result, despite the fact that most HCM patients have mild clinical symptoms, some patients may suffer from SCD and end-stage heart failure. Therefore, it is important to define the individual variants for a strong genotype-phenotype correlation of HCM observed incomplete penetrance and variable expressivity. On the other hand, experimental studies using approaches such as gene-editing and allele-specific RNA silencing on HCM treatment in recent years are promising (29, 30).

In conclusion, novel variants related with familial HCM and SCD history were identified but the association of these pathogenic variants and SCD was not clear. Variants rather helped other family members to get a clinical diagnosis. Determination of all individual variants in HCM-related genes may be important to demonstrate the inter variant effects on the clinical and structural differences of HCM families with the same pathogenic variant.

Acknowledgments: We would like to thank TUBITAK-IGBAM for in-house database screening.

Ethics Committee Approval: This study was approved by the Ethical Committee of the Istanbul University School of Medicine (11.02.2011/315).

Informed Consent: Written consent was obtained from the participants.

Peer Review: Externally peer-reviewed.

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

Conflict of Interest: Authors declared no conflict of interest.

Financial Disclosure: This study was supported by Turkish Society of Cardiology (Project No: 2013/2) and the Scientific Research Projects Coordination Unit of Istanbul University (Project numbers: 42173 and 53021).

Teşekkür: Kurum için veri tabanı taraması için TUBİTAK-İGBAM'a teşekkür ederiz.

Etik Komite Onayı: Bu çalışma için etik komite onayı İstanbul Üniversitesi Tıp Fakültesi Etik Kurulu'ndan alınmıştır (11.02.2011/315).

Bilgilendirilmiş Onam: Katılımcılardan bilgilendirilmiş onam alınmıştır.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Çalışma Konsepti/Tasarım- E.K.B., F.B.; Veri Toplama- F.G.G., G.K.; Veri Analizi/Yorumlama- E.K.B., F.B.; Yazı Taslağı- E.K.B.; İçeriğin Eleştirel İncelemesi- F.B.; Son Onay ve Sorumluluk- E.K.B., F.G.G., G.K., F.B.

Çıkar Çatışması: Yazarlar çıkar çatışması beyan etmemişlerdir.

Finansal Destek: Bu çalışma Türk Kardiyoloji Derneği (Proje No: 2013/2) ve İstanbul Üniversitesi Bilimsel Araştırma Projeleri Koordinasyon Birimi (Proje No: 42173 ve 53021) tarafından desteklenmiştir.

REFERENCES

1. Maron BJ, Gardin JM, Flack JM, Gidding SS, Kurosaki TT, Bild DE. Prevalence of hypertrophic cardiomyopathy in a general population of young adults. Echocardiographic analysis of 4111 subjects in the CARDIA Study. Coronary Artery Risk Development in (Young) Adults. Circulation 1995;92:785-9. [CrossRef]
2. Maron BJ, Maron MS, Semsarian C. Genetics of hypertrophic cardiomyopathy after 20 years: clinical perspectives. J Am Coll Cardiol. 2012;60(8):705-15. [CrossRef]
3. Jordà P, García-Álvarez A. Hypertrophic cardiomyopathy: Sudden cardiac death risk stratification in adults. Glob Cardiol Sci Pract. 2018;2018(3):25. [CrossRef]
4. Jarcho JA, McKenna W, Pare JA, Solomon SD, Holcombe RF, Dickie S, et al. Mapping a gene for familial hypertrophic cardiomyopathy to chromosome 14q1. N Engl J Med 1989;321:1372-8. [CrossRef]
5. Rodríguez JE, McCudden CR, Willis MS. Familial hypertrophic cardiomyopathy: basic concepts and future molecular diagnostics. Clin Biochem 2009;42(9):755-65. [CrossRef]
6. Meder B, Haas J, Keller A, Heid C, Just S, Borries A, et al. Targeted next-generation sequencing for the molecular genetic diagnostics of cardiomyopathies. Circ Cardiovasc Genet. 2011;4(2):110-22. [CrossRef]
7. Millat G, Chanavat V, Rousson R. Evaluation of a new NGS method based on a custom AmpliSeq library and Ion Torrent PGM sequencing for the fast detection of genetic variations in cardiomyopathies. Clin Chim Acta 2014;433:266-71. [CrossRef]
8. Waldmüller S, Schroeder C, Sturm M, Scheffold T, Imbrich K, Junker S, et al. Targeted 46-gene and clinical exome sequencing for mutations causing cardiomyopathies. Mol Cell Probes 2015; 29:308-14. [CrossRef]
9. Mendes de Almeida R, Tavares J, Martins S, Carvalho T, Enguita FJ, Brito D, et al. Whole gene sequencing identifies deep-intronic variants with potential functional impact in patients with hypertrophic cardiomyopathy. PLoS One 2017;12(8):e0182946. [CrossRef]
10. Bottillo I, D'Angelantonio D, Caputo V, Paiardini A, Lipari M, De Bernardo C, et al. Molecular analysis of sarcomeric and non-sarcomeric genes in patients with hypertrophic cardiomyopathy. Gene 2016;577:227-35. [CrossRef]
11. Ho CY, Charron P, Richard P, Girolami F, Van Spaendonck-Zwarts KY, Pinto Y. Genetic advances in sarcomeric cardiomyopathies: state of the art. Cardiovasc Res 2015;105:397-408. [CrossRef]

12. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405-24. [CrossRef]
13. Kucukkates E, Ersanli M, Gultekin N, Sayhan N, Mutlu H, Kalfoglu E, et al. Clinical Investigations 403Arg-Gln Missence Point Mutation of ß-Myosin-Heavy-Chain in Hypertrophic Cardiomyopathy Families in a Diverse Turkish Population and its Relation with Sudden Cardiac Death. Turk Kardiyol Dern Ars 1999;27:664-71.
14. Cam FS, Terzioglu O, Sagan A, Nalbantgil I, Ozerkan F, Ozdamar M, et al. Detection of Arg403Gln, Arg453Cys, Arg719Trp and Arg719Gln Mutations in the ß-Myosin Heavy Chain Gene (ß-Mhc) Causing to Hypertrophic Cardiomyopathy. Turk Kardiyol Dern Ars 2002;30:30-5.
15. Hallioglu Kilinc O, Giray D, Bisgin A, Tug Bozdogan S, Karpuz D. Familial hypertrophic cardiomyopathy: A case with a new mutation in the MYBPC3 gene. Turk Kardiyol Dern Ars 2017;45:450-3. [CrossRef]
16. Christiaans I, Nannenberg EA, Dooijes D, Jongbloed RJ, Michels M, Postema PG, et al. Founder mutations in hypertrophic cardiomyopathy patients in the Netherlands. Neth Heart J 2010;18(5):248-54. [CrossRef]
17. Landstrom AP, Ackerman MJ. Mutation type is not clinically useful in predicting prognosis in hypertrophic cardiomyopathy. Circulation 2010;122(23):2441-9; discussion 2450. [CrossRef]
18. Mohiddin SA, Begley DA, McLam E, Cardoso JP, Winkler JB, Sellers JR, Fananapazir L. Utility of genetic screening in hypertrophic cardiomyopathy: prevalence and significance of novel and double (homozygous and heterozygous) beta-myosin mutations. Genet Test 2003;7(1):21-7. [CrossRef]
19. Fujino N, Konno T, Hayashi K, Hodatsu A, Fujita T, Tsuda T, et al. Impact of systolic dysfunction in genotyped hypertrophic cardiomyopathy. Clin Cardiol 2013;36(3):160-5. [CrossRef]
20. Wang J, Smith PJ, Krainer AR, Zhang MQ. Distribution of SR protein exonic splicing enhancer motifs in human protein-coding genes. Nucleic Acids Res 2005;33(16):5053-62. [CrossRef]
21. Lopes LR, Zekavati A, Syrris P, Hubank M, Giambartolomei C, Dalageorgou C, et al. Genetic complexity in hypertrophic cardiomyopathy revealed by high-throughput sequencing. J Med Genet 2013;50(4):228-39. [CrossRef]
22. Girolami F, Olivotto I, Passerini I, Zachara E, Nistri S, Re F, et al. A molecular screening strategy based on beta-myosin heavy chain, cardiac myosin binding protein C and troponin T genes in Italian patients with hypertrophic cardiomyopathy. J Cardiovasc Med (Hagerstown) 2006;7(8):601-7. [CrossRef]
23. Bortot B, Athanasakis E, Brun F, Rizzotti D, Mestroni L, Sinagra G, Severini GM. High-throughput genotyping robot-assisted method for mutation detection in patients with hypertrophic cardiomyopathy. Diagn Mol Pathol 2011;20(3):175-9. [CrossRef]
24. Anan R, Greve G, Thierfelder L, Watkins H, McKenna WJ, Solomon S, Vecchio C, Shono H, et al. Prognostic implications of novel beta cardiac myosin heavy chain gene mutations that cause familial hypertrophic cardiomyopathy. J Clin Invest 1994;93(1):280-5. [CrossRef]
25. Ingles J, Doolan A, Chiu C, Seidman J, Seidman C, Semsarian C. Compound and double mutations in patients with hypertrophic cardiomyopathy: implications for genetic testing and counselling. J Med Genet 2005;42(10):e59. [CrossRef]
26. Van Driest SL, Jaeger MA, Ommen SR, Will ML, Gersh BJ, Tajik AJ, Ackerman MJ. Comprehensive analysis of the beta-myosin heavy chain gene in 389 unrelated patients with hypertrophic cardiomyopathy. J Am Coll Cardiol 2004;44(3):602-10. [CrossRef]
27. Restrepo-Cordoba AM, Campuzano O, Ripoll-Vera T, Cobo-Marcos M, Mademont-Soler I, Gámez JM, et al. Usefulness of Genetic Testing in Hypertrophic Cardiomyopathy: an Analysis Using Real-World Data. J Cardiovasc Transl Res 2017;10(1):35-46. [CrossRef]
28. Ingles J, McGaughran J, Scuffham PA, Atherton J, Semsarian C. A cost-effectiveness model of genetic testing for the evaluation of families with hypertrophic cardiomyopathy. Heart 2012;98(8):625-30. [CrossRef]
29. Jiang J, Wakimoto H, Seidman JG, Seidman CE. Allele-specific silencing of mutant Myh6 transcripts in mice suppresses hypertrophic cardiomyopathy. Science 2013;342(6154):111-4. [CrossRef]
30. Ma H, Marti-Gutierrez N, Park SW, Wu J, Lee Y, Suzuki K, et al. Correction of a pathogenic gene mutation in human embryos. Nature 2017;548(7668):413-9. [CrossRef]

İSTANBUL ÜNİVERSİTESİ YAYINEVİ

RESEARCH / ARAŞTIRMA
DOI: 10.26650/IUITFD.2019.0082
İst Tıp Fak Derg 2020 / J Ist Faculty Med 2020

VARIABLE ANATOMY OF THE INFRATROCHLEAR AND SUPRATROCHLEAR TRIANGLES AND THEIR MICRONEUROSURGICAL IMPORTANCE

İNFRATROKLEAR VE SUPRATROKLEAR ÜÇGENLERİN VARYATİF ANATOMİSİ VE MİKRONÖROCERRAHİ ÖNEMİ

İlke Ali GÜRSES[1], Osman COŞKUN[1], Özcan GAYRETLİ[1], Ayşin KALE[1], Adnan ÖZTÜRK[1]

[1]Istanbul University, Istanbul Faculty of Medicine, Department of Anatomy, Istanbul, Turkey

ORCID IDs of the authors: İ.A.G. 0000-0001-9188-4662; O.C. 0000-0002-0337-4927; Ö.G. 0000-0001-7958-3170; A.K. 0000-0002-2305-420X; A.Ö. 0000-0002-5819-0543

Cite this article as: Gurses IA, Coskun O, Gayretli O, Kale A, Ozturk A. Variable anatomy of the infratrochlear and supratrochlear triangles and their microneurosurgical importance. J Ist Faculty Med 2020;83(4):355-62. doi: 10.26650/IUITFD.2019.0082

ABSTRACT

Objective: Supratrochlear and infratrochlear triangles are surgical corridors for approaching the lateral wall of the cavernous sinus. The literature provides conflicting results for the morphology and morphometry of these triangles. Additionally, the possible effects of vascular structures that drain into the cavernous sinus in unknown. This study aimed to investigate the morphology, morphometry, and vascular relationships of the supratrochlear and infratrochlear triangles of the cavernous sinus.

Material and Method: Cranial bases of 25 cadavers were dissected bilaterally under a surgical microscope. Five of the cadavers were injected with colored silicone for vascular evaluation. The morphology of supratrochlear and infratrochlear triangles were classified according to the course of the trochlear nerve. Photogrametric measurements were used for evaluating the areas of both triangles with the ImageJ software. The triangular morphology was also investigated in regard to the drainage patterns of the superficial middle cerebral vein and cranial base dural sinuses.

Results: Type A, B, C, and D triangle morphology was present on 23 (46%), 9 (18%), 10 (20%), and 8 (16%) sides, respectively. The average areas for supratrochlear and infratrochlear triangles were 22.2 (±11.7) mm^2 and 78.4 (±27.7) mm^2, respectively. The supratrochlear triangle was significantly larger in Type D triangles. On 71.4% of injected specimens, the superior petrosal sinus contributed the cavernous sinus and formed a Type A triangle.

Conclusion: The anatomy of the supratrochlear and infratrochlear triangles are highly variable than previously reported. Introducing the knowledge regarding these variations to neurosurgical residency education programs and daily surgical practice could be valuable.

Keywords: Cavernous sinus, supratrochlear triangle, infratrochlear triangle

ÖZET

Amaç: Supratroklear ve infratroklear üçgenler, sinus cavernosus'un lateral duvarına ulaşmak için kullanılabilecek cerrahi koridorlardır. Literatürde bu üçgenlerin morfolojisi ve morfometrisi hakkında çelişkili bilgiler bulunmaktadır. Ayrıca, sinus cavernosus'a drene olan vasküler yapıların anatomiye olan etkileri bilinmemektedir. Bu çalışma sinus cavernosus üçgenlerinde olan supratroklear ve infratroklear üçgenlerin morfolojisini, morfometrisini ve vasküler komşuluklarını incelemeyi amaçlamıştır.

Gereç ve Yöntem: Yirmibeş kadavranın kafa tabanı cerrahi mikroskop altında bilateral olarak disseke edildi. Vasküler yapıların değerlendirilmesi amacıyla 5 kadavrada renkli silikon enjeksiyonu yapıldı. Supratroklear ve infratroklear üçgen morfolojisi, n. trochlearis'in uzanımına göre sınıflandırıldı. Her iki üçgenin alanları, ImageJ yazılımı kullanılarak fotogrametrik yöntem ile ölçüldü. Üçgenlerin morfolojisi ile v. cerebri media superficialis ve kafa tabanı dural sinüslerinin drenaj paterni karşılaştırıldı.

Bulgular: Tip A, B, C ve D üçgen morfolojisi sırasıyla 23 (%46), 9 (%18), 10 (%20) ve 8 (%16) tarafta gözlemlendi. Supratroklear ve infratroklear üçgenlerin ortalama alanları 22,2 (±11,7) mm^2 ve 78,4 (±27,7) mm^2 ölçüldü. Tip D üçgenlerde supratroklear üçgenin daha büyük olduğu görüldü. İnjeksiyon yapılan örneklerin %71,4'ünde sinus petrosus superior, sinus cavernosus'a drene olmakta ve belirgin Tip A üçgen meydana getirmekteydi.

Sonuç: Supratroklear ve infratroklear üçgenler, literatürde raporlandığının aksine çok varyatifti. Bu çeşitlilik bilgisinin beyin cerrahisi uzmanlık eğitimi ve günlük cerrahi uygulamaya aktarılması düşünülebilir.

Anahtar Kelimeler: Sinus cavernosus, supratroklear üçgen, infratroklear üçgen

Corresponding author/İletişim kurulacak yazar: iagurses@gmail.com

Submitted/Başvuru: 27.09.2019 • **Revision Requested/Revizyon Talebi:** 24.03.2020 •
Last Revision Received/Son Revizyon: 16.04.2020 • **Accepted/Kabul:** 20.06.2020 • **Published Online/Online Yayın:** 05.10.2020

INTRODUCTION

The oculomotor (CN-III), trochlear (CN-IV), and ophthalmic (CN-V1) nerves confine two triangular surgical corridors at the lateral wall of the cavernous sinus (CS) (1). The supratrochlear (STT) and infratrochlear triangles (ITT), which are named after their relative location to the CN-IV, are commonly used in lateral approaches to the CS (1-6).

Although the anatomy and morphometry of the ITT, also known as the Parkinson's triangle, had attracted many researchers (2, 7-13), morphologic studies that investigated both triangles are limited (9-11). The CN-IV constitutes borders for both triangles, therefore it is the major anatomical structure that affects the morphology. Lang and Reiter (10) classified the morphological patterns of these triangles according to the course of the CN-IV and found considerable variability (Figure 1). Studies that investigated the morphology of both triangles on different populations provide variable frequencies for morphological types, which could be attributed to ethnicity (9-11). Additionally, some studies that evaluated the morphometry of both triangles provide very similar averages for STT and ITT areas with little variability (2, 13). Apparently, there is a paucity of information on the morphology and morphometry of both triangles.

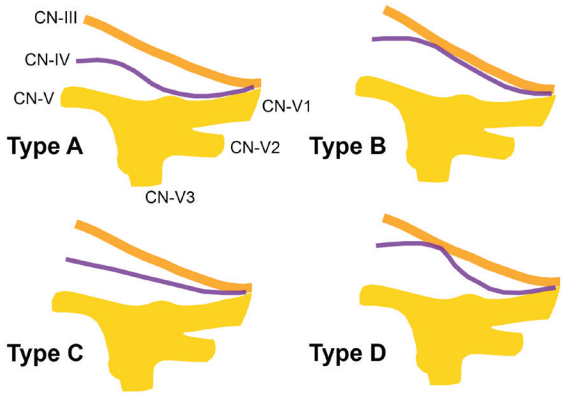

Figure 1: Schematic illustration depicting morphological types of supratrochlear and infratrochlear triangles. CN-III: Oculomotor nerve, CN-IV: Trochlear nerve, CN-V: Trigeminal nerve, CN-V1: Ophthalmic nerve, CN-V2: Maxillary nerve, CN-V3: Mandibular nerve. The figure is based on the classification of Lang and Reiter (10).

Additionally, it is well known that the CS is also a drainage point for the superficial middle cerebral vein (SMCV) (14, 15). Despite the anatomic detail provided on the STT and ITT in previous studies (2, 7-13), triangle anatomy in different venous patterns was not studied.

Therefore, this study was aimed to investigate the morphology and morphometry of the STT and ITT and to outline the relationships between these triangles and adjacent venous structures.

MATERIAL AND METHOD

Specimens and vascular injections

After ethical committee approval (date: 10.01.2018, number: 11), the morphology and morphometry of the STT and ITT were evaluated on 8 female and 17 male (total 25) cadavers. Sixteen cadavers were donors and 9 were of unclaimed origin. All specimens were obtained from the Department of Anatomy of Istanbul Faculty of Medicine either through the Body Donation Program of the department or legally procured under Act 2238 (16). All cadavers were preserved with a formalin-ethanol-glycerine-phenol solution and kept in cold storage (5-8 °C) after embalming. Average age at death was 60.9 (ranged between 26 and 85 years).

Among the sample, 5 cadavers were injected with colored silicone into the arteries (red) and veins (blue) according to the protocol reported by Sanan et al. (17) for evaluating the vascular anatomy of the CS.

Dissection protocol and morphological evaluation

All dissections were performed under 6x to 25x magnification of a surgical microscope (D.F. Vasconcellos S.A., M 1222, Sao Paolo, Brazil). After removing the calvaria with an oscillating saw, the cerebrum was removed with great care and the dura covering the CS was kept intact. Cranial nerves from two through to six were cut at subarachnoid level and their dural entry points were preserved. For vascular evaluation, the bridging veins connecting the SMCV to the CS were cut after identification of that given vein. In most of the cases, the anterior clinoid process was drilled in order to evaluate anterior sections of both triangles. Finally, outer dura covering the CS was dissected with care. Dissection of the inner dural layer was only preferred if exposure of cranial nerves were not adequate. Dural entry points of CN-III, CN-IV, and CN-V1 to the lateral wall of the CS were preserved for morphometric evaluation.

The morphology of the STT and ITT was classified according to Lang and Reiter (10). In Type A, the CN-IV had a curved course at the CS and formed two ill defined triangles. In Type B, the CN-IV approximates superiorly to the CN-III and the STT was smaller than the ITT. In Type C, the CN-IV had a linear course at the CS and formed two well defined triangles. In Type D, the CN-IV was close to the CN-III proximally and to the CN-V1 distally. In this type, the STT had a bow shape, rather than a triangle (Figure 1).

Drainage pattern of the SMCV was evaluated according to the classification of Suzuki and Matsumoto (15) as sphenoparietal, cavernous, emissary, superior petrosal, basal, squamosal, and combined (Figure 2).

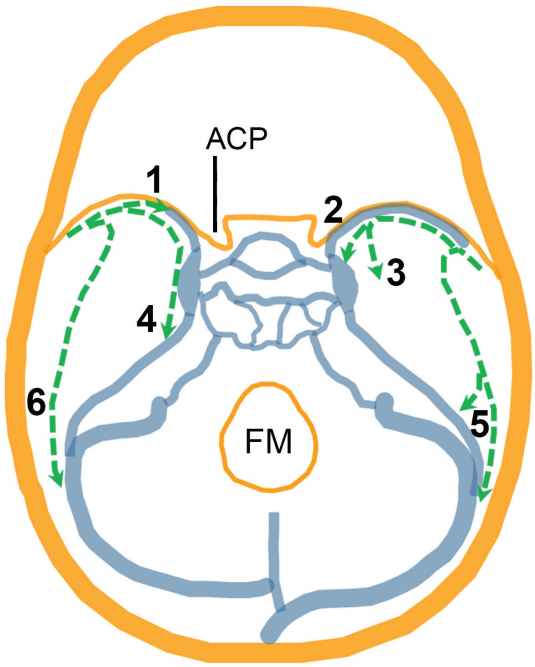

Figure 2: Schematic illustration of the cranial base summarizing the drainage patterns of the SMCV. Dotted green lines outline variated course of the SMCV. ACP: Anterior clinoid process, FM: Foramen magnum. The SMCV courses medial to the oval foramen and can drain into the sphenoparietal (1), cavernous (2), and the superior petrosal (4) sinuses directly. In emissary pattern (3), the SMCV drains into the emissary veins around the oval foramen. The SMCV can pass lateral to the oval foramen at the base of the middle cerebral fossa and drain into either the superior petrosal or the transverse sinuses in basal pattern (5). In squamous (6) pattern, the SMCV passes more laterally at the squama temporalis and drains into the transverse sinus. The illustration is based on the classification of Suzuki and Matsumoto (15).

Measurements and statistical analysis

Morphometry of the triangles were evaluated with photogrametry. Digital photographs of the CSs were obtained from a distance of 20 cm for each specimen. A tape measure of 10 mm was placed in every CS for calibration of the image with an editing software. ImageJ (v1.46r, 2013, NIH, USA) (18) software was used to measure the areas of both triangles (Figure 3).

The data obtained from ImageJ was transferred to an excel file and statistical analysis was performed with SPSS v. 21.0 (IBM SPSS Statistics for Windows, NY; IBM Corp., 2012). Morphometrical differences between sexes, sides, and morphological triangle types were evaluated with Mann-Whitney U test. Values with $p<0.05$ was considered as statistically significant.

RESULTS

Morphology and morphometry

Type A, B, C, and D triangles were observed on 23 (46%), 9 (18%), 10 (20%), and 8 (16%) sides, respectively (Figure 4) (Table 1).

The average areas for STT and ITT were 22.2 (±11.7) mm^2 and 78.4 (±27.7) mm^2, respectively. The differences for both triangles among sexes and sides were not statistically significant (Table 2). For STT, Type D triangles had a statistically larger area compared to Types A ($p=0.03$), B ($p=0.002$), and C ($p=0.01$) triangles, respectively. For ITT, differences between areas among morphological types did not reach statistical significance (Table 3).

Vascular relationships

Of 5 cadavers injected with colored silicone, optimal evaluation was possible on 9 sides. The SMCV drainage to the CS was cavernous type on 6 (66.7%), superior petrosal type on 1 (11.1%), emissary type on 1 (11.1%), and combined type on 1 (11.1%) side (Figure 5). The cavernous types drained into the CS through the anteromedial

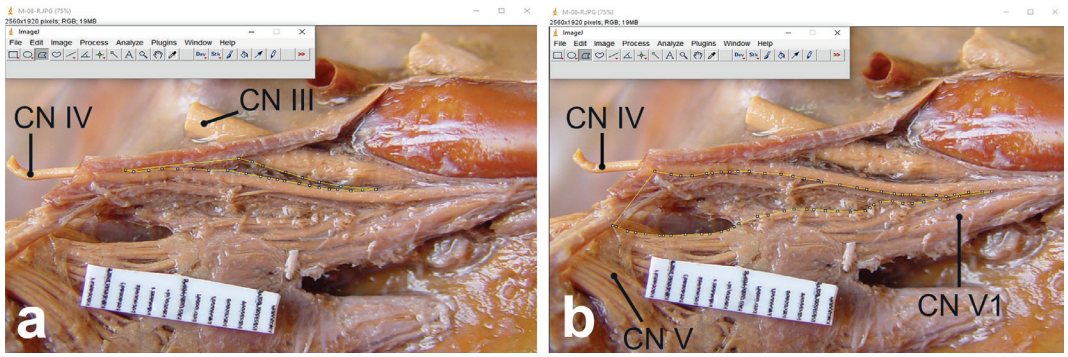

Figure 3: Figure shows area measurements for the supratrochlear (a) and infratrochlaer (b) triangles with the ImageJ software using the poligon selection tool. CN-III: Oculomotor nerve, CN-IV: Trochlear nerve, CN-V: Trigeminal nerve, CN-V1: Ophthalmic nerve.

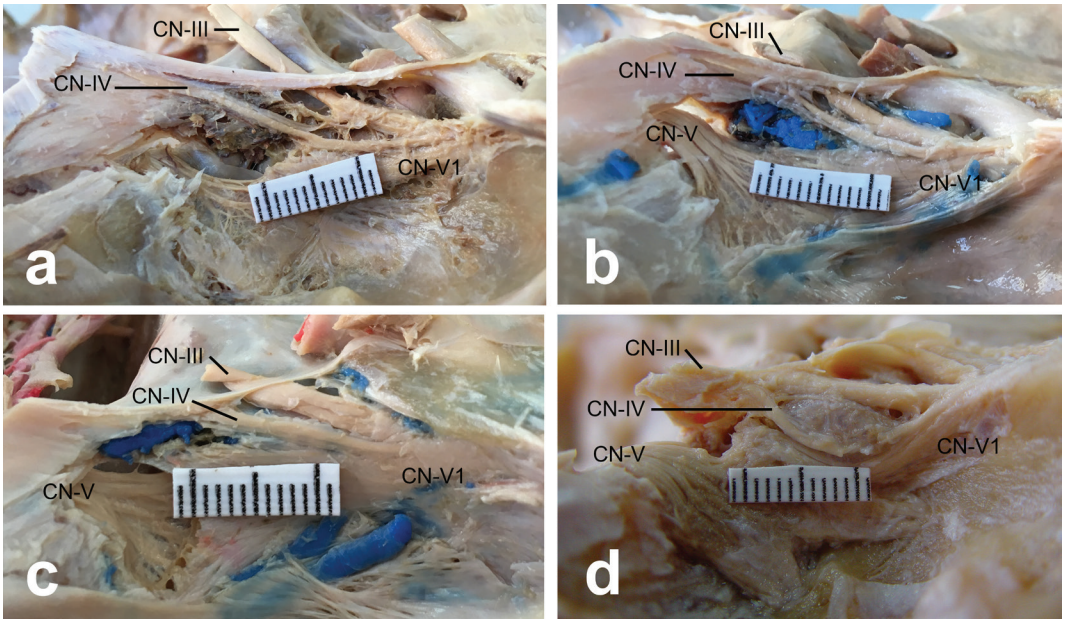

Figure 4: Type A (a), B (b), C (c), and D (d) triangle morphologies. CN-III: Oculomotor nerve, CN-IV: Trochlear nerve, CN-V: Trigeminal nerve, CN-V1: Ophthalmic nerve.

Table 1: Comparison of previous studies that evaluated supratrochlear and infratrochlear triangle morphology.

Previous study, year	Number of cadavers / sides used	Morphological triangle type				
		Type A	Type B	Type C	Type D	Type E
Lang and Reiter, 1984 (10)	86 sides	53.5%	31.4%	8.1%	7%	-
Miyazaki et al., 1994 (11)	50 sides (10 adults, 20 tissue blocks)	15%	60%	15%	5%	5%
Kayalioglu et al., 1999 (9)	108 sides (54 cadavers)	35.2%	30.8%	18.5%	6.5%	-
Present study, 2019	50 sides (25 cadavers)	46%	18%	20%	16%	-

Table 2: Comparison of supratrochlear and infratrochlear triangle areas regarding sex and side.

	Sex		Significance (p)
	Female	Male	
Supratrochlear	24.2±12.9	21.5±15.4	>0.05
Infratrochlear	79.1±29.7	78.1±27.3	>0.05
	Side		
	Right	**Left**	
Supratrochlear	25.5±16.4	19±12.3	>0.05
Infratrochlear	76.8±28	79.9±27.9	>0.05

triangle (Figure 5). The combined type had two SMCVs, one with cavernous and the other with superior petrosal drainage. Additionally, on 7 sides the superior petrosal sinus had contributed to the CS. On 5 out of 7 cases (71.4%) the CN-IV was relocated superiorly and formed a Type A triangle (Figure 5). Nevertheless, the sample size of vascular specimens was not large enough for statistical evaluation.

Table 3: Comparison of supratrochlear and infratrochlear triangle areas regarding morphological types.

	Supratrochlear[a]	Infratrochlear[b]
Type A	22.7±14.7	77±26.1
Type B	14.1±6.4	83.4±28.2
Type C	17.1±8.7	83.4±37
Type D	36.3±18.3	70.3±20.1

[a]Areas of Type D triangles were significantly larger than Type A (p=0.03), B (p=0.002), and C (p=0.01) triangles.
[b]Area differences between morphological types did not reach significance level.

IV and the CN-V1 enables the surgeon to enlarge both triangles and expose the abducens nerve and the lateral venous space (5, 19). Moreover, Knosp et al. (23) argued that approaching CS meningiomas through the ITT provided more space to work for the neurosurgeon. Therefore, the course of the CN-IV gains importance for the microneurosurgical approaches to the cavernous sinus.

There are a few preferred surgical approaches for resecting tumors in and around the CS (20). A cranio-orbito-zygomatic approach allows wide exposure of the entire CS with minimal brain retraction (20, 21), while an extended middle fossa approach is used in cases with a tumor at

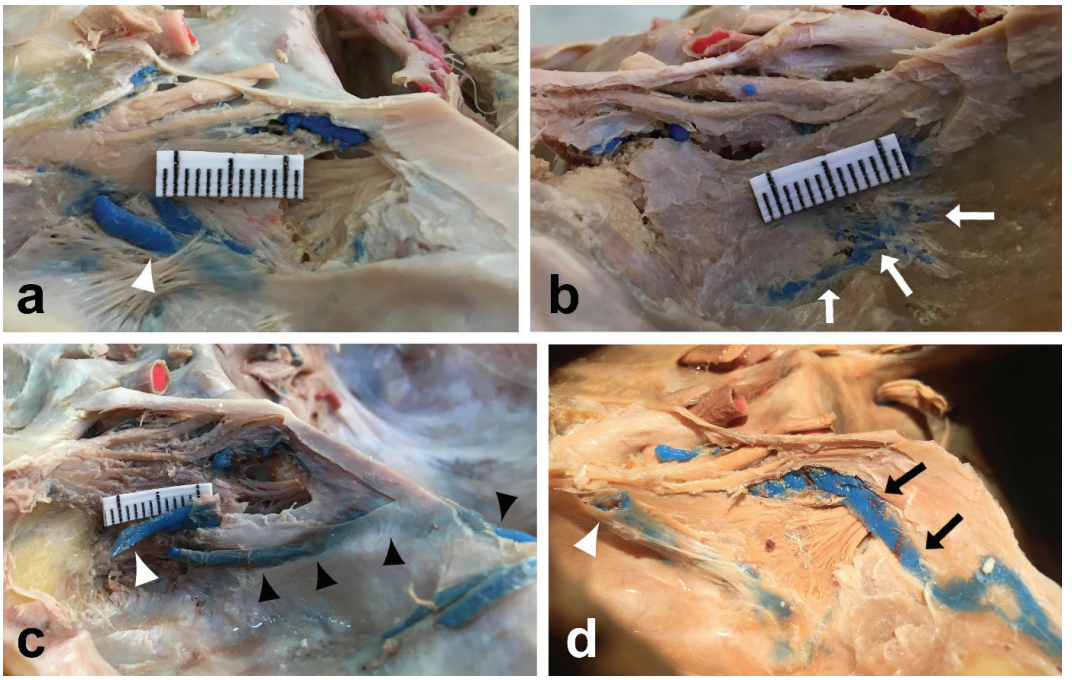

Figure 5: Cavernous (a), emissary (b) and combined (c) venous drainage patterns observed in the study. When the superior petrosal sinus contributed to the CS (black arrows), CN-IV was pushed superiorly and formed a Type A triangle morphology (d). White arrowheads depict cavernous, black arrowheads depict superior petrosal, and white arrows depict emissary drainage patterns.

DISCUSSION

The ITT, which was described by Parkinson (3, 4) as a direct entry route to the CS, forms a common surgical corridor at the lateral wall of the CS (5, 19). Through this triangle, it is possible to expose the posterior vertical segment, posterior bend, and the horizontal segment of the internal carotid artery, the meningohypophyseal trunk, posterosuperior and lateral venous spaces, and the lateral wall of the pituitary gland (2, 5, 19-22). Conversely, the STT is a narrow triangle that could be used for accessing the branches of the meningohypophyseal trunk (5). Despite the STT being narrow, dissection and retraction the CN-

the posterior CS (21, 24). An additional lateral sphenoidectomy, extended middle fossa approach also provides tumor resection in cases with petrous apex or Meckel's cave involvement (24). A lateral intradural approach allows wide exposure of the CS, especially where the CS is occupied and/or compressed by tumor resulting in lateral wall distortion or enlargement (20). However, this approach prevents direct visualization of the entry points of the cranial nerves at the lateral CS wall, thus increasing postoperative complications (5, 6, 19, 20). Conversely, an extradural approach provides a complete exposure of the tumor bed (20-22). Additionally, working between the

outer and inner dural layers of the lateral wall enables tumor resection without compromising the blood supply of adjacent cranial nerves (20, 21). In selected patients, a medial (transsellar) or lateral (transpterygoid) extradural endoscopic endonasal approach has been reported to yield higher clinical symptom recovery and cranial neuropathy improvement rate especially in cases with invasive adenomas of the CS (25-28).

Although classical texts (5, 19) mention the STT being the smaller triangle, previous literature reports either similar (13) or larger (2) areas for the STT (Table 4). Isolan et al. (2) also reported minimal variability for STT and ITT areas. The results of this study contradicts previous studies and found that the STT was larger and areas for both triangles were highly variable (Table 4). Given the complex and variable course of the CN-IV (10), it is possible for previous studies, which only provided linear measurements for nerves that do not traverse the lateral wall of the CS linearly, to miscalculate triangle areas. The variable anatomy of both triangles described by Lang and Reiter (10), urged the researchers to use a more reliable method of measurement (i.e. photogrametry) (18). Therefore, this study proposes that the areas for both triangles may be larger than previously reported. Moreover, this study showed that, especially in Type D morphology, the STT was siginificantly larger. During surgery, a relatively smaller ITT might require additional CN-IV dissection and retraction. Transient and permanent nerve damage due to CS surgery is uncommon (30). The major factors responsible for nerve damage are anatomical distortion of the region or direct involvement of neurovascular structures due to underlying pathologies such as meningiomas and aneurysms (20, 23, 30). Nevertheless, extensive manupilation of cranial nerves for imperative exposure during CS surgery might cause new nerve damages (30). In order to prevent this, combining different approaches such as extradural-lateral or extradural-superior approaches could be utilized for distorted cranial nerves with preoperative planning (20).

The CS is the primary venous drainage crossroad for the perisylvian cerebral regions, orbit, and the skull base (14). The SMCV constitutes the major venous structure that drains at the lateral wall of the CS (15, 31). It can drain into the anterior venous space via the sphenopareital sinus, the lateral venous space directly, or the posterosuperior venous space via the superior petrosal sinus (14, 15, 31). In this study, the superior petrosal type SMCV drainage was observed only in one case. Nevertheless, in the majority of cases (71.4%) where the superior petrosal sinus contributed to the cavernous sinuses, Type A morphology was observed. This might indicate that the venous drainage pattern of superficial veins into the CS might affect the CN-IV anatomy. However, this study could not support this finding with statistical significance. Thus, studies with larger sample sizes investigating whether the venous anatomy involves in triangle morphology are needed in the future.

Apart from therapeutic applications, understanding the complex anatomy of the CS has educational challeng-

Table 4: Comparison of anatomic studies that investigated the morphometry of infratrochlear and supratrochlear triangles.

Previous study, year	Medial border[a]	Lateral border[a]	Base[a]	Area[b]
Supratrochlear triangle				
Watanabe et al., 2003 (13)	10.9±4.1	14±3.8	7±2.1	34.1±15.1
Isolan et al., 2007 (2)	13.18±3.19	14.27±1.35	5.51±0.82	36.46±4.34
Present study, 2019	-	-	-	22.2±11.7
Infratrochlear triangle				
Harris & Rhoton, 1976 (7)	13	14	6	-
Sekhar et al., 1987 (12)	17	17	5	-
Inoue et al., 1990 (8)	16.4	16.5	4.5	-
Miyazaki et al., 1994 (11)	12	13	4	-
Day & Tschabitscher, 1996 (29)	10.48	11.79	6.94	-
Watanabe et al., 2003 (13)	13.1±2.8	16.4±3.2	6.2±1.4	35.6±14.6
Isolan et al., 2007 (2)	10.29±0.99	12.33±1.19	4.34±0.44	21.06±4.56
Present study, 2019	-	-	-	78.4±27.7

[a]Values for the borders of both triangles are presented as Mean±SD in mm.
[b]Values for triangle areas are presented as Mean±SD in mm^2.

es, such as low accessibility of cadaveric specimens for training, available educational material with poor detail or orientation, and decreased observation opportunities during noninvasive approaches (16, 20). Therefore, new educational models that has more accurate anatomical knowledge, are easily accessible, and are reusable were needed. Chung et al. (32) had provided a detailed schematic and a three dimensional model of the surgical triangles around the CS based on the segmentation of sectional cadaveric data (33, 34) and previous cadaveric studies (1, 2, 13, 35). This model allowed neurosurgery residents to simulate and study interhemispheric, pterional, middle cranial fossa, and retrosigmoid suboccipital approaches (32). Similarly, Qian et al. (36) have produced a three dimensional virtual reality model of the sellar region from cadaveric computed tomography data for neurosurgical residency training. Both educational models provide a convenient opportunity to observe complex spatial relationships and to perform basic skills. Nevertheless, adding further detail, including the variable anatomy of both triangles and venous structures into educational models may present an opportunity for advancing the neurosurgical training by increasing the anatomical accuracy and individuality.

A limitation of this study was its relative small sample size that was used to evaluate vascular anatomy. Some of reported venous drainage patterns were not observed. Additionally, a statistical comparison was not possible between venous drainage types and triangle morphologies. Therefore, the results should be taken as descriptive rather than definitive.

CONCLUSION

The morphology and morphometry of supratrochlear and infratrochlear triangles show siginificant variability. The supra-trochlear triangle is larger in cases with Type D triangle morphology. Although Type A triangle morphology was frequent in cases with superior petrosal contribution to the CS, future studies that compares different venous drainage patterns and triangle morphologies are needed.

Acknowledgements: The authors are deeply grateful to the (unclaimed and donated) cadavers for providing this research opportunity.

Ethics Committee Approval: This study was approved by the Ethical Committee of the Istanbul University Istanbul Faculty of Medicine (Date: 10.01.2018, Number: 11).

Informed Consent: Written consent was not obtained from the participants. (Cadaver study)

Peer Review: Externally peer-reviewed.

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

Conflict of Interest: Authors declared no conflict of interest.

Financial Disclosure: This work was supported by the grant of Scientific Research Projects Coordination Unit of Istanbul University (Project No: TSA-2016-20144).

Teşekkür: Yazarlar olarak, bu araştırma imkanını sağlayan kimsesiz ve bağış kadavralara gönülden minnettarız.

Etik Komite Onayı: Bu çalışma için etik komite onayı İstanbul Üniversitesi İstanbul Tıp Fakültesi Etik Kurulu'ndan alınmıştır (Tarih: 10.01.2018, Numara: 11).

Bilgilendirilmiş Onam: Katılımcılardan bilgilendirilmiş onam alınmamıştır. (Kadavra çalışması)

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Çalışma Konsepti/Tasarım- İ.A.G., O.C.; Veri Toplama- İ.A.G., Ö.G.; Veri Analizi/Yorumlama- A.K., A.Ö.; Yazı Taslağı- İ.A.G., O.C.; İçeriğin Eleştirel İncelemesi-Ö.G., A.K., A.Ö.; Son Onay ve Sorumluluk- İ.A.G., O.C., Ö.G., A.K., A.Ö.

Çıkar Çatışması: Yazarlar çıkar çatışması beyan etmemişlerdir.

Finansal Destek: Bu çalışma İstanbul Üniversitesi Bilimsel Araştırma Projeleri Koordinasyon Birimi hibesi ile desteklenmiştir (Proje Numarası: TSA-2016-20144).

REFERENCES

1. Dolenc V. Direct microsurgical repair of intracavernous vascular lesions. J Neurosurg 1983;58(6):824-31. [CrossRef]
2. Isolan GR, Krayenbühl N, de Oliviera E, Al-Mefty O. Microsurgical anatomy of the cavernous sinus: measurements of the triangles in and around it. Skull Base 2007;17(6):357-67. [CrossRef]
3. Parkinson D. A surgical approach to the cavernous portion of the carotid artery: anatomical studies and case report. J Neurosurg 1965;23(5):474-83. [CrossRef]
4. Parkinson D. Transcavernous repair of carotid cavernous fistula. J Neurosurg 1967;26(4):420-4. [CrossRef]
5. Rhoton ALjr: The middle cranial base and cavernous sinus. In Dolenc VV, Rogers L (eds), Cavernous Sinus: Developments and Future Perspectives. Wien, Springer Verlag, 2009;3-25. [CrossRef]
6. Romero FR, Ramires D, Cristiano LC, Silva MP, Vieira RB. Endoscopic and microsurgical approaches to the cavernous sinus - Anatomical review. Arq Bras Neurocir 2017;36(3):160-6. [CrossRef]
7. Harris FS, Rhoton ALjr. Anatomy of the cavernous sinus: a microsurgical study. J Neurosurg 1976;45(2):169-80. [CrossRef]
8. Inoue T, Rhoton ALjr, Theele D, Barry ME. Surgical approaches to the cavernous sinus: a microsugical study. Neurosurgery 1990;26(6):903-932. [CrossRef]

9. Kayalioglu G, Govsa F, Erturk M, Pinar Y, Ozer MA, Ozgur T. The cavernous sinus: topographic morphometry of its contents. Surg Radiol Anat 1999;21(4):255-60. [CrossRef]
10. Lang J, Reiter U. Über den verlauf der hirnnerven in der seitenwand des sinus cavernosus. Neurochirurgia 1984;27(4):93-7. [CrossRef]
11. Miyazaki Y, Yamamoto I, Shinozuka T, Sato O. Microsurgical anatomy of the cavernous sinus. Neurol Med Chir 1994;34(3):150-63. [CrossRef]
12. Sekhar LN, Burges J, Akın O. Anatomical study of the cavernous sinus emphasizing operative approaches and related vascular and neural reconstruction. Neurosurgery 1987;21(6):806-16. [CrossRef]
13. Watanabe A, Nagaseki Y, Ohkubo S, Ohhashi Y, Horikoshi T, Nishigaya K et al. Anatomical variations of the ten triangles around the cavernous sinus. Clin Anat 2003;16(1):9-14. [CrossRef]
14. Keller JT, Leach JL, van Loveren HR, Abdel Aziz KM, Froelich S: Venous anatomy of the lateral sellar compartment. In Dolenc VV, Rogers L (eds), Cavernous Sinus: Developments and Future Perspectives. Wien, Springer Verlag, 2009;35-51. [CrossRef]
15. Suzuki Y, Matsumoto K. Variations of the superficial middle cerebral vein: Classification using three-dimensional CT angiography. Am J Neuroradiol 2000;21(5):932-8.
16. Gürses İA, Coşkun O, Öztürk A. Current status of cadaver sources in Turkey and a wake-up call for Turkish Anatomists. Anat Sci Educ 2018;11(2):155-65. [CrossRef]
17. Sanan A, Abdel Aziz KM, Janjua RM, van Loveren HR, Keller JT. Colored silicone injection for use in neurosurgical dissections: Anatomic technical note. Neurosurgery 1999;45(5):1267-71. [CrossRef]
18. Rasband WS. ImageJ, U.S. National Institutes of Health, Bethesda, Maryland, USA, 1997-2016 https://imagej.nih.gov/ij/ [accessed 21 September 2019]
19. Dalgıç A, Boyacı S, Aksoy K. Anatomical study of the cavernous sinus emphasizing operative approaches. Turk Neurosurg 2010;20(2):186-204. [CrossRef]
20. Chotai S, Liu Y, Qi S. Review of surgical anatomy of the tumors involving cavernous sinus. Asian J Neurosurg 2018;13(1):1-8. [CrossRef]
21. Heth JA, Al-Mefty O. Cavernous sinus meningiomas. Neurosurg Focus 2003;14(6):e3. [CrossRef]
22. Komatsu F, Komatsu M, Inoue T, Tschabitscher M. Endoscopic supraorbital extradural approach to the cavernous sinus: A cadaver study. J Neurosurg 2011;114(5):1331-7. [CrossRef]
23. Knosp E, Perneczky A, Koos WT, Fries G, Matula C. Meningiomas of the space of the cavernous sinus. Neurosurg 1996;38(3):434-44. [CrossRef]
24. Sun DQ, Menezes AH, Howard MA, Gantz BJ, Hasan DM, Hansen MR. Surgical management of tumors involving Meckel's cave and cavernous sinus: Role of an extended middle fossa and lateral sphenoidectomy approach. Otol Neurotol 2018;39(1):82-91. [CrossRef]
25. Dolci RLL, Upadhyay S, Filho LFSD, Fiore ME, Buohliqah L, Lazarini PR et al. Endoscopic endonasal study of the cavernous sinüs and quadrangular space: Anatomic relationships. Head Neck 2016;38(Suppl-1):E1680-7. [CrossRef]
26. Koutourousiou M, Filho FVG, Fernandez-Miranda JC, Wang EW, Stefko ST, Snyderman CH et al. Endoscopic endonasal surgery for tumors of the cavernous sinus: A series of 234 patients. World Neurosurg 2017;103:713-32. [CrossRef]
27. Theodosopoulos PV, Cebula H, Kurbanov A, Cabero AB, Osorio JA, Zimmer LA et al. The medial extra-sellar corridor to the cavernous sinus: Anatomic description and clinical correlation. World Neurosurg 2016;96:417-22. [CrossRef]
28. Woodworth GF, Patel KS, Shin B, Burkhardt JK, Tsiouris AJ, McCoul ED et al. Surgical outcomes using a medial-to-lateral endonasal endoscopic approach to pituitary adenomas invading the cavernous sinus. J Neurosurg 2014;120(5):1086-94. [CrossRef]
29. Day JD, Tschabitscher M: Microsurgical dissection of the cranial base. Churchill Livingstone, New York, 1996.
30. Samii M, Gerganov VM: Surgery of cavernous sinus meningiomas: Advantages and disadvantages. In Dolenc VV, Rogers L (eds), Cavernous Sinus: Developments and Future Perspectives. Wien, Springer Verlag, 2009;153-62. [CrossRef]
31. Tanoue S, Kiyosue H, Okahara M, Sagara Y, Hori Y, Kashiwagi J et al. Para-cavernous sinus venous structures: Anatomic variations and pathologic conditions evaluated on fat-suppressed 3D fast gradient-echo MR images. Am J Neuroradiol 2006;27(5):1083-89.
32. Chung BS, Ahn YH, Park JS. Ten triangles around cavernous sinus for surgical approach, described by schematic diagram and three dimensional models with the sectioned images. J Korean Med Sci 2016;31(9):1455-63. [CrossRef]
33. Park JS, Chung MS, Shin DS, Har DH, Cho ZH, Kim YB et al. Sectioned images of the cadaver head including the brain and correspondences with ultrahigh field 7 T MRIs. Proc IEEE 2009;97(12):1988-96. [CrossRef]
34. Park HS, Chung MS, Shin DS, Jung YW, Park JS. Whole courses of the oculomotor, trochlear, and abducens nerves, identified in sectioned images and surface models. Anat Rec 2015;298(2):436-43. [CrossRef]
35. Hakuba A, Tanaka K, Suzuki T, Nishimura S. A combined orbitozygomatic infratemporal epidural and subdural approach for lesions involving the entire cavernous sinus. J Neurosurg 1989;71(5Pt1):699-704. [CrossRef]
36. Qian ZH, Feng X, Li Y, Tand K. Virtual reality model of the three-dimentional anatomy of the cavernous sinus based on a cadaveric image and dissection. J Craniofac Surg 2018;29(1):163-6. [CrossRef]

İSTANBUL ÜNİVERSİTESİ YAYINEVİ

RESEARCH / ARAŞTIRMA
DOI: 10.26650/IUITFD.2019.0080
İst Tıp Fak Derg 2020 / J Ist Faculty Med 2020

PSYCHIATRIC COMORBIDITY AND SLEEP PROBLEMS IN CHILDREN AND ADOLESCENTS WITH ATTENTION DEFICIT HYPERACTIVITY DISORDER IN RELATION TO ATTENTION DEFICIT HYPERACTIVITY DISORDER PRESENTATION, AGE AND GENDER

DİKKAT EKSİKLİĞİ HİPERAKTİVİTE BOZUKLUĞU TANILI ÇOCUK VE ERGENLERDE DİKKAT EKSİKLİĞİ HİPERAKTİVİTE BOZUKLUĞU GÖRÜNÜMÜ; YAŞ VE CİNSİYETE GÖRE PSİKİYATRİK KOMORBİDİTE VE UYKU PROBLEMLERİ

Murat COŞKUN[1], Gökçen GÜVEN[1], Alper ALNAK[1], Ali KARAYAĞMURLU[1]

Istanbul University, Istanbul Faculty of Medicine, Department of Child and Adolescent Psychiatry, Istanbul, Turkey

ORCID IDs of the authors: M.C. 0000-0002-4808-5870; G.G. 0000-0001-7557-0778; A.A. 0000-0002-3515-8217; A.K. 0000-0001-5464-2891

Cite this article as: Coskun M, Guven G, Alnak A, Karayagmurlu A. Psychiatric comorbidity and sleep problems in children and adolescents with Attention deficit hyperactivity disorder in relation to Attention deficit hyperactivity disorder presentation, age and gender. J Ist Faculty Med 2020;83(4):363-72. doi: 10.26650/IUITFD.2019.0080

ABSTRACT

Objective: This study aimed to investigate psychiatric comorbidity and sleep problems and their relationships with ADHD presentation, age and gender in a clinical sample of children and adolescents with ADHD.

Material and Method: One hundred fifty-four subjects aged 6-17 were included in the study. A semi-structured diagnostic interview was conducted to screen psychiatric disorders. The Child Depression Inventory (CDI), Screen for Child Anxiety Related Emotional Disorders (SCARED) and Children's Sleep Habits Questionnaire (CSHQ) were used to investigate internalizing difficulties and sleep problems.

Results: Overall high rates of comorbid disorders (78%) and sleep problems (97%) were found. ADHD-C was significantly more frequent in males and ADHD-I was more frequent in females ($p<0.001$). While oppositional defiant disorder (ODD), enuresis and encopresis were more frequent in subjects with ADHD-C ($p<0.05$), generalized anxiety (GAD) and social anxiety (SAD) disorders were more frequent in subjects with ADHD-I ($p<0.05$). Females, compared to males, had more frequent diagnoses of depression ($p=0.021$) and SAD ($p=0.03$). The majority of subjects (96.7%) scored above the cut off score of 41 in CSHQ (50.51±5.86). The ADHD-C group had significantly higher CSHQ total scores than the ADHD-I group ($p<0.05$). There was a significant positive correlation between CSHQ total scores and the

ÖZET

Amaç: Bu çalışmada, DEHB tanılı çocuk ve ergenlerden oluşan klinik bir örneklemde psikiyatrik komorbidite, uyku problemleri ve bunların DEHB görünümü, yaş ve cinsiyetle olan ilişkilerinin incelenmesi amaçlanmış.

Gereç ve Yöntem: Çalışmaya 6-17 yaş arası 154 katılımcı dahil edildi. Psikiyatrik bozuklukları değerlendirmek amacıyla yarı yapılandırılmış bir klinik görüşme gerçekleştirildi. İçselleştirme ve uyku sorunlarını değerlendirmek için Çocuklar için Anksiyete Bozuklukları Tarama Ölçeği (ÇATÖ), Çocuk Depresyon Envanteri (ÇDE) ve Çocuk Uyku Alışkanlıkları Anketi (ÇUAA) kullanıldı.

Bulgular: Katılımcılar arasında komorbid hastalık (%78) ve uyku problemleri (%97) sıklıkları oldukça yüksekti. DEHB bileşik görünüm erkeklerde daha sık olarak tespit edildi ($p<0,001$) ve karşıt olma-karşı gelme bozukluğu, enürezi ve enkoprezi bileşik görünümdeki olgularda daha sıktı ($p<0,05$). Dikkatsizlik görünümündeki olgularda ise sosyal anksiyete bozukluğu ve yaygın anksiyete bozukluğu görülme sıklıkları bileşik görünümdeki olgulara göre daha fazlaydı ($p<0,05$). Erkeklerle karşılaştırıldığında, kızlarda sosyal anksiyete bozukluğu ($p=0,03$) ve depresyon ($p=0,021$) daha sık olarak görülmekteydi. Katılımcıların %96,7'si ÇUAA'nde kesme puanı olan 41'in üzerinde skora sahipti (50,51±5,86). Bileşik görünümdeki olguların ÇUAA skorları dikkatsizlik görünümündeki olgulardan anlamlı olarak daha yüksekti ($p<0,05$). ÇUAA skorları ile toplam yaşam boyu psikiyatrik hastalık sayısı ve

Corresponding author/İletişim kurulacak yazar: drmc78@gmail.com

Submitted/Başvuru: 24.09.2019 • **Revision Requested/Revizyon Talebi:** 13.02.2020 •
Last Revision Received/Son Revizyon: 14.02.2020 • **Accepted/Kabul:** 03.02.2020 • **Published Online/Online Yayın:** 21.09.2020

number of lifetime comorbid diagnoses (p=0.006), self-reported anxiety (p=0.009) and depressive (p=0.004) symptoms.

Conclusion: Comorbidity and sleep problems may be common in young people with ADHD and may have complex reciprocal relations with several factors including ADHD presentation, age, and gender.

Keywords: ADHD, BMI, children, comorbidity, sleep

öz-bildirime dayalı depresyon (p=0,006) ve anksiyete (p=0,009) belirti şiddeti arasında pozitif yönde bir korelasyon saptandı.

Sonuç: DEHB'de psikiyatrik komorbid bozukluklar ve uyku problemleri yaygın olarak görülür ve DEHB görünümü, yaş ve cinsiyet gibi faktörler ile etkileşimleri kompleks ve karşılıklı olabilir.

Anahtar Kelimeler: DEHB, çocuk, uyku, komorbidite, VKİ

INTRODUCTION

Attention Deficit Hyperactivity Disorder (ADHD) is one of the most common psychiatric disorders of childhood with an estimated prevalence of 5% to 7% of school-age children worldwide (1). Left untreated ADHD may cause significant psychosocial, academic, and long-term negative consequences (2, 3). In addition to the impairment directly related to ADHD symptoms, a majority of subjects with ADHD have comorbid psychiatric disorders that warrant clinical attention (4–7). Comorbidity in ADHD is an important issue with multiple dimensions in terms of clinical practice and research (5, 7). It has been reported that there may be significant differences in terms of sociodemographic and clinical characteristics, family history, choice of optimal ADHD treatment, response to treatment and long-term outcome in subjects with and without comorbidity (5, 8, 9). The prevalence and patterns of comorbid disorders in ADHD may differ according to the study methodology (such as clinical vs epidemiological samples), different clinical presentations of ADHD and several sociodemographic variables such as age and gender. Comorbidity studies in clinical samples have reported more than 90 percent comorbidity rates in ADHD with externalizing (oppositional defiant and conduct disorders), anxiety, mood and learning disorders, and sleep problems as the most frequent comorbid conditions (5, 10-14). Meanwhile, sleep disorders or problems have been reported in the majority of young subjects, up to 70 percent, with ADHD (10, 11, 14-16). Presence of comorbidity, ADHD presentation, and medication treatment have been reported as important factors related to sleep problems in subjects with ADHD (10, 11, 14, 16). It is important to note that psychiatric comorbidity and sleep problems are important factors in all stages of ADHD management that includes diagnosis, choice of optimal treatment for ADHD, treatment response, and long term prognosis of ADHD (2, 8, 10, 14, 17). Therefore, it may be important to know the prevalence and patterns of psychiatric comorbidity and sleep problems and their relationship between clinical (such as ADHD presentation) and sociodemographic (such as age and gender) variables in young subjects with ADHD. Despite there having been several reports on the prevalence and patterns of comorbid psychiatric disorders in relation to different ADHD presentations, age and gender among adults with ADHD, there is a lack of studies on these issues among young subjects with ADHD (18, 19). In this study, the researchers investigated the prevalence and patterns of psychiatric comorbidity and sleep problems in relation to different ADHD presentations, age (children vs adolescents), and gender in a clinical sample of children and adolescents with ADHD.

MATERIAL AND METHOD

Participants and procedure

This study was conducted in the Child and Adolescent Psychiatry Department of Istanbul Medical Faculty, Istanbul University. Subjects in this study were among the patients who have been followed up with the diagnosis of ADHD in this center. Subjects (and parents) were approached for participation in the study during their clinical visits. Subjects and/or families who agreed to participate were then interviewed for their eligibility to take part in the study. Inclusion criteria were as follows; a) aged between 6 to 18 years old, b) having diagnosis of ADHD according to DSM-5 criteria, c) no evidence of intellectual disability or autism spectrum disorders during clinical interviews, d) having a score of 70 or above in psychometric tests and e) parents and subjects agreed to participate and signed informed consent. Participating subjects were then scheduled for a detailed interview for the study. Subjects were assessed using a semi-structured diagnostic instrument to investigate the presence of lifetime psychiatric disorders. Diagnosis of ADHD in these subjects was confirmed through several interviews with collateral information using parents' and teachers' reports. While subjects were asked to fill out self-reported scales for anxiety and depressive symptoms, parents filled out a sleep questionnaire regarding sleep habits of their children. The study was approved by the Istanbul Medical Faculty Ethical Committee.

Instruments

Schedule for affective disorders and schizophrenia for school-age children–present and lifetime version–Turkish version (K-SADS-PL-T)

K-SADS-PL is a semi-structured diagnostic interview schedule designed to assess major psychiatric disorders in children and adolescents based on DSM-IV criteria

(20). It has been used in several clinical and epidemiological studies in Turkey (21, 22). The Turkish version K-SADS-PL-T was used in this study (23).

The children's sleep habits questionnaire (CSHQ)
CSHQ is one of the most widely used parent-report measures of sleep for children (24). CSHQ characterizes sleep in a number of key domains including total sleep duration, sleep latency, sleep anxiety, parasomnias, bedtime behavior, night awakenings, sleep-disordered breathing, and daytime sleepiness. A cut-off total CSHQ score of 41 was accepted as clinically significant sleep problems. The Turkish version of CSHQ was used in the study (25).

Child depression inventory (CDI)
CDI is a 27 item self-report measure and is used commonly to measure depressive symptoms in children (26). The Turkish form was used in the study (27).

The screen for child anxiety related emotional disorders (SCARED)-child form
SCARED is a child and parent self-report instrument used to screen for childhood anxiety disorders including generalized anxiety disorder, separation anxiety disorder, panic disorder, and social phobia (28). In addition, it assesses symptoms related to school phobias. The Turkish form was used in the study (29).

Statistical analysis
SPSS 22.0 was used in statistical analysis. Descriptive statistics were used to report minimum, maximum, mean, standard deviation, frequency, and percentage data. Distribution of the variables was assessed with the Kolmogorov Smirnov test. The Mann-Whitney U test and an independent sample t-test were used in the analysis of quantitative data. The Chi-square test and Fischer test, when chi-square test conditions not met, were used in the analysis of qualitative data. The Spearman correlation test was used for correlation analysis.

RESULTS

A total of 154 subjects, aged 6 to 17 years, were included in the study (11.84±2.79 years). Of those, 115 subjects were male (74.7%) and 79 subjects were children below 12 years of age (51%). 91 subjects had a diagnosis of ADHD-combined presentation (ADHD-C) (59%) and 63 subjects had a diagnosis of ADHD-inattentive presentation (ADHD-I) (41%). There were no significant age differences between the two groups ($p>0.05$). ADHD-C was significantly more frequent in males and ADHD-I was more frequent in females ($p<0.001$). No subjects had ADHD-predominantly hyperactive/impulsive presentation. The body mass index (BMI) among the sample changed between 13.66-36.51 (20.24±4.14). There were no significant differences between ADHD-C and ADHD-I groups in terms of BMI ($p=0.098$). All subjects were on medications at the time of evaluation for the study. Methylphenidate (86.4%) was the most frequently used medication for ADHD. Sociodemographic and clinical characteristics of the subjects are shown in Table 1.

Lifetime comorbidity and sleep problems
One hundred twenty-one subjects received at least one diagnosis of comorbidity. Of those with a comorbid diagnoses (n=121), 42 subjects received two, 40 subjects received three and 39 subjects received more than three comorbid diagnoses. Prevalence of the lifetime comorbid diagnoses among the whole sample were (in decreasing order); enuresis (21.4%), generalized anxiety disorder (GAD) (20.8%), special phobia (19.5%), separation anxi-

Table 1: Sociodemographic and clinical characteristics of the subjects

Age	11.84±2.79 years (6-17)		
	n		**%**
Children (under 12 years)	79		51.3
Gender (male)	115		74.7
ADHD diagnosis	**ADHD-C**	**ADHD-I**	
Male (n=115)	n=78	n=37	***p<0.001**
Female (n=39)	n=13	n=26	
Maternal age (years)	38.5±6.1	39.0±6.5	**p=0.681
Paternal age (years)	42.3±7.0	43.7±6.9	**p=0.162
Diagnosed ADHD in first degree relatives (n=61)	n=40	n=21	p=0.167
Consanguineous marriage (n=14)	n=4	n=10	***p=0.015**
CSHQ scores	35 to 74		50.51±5.86

*X^2 test, **t test

ety disorder (18.8), oppositional defiant disorder (15.6%), social anxiety disorder (SAD) (14.9%), Tourette disorder (14.3%), obsessive compulsive disorder (11.7%), motor/vocal tic disorder (10.4%), encopresis (9.1%), depression (7.1%), post-traumatic stress disorder (3.2%), conduct disorder (2.6%), cigarette use disorder (1.3%), bipolar disorder (0.6%), panic disorder (0.6%), and bulimia nervosa (0.6%). Prevalence of lifetime psychiatric diagnoses in different gender, age, and ADHD presentation groups are shown in Table 2.

The majority of subjects (n=149; 96.7%) scored above the cutoff score of 41 in CSHQ (50.51±5.86) which is considered a clinically significant sleep problem. There was a significant positive correlation between the number of lifetime comorbid diagnoses and CSHQ total scores (r=0.224; p=0.006). In terms of current diagnosis, while participants with enuresis scored significantly higher on CSHQ than those without enuresis (58.66±6.5 vs. 53.57±5.94, p=0.022[m]), no such difference was found for other diagnoses including internalizing disorders (such as GAD, MDB and SAD) and externalizing disorders (ODD and CD).

Table 2: Prevalence of lifetime psychiatric diagnoses

Lifetime psychiatric diagnoses	n	%	Males (n=115)				Females (n=39)			
			Children (n=55)		Adolescents (n=60)		Children (n=24)		Adolescents (n=15)	
			ADHD-I n=16	ADHD-C n=39	ADHD-I n=21	ADHD-C n=39	ADHD-I n=15	ADHD-C n=9	ADHD-I n=11	ADHD-C n=4
Depression	11	7.1	0	1	2	2	3	0	2	1
Bipolar disorder	1	0.6	0	1	0	0	0	0	0	0
Psychosis	0	0	0	0	0	0	0	0	0	0
Panic disorder	1	0.6	0	0	0	1	0	0	0	0
Separation anxiety disorder	29	18.8	2	8	3	6	2	3	4	1
Social anxiety disorder	23	14.9	3	3	3	4	6	0	3	1
Specific phobia	30	19.5	1	7	4	8	6	0	3	1
Generalized anxiety disorder	32	20.8	4	4	6	6	5	2	3	2
Obsessive compulsive disorder	18	11.7	2	7	2	4	0	1	1	1
Enuresis	33	21.4	3	15	2	7	2	2	2	0
Encopresis	14	9.1	1	7	1	3	0	1	0	1
Anorexia nervosa	0	0	0	0	0	0	0	0	0	0
Bulimia nervosa	1	0.6	0	0	0	0	0	0	0	1
Oppositional defiant disorder	24	15.6	0	11	1	8	4	0	0	0
Conduct disorder	4	2.6	0	3	0	1	0	0	0	0
Tourette disorder	22	14.3	7	3	3	6	3	0	0	0
Motor/vocal tic disorder	16	10.4	2	3	4	5	1	1	0	0
Cigarette use	2	1.3	0	0	1	1	0	0	0	0
Alcohol use disorder	0	0	0	0	0	0	0	0	0	0
Substance use disorder	0	0	0	0	0	0	0	0	0	0
Post-traumatic stress disorder	5	3.2	0	1	0	2	0	0	2	0

However, there was a significant positive correlation between total CSHQ scores, self-reported anxiety (r=0.270, p=0.009), and depressive (r=0.300, p=0.004) symptoms.

Psychiatric comorbidity and sleep problems in relation to ADHD presentation, age and gender

In subjects with ADHD-I generalized anxiety (GAD) and social anxiety (SAD), disorders were found more frequently than subjects with ADHD-C (p<0.05). Subjects with ADHD-C were found to have more frequent diagnoses of oppositional defiant disorder (ODD), enuresis and encopresis than subjects with ADHD-I (p<0.05). There were no significant differences between the two groups regarding other comorbid diagnoses. Table 3 shows the prevalence and significance of lifetime comorbid diagnoses in ADHD-C or ADHD-I presentations.

Regarding the age and gender effects on lifetime comorbidity children as compared to adolescents, they have more frequent diagnosis of enuresis (p=0.046); females, compared to males, have more frequent diagnoses of depression (p=0.021) and social anxiety disorder (p=0.030). There were no significant differences in the mean number of comorbid diagnoses between different ADHD presentations, such as age and gender groups (p>0.05). The ADHD-C group had significantly higher CSHQ total scores than the ADHD-I group (p<0.05). Both groups did not differ significantly in subscales of CSHQ. There were no significant differences between children versus adolescents and males versus females with regard to total scores of CSHQ. Regarding BMI scores, while there was no significant difference between males vs females, mean scores (p<0.001) and rates of being overweight (≥25) (p=0.008) were higher in adolescents compared to children. Table 4 shows the number of comorbid diagnoses, CSHQ, and BMI scores in regard to ADHD presentation, age, and gender.

Table 3: Lifetime comorbid diagnoses in regard to different ADHD presentations

Lifetime diagnoses	ADHD-C		ADHD-I		p*
	n	%	n	%	
Depression	4	4.4	7	11.1	0.101
Bipolar disorder	1	1.1	0	0.0	0.410
Psychosis	0	0.0	0	0.0	-
Panic disorder	1	1.1	0	0.0	0.408
Separation anxiety disorder	18	19.8	11	17.5	0.777
Social anxiety disorder	8	8.8	15	23.8	***0.008***
Specific phobia	16	17.6	14	22.2	0.425
Generalized anxiety disorder	14	15.4	18	28.6	***0.038***
Obsessive compulsive disorder	13	14.3	5	7.9	0.251
Enuresis	25	27.5	8	12.7	***0.034***
Encopresis	12	13.2	2	3.2	***0.038***
Anorexia nervosa	0	0.0	0	0.0	-
Bulimia nervosa	1	1.1	0	0.0	0.410
Oppositional defiant disorder	24	26.4	0	0.0	***0.000***
Conduct disorder	4	4.4	0	0.0	0.096
Tourette disorder	14	15.4	8	12.7	0.687
Motor/vocal tic disorder	9	9.9	7	11.1	0.764
Cigarette use	1	1.1	1	1.6	0.777
Alcohol use disorder	0	0.0	0	0.0	-
Substance use disorder	0	0.0	0	0.0	-
Post-traumatic stress disorder	3	3.3	2	3.2	0.990

*X^2 test (Fisher's exact test)

Table 4: Number of comorbid diagnoses and CSHQ scores in regard to ADHD presentation, age and gender

	Mean±SD		Mean±SD	P
Number of comorbid diagnoses				
ADHD-C**	1.73±1.24	ADHD-I	1.58±1.43	0.468*
Children***	2.75±1.30	Adolescents	2.62±1.40	0.543*
Males***	2.68±1.30	Females	2.71±1.48	0.902*
CSHQ z				
ADHD-C**	50.80±9.10	ADHD-I	47.80±8.20	**0,033***
Children***	51.13±6.14	Adolescents	49.87±5.52	0.281*
Males***	50.78±6.02	Females	49.72±5.35	0.644*
BMI<25	53.78±6.12	BMI≥25	53.59±5.89	0.996*
Body mass index (BMI) (n=147)				
ADHD-C**	18.8±3.2	ADHD-I	19.4±3.7	0.098*
Children***	18.72±3.78	Adolescents	21.71±3.97	**<0.001***
BMI<25	67 (93.1%)	BMI<25	58 (77.3%)	
BMI≥25	5 (6.9%)	BMI≥25	17 (22.7%)	**0.008****
Males***	20.14±3.69	Females	20.55±5.36	0.732*
BMI<25	97 (87.4%)	BMI<25	28 (77.8%)	
BMI≥25	14 (12.6%)	BMI≥25	8 (22.2%)	0.160****

*Mann-Whitney U Test; **Comorbid diagnoses; ***All diagnoses including ADHD; ****X^2 test

DISCUSSION

Psychiatric comorbidity in ADHD is common in clinical practice and has significant implications on different dimensions of the disorder (2, 30). Psychiatric comorbidity tends to show variability, between individuals, throughout development in relation to individual and environmental factors (5, 8, 30). Sleep problems are also another area of concern that may have an influence on the clinical course and management of ADHD and even on its differential diagnosis as they might mimic ADHD-like symptoms (31). In clinical practice sleep problems are common, affecting up to 70 percent of children and adolescents with ADHD without any type of sleep problem specifically associated with ADHD (32, 33). The frequency and nature of sleep problems may be influenced by various factors including a predominant presentation of ADHD and a presence of comorbidity and medication status (34). Due to the complex associations between several aspects of ADHD, psychiatric comorbidity, sleep problems, and other individual factors such as gender, age, and BMI, it is important to enhance our understanding on the interplay between these factors. In this study, the researchers looked for these issues and found several important findings that may have clinical and research implications.

Lifetime comorbidity and sleep problems

both total number and distribution of comorbid diagnoses reported in children and adolescents with ADHD tend to show variability between studies depending on sample population characteristics, diagnostic instruments used, and other methodological issues. In general, a higher frequency of psychiatric comorbidity in children and adolescents with ADHD is reported in studies from clinical settings, ranging from 52 to 76 percent (30, 35, 36). In these clinic-based studies, oppositional defiant disorder was as frequently reported as comorbidity, followed by anxiety disorders (35). As consistent with the previous literature, 78 percent of participants included in our study met criteria for at least one comorbid diagnosis throughout their lifetime; while enuresis (21.4%), generalized anxiety disorder (GAD) (20.8%), special phobia (19.5%), separation anxiety disorder (18.8 %), oppositional defiant disorder (ODD) (15.6%), and social anxiety disorder (SAD) (14.9%) were the most frequent comorbid diagnoses. Subjects with ODD may be less adherent to clinical follow up and less volunteering to participate in time taking assessment than the subjects with anxiety. This may partly explain higher rates of anxiety disorders than ODD in our study (37). The higher frequency of enuresis found in our study may reflect a lifetime assessment of comorbidities, as enuresis tend to improve through age (38).

The effect of comorbid disorders on sleep is multifaceted and probably confounded by numerous other conditions (11, 15, 16). In general, an increased frequency of sleep disturbances have been reported in children with ADHD and comorbid disorder (11, 34). Anxiety disorders

are more consistently found to be associated with sleep anxiety and awakenings, while externalizing disorders are associated with bedtime resistance (11). As supporting in the literature, most of the participants in our study were having difficulties with sleep and there was a positive correlation between the number of lifetime psychiatric comorbidity and CSHQ scores. In terms of current diagnoses, participants with enuresis scored significantly higher on CSHQ than those without enuresis. No such difference was detected for any of the other comorbid diagnoses. Nevertheless, there was a positive correlation between self-reported anxiety/depressive symptoms and CSHQ total scores. This finding may underscore the importance of routine questioning about sleep problems in children with ADHD regardless of clinically evident anxiety or depressive disorders.

Psychiatric comorbidity and sleep problems in relation to ADHD presentation, age and gender

While psychiatric comorbidity profiles in individuals with ADHD may differ throughout their lifes according to gender and ADHD presentation, the researchers made an attempt to explore these effects. Predominant presentation of ADHD may alter the course and treatment of ADHD, as well as the comorbid disease profile (39, 40). In general, ODD and other disruptive behavioral problems are more common in individuals with ADHD-C than those with ADHD-I (39), while higher frequencies of anxiety disorders, both as a group and as particular subtypes, were reported with predominantly inattentive presentation. For example, in a study of adults with ADHD and SAD from Turkey, the authors concluded that SAD may have a more specific relationship with inattentive presentation than combined presentation (18). Another study conducted on 108 children and adolescents found a higher frequency of SAD in subjects with ADHD-I than those with ADHD-C (39). As consistent with previous literature, we found a higher frequency of ODD and lower frequency of SAD and GAD in ADHD-C compared to the ADHD-I group. Elimination disorders were also more frequent in the ADHD-C group than ADHD-I group. With respect to nocturnal enuresis, the effect of ADHD presentation is less investigated. In contrast to our study, two previous studies reported higher incidences of nocturnal enuresis in children and adolescents with ADHD-I (41, 42).

The effects of ADHD presentation on sleep problems are somewhat less investigated and inconclusive. In one study, sleep problems were found to be increased in children with ADHD-C than ADHD-I, and no difference was found between children with ADHD-I and healthy controls (34). However, more recent studies reported greater daytime sleepiness in subjects with ADHD-I than subjects with ADHD-C (31); and one study on adolescents with ADHD-I reported increased sleep disturbances as compared with subjects without ADHD (43). One recent study conducted on 83 children (aged between 6 and 12) reported higher sleep problems in children with ADHD-C when compared with ADHD-I (44). In this study, an increased burden of sleep problems reported on CSHQ was found in children and adolescents with ADHD-C than those with ADHD-I. Nevertheless, lower but still considerably high scores in subjects with ADHD-I should not be overlooked. In addition, the researchers couldn't detect any statistical difference at the level of subscales between the two groups.

Age may be another important mediator of comorbid disease profile in children and adolescents with ADHD (39). ODD is reported to be higher in children, while internalizing disorders such as depression and anxiety disorders more frequent in adolescents (45). However, a study by Yuce et al. reported a higher prevalence of ODD and several anxiety disorders such as special phobia and separation anxiety disorders in children with ADHD (39). In this study, the researchers looked for lifetime occurrence of psychiatric disorders and found that only enuresis was higher in children than adolescents. Since elimination disorders tend to improve throughout development, this finding was expected. There was no significant difference in total CSHQ scores between children and adolescents. This finding may reflect the fact that sleep problems are common in youth with ADHD during both childhood and adolescence; and this should encourage child mental health professionals to inquire about sleep related problems routinely during patients' clinic visits regardless of age.

Gender has also an effect on accompanying comorbidity in ADHD (46). The higher internalizing disorders ratio as a psychiatric comorbidity in females to males has been shown in literature with little controversy (39, 46). In accordance with this literature, we found higher frequencies of depression and SAD in females than males. It could be helpful for clinicians to pay particular attention when investigating comorbid internalizing disorders in young females with ADHD. Depressive and anxiety symptoms including concentration difficulties may be the first reason to engage in treatment-seeking behavior in females and may obscure underlying ADHD symptoms (47). Thus, clinicians should be aware of the possibility of underlying ADHD in female youth admitted to a clinic with complaints of anxiety and depression for the first time. There was no difference in CSHQ total score in terms of gender. This may suggest that other variables such as ADHD presentation, comorbidity, and medication status may affect sleep problems in young subjects with ADHD, rather than age and gender.

Meanwhile, the risk of being overweight doubled in subjects with ADHD and it has been reported that they may have higher BMI score compared to the non-ADHD peers

(48). Tendency for being overweight may be greater from age 10-12 onwards (48). As consistent with this, we found higher mean BMI scores in adolescents than children.

CONCLUSIONS AND LIMITATIONS

High rates of psychiatric comorbidity and sleep problems were found in children and adolescents with ADHD in this study. Subjects with ADHD-C had more frequent lifetime diagnosis of ODD, elimination disorders, and sleep problems compared to subjects with ADHD-I. In subjects with ADHD-I generalized anxiety (GAD) and social anxiety (SAD) disorders were found more frequently than with ADHD-C. Females, compared to males, have more frequent diagnoses of depression and SAD. There was a positive correlation between the mean number of comorbid diagnoses, self-reported anxiety/depressive symptoms, and CSHQ total scores. While BMI scores did show significant differences between ADHD and gender groups, adolescents had higher scores compared to children. Children and adolescents with ADHD should be routinely evaluated for psychiatric comorbidity and sleep problems regardless of ADHD presentation, age, or gender.

Regarding study limitations, it may be important to note that this study was conducted in a university hospital clinic with voluntary participation and all subjects were on medication. Findings of the study may be biased due to voluntary participation and medication use; and may not reflect ADHD subjects in general.

Acknowledgments: The authors would like to thank the children and their families for participation from the bottom of our hearts.

Ethics Committee Approval: This study was approved by the-Ethical Committee of the Istanbul University Istanbul Faculty of Medicine (2015/1857).

Informed Consent: Written consent was obtained from the participants.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- M.C, G.G., A.K.; Data Acquisition- M.C, G.G.; Data Analysis/Interpretation-M.C, G.G., A.A., A.K.; Drafting Manuscript- M.C., A.A., G.G.; Critical Revision of Manuscript- M.C., A.K., A.A.; Final Approval and Accountability- M.C, G.G., A.A., A.K.; Technical or Material Support- A.K., A.A., G.G.; Supervision- M.C.

Conflict of Interest: Authors declared no conflict of interest.

Financial Disclosure: Authors declared no financial support.

Teşekkür: Yazarlar, çocuklara ve ailelerine katılımlarından dolayı teşekkür eder.

Etik Komite Onayı: Bu çalışma için etik komite onayı İstanbul Üniversitesi İstanbul Tıp Fakültesi Etik Kurulu'ndan alınmıştır (2015/1857).

Bilgilendirilmiş Onam: Katılımcılardan bilgilendirilmiş onam alınmıştır.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Çalışma Konsepti/Tasarım- M.C, G.G., A.K.; Veri Toplama- M.C, G.G.; Veri Analizi/Yorumlama- M.C, G.G., A.A., A.K.; Yazı Taslağı- M.C., A.A., G.G.; İçeriğin Eleştirel İncelemesi-M.C., A.K., A.A.; Son Onay ve Sorumluluk- M.C, G.G., A.A., A.K.; Malzeme ve Teknik Destek- A.K., A.A., G.G.; Süpervizyon- M.C.

Çıkar Çatışması: Yazarlar çıkar çatışması beyan etmemişlerdir.

Finansal Destek: Yazarlar finansal destek beyan etmemişlerdir.

REFERENCES

1. Willcutt EG. The prevalence of DSM-IV attention-deficit/hyperactivity disorder: a meta-analytic review. Neurotherapeutics 2012;9:490-9. [CrossRef]
2. Dulcan M, Dunne JE, Ayres W, Arnold V, Benson RS, Bernet W, et al. Practice parameters for the assessment and treatment of children, adolescents, and adults with attention-deficit/hyperactivity disorder. J Am Acad Child Adolesc Psychiatry 1997;36:85S-121S. [CrossRef]
3. Agnew-Blais JC, Moffitt TE, Arseneault L, Polanczyk G V., Wertz J, Danese A. Young adult mental health and functional outcomes among individuals with remitted, persistent and late-onset ADHD. Br J Psychiatry 2018;213:526-34. [CrossRef]
4. Jensen PS, Martin D, Cantwell DP. Comorbidity in ADHD: Implications for research, practice, and DSM-V. J Am Acad Child Adolesc Psychiatry 1997;36:1065-79. [CrossRef]
5. Pliszka SR. Conceptual issues in understanding comorbidity in ADHD. Attention-Deficit Hyperact Disord Adults Child 2015:63-71. [CrossRef]
6. Inci SB, Ipci M, Akyol Ardıç U, Ercan ES. Psychiatric Comorbidity and Demographic Characteristics of 1,000 Children and Adolescents With ADHD in Turkey. J Atten Disord 2019;23:1356-67. [CrossRef]
7. Jensen PS, Hinshaw SP, Kraemer HC, Lenora N, Newcorn JH, Abikoff HB, et al. ADHD comorbidity findings from the MTA study: Comparing comorbid subgroups. J Am Acad Child Adolesc Psychiatry 2001;40:147-58. [CrossRef]
8. Cuffe SP, Visser SN, Holbrook JR, Danielson ML, Geryk LL, Wolraich ML, et al. ADHD and Psychiatric Comorbidity: Functional Outcomes in a School-Based Sample of Children. J Atten Disord 2015. pii: 1087054715613437. [Epub ahead of print] [CrossRef]
9. Takahashi K, Miyawaki D, Suzuki F, Mamoto A, Matsushima N, Tsuji H, et al. Hyperactivity and comorbidity in Japanese children with attention-deficit/hyperactivity disorder. Psychiatry Clin Neurosci 2007;61:255-62. [CrossRef]
10. Mick E, Biederman J, Jetton J, Faraone S V. Sleep Disturbances Associated with Attention Deficit Hyperactivity Disorder: The Impact of Psychiatric Comorbidity and Pharmacotherapy. J Child Adolesc Psychopharmacol 2002;10:223-31. [CrossRef]
11. Cortese S, Brown TE, Corkum P, Gruber R, O'Brien LM, Stein M, et al. Assessment and management of sleep problems in youths with attention-deficit/hyperactivity disorder. J Am Acad Child Adolesc Psychiatry 2013;52:784-96. [CrossRef]

12. Coskun M, Emine A, Ozturk M. Mirtazapine treatment for comorbid anxiety/depressive disorders in young subjects with attention-deficit hyperactivity disorder: Case series. Klin Psikofarmakol Bul 2010;20:246-51. [CrossRef]
13. Zorlu A, Unlu G, Cakaloz B, Zencir M, Buber A, Isildar Y. The Prevalence and Comorbidity Rates of ADHD Among School-Age Children in Turkey. J Atten Disord 2015. pii: 1087054715577991. [Epub ahead of print] [CrossRef]
14. Lambek R, Virring A, Jennum PJ, Thomsen PH, Møller LR. Sleep Problems and Daily Functioning in Children With ADHD: An Investigation of the Role of Impairment, ADHD Presentations, and Psychiatric Comorbidity. J Atten Disord 2014;21:731-40. [CrossRef]
15. Tarakçıoğlu MC, Kadak MT, Gürbüz H, Doğan E, Erdoğan F. Sleep Habits In Children And Adolescent Diagnosed With Attention Deficit Hyperactivity Disorder. Med J Haydarpaşa Numune Train Res Hosp 2016;56:71-7.
16. Öner P, Üneri ÖŞ, Rezaki B, Bodur Ş. Dikkat Eksikliği Hiperaktivite Bozukluğu olan Çocuklarda Uyku Sorunları ve Klinik Değişkenlerle İlişkisi. Kriz Derg 2008;16:25-32. [CrossRef]
17. Coskun M, Kaya İ. Dikkat Eksikliği Hiperaktivite Bozukluğu ve Diğer Komorbid Durumlar. Turkiye Klin J Child Psychiatry-Special Top 2015;1:68-76.
18. Koyuncu A, Çelebi F, Ertekin E, Kök BE, Tükel R. Clinical Effects of ADHD Subtypes in Patients With Social Anxiety Disorder. J Atten Disord 2019;23:1464-9. [CrossRef]
19. Soendergaard HM, Thomsen PH, Pedersen E, Pedersen P, Poulsen AE, Winther L, et al. Associations of Age, Gender, and Subtypes With ADHD Symptoms and Related Comorbidity in a Danish Sample of Clinically Referred Adults. J Atten Disord 2016;20:925-33. [CrossRef]
20. Kaufman J, Birmaher B, Brent D, Rao U, Flynn C, Moreci P, et al. Schedule for affective disorders and schizophrenia for school-age children-present and lifetime version (K-SADS-PL): Initial reliability and validity data. J Am Acad Child Adolesc Psychiatry 1997;36:980-8. [CrossRef]
21. Coskun M, Hajdini A, Alnak A, Karayagmurlu A. Internet Use Habits, Parental Control and Psychiatric Comorbidity in Young Subjects with Asperger Syndrome. J Autism Dev Disord 2020;50:171-9. [CrossRef]
22. Ercan ES, Polanczyk G, Akyol Ardıc U, Yuce D, Karacetın G, Tufan AE, et al. The prevalence of childhood psychopathology in Turkey: a cross-sectional multicenter nationwide study (EPICPAT-T). Nord J Psychiatry 2019;73:132-40. [CrossRef]
23. Gokler B, Unal F, Pehlivantürk B, Cengel Kultur E, Akdemir D. Reliability and validity of schedule for affective disorders and Schizophrenia for school age children-present and lifetime version-Turkish version (K-SADS-PL-T) [in Turkish]. Turkish J Child Adolesc Ment Heal 2004;11:109-16.
24. Owens JA, Spirito A, McGuinn M. The Children's Sleep Habits Questionnaire (CSHQ): Psychometric Properties of A Survey Instrument for School-Aged Children. Sleep 2017;23:1-9. [CrossRef]
25. Fis NP, Arman A, Ay P, Topuzoglu A, Gueler AS, Imren SG, et al. The validity and the reliability of Turkish Version of Children's Sleep Habits Questionnaire. Anatol J Psychiatry 2010;11:151-60. [CrossRef]
26. Kovacs M. Rating scales to assess depression in school-aged children. Int J Child Adolesc Psychiatry 1981;46:305-15.
27. Öy B. Çocuklar İçin Depresyon Ölçeği: Geçerlik ve Güvenirlik Çalışması. Türk Psikiyatri Dergisi 1991;2(2):132-136.
28. Birmaher B, Khetarpal S, Brent D, Cully M, Balach L, Kaufman J, et al. The Screen for Child Anxiety Related Emotional Disorders (SCARED): Scale construction and psychometric characteristics. J Am Acad Child Adolesc Psychiatry 1997;36:545-53. [CrossRef]
29. Çakmakçı F. Çocuklarda Anksiyete Bozukluklarını Tarama Ölçeği Geçerlik Ve Güvenirlik Çalışması [in Turkish]. Turkish J Child Adolesc Ment Heal 2004;11.
30. Jensen CM, Steinhausen H-C. Comorbid mental disorders in children and adolescents with attention-deficit/hyperactivity disorder in a large nationwide study. ADHD Atten Deficit Hyperact Disord 2015;7:27-38. [CrossRef]
31. Corkum P, Davidson F, MacPherson M. A Framework for the Assessment and Treatment of Sleep Problems in Children with Attention-Deficit/Hyperactivity Disorder. Pediatr Clin North Am 2011;58:667-83. [CrossRef]
32. Sung V, Hiscock H, Sciberras E, Efron D. Sleep problems in children with attention-deficit/hyperactivity disorder: Prevalence and the effect on the child and family. Arch Pediatr Adolesc Med 2008;162:336-42. [CrossRef]
33. Tarakcioglu MC, Akkin Gurbuz G, Kadak MT, poyraz BC, aksoy umut mert, erdogan firat. Is there a relationship between the symptoms of Attention Deficit Hyperactivity Disorder, ADHD, and chronotype in medication-free children? A preliminary study in children under 12 years of age. Arch Neuropsychiatry 2018;55:54-8. [CrossRef]
34. Mayes SD, Calhoun SL, Bixler EO, Vgontzas AN, Mahr F, Hillwig-Garcia J, et al. ADHD subtypes and comorbid anxiety, depression, and oppositional-defiant disorder: Differences in sleep problems. J Pediatr Psychol 2009;34:328-37. [CrossRef]
35. Souza I, Pinheiro MA, Denardin D, Mattos P, Rohde LA. Attention-Deficit/Hyperactivity Disorder and comorbidity in Brazil: Comparisons between two referred samples. Eur Child Adolesc Psychiatry 2004;13:243-8. [CrossRef]
36. Ahmad G. Psychiatric comorbidity differences in clinic-referred children and adolescents with ADHD according to the subtypes and gender. J Child Neurol 2009;24:679-84. [CrossRef]
37. Thiruchelvam D, Charach A, Schachar RJ. Moderators and mediators of long-term adherence to stimulant treatment in children with ADHD. J Am Acad Child Adolesc Psychiatry 2001;40:922-8. [CrossRef]
38. Franco I, Von Gontard A, De Gennaro M. Evaluation and treatment of nonmonosymptomatic nocturnal enuresis: A standardization document from the international children's continence society. J Pediatr Urol 2013;9:234-43. [CrossRef]
39. Yüce M, Zoroglu SS, Ceylan MF, Kandemir H, Karabekiroglu K. Psychiatric comorbidity distribution and diversities in children and adolescents with attention deficit/hyperactivity disorder: A study from Turkey. Neuropsychiatr Dis Treat 2013;9:1791-9. [CrossRef]
40. Ushijima H, Usami M, Saito K, Kodaira M, Ikeda M. Time course of the development of depressive mood and oppositional defiant behavior among boys with attention deficit hyperactivity disorder: Differences between subtypes. Psychiatry Clin Neurosci 2012;66:285-91. [CrossRef]

41. Baeyens D, Roeyers H, Hoebeke P, Verté S, Van Hoecke E, Vande Walle J. Attention deficit/hyperactivity disorder in children with nocturnal enuresis. J Urol 2004;171:2576-9. [CrossRef]
42. Khazaie H, Eghbali F, Amirian H, Moradi MR, Ghadami MR. Risk Factors of Nocturnal Enuresis in Children with Attention Deficit Hyperactivity Disorder. Shanghai Arch Psychiatry 2018;30(1):20-6.
43. Lin L-Y, Soong W-T, Tai Y-M, Shang C-Y, Chiu Y-N, Gau SS-F, et al. Association between symptoms and subtypes of attention-deficit hyperactivity disorder and sleep problems/disorders. J Sleep Res 2010;19:535-45. [CrossRef]
44. Eyuboglu M, Eyuboglu D. Behavioural sleep problems in previously untreated children with attention deficit hyperactivity disorder. Psychiatry Clin Psychopharmacol 2018;28:19-24. [CrossRef]
45. Connor DF, Edwards G, Fletcher KE, Baird J, Barkley RA, Steingard RJ. Correlates of comorbid psychopathology in children with ADHD. J Am Acad Child Adolesc Psychiatry 2003;42:193-200. [CrossRef]
46. Wilens TE, Biederman J, Faraone S V., Martelon M, Westerberg D, Spencer TJ. Presenting ADHD symptoms, subtypes, and comorbid disorders in clinically referred adults with ADHD. J Clin Psychiatry 2009;70:1557-62. [CrossRef]
47. Quinn PO, Madhoo M. A review of attention-deficit/hyperactivity disorder in women and girls: Uncovering this hidden diagnosis. Prim Care Companion J Clin Psychiatry 2014;16:PCC.13r01596. [CrossRef]
48. Fliers EA, Buitelaar JK, Maras A, Bul K, Höhle E, Faraone S V., et al. ADHD is a risk factor for overweight and obesity in children. J Dev Behav Pediatr 2013;34:566-74. [CrossRef]

İSTANBUL ÜNİVERSİTESİ YAYINEVİ

RESEARCH / ARAŞTIRMA
DOI: 10.26650/IUITFD.2020.0013
İst Tıp Fak Derg 2020 / J Ist Faculty Med 2020

THE TOTAL ANTIOXIDANT CAPACITY MAY NOT BE RELATED TO BILIRUBIN AND URIC ACID LEVEL IN PATIENTS WITH BETA THALASSEMIA

BETA TALASEMİ HASTALARINDA TOTAL ANTİOKSİDAN KAPASİTESİ BİLİRUBİN VE ÜRİK ASİT SEVİYESİ İLE İLİŞKİLİ DEĞİLDİR

Zeynep KARAKAŞ[1], Yasin YILMAZ[1,2], Dolay Damla ÇELİK[3], Agageldi ANNAYEV[4], Serap ERDEM KURUCA[3]

[1]Istanbul University, Istanbul Faculty of Medicine, Department of Pediatrics, Division Pediatrics Hematology, Thalassemia Center, Turkey
[2]İstanbul University, Institute of Health Sciences, Aziz Sancar Institute of Experimental Medicine, Department of Neuroscience, Istanbul, Turkey
[3]Istanbul University, Istanbul Faculty of Medicine, Department of Physiology, Turkey
[4]Acıbadem Hospital, Department of Pediatrics, Istanbul, Turkey

ORCID IDs of the authors: Z.K. 0000-0002-8835-3235; Y.Y. 0000-0002-4811-5750; D.D.Ç. 0000-0002-7523-8465; A.A. 0000-0002-9780-4843; S.E.K. 0000-0002-5566-7844

Cite this article as: Karakas Z, Yilmaz Y, Celik DD, Annayev A, Erdem Kuruca S. The total antioxidant capacity may not be related to bilirubin and uric acid level in patients with beta thalassemia. J Ist Faculty Med 2020;83(4):373-7. doi: 10.26650/IUITFD.2020.0013

ABSTRACT

Objective: Iron burden resulting from ineffective erythropoiesis and multiple transfusions can cause oxidative stress in patients with ß-thalassemia. Here we aimed to examine the total antioxidant and oxidant capacity (TAC and TOC) along with its relation to endogenous antioxidants (bilirubin and uric acid) in patients with ß-thalassemia.

Material and Method: Forty-five patients with transfusion-dependent (TDT) (n=30) and non-transfusion-dependent (NTDT) (n=15) ß-thalassemia and 20 healthy subjects were enrolled in the study. Analyses were done using Total Antioxidant Status (TAS) and Total Oxidant Status (TOS) kits.

Results: The TAC level of the patients was significantly increased compared to healthy subjects (2.75 vs. 2.10 mmol/L; p=0.01). The total bilirubin level was significantly elevated in NTDT patients compared to TDT patients (5.7±3.3 vs.1.9±1.4; p<0.001). No significant relationship between endogenous antioxidants and total antioxidant capacity of patients was detected (p=0.20)

Conclusion: The total antioxidant capacity of patients with ß-thalassemia might not be directly related to endogenous anti-oxidative status.

Keywords: Beta-thalassemia, antioxidant capacity, oxidant capacity, endogenous antioxidant

ÖZET

Amaç: Beta talasemi hastalarında inefektif eritropoez ve çoklu transfüzyonlardan kaynaklanan demir birikimi, hastaları oksidatif strese maruz bırakmaktadır. Bu çalışmada, beta talasemi hastalarında total antioksidan ve oksidan kapasiteleri ile bunların endojen antioksidanlar (bilirubin, ürik asid) arasındaki ilişkinin değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntemler: Çalışmaya transfüzyon bağımlı 30, transfüzyon bağımlı olmayan 15 olmak üzere kırk beş beta talasemi hastası ve 20 sağlıklı birey katılmıştır. Analiz için total antioksidan ve total oksidan durum kitleri kullanılmıştır.

Bulgular: Hastaların total antioksidan kapasitesi (TAK) sağlıklı bireylere göre anlamlı olarak yüksek saptanmıştır (2,75 vs. 2,10 mmol/L; p=0,01). Total bilirubin seviyesi transfüzyon bağımlı olmayan hastalarda, transfüzyon bağımlı hastalara oranla anlamlı olarak yüksek bulunmuştur (5,7±3,3 vs.1,9±1,4; p<0,001). Total antioksidan kapasite ile endojen antioksidan seviyeleri arasında anlamlı ilişki saptanmamıştır (p=0,20)

Sonuç: Beta talasemi hastalarının total antioksidan kapasitesi, hastaların endojen antioksidan durumları ile direkt ilişkili olmayabilir.

Anahtar Kelimeler: Beta talasemi, antioksidan kapasitesi, oksidan kapasitesi, endojen antioksidan

Corresponding author/İletişim kurulacak yazar: zeynepkar@hotmail.com

Submitted/Başvuru: 24.09.2019 • **Revision Requested/Revizyon Talebi:** 13.02.2020 •
Last Revision Received/Son Revizyon: 14.02.2020 • **Accepted/Kabul:** 02.03.2020 • **Published Online/Online Yayın:** 21.09.2020

INTRODUCTION

ß-thalassemia (BT) is a common monogenic disease. The Thalassemia International Federation (TIF) classifies thalassemia syndromes as either transfusion-dependent (TDT) or non-transfusion-dependent thalassemia (NTDT) (1).

Transfusion-dependent thalassemia patients need lifelong periodic blood transfusions. However, the complications of transfusions (mainly iron deposition) cause oxidative reactions, and iron chelation treatment is used to avoid iron-originated oxidative damage (2). To overcome oxidative stress, the body also uses endogenous (uric acid, bilirubin, and enzymes) antioxidant mechanisms.

Recently, total antioxidant capacity assays have become readily available and widely used (3). To use simple kits is much more convenient than attempting to measure the separate antioxidant statuses of the body. Our aim in this study was to evaluate the antioxidant and oxidant capacity of thalassemic patients and to reveal its association with endogenous antioxidants (uric acid, bilirubin).

MATERIAL AND METHOD

Participants

Twenty female and 25 male patients with ß-thalassemia [mean (±SD) age=27.3±9 years; range, 12–59 years] (non-transfusion-dependent n=15, transfusion-dependent n=30) from Istanbul Medical Faculty Thalassemia Center, along with a healthy control group comprised of 15 women and five men [mean (±SD) age=31.9±8 years; range, 18–45 years)], were recruited into the study. Eighty-four percent of patients were treated with deferasirox (DFX), and 11% of them were taking deferiprone (DFP). Two patients used no chelators. Fifty-three percent of the patients had undergone splenectomy. The mean ferritin level was 1410±1087 ng/mL, and almost half of the patients (48%) had a ferritin level of less than 1000 ng/ml.

The patients were recruited from a group of regularly followed-up outpatient patients who regularly came into the clinic for check-ups. During the study period, which was open for 3 months, patients with ß-thalassemia were asked to participate in the study. Researcher was in the clinic one day a week and met with the informed patients. Patients with acute infections (e.g., respiratory, gastrointestinal) and recent surgery history were not included in the study. Each patient's serum analysis (TAC and TOC level) was checked at the end of the study period. The healthy control group was recruited from among hospital staff (without any infection or chronic disorder). As the study period was restricted, only a limited number of healthy people participated in the trial.

Oxidant and antioxidant kits

TAC was used clinically as a biomarker measuring the antioxidant status of the body and was seen as more practical than measuring antioxidants individually owing to their synergistic interaction (4, 5). The total antioxidant and oxidant capacity were analyzed using Rel Assay Diagnostics kits (Turkey). The kits had Trolox Equivalent Antioxidant Capacity (TEAC) to analyze samples (6). Blood samples (serum) were gathered from patients and analyzed in a laboratory. To prevent the transfusion-related acute changes, blood sampling was carried out just before transfusion.

Statistics

The SPSS 21.0 (IBM Corp) software package was used for statistical analysis. For the evaluation of categorical variables, the Chi-square test was used. For the comparison of the two groups, the Mann-Whitney U test and Student's t-test were used. For the relationship between continuous variables, Pearson's and Spearman's tests were performed. P-values of <0.05 were considered significant.

RESULTS

Patients with transfusion-dependent and non-dependent thalassemia did not differ in terms of total antioxidant capacity (TAC) (2.76±0.65 and 2.72±1.25 mmol/L, p=0.90, respectively). The TOC levels of patients with TDT and NTDT were 18.76±15.05 and 31.96±33.02 µmol/L, respectively (p=0.15) (Figure 1).

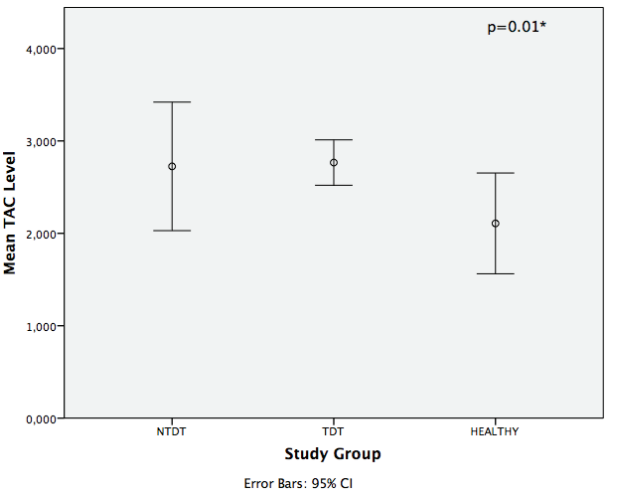

Figure 1: The mean TAC level in study group.
*p value lower than 0.05 is significant

The TAC level of the patients was significantly higher than in the healthy group (2.75±0.88 vs. 2.10±1.16 mmol/L, p=0.01). However, the TOC level did not significantly differ between the patients and the healthy group (23.16±23.15 vs. 16.79±11.87 µmol/L, p=0.25) (Figure 1). The total bilirubin level was significantly higher in the

Table 1. Laboratory values of study groups.

Characteristics	NTDT group	TDT group	Statistics
Ferritin	796±718 ng/ml	1728±1119 ng/ml	p=0.002*
Bilirubin	5.7±3.3 mg/dl	1.9±1.4 md/dl	p<0.001*
Uric Acid	3.8±1.2 mg/dl	4.2±1.2 md/dl	p=0.52
Albumin	4.9±0.4 g/dl	4.8±0.2 g/dl	p=0.51
ALT	22.6±19.6 U/l	30.2±25.0 U/l	p=0.32
AST	24.1±8.8 U/l	27.7±11.6 U/l	p=0.30

*p value lower than 0.05 is significant

NTDT group than the TDT group (5.7±3.3 vs. 1.9±1.4 mg/dl, p<0.001). The uric acid level did not differ between the NTDT and TDT groups (3.8±1.2 vs. 4.2±1.2 mg/dl; p=0.52)(Table 1). We detected no significant correlations between TAC and TOC levels and endogenous antioxidants (uric acid and bilirubin).

A simple linear regression was used to predict the TAC level based on the serum uric acid level. No significance was found (F(1,24)=1.71, p=0.20) with an R^2 of 0.06. Likewise, linear regression was also done to predict TOC level based on uric acid, and no significance was found (F(1,24)=0.01, p=0.91) with an R^2 of -0.04. Similar regression results were obtained for the correlation between total bilirubin level with TAC and TOC level (p=0.94 and p=0.27, respectively).

The mean WBC of patients was 13,030±13,100/mm^3, and the mean hemoglobin level was 9.2±1.5 gr/dl. There was no correlation between the TAC level with WBC and hemoglobin level (p=0.28 and p=0.27, respectively).

Both the TAC and TOC levels were higher (non-significantly) in the DFX group than in the DFP group of all

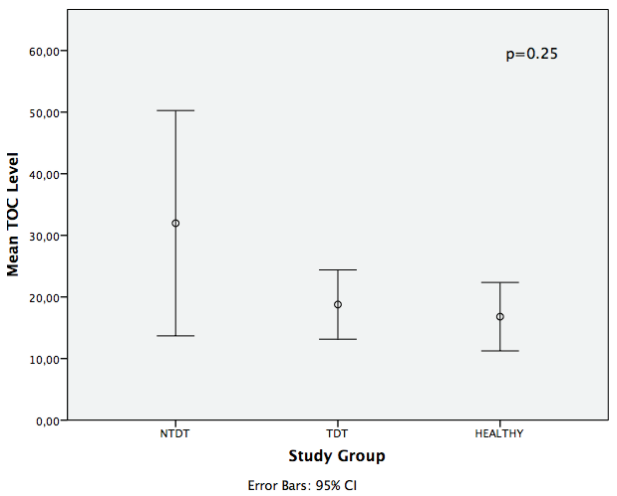

Figure 2: The mean TOC level in study group.

ß-thalassemia patients (2.77 vs. 2.48 mmol/L for TAC and 24.36 vs. 17.94 µmol/L for TOC). We detected no significant associations between TAC and TOC levels with age, gender, splenectomy, or ferritin level.

DISCUSSION

In this study, we found that TAC levels were significantly increased in ß-thalassemia patients compared to the control group. Among thalassemic patients, the TDT group had higher TAC levels, and the NTDT group had higher TOC levels, but these differences did not reach statistical significance. The endogen antioxidant and total bilirubin levels were significantly elevated in the NTDT group.

Iron overload continues to be the main problem in patients with thalassemia. Frequent transfusions, ineffective erythropoiesis, and inadequate iron excretory pathways cause iron deposition (7). Accumulation of iron can cause reactive oxygen species (ROS), which, in turn, leads to oxidative stress (8).

Several studies have investigated the oxidant-antioxidant status of patients with thalassemia (9-13). Asif et al. (9) detected high levels of TOC and TAC in children with thalassemia. In our study, we also found high levels of TAC in the patient group. However, we detected no increase in TOC levels in the patient group. This controversial data led us to speculate that antioxidant status might not be dependent on the body oxidant status of endogenous body antioxidant (bilirubin and uric acid) status.

Ferro et al. (10) argued that the higher hemoglobin levels provided by regular transfusions help limit oxidative damage. However, we detected no difference in the hemoglobin levels in the thalassemic group and no correlations between hemoglobin level and TAC and TOC levels.

Another study (11) reported high levels of TAC in patients with thalassemia. According to this author's proposals,

more transfusions necessitate higher antioxidant status. In our study, TDT showed a higher, but insignificant, antioxidant capacity. We also found that bilirubin (endogenous antioxidant molecule) was elevated in the NTDT patients.

Several studies found controversially low levels of TAC in patients with thalassemia. Cakmak et al. reported non-significant differences in TAC levels (12). Hamed et al. found decreased TAC levels in patients receiving chelation therapy (13). They also showed that TAC levels were significantly lower than those without chelation therapy. We detected no differences between the two chelators in terms of TAC levels, possibly because of a disproportionate number of patients using chelation (38 vs. 5). On the other hand, there were only two patients who did not use chelation. Therefore, it is difficult to speculate about the chelation effect on antioxidant capacity.

Interestingly, a recent meta-analysis showed that TAC levels were decreased in patients with thalassemia (14). Taken together, the findings of these studies suggest that the antioxidant capacity of patients with thalassemia is more complicated than we currently think. A limitation of this study was the small sample size. Insufficient data on the patients' dietary intake is a further limitation.

CONCLUSION

Here we report an increase in the anti-oxidative status in patients with thalassemia. Many factors influence anti-oxidative mechanisms, such as high levels of endogenous antioxidants and chelation therapy. These controversial results show that more studies are necessary to delineate the antioxidant mechanism of beta-thalassemia patients.

Acknowledgments: We thank Mr. David Chapman for copyediting the manuscript in English language.

Ethics Committee Approval: This study was approved by Istanbul University Istanbul Faculty of Medicine Clinical Research Ethics Committee (05/2014).

Informed Consent: Written consent was obtained from the participants.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- Z.K., A.A., S.E.K.; Data Acquisition- D.D.Ç., A.A.; Data Analysis/Interpretation- Z.K., Y.Y., D.D.Ç.; Drafting Manuscript- Y.Y., A.A., D.D.Ç.; Critical Revision of Manuscript- Z.K., S.E.K.; Final Approval and Accountability- Z.K., Y.Y., D.D.Ç., A.A., S.E.K.; Technical or Material Support- Z.K., D.D.Ç.; Supervision- Z.K.

Conflict of Interest: Authors declared no conflict of interest.

Financial Disclosure: This study was supported by Istanbul University Scientific Research Projects.

Teşekkür: Yazıyı ingilizce dili açısından düzenlediği için David Chapman'a teşekkür ederiz.

Etik Komite Onayı: Bu çalışma için etik kurul onayı İstanbul Üniversitesi İstanbul Tıp Fakültesi Klinik Araştırmalar Etik Kurulu'ndan alınmıştır (05/2014).

Bilgilendirilmiş Onam: Katılımcılardan bilgilendirilmiş onam alınmıştır.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Çalışma Konsepti/Tasarım- Z.K., A.A., S.E.K.; Veri Toplama- D.D.Ç., A.A.; Veri Analizi/Yorumlama- Z.K., Y.Y., D.D.Ç.; Yazı Taslağı- Y.Y., A.A., D.D.Ç.; İçeriğin Eleştirel İncelemesi- Z.K., S.E.K.; Son Onay ve Sorumluluk- Z.K., Y.Y., D.D.Ç., A.A., S.E.K.; Malzeme ve Teknik Destek- Z.K., D.D.Ç.; Süpervizyon- Z.K.

Çıkar Çatışması: Yazarlar çıkar çatışması beyan etmemişlerdir.

Finansal Destek: Bu çalışma İstanbul Üniversitesi Bilimsel Araştırma Projeleri kapsamında desteklenmiştir.

REFERENCES

1. Viprakasit V, Origa R. Genetic basis, pathophysiology and diagnosis. In: Cappellini MD, Cohen A, Porter J, Taher A, Viprakasit V, eds. Guidelines for the management of transfusion dependent thalassaemia (TDT). Nicosia: Thalassaemia International Federation (TIF) Publication, No. 20, third ed; 2014. p. 14-26.
2. Rund D, Rachmilewitz E. Beta-thalassemia. N Engl J Med 2005;353:1135-46. [CrossRef]
3. Bartosz G. Non-enzymatic antioxidant capacity assays: Limitations of use in biomedicine. Free Radic Res 2010;44(7):711-20. [CrossRef]
4. Apak R, Gorinstein S, Böhm V, Schaich KM, Ozyurek M, Guclu K. Methods of measurement and evaluation of natural antioxidant capacity/activity (IUPAC Technical Report). Pure Appl. Chem 2013;85:957-98. [CrossRef]
5. Niki E. Assessment of Antioxidant Capacity in vitro and in vivo. Free RadicBiol Med 2010;49:503-15. [CrossRef]
6. Dilis V, Trichopoulou A. Assessment of antioxidants in foods and biologicalsamples: a short critique. Int J Food Sci Nutr 2010;61(5):441-8. [CrossRef]
7. Rachmilewitz EA, Giardin PJ. How I treat thalassemia. Blood 2011;118:3479-88. [CrossRef]
8. Taher AT, Weatherall DJ, Cappellini MD. Thalassaemia. Lancet 2018;391:155-67. [CrossRef]
9. Asif M, Manzoor Z, Farooq MS, Munawar SH, Aziz A, Khan IA. Status of oxidant, antioxidantand serum enzymes in thalassaemic children receiving multiple blood transfusions. J Pak Med Assoc 2015;65(8):838-43.
10. Ferro E, Visalli G, La Rosa MA, Civa R, Papa GR, D'Ascola DG, et al. The role of anaemia in oxidative and genotoxic damage in transfused ß-thalassaemic patients. Hematology 2017;22:183-91. [CrossRef]

11. Bazvand F, Shams S, BorjiEsfahani M, Koochakzadeh L, Monajemzadeh M, Ashtiani MT, et al. Total Antioxidant Status in Patients with Major ß-Thalassemia. Iran J Pediatr 2011;21(2):159-65.
12. Cakmak A, Soker M, Koc A, Erel O. Paraoxonase and arylesteraseactivity with oxidative status in children with thalassemia major. J Pediatr Hematol Oncol 2009;31:583-7. [CrossRef]
13. Hamed EA, El Melegy NT. Renal functions in pediatric patients withbeta-thalassemia major: relation to chelation therapy: originalprospective study. Ital J Pediatr 2010;36:39-42. [CrossRef]
14. Manafikhi H, Drummen G, Palmery M, Peluso I. Total Antioxidant Capacity in beta-thalassemia: A systematic review and meta-analysis of case-control studies. Crit Rev Oncol Hematol 2017;110:35-42. [CrossRef]

İSTANBUL ÜNİVERSİTESİ YAYINEVİ

RESEARCH / ARAŞTIRMA
DOI: 10.26650/IUITFD.2020.0079
İst Tıp Fak Derg 2020 / J Ist Faculty Med 2020

THE PREDICTIVE VALUE OF ULTRASONOGRAPHY FOR POTENTIALLY MALIGNANT THYROID NODULES

POTANSİYEL MALİGN TİROİD NODÜLLERİ İÇİN ULTRASONOGRAFİNİN TAHMİN DEĞERİ

İlker ÖZGÜR[1], Ahmet Yalın İŞCAN[1], İsmail Cem SORMAZ[1], Fatih TUNCA[1], Artur SALMASLIOGLU[2], Aysel BAYRAM[3], Gülçin YEĞEN[3], Yasemin Giles ŞENYÜREK[1]

Istanbul University, Istanbul Faculty of Medicine, [1]Department of General Surgery, [2]Department of Radiology, [3]Department of Pathology, Istanbul, Turkey

ORCID IDs of the authors: İ.Ö. 0000-0003-1499-0503; A.Y.İ. 0000-0002-5576-9496; İ.C.S. 0000-0001-6907-978X; F.T. 0000-0001-6889-3894; A.S. 0000-0002-8576-7942; A.B. 0000-0002-5014-0074; G.Y. 0000-0003-2497-219X; Y.G.Ş. 0000-0001-5339-1840

Cite this article as: Ozgur I, Iscan AY, Sormaz IC, Tunca F, Salmaslioglu A, Bayram A, et al. The predictive value of ultrasonography for potentially malignant thyroid nodules. J Ist Faculty Med 2020;83(4):378-83. doi: 10.26650/IUITFD.2020.0079

ABSTRACT

Objective: In this study, we aimed to predict the malignancy potential of a thyroid nodule based on ultrasonography (US) features.

Material and Method: The data of 726 nodules in 619 patients who underwent thyroidectomy between 2005 and 2012 at Istanbul University, Istanbul Medical Faculty were retrospectively analyzed. US features of nodules were recorded and matched with pathology reports and each US feature was evaluated for diagnostic value.

Results: The study group consisted of mainly female patients (86.9%) and the mean age was 46,3 years. Thyroid cancer was detected in 374 (51.7%) nodules and the most common type was papillary thyroid cancer 97% (n=341). Microcalcifications (p=0.0001), irregular margin (p=0.001) and hypoechogenicity (p=0.038) were correlated with malignancy. The absence of any of these suspicious US features significantly predicted benign disease (p=0.0001). An increasing number of suspicious features predicted malign disease with increasing specificity, positive predictive value, and likelihood ratio. Microcalcifications and irregular borders had high specificity (>80%) to predict thyroid cancer.

Conclusion: The presence of microcalcifications, irregular margin, and hypoechogenicity in a thyroid nodule significantly correlates with malignant disease. Microcalcifications and irregular borders had high specificity to predict thyroid cancer. The probability of thyroid cancer increases with an increasing number of suspicious US features.

Keywords: Thyroid nodules, ultrasonography, thyroid cancer

ÖZET

Amaç: Bu çalışmada tiroid nodüllerindeki malignite olasılığını ultrasonografi (US) özelliklerinin öngörme seviyesini araştırmayı amaçladık.

Gereç ve Yöntem: İstanbul Üniversitesi İstanbul Tıp Fakültesi'nde, 2005 ila 2012 yılları arasında total tiroidektomi ameliyatı olan 619 hastada 726 nodül geriye dönük olarak incelendi. Tiroid nodüllerinin US özellikleri kaydedildi, patoloji raporları ile karşılaştırılarak eşleştirildi ve US özelliklerin tanısal değeri hesaplandı.

Bulgular: Çalışmaya alınan hastalarda kadın oranı (%86,9) olup, ortalama yaş 46,3 olarak hesaplandı. Tiroid kanseri 374 (%51,7) nodülde tespit edilirken, en sık kanser türü %97 (n=341) oranla papiller tiroid kanseriydi. Tiroid nodülünde mikrokalsifikasyon (p=0,0001), düzensiz sınır (p=0,001) ve hipoekojenite (p=0,038) varlığı malignite ile korelasyon gösterdi. Mikrokalsifikasyon ve düzensiz sınır özelliklerinin kanser öngörüsü bakımından özgüllüğü yüksek (>%80) bulundu. Nodülde bu şüpheli US özelliklerinden hiçbirinin olmaması selim hastalığı (p=0,0001) tahmin etti. Artan US şüpheli özelliklerin sayısı, artan özgüllük, pozitif prediktif değer ve olasılık oranı ile malign hastalığı öngördü. Mikro kalsifikasyonlar ve düzensiz sınır tiroid kanserini öngörmek için yüksek özgüllüğe (>%80) sahipti.

Sonuç: Bir tiroid nodülünde mikrokalsifikasyon, düzensiz sınır ve hipoekojenite varlığında tiroid kanseri olasılığı anlamlı şekilde yüksektir. Malignite öngörüsü açısından özgüllüğü en yüksek bulgular mikrokalsifikasyon ve düzensiz sınırdır. Bir nodüldeki şüpheli US özelliklerinin sayısı arttıkça malignite olasılığı da paralel olarak anlamlı şekilde artar.

Anahtar Kelimeler: Tiroid nodülleri, ultrasonografi, tiroid kanseri

Corresponding author/İletişim kurulacak yazar: dr.ilkerozgur@gmail.com

Submitted/Başvuru: 20.06.2020 • **Accepted/Kabul:** 21.07.2020 • **Published Online/Online Yayın:** 19.10.2020

INTRODUCTION

The prevalence of thyroid nodules in the general population ranges between 3 to 7% for palpable nodules and up to 50 to 67% for those detected by ultrasonography (US) (1). The increasing detection rate of thyroid nodules with the use of high-resolution US has necessitated a careful diagnostic approach to identify nodules with malignant potential. Fine needle aspiration biopsy (FNAB) is the primary diagnostic modality for patients with a thyroid nodule and with its utility, unnecessary surgery rates decreased. The US is also used to determine whether nodules have an increased risk for malignancy. Several sonographic features are associated with an increased risk of thyroid cancer, including hypoechogenicity, microcalcifications, irregular margin, intranodular hypervascularity, and taller rather than wider shape (2, 3). FNAB performed under US guidance improves the diagnostic accuracy of the procedure (4). FNAB is reported to have high sensitivity (68-98 %) and varying specificity (54-90%) (5-7). Nevertheless, false negative (FN) FNAB cytology results are possible (8, 9) due to the likelihood of having an FN result with nodules ≥4 cm (10,11), cystic lesions (12), follicular neoplasms (13), or a follicular variant of papillary thyroid carcinoma (PTC) (14). There is also interobserver variation in the interpretation of some sonographic features, such as microcalcifications, in thyroid US examination (15). New sonographic reporting systems were suggested to stratify the malignancy risk of thyroid nodules according to the certain sonographic features and to facilitate communication among the clinicians (16). This study aimed to determine the US features that were correlated with malignant nodules and to investigate the diagnostic value of these sonographic features to predict malignancy.

MATERIAL AND METHOD

The records of the patients who underwent thyroid surgery from January 2007 to May 2012 at the Department of General Surgery, Istanbul Medical Faculty were retrospectively analyzed. Six-hundred and nineteen patients who had accessible full clinical data and sonography images and/or reports were included. In these 619 patients, 726 index thyroid nodules were evaluated by thyroid ultrasonography (US). Of 619 patients, 494 (80%) had at least two nodules and 125 (20%) solitary thyroid nodule. All patients were evaluated with thyroid function tests. Patients who had depressed levels of serum thyroid-stimulating hormone (TSH) underwent thyroid scintigraphy. Neck US was done in all patients preoperatively by using 13-5 MHz linear array transducer (Sonoline Antares, Siemens, Erlangen, Germany). The US was performed by a single radiologist experienced in thyroid imaging. Nodules were described for size, echographic structure (solid vs cystic), echogenicity (hyper-, hypo-), calcification (micro-, macro-, eggshell or absence), and margin (circumscribed or irregular/lobulated) from US reports and/or images. Taller-than-wider feature was not reported in all of the US reports, and this feature was not included for statistical analysis.

Analysis of US findings

Sonographic and final histopathological findings were correlated in all of the 726 nodules. The records of US findings of index nodules were correlated with the macroscopic and microscopic description of thyroidectomy specimens in the histopathological reports to assess accurate nodule matching. Individual sonographic features that were significantly associated with thyroid cancer at the final histopathological examination were determined as the US features suspicious of thyroid cancer. The diagnostic value of the US to predict thyroid cancer was evaluated according to the presence of single or more suspicious sonographic findings in a thyroid nodule. The presence of single or more suspicious sonographic findings in a nodule was defined as true positive (TP) or false positive (FP) US result in case the nodule proved to be malignant or benign, respectively. The absence of suspicious sonographic findings in a nodule was defined as true negative (TN) or false negative (FN) US result in case the nodule proved to be benign or malignant, respectively. The sensitivity (TP/(TP+FN), specificity (TN/(TN+FP), positive predictive value (TP/(TP+FP), and negative predictive value (TN/(TN+FN) of suspicious US findings for preoperative diagnosis of thyroid cancer were determined.

Statistical evaluation was performed with SPSS Windows Ver. 21.0. The chi-square test was used to determine the differences in the frequency of thyroid cancer in different US features groups. Student's T-test was used to compare the continuous data between the groups. The significance level was set as $p<0.05$.

RESULTS

Of 619 patients, 538 (86.9%) were female and 81 (13.1%) were male with a mean age of 46.3 years. The final histopathological examination revealed thyroid cancer in 374 (51.7%) of 726 nodules. The frequency of papillary thyroid cancer was 97% (n=341), Hurthle cell cancer 1.5% (n=3), follicular cancer 1% (n=2) and medullary cancer 0.5% (n=1) in all patients. The mean tumor size was 23.6±12.8 mm. The diameters of nodules measured by the US ranged from 12 to 80 mm with a mean of 25.3±11.4 mm. There was no statistical significance in nodule size between malignant and benign nodules (19.6±16 mm vs. 18.9±14 mm, $p>0.05$).

Correlation of US features with final histopathology

Of 726 nodules, 365 (50.3%) were solid and 361 (49.7%) had solid-cystic mixed structure. The majority (n=477, 65.7%) of the nodules was hypoechoic. The frequency of

irregular/lobulated margin, macrocalcifications and microcalcifications was 22.2% (n=161), 27.1% (n=197), and 21.3% (n=155), respectively. Seven (0.9%) nodules had egg-shell calcification 0.9%. The final histopathological examination revealed thyroid cancer in 374 (51.5%) of 726 nodules. The rate of malignancy in solid and solid-cystic mixed nodules showed no significant difference (Table 1). Hypoechogenicity, microcalcifications, and irregular/lobulated margin of a nodule were found to be significantly correlated with thyroid cancer (Table 1). These three US features were defined as suspicious for malignancy. The rate of malignancy in hypoechoic nodules and nodules with microcalcifications and irregular/lobulated border was 54.3%, 63.2%, and 71.4%, respectively.

The sensitivity, specificity, PPV, and NPV of suspicious sonographic criteria for preoperative diagnosis of thyroid cancer were shown in Table 2. Hypoechogenicity had the highest sensitivity (69%) but the lowest specificity (38%). The presence of microcalcifications and irregular/lobulated had both high specificity (84% and 87%, respectively) but low sensitivity for preoperative diagnosis of thyroid cancer.

Of 726 nodules, 160 (22%) had no suspicious sonographic features, whereas one, two, or three suspicious sonographic features were present in 374 (51.5%), 164 (22.6%), and 28 (3.9%) nodules, respectively. The absence of suspicious sonographic findings was significantly associated with benign nodule with a likelihood ratio of 22.6 (Table 3). The negative predictive value of US in nodules with no suspicious features was 65%.

The presence of one suspicious feature in a nodule was not associated with increased frequency of malignancy, whereas the presence of two or three suspicious features

Table 1: Correlation of final histopathological diagnosis and US feature groups.

US features	Final histopathology of nodules		*p*
	Malignant (n=374) n (%)	Benign (n=352) n (%)	
Hypoechogenity	259 (69.2)	218 (62)	0.038
Solid nodule	197 (53)	168 (48)	0.2
Mixed structure	177 (47)	184 (52)	0.1
Macro calcifications	92 (24.6)	105 (30)	0.1
Micro calcifications	98 (26)	57 (16)	0.001
Irregular/lobulated margin	115 (30.7)	46 (13)	0.0001
Egg shell calcifications	4 (1)	3 (0.85)	0.1

Table 2: Binary classification of US findings.

US features	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Hypoechogenity	69	38	54	54
Microcalcifications	26	84	63	51
Irregular/lobulated border	31	87	71	54

Table 3: Malignancy and number of suspicious features correlation.

Nodule US features	Final histopathology of nodules		*p*	Likelihood ratio
	Malignant (n=374) n (%)	Benign (n=352) n (%)		
No suspicious feature	56 (15)	104 (29.5)	0.0001	22.6
One suspicious feature	191 (51)	183 (52)	0.8	0.06
Two suspicious feature	103 (27.5)	61 (17.3)	0.001	11
Three suspicious feature	24 (6.4)	4 (1.1)	0.0001	15

Table 4: Binary classification of an increasing number of suspicious US findings.

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
One suspicious feature	51	48	51	48
Two suspicious feature	27.5	82.6	62.8	51.7
Three suspicious feature	6.4	98.8	85.7	50

was significantly associated with cancer with a likelihood ratio of 11 and 15, respectively (Table 3). The probability of malignancy in nodules with one, two, and three suspicious US features was 13.6%, 62.8%, and 85.7%, respectively.

The specificity and PPV of the US for preoperative diagnosis of thyroid cancer in case of the presence of two suspicious US features in a nodule were 82.6% and 62.8%, respectively. The specificity and PPV increased to 98.8% and 85.7%, respectively, if three suspicious US features were observed in the same nodule (Table 4).

DISCUSSION

In this study, we investigated the diagnostic value of thyroid US for preoperative prediction of malignancy in thyroid nodules. We found out that the presence of microcalcifications, an irregular/lobulated margin, and marked hypoechogenicity in a nodule were significantly associated with histopathological diagnosis of thyroid cancer, and microcalcifications and irregular/lobulated margin were the US features which were highly specific for thyroid cancer. The likelihood of thyroid cancer increased as the number of suspicious US features increased in an index nodule.

Thyroid nodules are encountered commonly in daily practice due to the increased detection rate with the use of high-resolution US. It is important to differentiate between malignant and benign nodules for further management of the patient. Uncomplicated benign nodules can be followed up whereas suspicion of malignancy in a nodule constitutes the main indication for surgery. Thyroid US is the most valuable imaging method to determine the morphological characteristics of thyroid nodules, and FNAB is the primary diagnostic tool to predict the malignant potential of nodules (1, 17). The patients with nodular goiter may have solitary or multiple nodules and the likelihood of thyroid cancer per patient is independent of the number of nodules (18). Frates et al. determined that a strategy to biopsy the largest nodule in thyroid with multiple nodules would miss 14% of patients with two nodules who had cancer and approximately 50% of patients with three or more nodules who had cancer (18). The authors suggested the use of sonographic characteristics to prioritize nodules for FNA based on their risk of cancer. Nodule size is not predictive for malignancy (18, 19). Thyroid US is recommended to stratify the risk of malignancy in thyroid nodules and decide whether FNAB is indicated (1). Solid component, hypoechogenicity, microlobulated or irregular margins, microcalcifications, and taller-than-wide shape in a nodule were found to be significantly associated with thyroid cancer (16, 20). In a recent meta-analysis, the specificities of microcalcifications, irregular margins, and a taller than wide shape were found as 87.8%, 83.1%, and 96.6%, respectively (21).

The composition of a nodule is defined as cystic, solid, spongiform, predominantly cystic/solid. The incidence of malignancy was found significantly higher in solid and predominantly solid nodules compared to predominantly cystic nodules (15, 16, 18, 22). Pure cystic nodules have a very low risk of malignancy (15, 22). We found no significant difference in the incidence of thyroid cancer between solid and mixed nodules. A possible explanation for this result may be that the majority of the mixed nodules in our study were predominantly solid and there were no pure cystic nodules included in the study.

The echogenic structure of a nodule refers to the echogenic level of a non-calcified solid portion of the nodule relative to surrounding thyroid parenchyma. A nodule can be described as either entirely or predominantly hyper-, iso-, hypo- or very hypoechoic. The hypoechoic structure was found to have a stronger relationship with cancer compared to iso- or hyperechoic nodules (16, 22). However, the sensitivity and specificity of hypoechogenicity to predict malignancy are not high and reported to be about 62% (21). This finding is similar to our results. We found the sensitivity of hypoechogenicity 69%, but the specificity was 38%.

Calcifications of the thyroid gland are classified as microcalcifications, macrocalcifications, and peripheral rim calcifications so-called as eggshell calcifications. Macrocalcifications alone are not specific for malignancy (23). Macrocalcifications are usually part of the degenerative process and together with eggshell calcifications, they are rarely reported with thyroid carcinoma (16, 24). Although colloid crystals, fibrin debris, or microcystic areas may be observed as echogenic foci by the US mimicking microcalcification, these are benign features (23). True microcalcifications correspond to psammoma bodies and highly specific for malignancy (1, 2, 16, 23). In our study, we found the specificity of microcalcifications to predict malignancy 84% which was compatible with the reported rates in the literature (21).

Irregular or lobulated margins were significantly associated with thyroid cancer compared to well-circumscribed nodules (16). Irregular margins are reported to have low sensitivity but high specificity to predict cancer (1, 25, 26). Previous studies reported the ill-defined borders to be associated with malignancy (18, 25, 26). The arising new technologies and better sonographic ability of ultrasound devices led to better discrimination of borders. The well-known condition of thyroiditis with benign hyperplastic nodules which are reported to have ill-defined margins is a common finding and is not associated with malignancy (16, 29). A taller-than-wide shape is highly specific for malignancy, and this feature was reported to have the highest specificity compared to other US features to predict malignancy (21).

Risk stratification of thyroid nodules according to the sonographic characteristics is crucial to select the nodules for FNAB. Appropriate reporting systems with standard definitions have been necessitated for better communication between clinicians. Several Thyroid Imaging Reporting and Data Systems (TIRADS) such as EU-TIRADS, ACR-TIRADS, Kwak-TIRADS, and ATA US scores were developed (1, 16, 20, 21, 23, 30). All of these reporting systems classify the nodules according to the presence and number of suspicious US features. The risk of malignancy increases as the score or number of suspicious features increases in all of these risk classification systems. ACR-TIRADS is a more complicated system that gives points for each sonographic feature and score and classifies the nodules according to the sum of these points. Numerous studies showed that the diagnostic performance of these reporting systems is similar. In our study, we found that the likelihood of thyroid cancer significantly increased in a nodule when the number of suspicious US features increased. In nodules with two and three suspicious US features, the probability of malignancy increased by about 6 and 8 folds compared to nodules with one suspicious feature. We found that the specificities of the presence of two and three suspicious the US feature in a nodule to predict malignancy was 82.6% and 98.8%, respectively.

There are some limitations to our study. This is a retrospective study. Sonographic data were not collected prospectively. Although it is highly specific for malignancy, the taller-than-wider feature was not available in all sonography reports and it could not be included in statistical analysis. For the same reason, we were unable to classify the nodules and evaluate the malignancy rate according to recently defined reporting systems.

CONCLUSION

The suspicious US features in a thyroid nodule are microcalcification, hypoechogenicity, and irregular/ border. The presence of microcalcification or irregular border predicts malignancy with a specificity higher than 80%. The probability of malignancy in a thyroid nodule significantly increases with the number of suspicious US features.

Ethics Committee Approval: Ethics committee approval was not received due to the retrospective nature of the study.

Informed Consent: Informed consent was not received due to the retrospective nature of the study.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- İ.Ö., Y.G.Ş., F.T.; Data Acquisition- İ.Ö., A.Y.İ., İ.C.S., F.T., A.S., A.B., G.Y., Y.G.Ş..; Data Analysis/Interpretation- İ.Ö., G.Y., Y.G.; Drafting Manuscript-İ.Ö., A.Y.İ., A.S., A.B., Y.G.Ş.; Critical Revision of Manuscript- - İ.Ö., İ.C.S., F.T., Y.G.Ş.; Final Approval and Accountability- İ.Ö., A.Y.İ., İ.C.S., F.T., A.S., A.B., G.Y., Y.G.Ş..; Technical or Material Support- İ.Ö., Y.G.; Supervision- Y.G., F.T.

Conflict of Interest: Authors declared no conflict of interest.

Financial Disclosure: Authors declared no financial support.

Etik Komite Onayı: Retrospektif çalışma olduğundan etik komite onayı alınmamıştır.

Bilgilendirilmiş Onam: Retrospektif bir çalışma olduğundan bilgilendirilmiş onam alınmamıştır

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Çalışma Konsepti/Tasarım- İ.Ö., Y.G.Ş., F.T.; Veri Toplama- İ.Ö., A.Y.İ., İ.C.S., F.T., A.S., A.B., G.Y., Y.G.Ş.; Veri Analizi/Yorumlama- İ.Ö., G.Y., Y.G.; Yazı Taslağı- İ.Ö., A.Y.İ., A.S., A.B., Y.G.Ş.; İçeriğin Eleştirel İncelemesi- İ.Ö., İ.C.S., F.T., Y.G.Ş.; Son Onay ve Sorumluluk- İ.Ö., A.Y.İ., İ.C.S., F.T., A.S., A.B., G.Y., Y.G.Ş..; Malzeme ve Teknik Destek- İ.Ö., Y.G.; Süpervizyon- Y.G., F.T.

Çıkar Çatışması: Yazarlar çıkar çatışması beyan etmemişlerdir.

Finansal Destek: Yazarlar finansal destek beyan etmemişlerdir.

REFERENCES

1. Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, et. al. 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid 2016;26(1):1-133. [CrossRef]
2. Cappelli C, Castellano M, Pirola I, Cumetti D, Agosti B, Gandossi E, et. al. The predictive value of ultrasound findings in the management of thyroid nodules. QJM 2007;100(1):29-35. [CrossRef]
3. Hong YJ, Son EJ, Kim EK, Kwak JY, Hong SW, Chang HS. Positive predictive values of sonographic features of solid thyroid nodule. Clin Imaging 2010;34(2):127-33. [CrossRef]
4. Carmeci C, Jeffrey RB, McDougall IR, Nowels KW, Weigel RJ. Ultrasound-guided fine-needle aspiration biopsy of thyroid masses. Thyroid 1998;8(4):283-9. [CrossRef]

5. Tee YY, Lowe AJ, Brand CA, Judson RT. Fine-needle aspiration may miss a third of all malignancy in palpable thyroid nodules: a comprehensive literature review. Ann Surg 2007;246(5):714-20. [CrossRef]
6. Peng Y, Wang HH. A meta-analysis of comparing fine-needle aspiration and frozen section for evaluating thyroid nodules. Diagn Cytopathol 2008;36(12):916-20. [CrossRef]
7. Oertel YC, Miyahara-Felipe L, Mendoza MG, Yu K. Value of repeated fine needle aspirations of the thyroid: an analysis of over ten thousand FNAs. Thyroid 2007;17(11):1061-6. [CrossRef]
8. Lewis CM, Chang KP, Pitman M, Faquin WC, Randolph GW. Thyroid fine-needle aspiration biopsy: variability in reporting. Thyroid 2009;19(7):717-23. [CrossRef]
9. Yeh MW, Demircan O, Ituarte P, Clark OH. False-negative fine-needle aspiration cytology results delay treatment and adversely affect outcome in patients with thyroid carcinoma. Thyroid 2004;14(3):207-15. [CrossRef]
10. Pinchot SN, Al-Wagih H, Schaefer S, Sippel R, Chen H. Accuracy of fine-needle aspiration biopsy for predicting neoplasm or carcinoma in thyroid nodules 4 cm or larger. Arch Surg 2009;144(7):649-55. [CrossRef]
11. Yoon JH, Kwak JY, Moon HJ, Kim MJ, Kim EK. The diagnostic accuracy of ultrasound-guided fine-needle aspiration biopsy and the sonographic differences between benign and malignant thyroid nodules 3 cm or larger. Thyroid 2011;21(9):993-1000. [CrossRef]
12. Nguyen GK, Lee MW, Ginsberg J, Wragg T, Bilodeau D. Fine-needle aspiration of the thyroid: an overview. Cytojournal 2005;2(1):12. [CrossRef]
13. Faquin WC. Diagnosis and reporting of follicular-patterned thyroid lesions by fine needle aspiration. Head Neck Pathol 2009;3(1):82-5. [CrossRef]
14. Kesmodel SB, Terhune KP, Canter RJ, Mandel SJ, LiVolsi VA, Baloch ZW, et. al. The diagnostic dilemma of follicular variant of papillary thyroid carcinoma. Surgery 2003;134(6):1005-12. [CrossRef]
15. Malhi H, Beland MD, Cen SY, Allgood E, Daley K, Martin SE, et. al. Echogenic foci in thyroid nodules: significance of posterior acoustic artifacts. AJR Am J Roentgenol 2014;203(6):1310-6. [CrossRef]
16. Kwak JY, Han KH, Yoon JH, Moon HJ, Son EJ, Park SH, et. al. Thyroid imaging reporting and data system for US features of nodules: a step in establishing better stratification of cancer risk. Radiology 2011;260(3):892-9. [CrossRef]
17. Gharib H, Papini E, Paschke R, Duick DS, Valcavi R, Hegedüs L, et. al. AACE/AME/ETA Task Force on Thyroid Nodules. American Association of Clinical Endocrinologists, Associazione Medici Endocrinologi, and European Thyroid Association Medical guidelines for clinical practice for the diagnosis and management of thyroid nodules: executive summary of recommendations. Endocr Pract 2010;16(3):468-75. [CrossRef]
18. Frates MC, Benson CB, Doubilet PM, Kunreuther E, Contreras M, Cibas ES, et. al. Prevalence and distribution of carcinoma in patients with solitary and multiple thyroid nodules on sonography. J Clin Endocrinol Metab 2006;91(9):3411-7. [CrossRef]
19. Nam-Goong IS, Kim HY, Gong G, Lee HK, Hong SJ, Kim WB, et. al. Ultrasonography-guided fine-needle aspiration of thyroid incidentaloma: correlation with pathological findings. Clin Endocrinol (Oxf) 2004;60(1):21-8. [CrossRef]
20. Tessler FN, Middleton WD, Grant EG, Hoang JK, Berland LL, Teefey SA, et. al. ACR Thyroid Imaging, Reporting and Data System (TI-RADS): White Paper of the ACR TI-RADS Committee. J Am Coll Radiol 2017;14(5):587-95. [CrossRef]
21. Remonti LR, Kramer CK, Leitão CB, Pinto LC, Gross JL. Thyroid ultrasound features and risk of carcinoma: a systematic review and meta-analysis of observational studies. Thyroid 2015;25(5):538-50. [CrossRef]
22. Frates MC, Benson CB, Charboneau JW, Cibas ES, Clark OH, Coleman BG, et. al. Society of Radiologists in Ultrasound. Management of thyroid nodules detected at US: Society of Radiologists in Ultrasound consensus conference statement. Radiology 2005;237(3):794-800. [CrossRef]
23. Russ G, Bonnema SJ, Erdogan MF, Durante C, Ngu R, Leenhardt L. European Thyroid Association Guidelines for Ultrasound Malignancy Risk Stratification of Thyroid Nodules in Adults: The EU-TIRADS. Eur Thyroid J 2017;6(5):225-37. [CrossRef]
24. Khoo ML, Asa SL, Witterick IJ, Freeman JL. Thyroid calcification and its association with thyroid carcinoma. Head Neck 2002;24(7):651-5. [CrossRef]
25. Kim HG, Moon HJ, Kwak JY, Kim EK. Diagnostic accuracy of the ultrasonographic features for subcentimeter thyroid nodules suggested by the revised American Thyroid Association guidelines. Thyroid 2013;23(12):1583-9. [CrossRef]
26. Papini E, Guglielmi R, Bianchini A, Crescenzi A, Taccogna S, Nardi F, et. al. Risk of malignancy in nonpalpable thyroid nodules: predictive value of ultrasound and color-Doppler features. J Clin Endocrinol Metab 2002;87(5):1941-6. [CrossRef]
27. Wienke JR, Chong WK, Fielding JR, Zou KH, Mittelstaedt CA. Sonographic features of benign thyroid nodules: interobserver reliability and overlap with malignancy. J Ultrasound Med 2003;22(10):1027-31. [CrossRef]
28. Park JY, Lee HJ, Jang HW, Kim HK, Yi JH, Lee W, et. al. A proposal for a thyroid imaging reporting and data system for ultrasound features of thyroid carcinoma. Thyroid 2009;19(11):1257-64. [CrossRef]
29. Hoang JK, Lee WK, Lee M, Johnson D, Farrell S. US Features of thyroid malignancy: pearls and pitfalls. Radiographics 2007;27(3):847-60. [CrossRef]
30. Horvath E, Majlis S, Rossi R, Franco C, Niedmann JP, Castro A, et. al. An ultrasonogram reporting system for thyroid nodules stratifying cancer risk for clinical management. J Clin Endocrinol Metab 2009;94(5):1748-51. [CrossRef]

İSTANBUL ÜNİVERSİTESİ YAYINEVİ

RESEARCH / ARAŞTIRMA
DOI: 10.26650/IUITFD.2020.0041
İst Tıp Fak Derg 2020 / J Ist Faculty Med 2020

EFFECTS OF EXTREMELY LOW-FREQUENCY MAGNETIC FIELD ON HEALTHY FIBROBLASTS AND BREAST CANCER CELLS

ÇOK DÜŞÜK FREKANSLI MANYETİK ALANIN SAĞLIKLI FİBROBLAST VE MEME KANSERİ HÜCRELERİNE ETKİSİ

Handan KAYHAN[1], Babek ERDEBİLLİ[2], Sevim GÖNEN[3], Meriç Arda EŞMEKAYA[4], Ersen ERTEKİN[5], Ayşe Gülnihal CANSEVEN[4]

Gazi University, Faculty of Medicine, [1]Department of Adult Hematology, [3]Department of Nephrology, [4]Department of Biophysics, Ankara, Turkey
[2]Yıldırım Beyazıt University, Department of Industrial Engineering, Ankara, Turkey
[5]Adnan Menderes University, Faculty of Medicine, Department of Radiology, Aydın, Turkey

ORCID IDs of the authors: H.K. 0000-0003-4967-2740; B.E. 0000-0001-8860-3903; S.G. 0000-0002-2059-6193; M.A.E. 0000-0003-0469-4954; E.E. 0000-0001-7182-0725; A.G.C. 0000-0003-0716-7981

Cite this article as: Kayhan H, Erdebilli B, Gonen S, Esmekaya MA, Ertekin E, Canseven AG. Effects of extremely low-frequency magnetic field on healthy fibroblasts and breast cancer cells. J Ist Faculty Med 2020;83(4):384-9. doi: 10.26650/IUITFD.2020.0041

ABSTRACT

Objective: Modern people are exposed to many environmental factors, including Extremely Low-Frequency Magnetic Fields (ELF-MF). ELF-MFs are emitted from everything that produces electricity or that it passes through. Some studies have shown no significant detrimental effect of ELF-MFs on biological systems, while other studies have shown that ELF-MFs increase the risk of the development of childhood leukaemia (1). Moreover, the 40 Hz-7mT range ELF-MF has been used as a magnetotherapy instrument (2). In this study, we aimed to compare the effect of ELF-MF on healthy dermal fibroblasts and breast cancer cells (MCF7).

Material and Method: After the administration of 1 mT-50 Hz ELF-MF to healthy and cancer cells for different exposure times, apoptosis/necrosis levels were investigated and cell cycles and proliferation indexes were examined. The Analytic Hierarchy Process (AHP) was applied to find the most effective dose.

Results: The ELF-MF did not show any significant effect on MCF7 cells. However, it caused increased apoptosis and decreased proliferation of healthy fibroblast cells. Furthermore, no changes in the cell cycle were observed in either cell line. As a result of AHP, the most effective exposure was determined to be 1 hour.

Conclusion: Our results showed that while the ELF-MF reduces the viability of healthy fibroblasts, it has not observed therapeutic effect on breast cancer cells.

Keywords: Extremely low-frequency magnetic field, ELF-MF, apoptosis, proliferation, MCF, healthy fibroblasts

ÖZET

Amaç: Modern insan, çok düşük frekanslı manyetik alan (ELF-MF) da dâhil olmak üzere birçok çevresel etkene maruz kalmaktadır. ELF-MF, içinden elektrik geçen veya üreten her şeyden yayılır. Bazı çalışmalar ELF-MF'nin biyolojik sistemler üzerinde önemli bir zararlı etkisi olmadığını göstermişken, diğer çalışmalar ELF-MF'nin çocukluk çağı lösemisi geliştirme riskini arttırdığını göstermiştir (1). Dahası, 40 Hz-7mT gücündeki ELF-MF bir magnetoterapi aracı olarak kullanılmaktadır (2). Bu çalışmada, ELF-MF'nin sağlıklı dermal fibroblastlar ve meme kanseri hücreleri (MCF7) üzerindeki etkisinin karşılaştırılması amaçlanmıştır.

Gereç ve Yöntem: Farklı maruziyet süreleri için sağlıklı ve tümör hücrelerine 1 mT-50 Hz ELF-MF uygulandıktan sonra apoptoz/nekroz seviyeleri araştırılmıştır; hücre döngüleri ve proliferasyon indeksleri incelenmiştir. En etkili dozu bulmak için Analitik Hiyerarşi Süreci (AHP) uygulanmıştır.

Bulgular: ELF-MF, MCF7 hücreleri üzerinde herhangi bir anlamlı bir etki göstermemiştir. Bununla birlikte, sağlıklı fibroblast hücrelerinde apoptozun artmasına ve proliferasyonunu düşmesine sebep olmuştur. Ayrıca, her iki hücre hattında da hücre döngüsünde herhangi bir değişiklik gözlemlenmemiştir. AHP sonucunda en etkili maruziyetin 1 saat olduğu belirlenmiştir.

Sonuç: Elde ettiğimiz sonuçlar, ELF-MF'nin sağlıklı fibroblastların canlılığını azaltırken meme kanseri hücreleri üzerinde herhangi bir terapötik etki yaratmadığını göstermiştir.

Anahtar Kelimeler: Çok düşük frekanslı manyetik alan, ELF-MF, apoptoz, proliferasyon, MCF, sağlıklı fibroblast

Corresponding author/İletişim kurulacak yazar: handankayhan@gazi.edu.tr

Submitted/Başvuru: 23.04.2019 • **Revision Requested/Revizyon Talebi:** 22.05.2020 •
Last Revision Received/Son Revizyon: 04.06.2020 • **Accepted/Kabul:** 17.06.2020 • **Published Online/Online Yayın:** 19.10.2020

INTRODUCTION

Extremely low frequency (ELF) fields are electromagnetic fields (EMF) with frequencies below 300 Hz. They are included in the lower part of the electromagnetic spectrum. Many studies have reported that ELF exposure causes significant changes in cell survival, cell cycle progression, DNA integrity, and proliferation (3-8). However, there are many conflicting studies regarding the consequences of ELF-MF in cells (5). Some studies have shown that magnetic therapy can be utilized as an invasive method used in the treatment of several diseases (9-17). In contrast, other epidemiological studies have concluded that continuous environmental magnetic field exposure increases the risk of cancer (18, 19). The cellular response to ELF-MF may depend on many parameters including frequency, waveform, the strength and the exposure duration of the electromagnetic field, and genetic/biological characteristics of the cells (4, 20, 21).

The present study investigated and compared the effect of ELF-MF on healthy fibroblasts and breast cancer cells. Healthy and tumour cells were exposed to a 1 mT ELF-MF at 50 Hz frequency for 1, 3, and 5 hours, and then the cell cycle, proliferation, and apoptosis/necrosis were observed in comparison.

MATERIAL AND METHOD

In-vitro ELF-EMF exposure system

In vitro exposures were performed using the Helmholtz paired coil-based ELF exposure system (Figure 1) settled in a 5 % CO2 incubator at 37°C. A 50 Hz sinusoidal magnetic field, which was homogeneous throughout the axis of the coil pair, was generated with this system. The identical coils configured in the Helmholtz design were made up of 100 turns of copper wire with a diameter of 13 cm. The power generator which produced the AC magnetic field was connected to the coils. The coils were settled in a horizontal plane to produce a vertically oriented magnetic field. The distance between the coils was 6.5 cm. The electrical parameters of the coils are given in Table 1. The magnetic field was measured using a Hall-Effect Gaussmeter (Yokogawa, Tokyo, Japan). The petri dishes containing cell suspensions were located at the centre of the coil-based exposure system which had a homogenous ELF-EMF distribution. The cells were exposed to a 50 Hz ELF-MF with a field strength of 1 mT for 1, 3, and 5 hours.

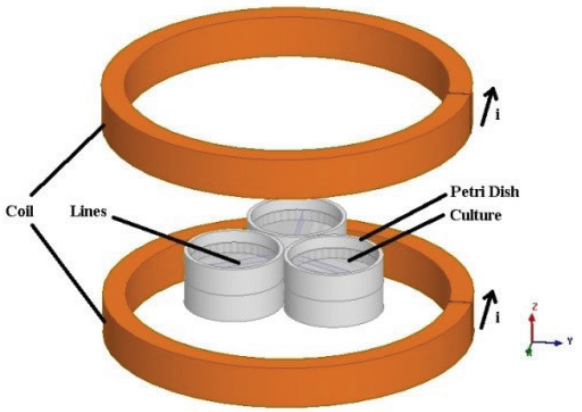

Figure 1: Schematic diagram of the Helmholtz coil-based ELF exposure system.

Table 1: The electrical parameters of the coils.

Coil Parameters	Value
Number of turns	100
Inner diameter (cm)	11.4
Outer diameter (cm)	13
Thickness (cm)	1.5
Resistance	5.15
Inductance	5.4

Cell culture

Human, neonatal, healthy primary dermal fibroblast (ATCC® PCS-201-010™) and MCF7 cell lines (ATCC® HTB-22™, human metastatic breast cancer cells) were maintained in Dulbecco's modified Eagle medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 50 U/ml penicillin and 50 µg/ml streptomycin. The cells were cultured in a 95% humidified atmosphere containing 5% CO2 at 37 °C. These adhesive cells were passaged every three days by trypsinization and washed with Ca and Mg-free phosphate-buffered saline (PBS) and fed with the same media. They were seeded on a 24-well plate 24 h before starting the experiments. 2×10^5 cells were plated for each well. The control group, consisting of both healthy fibroblasts and MCF7 cells, was kept in a separate incubator with the same conditions and was not exposed to an ELF-MF.

Cell survival

WST-1 Assay (Roche, Cell Proliferation Reagent WST-1) was applied for cell viability. This is a colorimetric test for the relative quantitation of cell proliferation in a culture medium. Briefly; after the exposures, 10000 cells/well in 90 µl DMEM were plated in 96-well plates and 10 µl of cell proliferation reagent WST-1 was added to each well and incubated for 2 hours. After incubation, the plate was read with the TECAN-Sunrise ELISA Reader (Switzerland) at 450 nm with a reference of 630 nm. The reference readings were subtracted from the original readings at 450 nm. The results were presented as a percent of cell viability (% of control). All experiments were performed in triplicate under blind conditions.

Annexin-V-FITC/ Propidium iodide (PI) Staining

The percentages of apoptotic and necrotic cells were analysed using Annexin-V-FITC and Propidium Iodide (PI)

staining (eBioscience, Annexin V-FITC Apoptosis Detection Kit) using a flow cytometer (BD Bioscience, FACSCalibur) in this study. The change in the plasma cell membrane is one of the earliest features of apoptosis. The exposure of phosphatidylserine (PS) from the cytoplasmic surface of the plasma cell membrane to the external surface is one of the hallmarks of early-phase apoptosis. The membrane remains intact in this phase. The translocation PS to the external surface may be determined using several fluorescence probes. Annexin-V is a 35–36 k DA Ca^{2+} dependent anti-coagulant protein and has a high affinity for binding PS. Necrotic cells are permeable for Propidium Iodide (PI) which intercalates in DNA bases and emits red fluorescence.

Briefly, when ELF treatments were applied, the cells were washed and resuspended in 195 µl binding buffer at a density of $2x10^5$ cells/ml. Then, 5 µl Annexin-V-FITC (eBioscience) dye was added to each cell suspension (195 µl). The cells were gently vortexed and incubated for 10 min in the dark. Afterwards, the cells were washed at 1200 rpm for 5 minutes, the supernatant was discarded and the pellet was resuspended in 190 µl binding buffer. 10 µl PI (20 µg/ml) was added to each cell suspension and the samples were read and analysed using the FacsCalibur Flow Cytometer (Becton–Dickinson). All experiments were performed in triplicate under blind conditions.

Analytical Hierarchy Process (AHP)

AHP is a numerical method that allows you to sort decision options and select one of them according to the multiple criteria specified. To implement this method, the following steps are performed: first, each decision option is given numerical points showing how much it meets the set criteria. For example, we used the averages of our test results. Then, by making binary comparisons, a matrix is created for each alternative. These matrices are normalized and their consistency is checked. Then, with the help of matrix algebra, an average score is obtained for each alternative. The alternative with the highest score is the most appropriate alternative according to the decision maker's comparisons.

Statistical method

All data were analysed using the independent one-tailed Student's t-test. $p<0.05$ was considered as statistically significant.

RESULTS

Our data showed that the ELF-MF did not affect proliferation, apoptosis, and cell cycle of breast cancer cells (MCF7). However, the proliferation of healthy breast fibroblasts was decreased (Figure 2) and apoptosis (Figure 3) was increased significantly. For the first time, the effect of ELF-MF on healthy fibroblasts and breast cancer cells was investigated simultaneously.

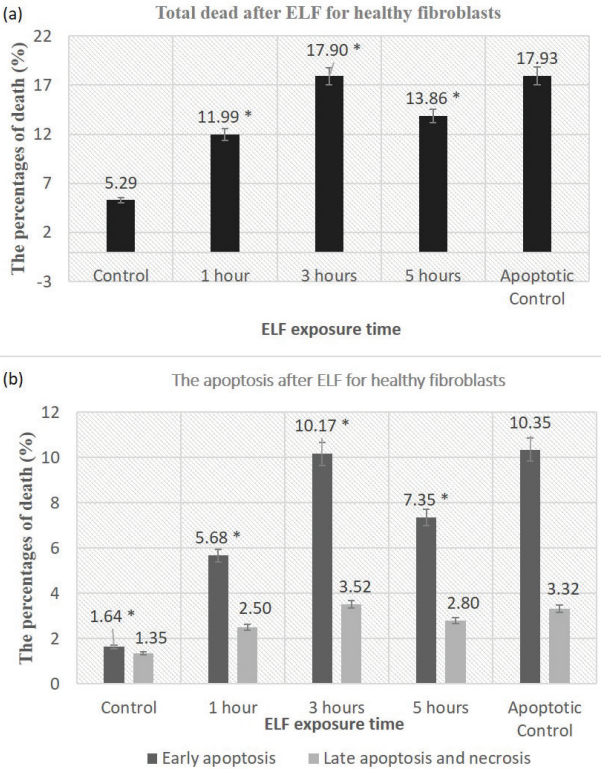

Figure 2: The total dead (a), early apoptosis, late apoptosis and necrosis (b) percentages after 1 mT-50 Hz ELF-MF exposures on healthy fibroblasts. It was observed that the ELF-MF triggers apoptosis but does not cause necrotic death. *The statistically significant percentages.

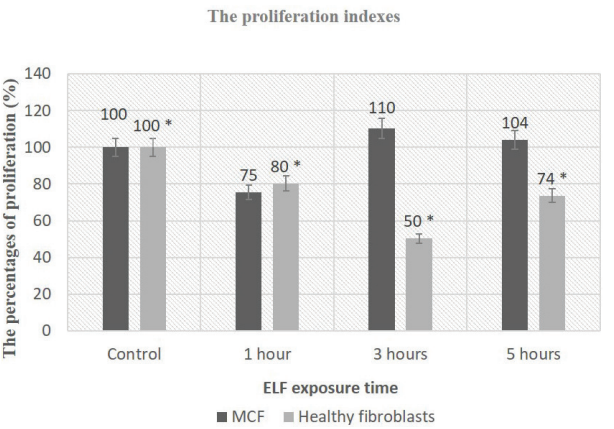

Figure 3: The figure shows viability rates in breast cancer cells and healthy fibroblasts after 1 mT-50 Hz ELF-MF exposure. *The statistically significant percentages.

The AHP method was applied and as a result of the AHP matrix, we concluded that 1-hour ELF-MF exposure was the most effective exposure time.

DISCUSSION

Studies have shown that ELF-MFs can have different effects in different tissues/cell lines. The biological mechanism of the cells' response to it has not been elucidated yet. After ELF-MF exposure, activation of inflammatory pathways or significant reactive oxygen species (ROS) accumulation occurs in some cell lines, while some other cell lines were found to have no significant effect. This may be due to genetic differences. Although ELF-MFs seem to have very low energy to cause mutations in DNA, they may make the cells more vulnerable to the harmful effects of the environment by epigenetic means and thus pave the way for tumour formation. Indeed, a study showed that ELF-MFs did not directly affect the methylation or histone modifications of leukaemia cells but made them susceptible to DNA and histone modifications by stabilizing active chromatins (1). Some groups showed that oxidative stress levels were increased and reactive oxygen species (ROS) accumulation occurred after ELF-MF exposure (22, 23). The increase in the amount of ROS formation may cause DNA damage implicitly and apoptotic cell death. ELF EMFs may affect the permeability of the plasma cell membrane which may cause dysfunction of ion channels existing on the membrane. Moreover, it has been observed that free radical scavengers have the ability to prevent genotoxic effects. These observations supported the idea the ELF-EMFs may induce ROS production which may lead to DNA damage (8, 24). The Fenton reaction in which hydroxyl radicals are generated may play an important role in free radical generation in ELF exposed systems (25). Moreover, it was indicated that the ELF-MFs altered some antioxidant genes such as *SOD2, GSTM3, MGST1,* and *MGST3* (26). Some groups showed that immune cell activation occurred due to the 50 Hz and 1 mT ELF-EMF exposure. ELF EMFs may also induce cytokine formation (IL-IB) in addition to free radicals in human monocytes (27-29).

In this study, we have seen the effects of ELF-MFs on two different cell lines, one healthy group and one tumour cells group, with a healthy primary dermal fibroblast and with human metastatic breast cancer cells (MCF7). The apoptotic activity of cancer cells exposed to ELF-MFs has been investigated in different tumour types in the literature. A 50 Hz, 45 mT ELF treatment for 1- 2.5 h induced apoptotic cell death in Human Myelogenous Leukemic cell lines (HL60 and ML-1) cells (30). No significant change was observed in the apoptotic rate of healthy peripheral leukocytes in the same exposure conditions (30). The number of apoptotic cells and micronucleus formation was increased significantly when SCL II (human squamous cell carcinoma) cells were treated with 50 Hz and 0.8–1 mT ELF-EMF for 48 and 72 h (31). Chen et al (32) reported a significant reduction in viability of HeLa cells that were exposed to a 60 Hz, 1.2 mT ELF for 72 h. Similarly, in another study, the survival rate of PC–12 cells was decreased when they were exposed to the ELF at 60 Hz (33). The short term effects of exposure to a 1.5 mT, 1 Hz ELF field has been shown in human colon adenocarcinoma cells. The proliferation rate of cells was significantly decreased due to 360 min ELF exposure (33). Dexamethasone induced cell proliferation inhibition was observed in HCA–2 human adenocarcinoma cells that were exposed to 25 Hz, 1 mT ELF for durations of between 45 and 120 min (34). ELF EMFs may also be used in cancer treatment. A 0.5 mT (1 h per day) ELF-EMF exposure reduced implanted tumour growth in mice (35). Similarly, the survival rate of human colon and breast adenocarcinoma cells was decreased due to 1 mT ELF exposure (36).

The results of our study showed that the ELF-MF did not affect breast cancer cells. However, it led to the death of healthy cells. Also, the ELF-MF did not alter the cell cycle of healthy fibroblasts showing that it did not cause any arrest i.e. the DNA repair mechanism might not work. Although ELF-MFs have been shown to cause changes in inflammatory pathways, immune system, ROS accumulation, histones, and some genes, studies to date have still needed research on how the mechanism works. The study we conducted showed the damaging effect of ELF-MFs on healthy cells.

Ethics Committee Approval: Ethics committee approval is not required.

Informed Consent: Informed consent is not required.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- H.K.; Data Acquisition- H.K.; Data Analysis/Interpretation- H.K., S.G.; Drafting Manuscript- H.K., M.A.E.; Critical Revision of Manuscript- E.E., S.G.; Final Approval and Accountability- H.K., B.E., S.G., M.A.E., E.E., A.G.C.; Technical or Material Support- H.K., A.G.C., S.G.; Supervision- M.A.E.

Conflict of Interest: Authors declared no conflict of interest.

Financial Disclosure: Authors declared no financial support.

Etik Komite Onayı: Etik kurul onayı gerekmemektedir.

Bilgilendirilmiş Onam: Bilgilendirilmiş onam gerekmemektedir.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Çalışma Konsepti/Tasarım-H.K.; Veri Toplama- H.K.; Veri Analizi/Yorumlama- H.K., S.G.; Yazı Taslağı- H.K., M.A.E.; İçeriğin Eleştirel İncelemesi- E.E., S.G.; Son Onay ve Sorumluluk- H.K., B.E., S.G., M.A.E., E.E., A.G.C.; Malzeme ve Teknik Destek- H.K., A.G.C., S.G.; Süpervizyon- M.A.E

Çıkar Çatışması: Yazarlar çıkar çatışması beyan etmemişlerdir.

Finansal Destek: Yazarlar finansal destek beyan etmemişlerdir.

REFERENCES

1. Manser M, Sater MR, Schmid CD, Noreen F, Murbach M, Kuster N, et al. ELF-MF exposure affects the robustness of epigenetic programming during granulopoiesis. Sci Rep 2017;7:43345. [CrossRef]
2. Goraca A, Ciejka E, Piechota A. Effects of extremely low frequency magnetic field on the parameters of oxidative stress in heart. J Physiol Pharmacol 2010;61(3):333-8.
3. Lange S, Viergutz T, Simko M. Modifications in cell cycle kinetics and in expression of G1 phase-regulating proteins in human amniotic cells after exposure to electromagnetic fields and ionizing radiation. Cell Prolif 2004;37(5):337-49. [CrossRef]
4. Trillo MA, Martinez MA, Cid MA, Leal J, Ubeda A. Influence of a 50 Hz magnetic field and of all-trans-retinol on the proliferation of human cancer cell lines. Int J Oncol 2012;40(5):1405-13. [CrossRef]
5. Cid MA, Ubeda A, Hernandez-Bule ML, Martinez MA, Trillo MA. Antagonistic effects of a 50 Hz magnetic field and melatonin in the proliferation and differentiation of hepatocarcinoma cells. Cell Physiol Biochem 2012;30(6):1502-16. [CrossRef]
6. Wei M, Guizzetti M, Yost M, Costa LG. Exposure to 60-Hz magnetic fields and proliferation of human astrocytoma cells in vitro. Toxicol Appl Pharm 2000;162(3):166-76. [CrossRef]
7. Eleuteri AM, Amici M, Bonfili L, Cecarini V, Cuccioloni M, Grimaldi S, et al. 50 Hz extremely low frequency electromagnetic fields enhance protein carbonyl groups content in cancer cells: effects on proteasomal systems. J Biomed Biotechnol 2009;2009:834239. [CrossRef]
8. Lai H, Singh NP. Acute exposure to a 60 Hz magnetic field increases DNA strand breaks in rat brain cells. Bioelectromagnetics 1997;18(2):156-65. [CrossRef]
9. Kaszuba-Zwoinska J, Ciecko-Michalska I, Madroszkiewicz D, Mach T, Slodowska-Hajduk Z, Rokita E, et al. Magnetic field anti-inflammatory effects in Crohn's disease depends upon viability and cytokine profile of the immune competent cells. J Physiol Pharmacol 2008;59(1):177-87.
10. Sutbeyaz ST, Sezer N, Koseoglu F, Kibar S. Low-frequency Pulsed Electromagnetic Field Therapy in Fibromyalgia A Randomized, Double-blind, Sham-controlled Clinical Study. Clin J Pain 2009;25(8):722-8. [CrossRef]
11. Markov MS. Magnetic field therapy: A review. Electromagn Biol Med 2007;26(1):1-23. [CrossRef]
12. Lappin MS, Lawrie FW, Richards TL, Kramer ED. Effects of a pulsed electromagnetic therapy on multiple sclerosis fatigue and quality of life: A double-blind, placebo controlled trial. Altern Ther Health M 2003;9(4):38-48.
13. Bertolino G, Braga AD, Rosa KDLD, Brito LCD, de Araujo JE. Macroscopic and histological effects of magnetic field exposition in the process of tissue reparation in Wistar rats. Arch Dermatol Res 2006;298(3):121-6. [CrossRef]
14. Cane V, Botti P, Soana S. Pulsed Magnetic-Fields Improve Osteoblast Activity during the Repair of an Experimental Osseous Defect. J Orthopaed Res 1993;11(5):664-70. [CrossRef]
15. Cameron IL, Sun LZ, Short N, Hardman WE, Williams CD. Therapeutic Electromagnetic Field (TEMF) and gamma irradiation on human breast cancer xenograft growth, angiogenesis and metastasis. Cancer Cell Int 2005;5:23. [CrossRef]
16. Yamaguchi S, Ogiue-Ikeda M, Sekino M, Ueno S. The effect of repetitive magnetic stimulation on the tumor development. Ieee T Magn 2004;40(4):3021-3. [CrossRef]
17. Kirson ED, Gurvich Z, Schneiderman R, Dekel E, Itzhaki A, Wasserman Y, et al. Disruption of cancer cell replication by alternating electric fields. Cancer Res 2004;64(9):3288-95. [CrossRef]
18. Barbault A, Costa FP, Bottger B, Munden RF, Bomholt F, Kuster N, et al. Amplitude-modulated electromagnetic fields for the treatment of cancer: Discovery of tumor-specific frequencies and assessment of a novel therapeutic approach. J Exp Clin Cancer Res 2009;28(1):51. [CrossRef]
19. Chen G, Lu D, Chiang H, Leszczynski D, Xu Z. Using model organism Saccharomyces cerevisiae to evaluate the effects of ELF-MF and RF-EMF exposure on global gene expression. Bioelectromagnetics 2012;33(7):550-60. [CrossRef]
20. Tenuzzo B, Chionna A, Panzarini E, Lanubile R, Tarantino P, Di Jeso B, et al. Biological effects of 6 mT static magnetic fields: A comparative study in different cell types. Bioelectromagnetics 2006;27(7):560-77. [CrossRef]
21. Focke F, Schuermann D, Kuster N, Schar P. DNA fragmentation in human fibroblasts under extremely low frequency electromagnetic field exposure. Mutat Res-Fund Mol M 2010;683(1-2):74-83. [CrossRef]
22. Luukkonen J, Liimatainen A, Juutilainen J, Naarala J. Induction of genomic instability, oxidative processes, and mitochondrial activity by 50Hz magnetic fields in human SH-SY5Y neuroblastoma cells. Mutat Res 2014;760:33-41. [CrossRef]
23. Tiwari R, Lakshmi NK, Bhargava SC, Ahuja YR. Epinephrine, DNA integrity and oxidative stress in workers exposed to extremely low-frequency electromagnetic fields (ELF-EMFs) at 132 kV substations. Electromagn Biol Med 2015;34(1):56-62. [CrossRef]
24. Lai H, Singh NP. Melatonin and a spin-trap compound block radiofrequency electromagnetic radiation-induced DNA strand breaks in rat brain cells. Bioelectromagnetics 1997;18(6):446-54. [CrossRef]
25. Lai H, Singh NP. Magnetic-field-induced DNA strand breaks in brain cells of the rat. Environ Health Persp 2004;112(6):687-94. [CrossRef]
26. Mahmoudinasab H, Sanie-Jahromi F, Saadat M. Effects of extremely low-frequency electromagnetic field on expression levels of some antioxidant genes in human MCF-7 cells. Mol Biol Res Commun 2016;5(2):77-85.
27. Rollwitz J, Lupke M, Simko M. Fifty-hertz magnetic fields induce free radical formation in mouse bone marrow-derived promonocytes and macrophages. Biochim Biophys Acta 2004;1674(3):231-8. [CrossRef]
28. Frahm J, Lantow M, Lupke M, Weiss DG, Simko M. Alteration in cellular functions in mouse macrophages after exposure to 50 Hz magnetic fields. J Cell Biochem 2006;99(1):168-77. [CrossRef]
29. Lupke M, Rollwitz J, Simko M. Cell activating capacity of 50 Hz magnetic fields to release reactive oxygen intermediates in human umbilical cord blood-derived monocytes and in Mono Mac 6 cells. Free Radic Res 2004;38(9):985-93. [CrossRef]
30. Hisamitsu T, Narita K, Kasahara T, Seto A, Yu Y, Asano K. Induction of apoptosis in human leukemic cells by magnetic fields. Jpn J Physiol 1997;47(3):307-10. [CrossRef]

31. Simko M, Kriehuber R, Weiss DG, Luben RA. Effects of 50 Hz EMF exposure on micronucleus formation and apoptosis in transformed and nontransformed human cell lines. Bioelectromagnetics 1998;19(2):85-91. [CrossRef]
32. Chen YC, Chen CC, Tu W, Cheng YT, Tseng FG. Design and fabrication of a microplatform for the proximity effect study of localized ELF-EMF on the growth of in vitro HeLa and PC-12 cells. J Micromech Microeng 2010;20(12):125023. [CrossRef]
33. Ruiz-Gomez MJ, de la Pena L, Prieto-Barcia MI, Pastor JM, Gil L, Martinez-Morillo M. Influence of 1 and 25 Hz, 1.5 mT magnetic fields on antitumor drug potency in a human adenocarcinoma cell line. Bioelectromagnetics 2002;23(8):578-85. [CrossRef]
34. Ruiz-Gomez A, Ruiz-Gomez MJ, Pastor JM, De la Pena L, Martinez M. Stochastic simulation of logistic growth of a colon adenocarcinoma cell line. Europ J Cancer 1999;35:S28.
35. Hannan CJ, Jr., Liang Y, Allison JD, Pantazis CG, Searle JR. Chemotherapy of human carcinoma xenografts during pulsed magnetic field exposure. Anticancer Res 1994;14(4A):1521-4.
36. Tofani S, Barone D, Berardelli M, Berno E, Cintorino M, Foglia L, et al. Static and ELF magnetic fields enhance the in vivo anti-tumor efficacy of cis-platin against lewis lung carcinoma, but not of cyclophosphamide against B16 melanotic melanoma. Pharmacol Res 2003;48(1):83-90. [CrossRef]

İSTANBUL ÜNİVERSİTESİ YAYINEVİ

RESEARCH / ARAŞTIRMA
DOI: 10.26650/IUITFD.2020.0047
İst Tıp Fak Derg 2020 / J Ist Faculty Med 2020

DIAGNOSTIC VALUE OF SOLUBLE TRIGGERING RECEPTOR EXPRESSED ON MYELOID CELLS IN SPONTANEOUS BACTERIAL PERITONITIS

SPONTAN BAKTERİYEL PERİTONİTİN TANISINDA STREM-1 MOLEKÜLÜNÜN TANISAL DEĞERİ

Alpay MEDETALİBEYOĞLU[1], Seda ÇELİK[2], Rabia DENİZ[1], Emine Bilge ÇAPARALİ[1], Murat KÖSE[1], Timur Selçuk AKPINAR[1], Yücel ARMAN[3], Adem ATICI[4], Murat AKARSU[3], Şengül AYDIN YOLDEMİR[3], İbrahim Hakkı KÖKER[5], Tufan TÜKEK[1]

Istanbul University, Istanbul Faculty of Medicine, [1]Department of Internal Medicine, [4]Department of Cardiology Istanbul, Turkey
[2]Kafkas University, Faculty of Medicine, Biochemistry, Kars, Turkey
[3]Istanbul Okmeydani Training and Research Hospital, Department of Internal Medicine, Istanbul, Turkey
[5]Bezmialem Vakif University, Department of Gastroenterology, Istanbul, Turkey

ORCID IDs of the authors: A.M. 0000-0002-5829-9186; S.Ç. 0000-0003-2055-3537; R.D. 0000-0003-4537-894X; E.B.Ç. 0000-0001-9747-244X; M.K. 0000-0002-7487-9287; T.S.A. 0000-0002-9591-4475; Y.A. 0000-0002-9584-6644; A.A. 0000-0001-6580-7954; M.A. 0000-0002-2675-4252; Ş.A.Y. 0000-0003-4236-1181; İ.H.K. 0000-0002-4513-6927; T.T. 0000-0002-4237-1163

Cite this article as: Medetalibeyoglu A, Celik S, Deniz R, Caparali EB, Kose M, Akpinar TS, et al. Diagnostic value of soluble triggering receptor expressed on myeloid cells in spontaneous bacterial peritonitis. J Ist Faculty Med 2020;83(4):390-6.
doi: 10.26650/IUITFD.2020.0047

ABSTRACT

Objective: The aim of this study is to investigate the role of sTREM-1 molecule in the diagnosis of spontaneous bacterial peritonitis in patients with portal type ascites.

Material and Method: We included 122 patients with portal type ascites in the study, 58 had infected ascites (peritonitis) (F/M:27/31, mean age 61.6±15.0 years) and 64 had uninfected ascites (F/M:23/41, mean age 63.4±9.4). Complete blood count, albumin, and C-reactive protein (CRP) were defined in the blood samples. Additionally, neutrophil count, albumin, total protein, lactate dehydrogenase (LDH), and sTREM-1 levels were measured in the peritoneal fluid samples.

Results: There were significant differences in ascites neutrophil, serum neutrophil, CRP, ascites LDH, and ascites sTREM-1 levels between two groups. AUC values for ascites neutrophil, serum neutrophil, CRP, and sTREM-1 were 1.0 (95% CI 1.0-1.0), 0.676 (95% CI 0.580-0.771), 0.721 (95% CI 0.632-0.811), and 0.644 (95% CI 0.546-0.74), respectively. In females, sTREM-1 levels were positively correlated with ascites neutrophil, ascites LDH, ascites albumin, ascites total protein levels, and platelet count.

ÖZET

Amaç: Bu çalışmanın amacı portal tipte asidi olan hastalarda sTREM-1 molekülünün spontan bakteriyel peritonit tanısındaki rolünü araştırmaktır.

Gereç ve Yöntem: Çalışmaya portal tipte asiti olan 122 hasta dahil edildi, 58 hastada enfekte asit (K/E:27/31, ortalama yaş 61,6±15,0 yıl), 64 hastada enfekte olmayan asit (K/A:23/41, ortalama yaş 63,4±9,4) vardı. Hastalardan başvuru sırasında alınan kan örneklerinde tam kan sayımı, albümin ve C-reaktif protein (CRP) değerleri; eşzamanlı alınan periton sıvısı örneklerinde ise albümin, total protein, laktat dehidrojenaz, (LDH) ve sTREM-1 seviyeleri ölçüldü.

Bulgular: Enfekte asitli ve enfekte olmayan asitli hastalar karşılaştırıldığında asit nötrofil, serum nötrofil, CRP, asit LDH ve asit sTREM-1 düzeylerinde anlamlı farklılıklar görüldü. Asit nötrofil, serum nötrofil, CRP ve sTREM-1 için eğrinin altında kalan alanlar (EAA) sırasıyla 1,0 (%95 CI 1,0-1,0), 0,676 (%95 CI 0,580-0,771), 0,721 (%95 CI 0,632-0,811) ve 0,644 (%95 CI 0,546-0,74) idi. Ayrıca kadın hastalarda sTREM-1 değerleri; asit nötrofil, asit LDH, asit albümin, asit total protein değerleri ve trombosit sayısı ile pozitif korelasyon gösterdi.

Corresponding author/İletişim kurulacak yazar: dralpaymedet@gmail.com

Submitted/Başvuru: 25.04.2020 • **Revision Requested/Revizyon Talebi:** 09.06.2020 •
Last Revision Received/Son Revizyon: 16.06.2020 • **Accepted/Kabul:** 18.06.2020 • **Published Online/Online Yayın:** 25.09.2020

Conclusion: Infection of ascites is an important complication of portal hypertension and ascites neutrophil count measurement is the traditional method used for diagnosis. sTREM-1 can be an alternative marker to identify ascites infections, especially in the cases of peritonitis without an increase in neutrophil count.

Keywords: sTREM-1, spontaneous bacterial peritonitis, ascites

Sonuç: Asit enfeksiyonu portal hipertansiyonun önemli bir komplikasyonudur ve geleneksel tanı metodu asit nötrofil sayısı ölçümüdür. sTREM-1, özellikle nötrofil sayısında artış olmayan peritonit vakalarında, asit enfeksiyonlarını tanımlamak için alternatif bir belirteç olabilir.

Anahtar Kelimeler: sTREM-1, spontan bakteriyel peritonit, asit

INTRODUCTION

One of the most common complications in cirrhosis with ascites is spontaneous bacterial peritonitis (SBP) (1, 2). With early diagnosis and advanced treatment options, mortality rates of SBP have been decreased from 90% to approximately 20% (3). SBP is a serious problem for both outpatients and hospitalized patients with ascites, and the primary diagnostic tool is paracentesis (1, 4, 5). Although patients with SBP may have symptoms related to peritonitis, systemic inflammation, impaired liver and kidney functions, shock, and gastrointestinal hemorrhage; some of the patients with SBP might be asymptomatic and misdiagnosed especially in outpatient clinics (4-6).

Triggering receptor expressed on myeloid cells-1 (TREM-1) is a membrane molecule in the immunoglobulin super family. It is expressed on the surface of neutrophils and monocytes with the stimulation of bacteria and fungi. In addition to neutrophils and monocytes, it is found on dendritic cells, natural killer cells, T, and B lymphocytes as well as respiratory epithelial cells and hepatic endothelial cells. After encountering infectious agents, membrane-bound TREM-1 is upregulated. Subsequently, the soluble form of this molecule (sTREM-1) is released into body fluids such as plasma, bronchoalveolar fluid, pleural fluid, peritoneal fluid, and cerebrospinal fluid (7-12). Owing to that, sTREM-1 was thought to be used as a marker especially in bacterial infections; increased levels have been shown in sepsis, pneumonia, exacerbation of chronic obstructive pulmonary disease, secondary peritonitis and bacterial meningitis, so far (13-15). Although some studies suggested that sTREM-1 levels show no increase in uninfectious situations such as vasculitis, psoriasis, and ulcerative colitis (16), it has also been related with inflammatory and autoimmune diseases, like pancreatitis, gout, peptic ulcer, systemic lupus erythematosus in recent time (11, 12, 17).

In the present study, the ascites sTREM-1 levels were measured to identify the role of the sTREM-1 molecule in the diagnosis of SBP in patients with portal type ascites and compare it with the conventional diagnostic tool of spontaneous bacterial peritonitis, ascites neutrophil count. We also aimed to determine the sensitivity and specificity of sTREM-1 in the diagnosis of SBP.

MATERIAL AND METHOD

Study design and patients

We designed this study as a cross-sectional study involving consecutive patients admitted with ascites, between September 2014 and January 2016. Only patients whose ascites were drained for the diagnostic produce with suspected peritonitis and were found to be portal type (serum acid-albumin gradient ≥1.1) were included (18). The patients who are diagnosed with a non-portal type of ascites, any malignant neoplasm, or those using antibiotics, immunosuppressive therapy, or chemotherapeutic were excluded. Overall, 122 patients were included in the study, and 58 patients with an ascites neutrophil count greater than $250\times10^3/mm^3$ were identified as having infected ascites (19), 64 patients with an ascites neutrophil count less than $250\times10^3/mm^3$ were defined as having uninfected ascites (Figure 1). Ethical approval was obtained from the Institutional Ethics Review Board with the number of 2014-926/955, and all participants gave informed consent.

Sample and data collection

Data on demographic, clinical, and laboratory characteristics were recorded and both ascites and blood samples were collected. Ascites samples were collected before

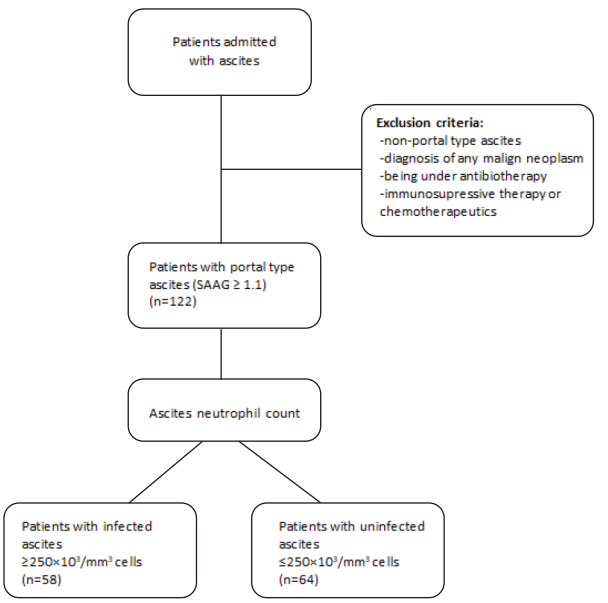

Figure 1: STARD diagram for study design.

any intervention via the insertion of a sterile needle into the left lower quadrant of the abdomen which is the opposite of McBurney point as in the classical paracentesis procedure to determine cell count and biochemical parameters (albumin, total protein, LDH, and sTREM-1). Venous blood samples were taken into both an EDTA anticoagulated tube for the complete blood count and a dry tube for the serum biochemical parameters (albumin and C-reactive protein). Complete blood count and plasma biochemistry analysis were done by our central biochemistry laboratory based on routine protocols. To determine the cell count and collect the supernatant, ascites samples were centrifuged at 1500 rpm for 10 minutes at +4°C, and the supernatant was kept at -80°C. The concentrations of sTREM-1 in ascites fluids were measured by following the manufacturer's instructions with commercial sandwich enzyme-linked immunosorbent assays (ELISA) in 96-well plates which have an assay range of 3 to 900 ng/L and sensitivity of 1.51 ng/L.

Statistical analysis

Distribution of the data was analyzed and continuous variables were presented as mean ± standard deviation (SD). The differences between groups and subgroups were analyzed by parametric Student's t-test and non-parametric Mann–Whitney U test as applicable. Correlation of sTREM-1 values with other diagnostic tests were analyzed by Spearman or Pearson correlation coefficients. To evaluate the diagnostic performance of sTREM-1, receiver operating characteristics (ROC) with corresponding areas under the curve (AUCs) were calculated. Since peritonitis is diagnosed by the presence of more than $250\times10^3/mm^3$ neutrophils in ascites fluid, it was accepted as the gold standard when compared to other diagnostic tests and sTREM-1.

P values less than 0.05 were considered statistically significant. Statistical analyses were performed by using SPSS 21.0.0.0 (SPSS Inc.), Graphpad Prism v5. (GraphPad, Software Inc.), and MedCalc v16.8.4 (MedCalc Software).

RESULTS

The characteristics of patients with infected ascites and uninfected ascites are presented in Table 1. Significant differences between two groups were seen on ascites neutrophil, serum neutrophil, CRP, ascites LDH, and asci-

Table 1: The patient characteristics for the patients with infected ascites and uninfected ascites

	Patients with uninfected ascites (n)			Patients with infected ascites (n)		
Group, (number)	**Female (23)**	**Male (41)**	**Total (64)**	**Female (27)**	**Male (31)**	**Total (58)**
Ascites albumin (g/dL)	1.16±1.0	1.05±0.8	1.09±0.9	1.20±1.0	1.31±0.8	1.26±0.9
Ascites total protein (g/dL)	2.07±1.4	2.07±1.3	2.07±1.3	2.48±1.7	2.41±1.1	2.44±1.4
Ascites LDH (u/L)	88.04±69.7	117.95±103.9	107.20±93.6	328.00±828.5	172.10±160.5	244.67±576.9
Ascites neutrophil ($/mm^3$)	108.70±65.1	124.66±56.6	118.92±59.8	1877.78±2732.0	2608.06±6807.3	2268.10±5284.4
sTREM-1 (ng/L)	381.79±174.6	380.38±190.7	380.89±183.7	513.78±198.5	423.81±208.1	465.69±206.9
Serum albumin (g/dL)	3.23±0.7	2.74±0.6	2.91±0.7	2.87±0.7	2.95±0.4	2.91±0.6
Serum CRP (mg/L)	26.93±30.5	38.39±44.6	34.27±40.2	70.35±67.5	92.89±87.2	82.40±78.8
Serum neutrophil ($/mm^3$)	4976.09±7028.2	5634.15±4095.4	5397.66±5291.3	7267.41±6112.5	8467.74±8764.3	7908.97±7604.5
Hemoglobin (g/dL)	9.90±1.8	9.86±1.62	9.87±1.7	9.80±1.9	10.39±1.9	10.11±1.9
Platelets (/µL)	143000±160100	168500±138925	159000±146000	149000±149300	149500±100100	149000±124000
MPV (fL)	8.63±1.3	8.96±1.1	8.84±1.2	8.75±1.3	8.58±1.3	8.66±1.3
MELD	11.70±5.6	11.20±5.1	11.38±5.3	11.56±4.9	12.26±4.0	11.93±4.4

tes sTREM-1 levels (p<0.0001, 0.001, <0.0001, 0.002, and 0.006, respectively).

Subgroups analysis for gender showed significant differences in the comparison of male patients with infected ascites and uninfected ascites for the ascites neutrophil, serum neutrophil, ascites LDH, and CRP (p<0.0001, 0.02, 0.02, and 0.01, respectively) while the difference in sTREM-1 values was insignificant (p=0.29). In females with infected ascites and uninfected ascites, there was a significant difference in the ascites neutrophil, CRP, ascites LDH, serum neutrophil, and sTREM (p<0.0001, 0.006, 0.015, 0.006, and 0.007, respectively).

Etiology of cirrhosis was found in patients as; HBV-related 31.1%, cryptogenic 20.5%, HCV-related 23.0%, alcoholic 11.5%, autoimmune 4.9%, and other 9.0% (including; Caroli's disease, portal vein thrombosis, non-alcoholic steatohepatitis, and hepatic fibrosis). There were no significant differences in the causes of cirrhosis on subgroup analysis for neither gender nor ascites infection status, however, all patients with autoimmune causes were female (n=6).

The predictive power of sTREM-1, ascites neutrophil, serum neutrophil, and CRP in the diagnosis of ascites infection is demonstrated as the area under the receiver operating characteristic curve (AUC) and given in Figure 2.

In ROC analysis to differentiate patients with infected ascites and uninfected ascites, AUC values for ascites neutrophil (gold standard criteria), serum neutrophil, CRP, and sTREM-1 were 1.0 (95% CI 1.0-1.0), 0.676 (95% CI

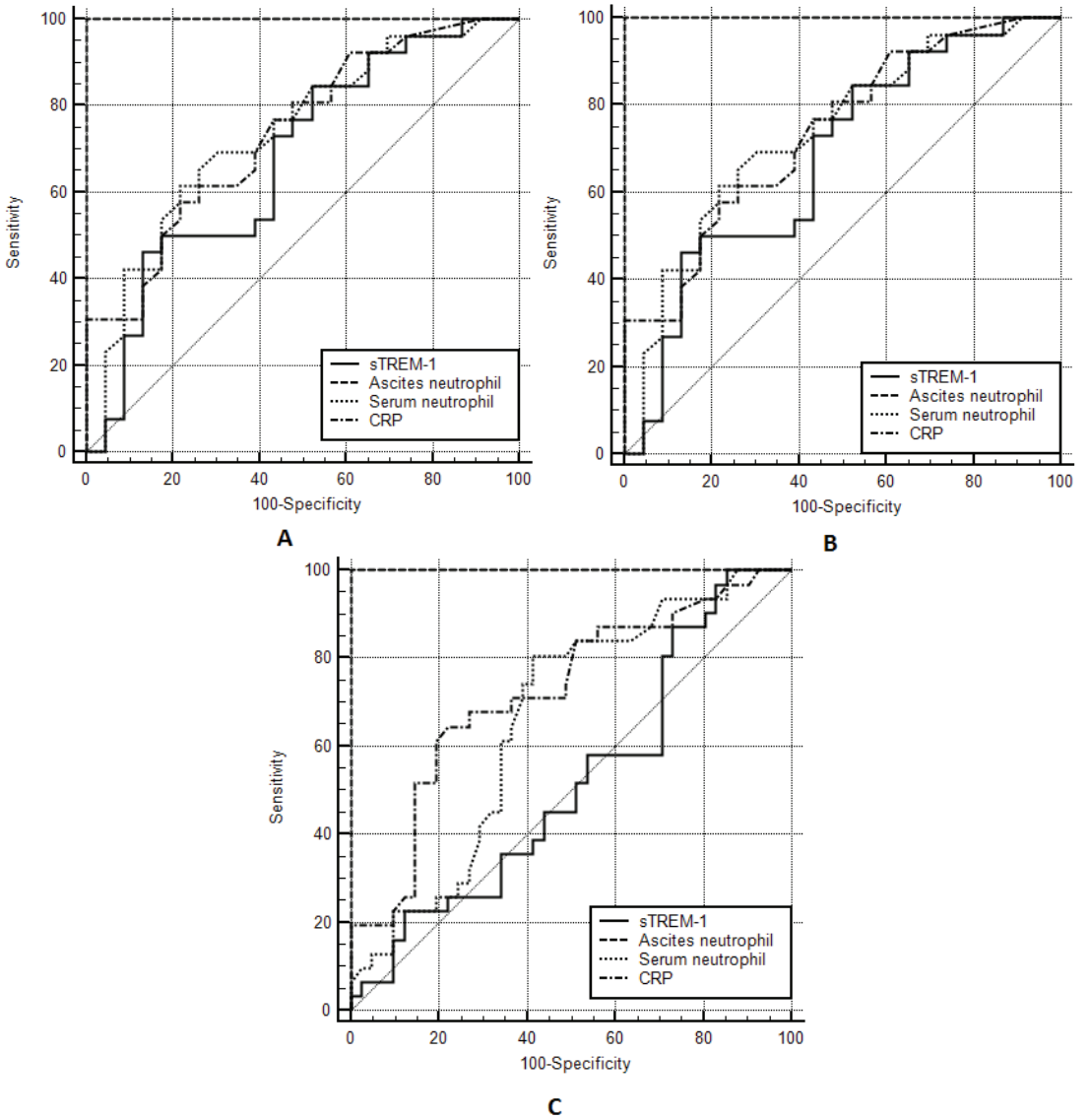

Figure 2A-C: ROC curve in the diagnosis of patients with infected ascites versus uninfected ascites A: for overall groups, B: female patients, C: and male patients.

Table 2: Spesificity, sensitivity, positive predictive value, and negative predictive value of different markers in the diagnosis of patients with infected ascites versus uninfected ascites

Variable	Threshold	Sensitivity (95% CI)%	Spesificity (95% CI)%	Positive predictive value	Negative predictive value	Positive Likelihood Ratio (95% CI)	P	AUC (95% CI)%
sTREM-1 (ng/L)	452	0.48 (0.35-0.62)	0.77 (0.64-0.86)	0.65	0.62	2.06 (1.2-3.5)	0.004	0.644 (0.556-0.744)
Serum CRP (mg/L)	47	0.59 (0.45-0.71)	0.77 (0.64-0.86)	0.69	0.67	2.5 (1.5-4.1)	<0.0001	0.721 (0.632-0.811)
Serum neutrophil ($x10^3/mm^3$)	4300	0.72 (0.59-0.83)	0.63 (0.50-0.74)	0.64	0.71	1.93 (1.4-2.8)	0.0003	0.676 (0.580-0.771)
Ascites neutrophil ($x10^3/mm^3$)	250	1.0	1.0	1.0	1.0	3.76 (2.5-5.7)	<0.0001	1.00 (1.0-1.0)

0.580-0.771), 0.721 (95% CI 0.632-0.811), and 0.644 (95% CI 0.546-0.744), respectively (Figure 2).

Additionally, subanalysis for ascites neutrophil, serum neutrophil, CRP, and sTREM-1 values according to gender were performed by determining AUC. In male patients results were 1.0 (95% CI 1.0-1.0), 0.660 (95% CI 0.533-0.787), 0.725 (95% CI 0.606-0.844), 0.574 (95% CI 0.440-0.707), and in female patients were 1.0 (95% CI 1.0-1.0), 0.727 (95% CI 0.585-0.869), 0.726 (95% CI 0.587-0.865), 0.721 (95% CI 0.575-0.868), respectively (Figure 2).

Sensitivity, specificity, positive predictive value, negative predictive value, and AUC of variables are given in Table 2.

Although it was not strong, a positive correlation was found between sTREM-1 and ascites neutrophil (r=0.24, p=0.009). In female patients, sTREM-1 was found positively correlated with ascites neutrophil, ascites LDH, ascites albumin, ascites total protein, and platelet count (r=0.37, p=0.007; r=0.27, p=0.05; r=0.272, p=0.06; r=0.30, p=0.04; and r=0.30, p=0.03, respectively), while there was no positive correlation between sTREM-1 and any parameters in male patients.

DISCUSSION

Triggering receptor expressed on myeloid cells-1 (TREM-1) and its soluble form sTREM-1 have increasing popularity in last years, notably for their potential role as a biomarker in the diagnosis of bacterial infections, as well as some inflammatory diseases. Studies of sTREM-1 molecule mainly focused on sepsis (7, 14, 15, 20, 21). Although there is a study investigating the effect of sTREM-1 molecule in diagnosing secondary peritonitis, (13), this is the first study in the literature investigating the role of sTREM-1 molecule for the diagnosis of SBP.

Ascites neutrophil count is accepted as the conventional diagnostic marker for the diagnosis of SBP, mainly due to the lower positive culture rates in ascites infections and relative difficulties of performing culture related to technique (22-24). However, these may cause misdiagnosis of SBP cases in which neutrophil count does not increase (25). Therefore, alternative markers may be required.

Similar to the present study, same parameters were investigated as a marker determining the infection in previous studies, but most of the data were obtained from studies focused on sepsis. Nevertheless, our results are consistent with the outcomes of those studies. For example, Barati et al. found AUC values of CRP, serum neutrophil count, and sTREM-1 for the diagnosis of infection in SIRS as 0.66 (95% CI 0.54-0.77) (p=0.00), 0.59 (95% CI 0.47-0.70) (p=0.14), and 0.65 (95% CI 0.53-0.76) (p=0.00), respectively (7). The only study investigating secondary peritonitis found the AUC for sTREM-1 as 0.76 (95% CI 0.63-0.90) (12).

In previous studies, various sensitivity and specificity values have been noted related to the sTREM-1. For example, Bayram et al. (14) found that the sensitivity and specificity of sTREM-1 molecule for sepsis were 81.8% and 73.2%, respectively. Moreover, Determann et al. (13) suggested a cut-off value of 160 pg/ml for sTREM-1 in secondary peritonitis; which is quite low compared to a cut-off value of 452 pg/ml that we found for SBP with 88% sensitivity (95% CI 0.73-0.95) and 67% specificity (95% CI 0.51-0.80). Their positive predictive value was moderate at 0.70 (95% CI 0.55–0.82), but the negative predictive value was high at 0.86 (95% CI 0.69–0.94). In addition, their positive likelihood ratio (LR+) was 2.63 (95% CI 1.65-4.19) and the negative likelihood ratio (LR-) was 0.19 (0.08–0.47).

In the study of Determann et al. (26), sTREM-1 use was suggested to be better than CRP in identifying infection.

In favor of this, Gibot et al. (9) found that sTREM-1 is even more accurate than any laboratory values or clinical findings detecting the presence of bacterial or fungal pneumonia (LR+, 10.38; sensitivity 98%; specificity 90%). On the contrary Latour-Perez et al. (18) suggested that sTREM-1 has a poor distinctive power to detect infection in critically ill patients with SIRS and does not have an additional diagnostic value to the diagnostic value provided by other commonly used clinical tests. Our results, on the other hand, showed a similar specificity, positive predictive value, and negative predictive value, as well as little lower sensitivity, AUC, and LR for sTREM-1 compared to CRP. Due to the fact that unless the causative pathogen is shown, no single predictor can not be the perfect diagnostic tool for the infections or SBP, sTREM-1 may be at least a contributor for the evaluation of the patient with the suspicion of SBP.

Another notable finding in our study is that sTREM-1 has been shown to be more specific in females compared to males. This has not been discussed in previous studies for sTREM-1. But the cause of this difference is not clear.

The study has some limitations. First, although we planned to evaluate the role of sTREM-1 in the diagnosis of SBP, we made the diagnosis based on the number of ascites neutrophil count, as it is still the gold standard. However, some SBP cases may be missed because the number of ascites neutrophils does not increase. Therefore, future studies are necessary in this regard. Second, the study population is relatively small to show possible significant differences in subgroup analysis. Both ROC and correlation analysis suggested that SBP has a more significant relationship with increased sTREM-1 levels in female patients. However, there is no known gender or hormone-based difference for the production and release of sTREM-1 on literature. These results we found may be due to small subgroup sizes. Therefore, further research is needed with larger populations to determine the role of the sTREM-1 molecule in the diagnosis and prognosis of peritonitis and clarify this possible gender effect.

In conclusion, infection of ascites is an important complication of portal hypertension, and the neutrophil count in ascites fluid is the traditional and widely used tool for the diagnosis. Recently, it has been shown that sTREM-1 molecule released from myeloid cells that encounter bacteria, can detect infections, similar to the well-known infection marker CRP. Therefore, sTREM-1 could be an alternative marker to identify ascites infections, especially in cases of peritonitis without an increase in the neutrophil count.

Ethics Committee Approval: This study was approved by the Ethical Committee of the Istanbul University, Istanbul Faculty of Medicine (Decision no: 2014-926/955).

Informed Consent: Written consent was obtained from the participants.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- A.M., S.Ç., R.D., M.K., M.A.; Data Acquisition- A.A., Ş.A.Y., İ.H.K., E.B.Ç.; Data Analysis/Interpretation- T.T., Y.A., T.S.A., E.B.Ç.; Drafting Manuscript- A.M., S.Ç., R.D., A.A., Ş.A.Y.; Critical Revision of Manuscript- T.T., Y.A., T.S.A., İ.H.K., M.K., M.A.; Final Approval and Accountability- A.M., S.Ç., R.D., E.B.Ç., M.K., T.S.A., Y.A., A.A., M.A., Ş.A.Y., İ.H.T., T.T.; Technical or Material Support- A.M., S.Ç., R.D., M.K., E.B.Ç.; Supervision- T.T., Y.A.

Conflict of Interest: Authors declared no conflict of interest.

Financial Disclosure: This study was supported by the Istanbul University Scientific Research Projects Fund (BAP). (Project Number: 46543).

Etik Komite Onayı: Bu çalışma için etik komite onayı İstanbul Üniversitesi, İstanbul Tıp Fakültesi Etik Kurulu'ndan alınmıştır (Karar no: 2014-926/955).

Bilgilendirilmiş Onam: Katılımcılardan bilgilendirilmiş onam alınmıştır.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Çalışma Konsepti/Tasarım- A.M., S.Ç., R.D., M.K., M.A.; Veri Toplama- A.A., Ş.A.Y., İ.H.K., E.B.Ç.; Veri Analizi/Yorumlama- T.T., Y.A., T.S.A., E.B.Ç.; Yazı Taslağı- A.M., S.Ç., R.D., A.A., Ş.A.Y.; İçeriğin Eleştirel İncelemesi- T.T., Y.A., T.S.A., İ.H.K., M.K., M.A.; Son Onay ve Sorumluluk- A.M., S.Ç., R.D., E.B.Ç., M.K., T.S.A., Y.A., A.A., M.A., Ş.A.Y., İ.H.T., T.T.; Malzeme ve Teknik Destek- A.M., S.Ç., R.D., M.K., E.B.Ç.; Süpervizyon- T.T., Y.A.

Çıkar Çatışması: Yazarlar çıkar çatışması beyan etmemişlerdir.

Finansal Destek: Bu çalışma İstanbul Üniversitesi Bilimsel Araştırma Projeleri Fonu (BAP) tarafından desteklenmiştir. (Proje Numarası: 46543).

REFERENCES

1. Rimola A, Garcia-Tsao G, Navasa M, Piddock LJ, Planas R, Bernard B, et al. Diagnosis, treatment and prophylaxis of spontaneous bacterial peritonitis: a consensus document. International Ascites Club. J Hepatol 2000;32(1):142-53. [CrossRef]
2. Rostkowska KA, Szymanek-Pasternak A, Simon KA. Spontaneous bacterial peritonitis - therapeutic challenges in the era of increasing drug resistance of bacteria. Clin Exp Hepatol 2018;4(4):224-31. [CrossRef]
3. Garcia-Tsao G. Current management of the complications of cirrhosis and portal hypertension: variceal hemorrhage, ascites, and spontaneous bacterial peritonitis. Gastroenterology 2001;120(3):726-48. [CrossRef]
4. European Association for the Study of the Liver. EASL Clinical Practice Guidelines on the management of ascites, spontaneous bacterial peritonitis, and hepatorenal syndrome in cirrhosis. J Hepatol 2010;53(3):397-417. [CrossRef]

5. Nousbaum JB, Cadranel JF, Nahon P, Khac EN, Moreau R, Thévenot T, et al. Diagnostic accuracy of the Multistix 8 SG reagent strip in diagnosis of spontaneous bacterial peritonitis. Hepatology 2007;45(5):1275-81. [CrossRef]
6. Evans LT, Kim WR, Poterucha JJ, Kamath PS. Spontaneous bacterial peritonitis in asymptomatic outpatients with cirrhotic ascites. Hepatology 2003;37(4):897-901. [CrossRef]
7. Barati M, Bashar FR, Shahrami R, Zadeh MH, Taher MT, Nojomi M. Soluble triggering receptor expressed on myeloid cells 1 and the diagnosis of sepsis. J Crit Care 2010;25(2):362.e1-6. [CrossRef]
8. Bouchon A, Dietrich J, Colonna M. Cutting edge: inflammatory responses can be triggered by TREM-1, a novel receptor expressed on neutrophils and monocytes. J Immunol 2000;164(10):4991-5. [CrossRef]
9. Gibot S, Cravoisy A, Levy B, Bene MC, Faure G, Bollaert PE. Soluble triggering receptor expressed on myeloid cells and the diagnosis of pneumonia. N Engl J Med 2004;350(5):451-8. [CrossRef]
10. Sun XG, Ma Q, Jing G, Wang GQ, Hao XD, Wang L. Increased levels of soluble triggering receptor expressed on myeloid cells-1 in cerebrospinal fluid of subarachnoid hemorrhage patients. J Clin Neurosci 2017;35:139-43. [CrossRef]
11. Ford JW, McVicar DW. TREM and TREM-like receptors in inflammation and disease. Curr Opin Immunol 2009;21(1):38-46. [CrossRef]
12. Pandupuspitasari NS, Khan FA, Huang CJ, Chen X, Zhang S. Novel Attributions of TREMs in Immunity. Curr Issues Mol Biol 2016;20:47-54. [CrossRef]
13. Determann RM, van Till JW, van Ruler O, van Veen SQ, Schultz MJ, Boermeester MA. sTREM-1 is a potential useful biomarker for exclusion of ongoing infection in patients with secondary peritonitis. Cytokine 2009;46(1):36-42. [CrossRef]
14. Bayram H, Tunger O, Civi M, Yuceyar MH, Ulman C, Dinc H, et al. Diagnostic and prognostic value of procalcitonin and sTREM-1 levels in sepsis. Turk J Med Sci 2015;45/3):578-86. [CrossRef]
15. Brenner T, Uhle F, Fleming T, Wieland M, Schmoch T, Schmitt F, et al. Soluble TREM-1 as a diagnostic and prognostic biomarker in patients with septic shock: an observational clinical study. Biomarkers 2017;22(1):63-9. [CrossRef]
16. Bouchon A, Facchetti F, Weigand MA, Colonna M. TREM-1 amplifies inflammation and is a crucial mediator of septic shock. Nature 2001;410(6832):1103-7. [CrossRef]
17. Bassyouni IH, Fawzi S, Gheita TA, Bassyouni RH, Nasr AS, El Bakry SA, et al. Clinical association of a soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) in patients with systemic lupus erythematosus. Immunol Invest 2017;46(1):38-47. [CrossRef]
18. Chubb SP, Williams RA. Biochemical Analysis of Pleural Fluid and Ascites. Clinical Biochemist Reviews 2018;39(2):39-50.
19. Velkey B, Vitális E, Vitális Z. Spontán bakteriális peritonitis [Spontaneous bacterial peritonitis]. Orvosi Hetilap 2017;158(2):50-7. [CrossRef]
20. Latour-Perez J, Alcala-Lopez A, Garcia-Garcia MA, Sánchez-Hernández JF, et al. Diagnostic accuracy of sTREM-1 to identify infection in critically ill patients with systemic inflammatory response syndrome. Clin Biochem 2010;43(9):720-4. [CrossRef]
21. Charles PE, Noel R, Massin F, Guy J, Bollaert PE, Quenot JP, et al. Significance of soluble triggering receptor expressed on myeloid cells-1 elevation in patients admitted to the intensive care unit with sepsis. BMC Infect Dis 2016;16(1):559. [CrossRef]
22. Al-Ghamdi H, Al-Harbi N, Mokhtar H, Daffallah M, Memon Y, Aljumah AA, et al. Changes in the patterns and microbiology of spontaneous bacterial peritonitis: analysis of 200 cirrhotic patients. Acta Gastroenterol Belg 2019;82(2):261-6.
23. Bal CK, Bhatia V, Daman R. Predictors of fifty days in-hospital mortality in patients with culture negative neutrocytic ascites. BMC Gastroenterol 2017;17(1):64. [CrossRef]
24. Sajjad M, Khan ZA, Khan MS. Ascitic Fluid Culture in Cirrhotic Patients with Spontaneous Bacterial Peritonitis. J Coll Physicians Surg Pak 2016;26(8):658-61.
25. Gong YX, Cui SN, Li L, Wang MM, Guo N. [Diagnostic value of human neutrophil peptide in spontaneous bacterial peritonitis]. Zhonghua Gan Zang Bing Za Zhi 2013;21(12):944-8.
26. Determann RM, Achouiti AA, El Solh AA, Bresser P, Vijfhuizen J, Spronk PE, et al. Infectious pleural effusions can be identified by sTREM-1 levels. Respir Med 2010;104(2):310e5. [CrossRef]

İSTANBUL ÜNİVERSİTESİ YAYINEVİ

RESEARCH / ARAŞTIRMA
DOI: 10.26650/IUITFD.2019.0074
İst Tıp Fak Derg 2020 / J Ist Faculty Med 2020

ANAEROBIC BACTERIA ISOLATED FROM CLINICAL SPECIMENS IN A UNIVERSITY HOSPITAL AND RESISTANCE OF ANAEROBIC GRAM-NEGATIVE RODS TO ANTIBIOTICS

BİR ÜNİVERSİTE HASTANESİNDE KLİNİK ÖRNEKLERDEN İZOLE EDİLEN ANAEROP BAKTERİLER VE ANAEROP GRAM-NEGATİF ÇOMAKLARIN ANTİBİYOTİKLERE DİRENCİ

Lütfiye ÖKSÜZ[1]

[1]Istanbul University, Istanbul Faculty of Medicine, Department of Medical Microbiology, Istanbul, Turkey

ORCID IDs of the authors: L.Ö. 0000-0002-6722-141X

Cite this article as: Oksuz L. Anaerobic bacteria isolated from clinical specimens in a university hospital and resistance of anaerobic Gram-negative rods to antibiotics. J Ist Faculty Med 2020;83(4):397-406. doi: 10.26650/IUITFD.2019.0074

ABSTRACT

Objective: Although anaerobic bacteria are normal microbiota members in humans, they can cause endogenous and exogenous infections. The empirical treatment of anaerobic infections is based on reports of susceptibility patterns reported in various studies. This study aims to identify the anaerobic bacteria isolated from clinical samples in 2018 and to determine the resistance of anaerobic Gram-negative rods to antibiotics and to compare the results obtained with the results of anaerobic Gram-negative rods isolated between 2015 and 2017 in the same unit in this study.

Material and Method: Specimens were inoculated on Schaedler Agar and Cooked Meat Broth and incubated in anaerobic conditions. Bacteria were identified by colony morphologies, conventional tests and anaerobic diagnostic discs. Antibiotic susceptibility tests were performed using the concentration gradient method and evaluated according to the criteria of CLSI.

Results: Of the 1630 clinical samples sent for anaerobic culture, 41 (2.5%) anaerobic bacteria were isolated. Most of the bacteria were isolated from the Department of Gynecology and Obstetrics (29%), Otorhinolaryngology (29%) clinics and mostly abscess specimens (49%). Seventy-one percent of the isolated anaerobic bacteria were Gram-negative and 29% Gram-positive bacteria. The most frequently isolated anaerobic bacteria were *Bacteroides fragilis* group (24%) and *Prevotella spp* (22%). Clindamycin resistance was quite high and there was no carbapenem resistance in anaerobic Gram-negative rods, but one third of the isolates were resistant to amoxicillin+clavulanic acid.

Conclusion: It was remarkable that more than half of the isolated anaerobic Gram-negative rods, especially the *B. fragilis* group,

ÖZET

Amaç: Anaerop bakteriler, insanda normal mikrobiyota üyesi olmakla birlikte endojen ve ekzojen enfeksiyonlara neden olabilmektedir. Anaerobik enfeksiyonların ampirik tedavisi, çeşitli araştırmalarda bildirilen duyarlılık paterni raporlarına dayanmaktadır. Bu çalışmada, 2018 yılında klinik örneklerden izole edilen anaerop bakterilerin tanımlanarak anaerop Gram-negatif çomakların antibiyotiklere direnç durumlarının belirlenmesi ve elde edilen sonuçların aynı birimde 2015-2017 yılları arasında izole edilen anaerop Gram-negatif çomaklara ait sonuçlarla karşılaştırılması amaçlanmıştır.

Gereç ve Yöntem: Klinik örnekler Schaedler agar ve kıymalı buyyon besiyerlerine ekilerek anaerop ortam sağlayıcı ile birlikte anaerop ortamda inkübe edilmiştir. Bakteri tanımlaması koloni morfolojisi, konvansiyonel testler ve anaerop tanı diskleri ile yapılmıştır. Antibiyotik duyarlılık deneyleri, konsantrasyon gradiyent yöntemi kullanılarak gerçekleştirilmiş ve CLSI kriterlerine göre değerlendirilmiştir.

Bulgular: Anaerop kültür için gönderilen 1630 klinik örnekten 41 (%2,5) anaerop bakteri izole edilmiştir. Anaerop bakteri izole edilen örneklerin en çok Kadın Hastalıkları ve Doğum Kliniği (%29) ve Kulak-Burun-Boğaz (%29) kliniklerinden ve en fazla abse (%49) örneklerinden izole edildiği belirlenmiştir. İzole edilen anaerop bakterilerin %71'inin Gram-negatif, %29'unun Gram-pozitif bakteriler olduğu, en sık izole edilen anaerop bakterilerin *Bacteroides fragilis* grubu (%24) ve *Prevotella* cinsi (%22) bakteriler olduğu bulunmuştur. Klindamisin direncinin oldukça yüksek olduğu, anaerop Gram-negatif çomaklarda karbapenem direncinin olmadığı, ancak suşların üçte birinin amoksisilin+klavulanik asite dirençli olduğu bulunmuştur.

Corresponding author/İletişim kurulacak yazar: oksuzl@istanbul.edu.tr

Submitted/Başvuru: 09.09.2019 • **Revision Requested/Revizyon Talebi:** 14.04.2020 •
Last Revision Received/Son Revizyon: 14.04.2020 • **Accepted/Kabul:** 16.06.2020 • **Published Online/Online Yayın:** 05.10.2020

were resistant to clindamycin and about a third of amoxicillin+ clavulanate. Increased resistance to these antibiotics used empirically in the treatment of infections caused by anaerobic Gram-negative rods is anticipated to limit antibiotic treatment regimens in the future. Routine monitoring of resistance is necessary for proper empirical treatment.

Keywords: Anaerobic bacteria, antibiotic resistance

Sonuç: Özellikle *B. fragilis* grubunda olmak üzere, izole edilen anaerop Gram-negatif çomakların yarısından fazlasının klindamisine ve yaklaşık üçte birinin amoksisilin+klavulanata dirençli olması dikkat çekicidir. Ampirik olarak kullanılan antibiyotiklere karşı artan direncin, gelecekteki antibiyotik tedavi rejimlerini kısıtlayacağı tahmin edilmektedir. Uygun ampirik tedavi için direncin rutin olarak izlenmesi gereklidir.

Anahtar Kelimeler: Anaerop bakteri, antibiyotik direnci

INTRODUCTION

Anaerobic bacteria, which are located in the majority of body surfaces and in the mucous membranes, are the important members of the human normal microbiota. These bacteria are mainly found on the skin, mouth, gastrointestinal system, and female genital system. Anaerobic bacteria cause endogenous infections when they increase in number in the region where they are found as normal microbiota or when they reach the sterile regions, and exogenous infections by entering into the body by accidents or traumas. Anaerobic bacteria may cause various infections in the sterile regions, such as wound infections, bacteremia, osteomyelitis, intra-abdominal infections, endophthalmitis, gas gangrene, or tooth and mouth infections, when the host's defense is weakened. They generally cause polymicrobial infections together with aerobic bacteria (1-3).

Treatment of polymicrobial anaerobic infections is generally performed by antibiotic treatment together with surgical drainage. Because most anaerobic infections are mixed infections, so antibiotics which are effective for both aerobic and anaerobic organisms must be used in treatment. Although clindamycin and metronidazole are known as the most effective drugs in the treatment of anaerobic infections, resistance to these drugs has developed in recent years. The alternative options are cefoxitin, beta lactam-beta lactamase inhibitors, carbapenems, and tigecycline (4, 5).

Identification of anaerobic bacteria and antibiotic susceptibility testing cannot be performed in most microbiology laboratories due to various reasons including technical difficulties, and empirical treatment of anaerobic infections were based on the susceptibility pattern reports of various research (6). Researchers have recently reported that antibiotic resistance is increasing rapidly in anaerobic bacteria as well as in aerobic bacteria (6, 7). In this study we aimed to identify the anaerobic bacteria isolated from clinical samples in 2018, to determine the resistance of anaerobic Gram-negative rods to antibiotics, and to compare our findings the susceptibility results of anaerobic Gram-negative rods isolated in the same unit between 2015 and 2017.

MATERIAL AND METHOD

We evaluated the anaerobic bacteria isolated from various samples (abscess, tissue, pleural fluid, peritoneal fluid, etc.) in our laboratory in 2018 and the resistance of anaerobic Gram-negative rods to antibiotics, then we compared with the data of the anaerobic bacteria isolated between 2015-2017 in the same unit. For the anaerobic culture, the samples were inoculated on 5% sheep blood Schaedler agar and cooked meat broth (Beckton Dickinson, USA), and were incubated for 48-72h at 37˚C in an anaerobic jar with an anaerobic environment provider (GasPak, Oxoid, UK). Following an aerotolerance test at the end of the incubation period, the colonies reproducing in only anaerobic environments were identified as anaerobic bacteria. Identification of anaerobic bacteria was performed with colony morphology, conventional tests, and using the anaerobic diagnostic discs (An-Ident-disk, Oxoid, UK) including kanamycin (1000 µg), colistin (10 µg), vancomycin (5 µg), penicillin (2 IU), erythromycin (60 µg), rifampicin (15 µg). Identifications were evaluated according to the recommendations of the manufacturing company and to scientific reference books (8). Bacterial suspensions were adjusted to 1 McFarland turbidity for antibiotic susceptibility tests and performed with a gradient test (E test, bioMerieux, France) on 5% sheep blood Brucella agar including hemin (5 µg/mL), and Vitamin K1 (1 µg/mL), for amoxicillin+ clavulanate, imipenem, cefoxitin, metronidazole, and clindamycin. Antibiotic susceptibility test results were evaluated according to Clinical & Laboratory Standards Institute(CLSI) criteria and the recommendations of the manufacturing company (9, 10, 38).

RESULTS

Forty-one anaerobic bacteria were isolated in 35 out of 1630 clinical samples (2.5 %) sent for anaerobic culture to our laboratory in 2018. We found that the anaerobic bacteria isolated samples were mostly submitted from Gynecology and Obstetrics Clinic (29%), and Otorhinolaryngology (29%) clinics, and the most of anaerobic bacteria were isolated from abscess (49%) samples. Seventy-one percent of the anaerobic bacteria isolated were Gram-negative bacteria, and 29% were Gram-positive, and most frequently isolated anaerobic bacteria were

Bacteroides fragilis group (24%), and *Prevotella* species (22%) (Table 1).

A total of 223 (4%) anaerobic bacteria were isolated from 143 (2.5%) clinical samples (n=5535) sent for anaerobic culture in a three-year period between 2015-2017 (11). One hundred sixty-one (72%) of these anaerobic bacteria were isolated from abscess, biopsy and tissue samples, 46 (21%) were isolated from sterile body fluids, and 16 (7%) were isolated from blood samples in a three-year period. Eighty percent of patients from whom anaerobic bacteria were isolated from their clinical samples were adults. A total of 223 anaerobic bacteria consisting of 152 (68%) Gram-negative, and 71 (32%) Gram-positive (35 anaerobic Gram-positive cocci, 36 anaerobic Gram-positive rod) were isolated from the clinical samples of a total of 143 patients. Twelve of the anaerobic Gram-positive rods were identified as *Cutibacterium acnes*, five were *Actinomyces* spp, and three were identified as *Clostridium* spp. (Table 1). Table 2 shows the antibiotic resistance rates of anaerobic Gram-negative rods isolated between 2015-2017, and in 2018.

DISCUSSION

Antimicrobial resistance has significantly increased in most pathogenic anaerobic bacteria in the last 20-30 years. However, antibiotic susceptibility tests for anaerobic bacteria cannot always be performed in most microbiology laboratories, owing to difficulties in the isolation of the anaerobic bacteria, lack of a standardised susceptibility methods, lack of correlation between the susceptibility test results and the clinical response. As a result of these, the antimicrobial resistance issue has usually been ignored by the clinicians and microbiologists due to necessity of immediate initiation of the empirical treatment (3, 12, 13).

Obtaining data about the antibiotic susceptibility of anaerobic bacteria is highly important for clinical laboratories because the treatment of anaerobic infections is usually performed empirically in the guidance of the studies reporting susceptibility patterns (6). An increase in morbidity, mortality, and hospitalization period occurs if initial antibiotic treatment is not appropriate in severe infections associated with anaerobic bacteria (3,12).

The Clinical and Laboratory Standards Institute (CLSI) does not recommend the performing of routine antibiotic susceptibility testing in anaerobic bacteria, owing to difficulties in susceptibility testings of anaerobic bacteria. It recommends the performing of antibiotic susceptibility testing in several conditions: in severe and life-threatening infections (bacteremia, endocarditis, brain abscess), for isolates of the resistant species, for anaerobic bacteria isolated from the sterile regions, in unresponsiveness to empirical treatment, in cases that require long term treatment, and in the isolation of high virulent bacteria (*Bacteroides* spp, *Prevotella* spp, and *Fusobacterium* spp) (9, 14). Researchers reported that routine susceptibility tests were not performed because the anaerobic Gram-positive cocci were generally sensitive to most of the antibiotics (15). However, resistance to penicillin and clindamycin has been reported as prevalent in anaerobic Gram-positive cocci in recent years, and the most effec-

Table 1: Isolated anaerobic bacteria in the period 2015-2017 and in 2018.

Isolated anaerobic bacteria	**2015-2017 n (%)**	**2018 n (%)**
Bacteroides fragilis group	60 (26.9)	10 (24.4)
Porphyromonas spp.	37 (16.6)	4 (9.8)
Prevotella spp.	36 (16.1)	9 (22)
Fusobacterium spp.	16 (7.2)	1 (2.4)
Other anaerobic Gram-negative rods	3 (1.3)	4 (9.8)
Anaerobic Gram-negative cocci	-	1 (2.4)
Peptococus niger	1 (0.4)	-
Other anaerobic Gram-positive cocci	34 (15.2)	4 (9.8)
Cutibacterium acnes	11 (4.9)	2 (4.8)
Clostridium spp.	3 (1.3)	1 (2.4)
Actinomyces spp.	5 (2.2)	-
Eubacterium spp.	1 (0.4)	-
Other anaerobic Gram-positive rods	16 (7.2)	5 (12.2)
Total	**223 (100)**	**41 (100)**

Table 2: Resistance to antibiotics by years in isolated anaerobic Gram-negative rods (%).

Bacteria/Antibiotics	**Years**			
***Bacteroides fragilis* group**	**2015**	**2016**	**2017**	**2018**
Amoxicillin+Clavulanate	18	18	21.4	30
İmipenem	0	4	0	0
Cefoxitin	33	-	46	10
Clindamycin	75	47	50	87
Metronidazole	6.2	9.5	-	-
***Fusobacterium* spp.**				
Amoxicillin+Clavulanate	20	5.8	33.3	-
İmipenem	0	0	0	-
Cefoxitin	0	0	0	-
Clindamycin	44.4	38	16.6	-
Metronidazole	25	25	20	-
***Porphyromonas* spp.**				
Amoxicillin+Clavulanate	11.1	0	0	33
İmipenem	12.5	0	0	0
Cefoxitin	0	0	0	66
Clindamycin	83,3	44.4	37.5	66
Metronidazole	11.1	28.5	0	-
***Prevotella* spp.**				
Amoxicillin+Clavulanate	20	0	0	33
İmipenem	0	0	0	0
Cefoxitin	0	0	0	0
Clindamycin	40	33.3	66.6	28

tive antibiotics in anaerobic Gram-positive rods were reported as penicillin, and carbapenems (16).

The basic approach in the management of severe anaerobic infections is antibiotic treatment combined with surgical intervention. As *Bacteroides* species constitute a significant part of normal microbiota, control of the disease is highly difficult in infections resulting from the endogenous dissemination of this organism. Antibiotic prophylaxis might be required when these organisms reach sterile regions, as a consequence of the deterioration of the natural barriers of mucosal surfaces with medical procedures. Almost all members of the *B. fragilis* group, many *Prevotella* and *Porphyromonas* species, and some *Fusobacterium* isolates produce the ß-lactamase enzyme which provides resistance to penicillin, and cephalosporins. Moreover plasmid-mediated resistance to clindamycin is common. Therefore the most effective antibiotics for Gram-negative anaerobic rods are metronidazole, carbapenems, and ß-lactam- ß-lactamase inhibitors (14, 17). Data concerning the resistance of anaerobic bacteria are limited to studies from reference laboratories or international large companies, except the surveillance studies in hospitals with good facilities, because in many centers susceptibility tests for anaerobic bacteria are rarely performed. Although there are differences between the regions, cities, and centers, in the studies performed in countries with approved methods, common tendencies may be detected, and tendencies affecting the empirical antibiotic treatment recommendations occur. The antibiotic susceptibility of these agents cannot be guessed before testing because there is inadequate local or national data for estimating the susceptibility due to the increased resistance against clindamycin, and cefoxitin, particularly in *B. fragilis* group (13, 14). Anaerobic bacteria had a standard susceptibility pattern 30 years ago, however, the efficacy of selected empirical treatment currently cannot be anticipated. It is known that the clinicians generally do not have adequate time for waiting for the results of antibiotic susceptibility testing in anaerobic infections. The

surveillance reports from Europe and the United States of America (USA) showed that resistance against all classes of antimicrobial agents has consistently been increasing, and even some antibiotics are not effective at all (14). In the paragraphs below, the resistance rates to antibiotics of anaerobic bacteria isolated in our study are compared with similar studies in the literature.

Metronidazole (5-nitroimidazole) is the first drug option, which is highly effective for anaerobes. Although the drug has been used since the 1960s, the resistance is rare, though metronidazole resistant *B. fragilis* isolates have recently been reported in 1-8% from different regions of the world (3, 13). Metronidazole resistance is associated with the *nim* genes which encode the production of a different nitroreductase enzyme. *Nim* genes were suggested to encode various homologous nitro imidazole reductases that can directly convert 5-nitroimidazole to inactive 5-aminoimidazole, and thus prevent the reduction of metronidazole to active nitro radical anion form (24). 5-nitroimidazole is a prodrug, and must be reduced by the intracellular transport protein ferredoxin after entry into the cell for its activation. Nitroimidazole reductases encoded by *nim* genes probably compete with this reduction, and convert the nitro group of the prodrug to an amin derivative that is not toxic for the bacteria (13). While nine different *nim* genes (A-I) have been identified so far, metronidazole resistance was also reported in isolates that did not have the *nim* gene (3). Resistance not associated with the *nim* gene was reported in a patient with a history of excessive use of metronidazole, though its mechanism is not completely known (13). The metronidazole resistance gene is found both on the chromosome and plasmid, and most of them are transferable (10). Metronidazole resistance was detected in 6-9% of the *B. fragilis* group, in 20-25%, of *Porphyromonas* species, in 11-28% of *Prevotella* species, and in 0-33% of *Fusobacterium* species of the anaerobic Gram-negative rods isolated in our hospital between 2015-2018 (Table 2). The investigation of similar studies in our country showed that Bahar et al., reported to have found no metronidazole resistant isolates in *B. fragilis, Porphyromonas* and *Prevotella* isolates (18, 19). Kiremitçi et al. reported only 18% in all anaerobic bacteria, and found the resistance to metronidazole in only one *B. fragilis* isolate (20). Ülger Toprak et al. (21), found no metronidazole resistance in their study investigating the carbapenem and metronidazole resistance in 66 *B. fragilis* isolates. These researchers found that all isolates were susceptible to metronidazole in their antimicrobial resistance study with 508 *Prevotella* isolates from 13 countries (22). Keşli et al. found no metronidazole resistance in *B. fragilis* groups in their study (23). To detect a very small rate of resistance to metronidazole or no metronidazole resistance in similar studies conducted in our country shows the significance of the metronidazole resistance of 6% and 9% detected in 2015 and 2016 in our study. The priority usage of metronidazole for empirical treatment in surgical clinics in our hospital may explain the higher metronidazole resistance rates detected in all Gram-negative rods. A study from South Africa reported that 8% of 23 *B. fragilis* isolates were highly resistant to metronidazole (>256 µg/mL). Researchers reported that only *nim* genes could not be blamed for resistance due to metronidazole highly resistant isolates having no *nim* gene (24). Syndman et al., reported to have detected metronidazole resistance in two isolates in their study conducted with 1957 *B. fragilis* groups isolated from eight centers in a period of four-years (25). Researchers reported that only one isolate was resistant to metronidazole in a seven-year surveillance study with 5,225 *B. fragilis* isolates from 10 centers in the USA (6).

Increased resistance rates to clindamycin, which is the classic anaerobic effective drug, have been reported in the last 20 years. The rates of resistance are 10-60% in *Bacteroides* species but it varies between countries. The resistance mechanism of clindamycin is known as the target change after methylation of 23S rRNAs. The clindamycin resistance gene is located on a transportable plasmid, which explains the rapid dissemination of clindamycin resistance (10). We found the resistance rates of clindamycin as 47-87% in *Bacteroides* spp, as 16-44% in *Fusobacterium* spp, 37-83% in *Porphyromonas* spp, and as 28-66% in *Prevotella* spp between 2015-2018 in the present study (Table 2). Investigation of similar studies in our country showed that Bahar et al. reported to have found the clindamycin resistance as 5-10% in *Prevotella* spp, and found no clindamycin resistant isolate in *Porphyromonas* spp (19). Kiremitçi et al. reported the clindamycin resistance as 53% in *B. fragilis* isolates (n=15) (20). Clindamycin resistance was reported as 36% in 45 *B. fragilis* group isolates in the study of Ülger Toprak et al. (26). Clindamycin resistance was detected as 28% in *B. fragilis* group, and as 11% in *Prevotella* species in the study of Keşli et al. (27). Gürler et al., reported the clindamycin resistance in *B. fragilis* isolates as 16% (11), and Liu et al. reported resistance as 37% (7). Clindamycin resistance was reported as 26% in a seven-year surveillance study with 5,225 *B. fragilis* isolates from 10 centers in the USA (6). Ülger Toprak et al. reported the clindamycin resistance as 34% in their antimicrobial resistance study with 508 *Prevotella* isolates from 13 countries. In addition, researchers emphasized that detection of antibiotic resistance against three or more antibiotics of 10% of *Prevotella* isolates was concerning (22). Mobile genetic elements were suggested to have a significant role in the dissemination of multiple resistance phenotypes in anaerobic bacteria (14). Syndman et al. (25), reported to have detected high resistance (60%) for clindamycin in their study with 1957 *B. fragilis* group isolates isolated from eight centers in a four-year period. The rate of

clindamycin resistance in our study are quite high, similar to the rates obtained both in our country and the other countries.

A different mechanism has been reported in the resistance against beta lactam-beta lactamase inhibitors. Beta lactam resistance develops when excessive cephalosporinase is produced, however the susceptibility to beta lactam-beta lactamase inhibitors remains. Resistance to beta lactam-beta lactamase inhibitor combinations may develop only in addition to another resistance mechanism such as porin loss (12). Researchers reported that the presence of *cep*A associated *IS*1124 increased the gene expression, resulting in excessive enzyme production, and the changes in porin proteins caused the resistance (28). Amoxicillin+clavulanate resistance in isolated anaerobic Gram-negative rods was detected as 18-30% in *B. fragilis* group, as *6-33*% in *Porphyromonas* species, as 11-33% in *Prevotella* species, and as 0-20% in *Fusobacterium* species in our study (Table 2). These rates were higher than the results of other studies. It has been suggested that the rate of resistance increased due to frequency of use, because anaerobic bacteria generally causes polymicrobial infections. Beta lactam-beta lactamase inhibitors are also effective antibiotics for facultative anaerobic bacteria, which produce an extended spectrum beta-lactamase. Bahar et al. reported to have detected no resistant isolates against amoxicillin+clavulanate in *B. Fragilis* group isolates (n=25) isolated from the abscess samples of pediatric patients in their study (18). Bahar et al. reported to have found no ampicillin +sulbactam resistance in *Porphyromonas* and *Prevotella* isolates in another study (19). Ülger Toprak et al. reported the amoxicillin+clavulanic acid resistance as 2% in 45 *B. fragilis* group isolates in their study (26). Less than 10% resistance to beta lactam-beta lactamase inhibitors has been reported in studies from different countries (4, 13, 29). Conversely, high resistance rates (37%) for ampicillin +sulbactam were reported in *B. fragilis* isolates from a study reported from Taiwan (37). In addition, Liu et al. reported the amoxicillin+clavulanic acid resistance as 23% in *B. fragilis* isolates (7). These rates were similar with our results.

Carbapenems are generally stable against most carbapenemases, but resistant strains are rarely reported. Carbapenems and piperacillin+tazobactam were reported as the most active agents with resistance rates of 0.9%, and 2.3% in *B. fragilis* isolates in a three-year surveillance study (30). Imipenem resistant *B. fragilis* isolates were first reported in Japan (31), later different studies also reported the imipenem resistance in *B. fragilis* isolates (25). Carbapenem resistance in the *B. fragilis* group depends on the production of B class metallo beta lactamase, from one of the *cfi*A/*ccr*A genes, and confers resistance to all beta lactams including the beta lactamase inhibitor combinations (13). Imipenem resistance in Turkey was first reported in the *B. fragilis* group in 1999, and it was shown that the resistance increased from 2% to 10% in a five-year period (21, 32). However, in two different studies, Bahar et al. reported no imipenem resistance in *B. fragilis, Prevotella, and Porphyromonas* isolates (18, 19). Similarly, we detected no carbapenem resistance in 2015, 2017, and 2018. In the present study however, 4% imipenem resistance was detected in *B. fragilis* group bacteria from 2016. Kiremitçi et al. detected imipenem resistance in one (6.6%) of the *B. fragilis* isolates (n=15) in their study investigating the susceptibility in anaerobic bacteria (20). Imipenem resistance was detected as 2% in 45 *B. fragilis* group isolates in the study of Ülger Toprak et al. (26). The same researchers reported that 27% of 66 *B. fragilis* isolates had the *cfi*A gene, 32% had the IS*1187* insertion sequence, and five isolates had both the *cfiA* gene, and IS*1187* insertion sequence, and these isolates were reported to have resistance to carbapenems. Researchers indicated, in accordance with the results of this study, that the presence of the IS element was important in determining the high level of carbapenem resistance (21). All isolates were sensitive to carbapenems in the resistance study of the same researchers with 508 *Prevotella* isolates from 13 countries (22). No imipenem resistance was detected in *B. fragilis* group bacteria in the study of Keşli et al. (23). Liu et al. reported an increase in carbapenem resistance of 207 *B. fragilis* strains isolated from the blood cultures between 2002-2006. The rates of non-susceptibility for imipenem and meropenem in *B. fragilis* isolates which were different than our study were indicated as 7%, and 12%, respectively (7). This rate was significantly high for carbapenems. Researchers in a study in South Africa reported that 8% of 23 *B. fragilis* isolates were highly resistant to imipenem (>256 µg/mL) (24). Carbapenem resistance was detected as approximately 1% in a seven-year surveillance study with 5225 *B. fragilis* isolates from 10 centers in the USA (6). Syndman et al., indicated that while the rate of carbapenem resistance was detected as 1.1-2.5% in 1957 *B. fragilis* group isolates isolated from eight centers in a four-year period, isolates with high MIC for carbapenems had no *cfi*A gene (25). In a study performed using the agar dilution method with anaerobic bacteria isolated from 521 clinic samples between 2014-2016 in South Korea, imipenem resistance was detected in 5% of *B. fragilis* strains, and as 14% in other *Bacteriodes* strains (33). Recently, detection of resistance to carbapenems, the most wide-spectrum antibiotic, in anaerobic Gram-negative rods might be the indicator of an alarming condition.

Cefoxitin is in group C among antibiotics recommended by CLSI for anaerobic bacteria and is recommended as an alternative in strains that are resistant to most primary drugs (9). In addition, cefoxitin is not recommended for intra-abdominal infections caused by *Bacteroides spe-*

cies (3). Beta lactamase gene encoded by *cep*A and *cfx*A, which may be transferred by plasmid or a mobile transposon, is responsible from the resistance of cefoxitin (13), and most *B. fragilis* isolates and two-thirds of *Prevotella* species are known to produce beta lactamase (7). The rate of resistance to cefoxitin in *Bacteroides* species was found as 10-33%, as 0-66% in *Porphyromonas* species, and no cefoxitin resistance was detected in *Fusobacterium* and *Prevotella* species in current study. Bahar et al. reported the cefoxitin resistance as 32% in the *B. fragilis* group isolated from the abscess samples of pediatric patients (n=25) in compliance with the results of this study (18). The same researchers reported the cefoxitin resistance as 3% in *Porphyromonas* isolates (19). Kiremitçi et al. reported the cefoxitin resistance as 7% in *B. fragilis* isolates (n=15) (20). Ülger Toprak et al. in a study reported the cefoxitin resistance as 11% in 45 *B. fragilis* group isolates (26). Keşli et al. found the cefoxitin resistance as 36% in the *B. fragilis* group, and as 11% in *Prevotella* species (23). Gürler et al. found the cefoxitin resistance as 16% in *B. fragilis* isolates (27). Researchers reported in a study conducted in South Africa that 8% of 23 *B. fragilis* isolates were highly resistant to cefoxitin (>256 µg/mL) (24). Cefoxitin resistance was reported as 10% in a seven-year surveillance study performed with 5225 *B. fragilis* isolates from ten centers in the USA (6). Cefoxitin resistance was detected as 7% in compliance with our results in 2018 (10%), in accordance with susceptibility results performed using the agar dilution method in anaerobic bacteria isolated from 521 clinical samples in a study conducted between 2014-2016 in South Korea (33). The resistance rate of cefoxitin is increasing in anaerobic Gram-negative bacilli with respect to rates in our country and in the world.

Investigation of studies associated with antibiotic susceptibility in anaerobic bacteria showed that most of the research was focused on the *B. fragilis* group, and antibiotic resistance has been gradually increasing. The resistance rates to antibiotics in the *B. fragilis* group indicated in various studies are given in Table 3. The most comprehensive study associated with the antimicrobial resistance of *B. fragilis* group was the study of Nagy et al., which included 824 isolates from 13 European countries (34). Researchers indicated that the significant increase in antibiotic resistance was detected for cefoxitin, clindamycin, and moxifloxacin, and total resistance to cefoxitin was reported as 17%, resistance to clindamycin as 32%, resistance to moxifloxacin as 13%, resistance to amoxicillin+clavulanic acid as 3%, resistance to piperacillin+tazobactam as 10%, resistance to imipenem as 1.2%, and resistance to metronidazole was reported as less than 1%. Researchers emphasized that isolates which were not sensitive to imipenem and metronidazole were more resistant to the other antibiotics. Piperacillin+tazobactam was reported to be more active compared with amoxicillin+clavulanic acid. Antibiotic resistance was reported to differ between the regions; resistance to moxifloxacin (21%) in Scandinavian countries, and resistance to metronidazole (42%) in Mediterranean countries were reported to be higher. As Turkey is in the Mediterranean region,

Table 3: Antibiotic resistance rates in *B. fragilis* group isolates in various studies in the literature (%).

Research	Amox+Clavulanate	Imipenem	Cefoxitin	Clindamycin	Metronidazole
Gurler 1997	0	0	16	16	0
Bahar 2002	0	0	32	0	0
Ulger 2004	2	2	11	36	0
Syndman 2007	-	1	10	25	1 isolate
Kiremitci 2008	-	1 isolate	7	53	1 isolate
Liu 2008	23	7-12	13	37	0
Syndman 2010		0,9			
Bouchillon 2010	-	0-5	10-27	10-29	0-1.2
Galvao 2011	-	8	8	0	8
Nagy 2011	10	1.2	17	32	<1
Syndman 2011		1.1-2.5	7	30	2 isolate
Ulger 2012	-	1,5	-	-	0
Keşli 2018	-	0	36	28	0
Rodloff 2018	-	1.7	-	22	0.5
Oksuz 2018	17.8	2	17.4	54	4.6
Present study	**30**	**0**	**10**	**87**	**-**

this result shows that our results were compatible with the rates of Nagy. Piperacillin+tazobactam resistance was also found to be higher in Scandinavian countries. When the researchers compared the data of two multi-center studies (15 countries with 1289 isolates, and 19 countries with 1,284 isolates) conducted in Europe in previous years with their own studies, they pointed out that resistance has increased over the years.

In accordance with the results of "The Tigecycline European Surveillance Trial (TEST)" presented in 2010 in the European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), resistance to cefoxitin was reported as 10-27%, to clindamyc in as 10-29%, to metronidazole as 0-1.2%, to meropenem as 0-5%, and to piperacillin+tazobactam as 0-12% in 1420 *B. fragilis* isolates (35). Antimicrobial resistance was investigated in 7008 anaerobic bacteria in the TEST study conducted in 2018, and most of the anaerobic bacteria were reported to be susceptible to meropenem, metronidazole, and tigecycline, but resistance to clindamycin in Gram-negative bacteria (28-48%) was alarming (36). In addition, in the TEST study, 22% of the strains were shown to be resistant to clindamycin, 1.7 % to meropenem, and 0.5% to metronidazole in *B. fragilis* isolates; however, clindamycin resistance was between 10-30%, meropenem resistance 0.2-0.6%, and metronidazole resistance 0.6-1.9% in *Prevotella* species (36). The data of this recent study were similar to our results and highlight the increase in carbapenem resistance.

One of the limitations of our study was that full identification could not be performed for all isolated anaerobic bacteria due to financial issues, and antibiotic resistance of anaerobic Gram-positive bacteria could not be investigated. In addition, metronidazole resistance could not be investigated in anaerobic Gram-negative rods in 2018 due to the same financial issues. Although the number of anaerobic bacteria isolated is low, it can be said that the resistance rates obtained in 2018 are still high, and the high resistance rates for clindamycin in particular could not be ignored. Another limitation of our study was that susceptibility tests could not be performed using the gold standard method of agar dilution due to the study consisting of retrospective data. Unfortunately, this method requires intensive labour, and could not be performed during routine laboratory workflow. Researchers in a study in Switzerland reported that a concentration gradient test was compatible with the agar dilution test, and the rate of major and very major error was less than 1% (37). Furthermore, in a study which compared the agar dilution, disk elucion in broth, and gradient test methods for susceptibility tests of anaerobic bacteria (n=86) in our department, no statistically significant difference between three methods was reported (11). In addition, the rate of production of beta lactamase could not be determined in our study. Whether or not to use beta lactam antibiotic in treatment in centers where no routine antibiotic susceptibility tests are performed is determined by detecting the presence of beta lactamase in anaerobic bacteria. We did not perform a beta lactamase test in our study because many anaerobic Gram-negative rods, primarily the *B. fragilis,* are known to produce high rates of beta lactamase. We performed antibiotic susceptibility tests for all pathogenic anaerobic bacteria isolated. In addition, mechanisms other than the beta lactamase production may also develop resistance to antibiotics in anaerobic Gram-negative rods. Anaerobic bacteria without beta-lactamase enzyme, but resistant to ampicillin, have been reported rarely. We suggest that all these limitations may be eliminated with well planned prospective studies using the gold standard methods.

CONCLUSION

It was striking to find out in our study that more than half of the anaerobic Gram-negative rods primarily the *B. fragilis* group were resistant to clindamycin, and approximately one third were resistant to amoxicillin+clavulanate. It is estimated that the increased resistance to these antibiotics, which are empirically used in the treatment of anaerobic Gram-negative rods associated infections will limit the antibiotic treatment regimes in the future. The routine monitoring of resistance is highly important for providing appropriate empirical treatment.

Acknowledgments: I would like to thank my Professor Dr. Nezahat GÜRLER who has supported me in my interest in anaerobic bacteria since I first was introduced to microbiology, to Masters student Rama Al Hamwi, who compiling the isolated anaerobic bacteria in 2018, and to Prof. Dr. Arif KAYGUSUZ for his contributions in the post reading of the article.

Ethics Committee Approval: Ethics committee approval was not received due to the retrospective nature of the study

Informed Consent: Informed consent was not received due to the retrospective nature of the study.

Peer Review: Externally peer-reviewed.

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

Conflict of Interest: Authors declared no conflict of interest.

Financial Disclosure: Authors declared no financial support.

Teşekkür: Mikrobiyolojiyle ilk tanışmamdan bu yana anaerobik bakterilere olan ilgimde beni destekleyen Prof. Dr. Nezahat Gürler'e, 2018 yılında izole anaerobik bakterilerin derlenmesindeki

katkılarından dolayı Yüksek lisans öğrencisi Rama Al Hamwi'ye ve Prof. Dr. Arif KAYGUSUZ'a makalenin son okumasındaki katkılarından dolayı teşekkür ederim.

Etik Komite Onayı: Retrospektif çalışma olduğundan etik komite onayı alınmamıştır.

Bilgilendirilmiş Onam: Retrospektif bir çalışma olduğundan bilgilendirilmiş onam alınmamıştır

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Çalışma Konsepti/Tasarım- L.Ö.; Veri Toplama- L.Ö.; Veri Analizi/Yorumlama- L.Ö.; Yazı Taslağı- L.Ö.; İçeriğin Eleştirel İncelemesi- L.Ö.; Son Onay ve Sorumluluk- L.Ö.

Çıkar Çatışması: Yazarlar çıkar çatışması beyan etmemişlerdir.

Finansal Destek: Yazarlar finansal destek beyan etmemişlerdir.

REFERENCES

1. Baysallar M, Bedir O. Anaerobic Bacteriology. In: Garcia LS, Isenberg HD, editors. The Handbook of Clinical Microbiology Methoods (Translate eds: Başustaoğlu A, Yıldıran ŞT). ASM Press: Washington; 2014, s. 4.0.1.
2. Jousimies-Somer HR, Summanen P, Citron DM, Baron EJ, Wexler HM, Finegold SM, editors. Waddsworth-KTL Anaerobic Bacteriology Manual. Chapter 1: Introduction to Anaerobic Bacteriology. Star Publishing Company: California; 2002, p.1.
3. Tunçkanat F. Anaerobic bacteria: General characteristics. In: Topçu AW, Söyletir G, Doğanay M, editors. Infectious Diseases and Microbiology. Nobel Medical Bookstores: İstanbul; 2017, s.2036.
4. Cohen-Poradosu R, Kasper DL. Anaerobic Infections: General concepts. In: Bennett JE, Dolin R, Blaser MJ editors. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. Elseiver: USA; 2015, s.2736-43.
5. Yenen OŞ. Infections of anaerobic bacteria. In: Brooks GF, Carroll KC, Butel JS, Morse SA, Mietzner TA, editors. Jawetz, Melnick, Adelberg Medical Microbiology. Nobel Medical Bookstores: İstanbul; 2014, s.295.
6. Syndman Dr, Jacobus NV, Mc Dermott LA, Ruthazer R, Golan Y, Goldstein EJ, et al. National Survey on the Susceptibility of Bacteroides fragilis Group: Report and Analysis of Trends in the United States from 1997 to 2004. Antimicrob Agents Chemother 2007;51(5):1649-55. [CrossRef]
7. Liu CY, Huang YT, Liao CH, Yen LC, Lin HY, Hsueh PR. Increasing Trends in Antimicrobial Resistance among Clinically Important Anaerobes and Bacteroides fragilis Isolates Causing Nosocomial Infections: Emerging Resistance to Carbapenems. Antimicrob Agents Chemother 2008;52(9):3161-8. [CrossRef]
8. Procop GW, Church DL, Hall GS, William M. Janda WM, Koneman EW, Schreckenberger PC, Woods GL, editors. Koneman's Color Atlas & Textbook of Diagnstic Microbiology. The anaerobic bacteria. Lipincott Williams & Wilkins: Philadelphia; 2017, p.983-1071.
9. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. 27th ed. CLSI supplement M100, 2017. S-26.
10. Jousimies-Somer HR, Summanen P, Citron DM, Baron EJ, Wexler HM, Finegold SM, editors. Waddsworth-KTL Anaerobic Bacteriology Manual. Chapter 8. Susceptibility testing of anaerobic bacteria. Star Publishing Company: California; 2002, s.143.
11. Öksüz L, Gürler N. Resistance of anaerobic bacteria isolated from clinical specimens to antibiotics: A three-year surveillance. 33. Antimicrobial Chemothearpy Congress, Oral presentation, S130, 2-6 May 2018, Fethiye.
12. Dubreuil L, Odou MF. Anaerobic bacteria and antibiotics: What kind of unexpected resistance could I find in my laboratory tomorrow? Anaerobe 2010;16:555-9. [CrossRef]
13. Hecth DW. Anaerobes: Antibiotic resistance, clinical significance, and the role of susceptibility testing. Anaerobe 2006;12:115-21. [CrossRef]
14. Gajdacs M, Spengler G, Urban E. Identification and Antimicrobial Susceptibility Testing of Anaerobic Bacteria: Rubik's Cube of Clinical Microbiology? Antibiotics 2017;6:1-29. [CrossRef]
15. Gürler N. Anaerobic cocci. In: Topçu AW, Söyletir G, Doğanay M. Infectious Diseases and Microbiology. s. 2046. Nobel Medical Bookstores: İstanbul; 2017.
16. Könönen E, editor. Anaerobic cocci and anaerobic Gram-positive nonsporulating bacilli. In: Bennett JE, Dolin R, Blaser MJ. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. Elseiver: USA; 2015, s.2781-6.
17. Murray PR, Rosenthal KS, Pfaller MA, editors. Medical Microbiology. Philadephia; 2009, s.399-404.
18. Bahar H, Mamal Torun M, Demirci M, Sever N, İstanbullu A. Anaerobic bacteria isolated from clinical specimens of pediatric patients and resistance to antibiotics. ANKEM Derg 2002;16(4):486-91.
19. Bahar H, Mamal Torun M, Demirci M, Kocazeybek B. Antimicrobial resistance and beta lactam production of clinical isolates of Prevotella and Porphyromonas species. Chemotherapy 2005;51:9-14. [CrossRef]
20. Kiremitçi A, Türkkan AA, Akgün Y, Durmaz G, Kaşifoğlu N. Isolation of anaerobic bacteria from clinical samples and determination of antibiotic susceptibility. Bulletin of ANKEM 2008;22(3):132-44.
21. Ülger Toprak N, Uzunkaya ÖD, Soki J, Söyletir G. Susceptibility profiles and resistance genes for carbapenems (cfiA) and metronidazole (nim) among Bacteroides species in a Turkish University Hospital. Anaerobe 2012;18:169-71. [CrossRef]
22. Ulger Toprak N, Veloo ACM, Urban E, et al. A multicenter survey of antimicrobial susceptibility of Prevotella species as determined by E test methodology. Anaerobe 2018;52:9-15. [CrossRef]
23. Keşli R, Demir C. Identification of Anaerobic Gram-Negative Bacilli Isolated from Various Clinical Specimens and Determination of Antibiotic Resistance Profiles with E-Test Methods. Mikrobiyol Bul 2018;52(1):72-9. [CrossRef]
24. Galvao BPGV, Meggersee RL, Abratt VR. Antibiotic resistance and adhesion potential of Bacteroides fragilis clinical isolates from Cape Town, South Africa. Anaerobe 2011;17:142-6. [CrossRef]
25. Syndman DR, Jacobus NV, Mc Dermott LA, Golan Y, Goldstein EJ, Harrel L, et al. Update on resistance of Bacteroides fragilis group and related species with special attention to carbapenems 2006-2009. Anaerobe 2011;17:147-51. [CrossRef]

26. Ülger Toprak N, Çelik C, Çakıcı Ö, Söyletir G. Antimicrobial susceptibilities of Bacteroides fragilis and Bacteroides thetaiotamicron strains isolated from clinical specimens and human intestinal microbiota. Anaerobe 2004;10(5):255-9. [CrossRef]
27. Gürler N, Zandi H, Töreci K. Comparison of agar dilution, E test and broth disc elution methods in determining the susceptibility of anaerobic bacteria. Bulletin of ANKEM 1997;11(4):487-92.
28. Tunçkanat F. Susceptibility tests of anaerobic bacteria and resistance status. Alıntılama tarihi: 09.07.2018. Available from: http://www.tmc-online.org/userfiles/file/AKG_Sunumlar/22nisan/ferda_tunckanat_anaerop_bakterilerin_duyarlilik_testleri.pdf.
29. Garrett WS, Onderdonk AB. Bacteroides, Prevotella, Porphyromonas, and Fusobacterium Species (and Other Medically Important Anaerobic Gram-Negative Bacilli). In: Bennett JE, Dolin R, Blaser MJ, editors. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. Elseiver: USA; 2015, s.2773-80.
30. Syndman DR, Jacobus NV, McDermott LA, Golan Y, Hecht DW, Goldstein EJ, et al. Lessons Learned from the Anaerobe Survey: Historical Perspective and Review of the Most Recent Data (2005-2007) Clin Infect Dis 2010;50(Suppl 1):S26-33. [CrossRef]
31. Yamazoe K, Kato N, Kato H, et al. Distribution of the cfiA Gene among Bacteroides fragilis Strains in Japan and Relatedness of cfiA to Imipenem Resistance. Antimicrob Agent Chemother 1999;43(11):2808-10. [CrossRef]
32. Ülger Toprak N. Susceptibility tests and data Turkey: Anaerobes. Gülhane Microbiology Days, Antimicrobial Chemotherapy Laboratory Applications and Innovations. 20-22 Nisan 2010, İstanbul.
33. Byun BH, Kim M, Lee Y, Lee K, Chong Y. Antibiotic susceptibility patterns of anaerobic bacterial clinical isolates from 2014-2016, including recently named or renamed species. Ann Lab Med 2019;36:190-9. [CrossRef]
34. Nagy E, Urban E, Nord CE. Antimicrobial susceptibility of Bacteroides fragilis group isolates in Europe: 20 years of experience. Clin Microb Infect 2011;17:371-9. [CrossRef]
35. Bouchillon S, Renteria M, Hackel M, Johnson B, Dowzicky M. Gram-negative anaerobe susceptibility rates in six European countries, 2007-2009. 20th ECCMID Congress, P1293, Vienna, 10-13 April 2010.
36. Rodloff AC, Dowzicky MJ. In vitro activity of tigecycline and comparators against a European collection of anaerobes collected as part of the Tigecycline Evaluation and Surveillance Trial (T.E.S.T.) 2010-2016. Anaerobe 2018;51:78-88. [CrossRef]
37. Wüst J, Hardegger U. Comparison of the E test and a reference agar dilution method for susceptibility testing of anaerobic bacteria. Eur J Clin Microbiol Infect Dis 1992;11(12):1169-73. [CrossRef]
38. Biomerieux, E test application guide. 2012/07. Available from: https://www.biomerieux-usa.com/sites/subsidiary_us/files/supplementary_inserts_-_16273_-_b_-_en_-_eag_-_etest_application_guide-3.pdf Alıntılama tarihi: 15.05.2020.

İSTANBUL ÜNİVERSİTESİ YAYINEVİ

RESEARCH / ARAŞTIRMA
DOI: 10.26650/IUITFD.2020.0005
İst Tıp Fak Derg 2020 / J Ist Faculty Med 2020

NON-ALBICANS SPECIES: MAIN ACTORS OF CANDIDEMIA? SEVEN-YEAR EXPERIENCE FROM A SINGLE CENTRE

KANDİDEMİLERİN BAŞ AKTÖRÜ ALBİCANS DIŞI TÜRLER MİDİR? YEDİ YILLIK TEK MERKEZ DENEYİMİ

Zehra Çağla KARAKOÇ[1]

Istinye University, Faculty of Medicine, Infectious Diseases and Clinical Microbiology, Istanbul, Turkey

ORCID IDs of the authors: Z.Ç.K. 0000-0002-1618-740X

Cite this article as: Karakoc ZC. Non-albicans species: main actors of candidemia? Seven-year experience from a single centre. J Ist Faculty Med 2020;83(4):407-12. doi: 10.26650/IUITFD.2020.0005

ABSTRACT

Objective: Candidemia is a major cause of mortality among healthcare-associated infections. Considering the increase in non-*albicans* species in recent years, it is important to define the treatment approach by identifying *Candida* at the species level. The aim of this study was to evaluate the epidemiological characteristics, risk factors and mortality of patients with candidemia in our hospital.

Material and Method: Forty-four patients with *Candida* species isolated from at least one bottle of blood culture taken during hospitalization between January 2013 and October 2019 were included in the study. Patients' demographic information, comorbidities, duration of hospitalization and ward, neutropenia, total parenteral nutrition (TPN), steroid administration and invasive device use, antimicrobial treatments used in the last month, source of candidemia, acute phase indicators, *Candida* species and antifungal resistance, antifungal treatment, clinical response and mortality were evaluated retrospectively. *Candida* species and antifungal susceptibilities were identified using the automated system VITEK®2 (bioMérieux, Marcy l'Etoile, France).

Results: A total of 44 patients with candidemia participated; the median age was 57, and 27 (61.3%) were male. The median length of stay was 33.5 days. Forty-two (95.4%) of the cases were accompanied by multiple comorbidities, and the most common aetiology was malignancy (59%). Most (97.7%) of the patients had received broad-spectrum antibiotic treatment in the last month. Central venous catheters (CVCs) were used in 35 (79.5%) of the cases and 50% of them were treated with TPN. *Candida albicans* (54.6%) was the most common species, followed by *Candida tropicalis* (18.2%). Non-albicans species were observed to increase over time. Thirty-day mortality was 36.3%.

ÖZET

Amaç: Kandidemiler sağlık bakımı ile ilişkili infeksiyonlar arasında mortalitesi yüksek olan bir grubu oluşturmaktadır. *Albicans* dışı türlerin sebep olduğu kandidemilerin insidansındaki artış, tür düzeyinde tanımlamanın ve bu sayede etkin antifungal tedaviyi uygulamanın önemini ortaya koymuştur. Çalışmamızda; hastanemizdeki kandidemi olgularının epidemiyolojik özellikleri, risk faktörleri ve mortaliteleri açısından değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntem: Ocak 2013-Ekim 2019 tarihleri arasında yatışı sırasında alınan en az bir şişe kan kültüründe *Candida* türlerinin izole edildiği 44 hasta çalışmaya dahil edildi. Hastaların demografik bilgileri, komorbiditeleri, yatış süresi-yattığı ünite, son bir ayda yapılan cerrahi girişimler, nötropeni, total parenteral beslenme (TPN), steroid uygulaması ve invazif araç kullanımı, son bir ayda ve ayrıca kandidemi sırasında kullandığı antimikrobiyal tedaviler, kandideminin kaynağı, akut faz göstergeleri, *Candida* türü ve antifungal direnci, antifungal tedavi türü ve süresi, tedaviye klinik yanıt ve gelişen mortaliteler retrospektif olarak değerlendirildi. *Candida* türleri ve antifungal duyarlılıkları VITEK®2 (bioMérieux, Marcy l'Etoile, Fransa) otomatize sistemi ile tanımlandı.

Bulgular: Toplam 44 kandidemili hastanın; ortanca yaşı 57 (0-87), 27 (%61,3)'si erkek, 17 (%38,6)'si kadındı. Ortanca yatış süresi 33,5 gündü. Olguların 42 (%95,4)'sine çeşitli komorbiditeler eşlik etmekte olup, en sık etiyoloji malignite (%59) olarak saptandı. Hastaların 43 (%97,7)'ü son bir ayda geniş spektrumlu antibiyotik tedavisi almıştı. Olguların 35 (%79,5)'inde SVK vardı ve yarısına TPN tedavisi uygulanıyordu. Tür dağılımında en sık *Candida albicans* (%54,6) görülürken, ikinci sırayı *Candida tropicalis* (%18,2) izliyordu ve zamanla *albicans* dışı türlerin arttığı gözlendi. Otuz günlük mortalite %36,3 olarak saptandı.

Corresponding author/İletişim kurulacak yazar: ckarakoc@gmail.com

Submitted/Başvuru: 20.01.2020 • **Revision Requested/Revizyon Talebi:** 29.01.2020 •
Last Revision Received/Son Revizyon: 31.01.2020 • **Accepted/Kabul:** 10.02.2020 • **Published Online/Online Yayın:** 17.09.2020

Conclusion: Non-*albicans* candidemia was found to have increased over the years in our study. The main risk factors for candidemia were determined as the presence of comorbidities, especially malignancy, prior broad-spectrum antibiotherapy use, TPN treatment and the presence of CVC. The mortality rate in this study was also consistent with the literature.

Keywords: Candidemia, epidemiology, risk factors

Sonuç: Çalışmamızda tek merkezin kandidemi sonuçları irdelenmiş; yıllar içinde *albicans* dışı kandidemilerin artış gösterdiği saptanmıştır. Kandidemi için başlıca risk faktörlerinin malignite başta olmak üzere komorbiditelerin varlığı, öncesinde geniş spektrumlu antibiyoterapi uygulanması, TPN tedavisi ve SVK varlığı olarak belirlenmiştir. Mortalite oranı da literatürle uyumludur.

Anahtar Kelimeler: Kandidemi, epidemiyoloji, risk faktörleri

INTRODUCTION

Candidemia, which constitutes an important part of invasive *Candida* infections, is an important cause of mortality in hospitalized patients and is the fourth most common cause of bloodstream infections in hospitals. Nowadays, increasingly complex surgical interventions, patients at high risk of infection, and changing demographic characteristics of patients have increased the frequency of candidemia (1). Two important groups of patients are at risk for candidemia: immunosuppressed patients and intensive care unit patients. Neutropenia, chemotherapy, broad-spectrum antibiotherapy, invasive device use, total parenteral nutrition (TPN) treatment, comorbidities (chronic kidney failure, advanced age, etc.) and surgery are considered predisposing risk factors for candidemia in these patients (2).

Candidemia can originate endogenously from the gastrointestinal tract, skin and mucous membranes. In the literature, it has been determined that the most important risk factor for dissemination of infection into the bloodstream is *Candida* colonization in the mucous membranes. In fact, in 80% of patients who develop a bloodstream infection due to *Candida albicans* and *Candida glabrata*, mucous membrane colonization develops before the development of candidemia (3, 4). In addition, intravenous catheter use, contaminated TPN and hands of healthcare workers have also been associated with exogenous candidemia (4).

Candidemia can cause acute sepsis, which is usually indistinguishable from bacterial bloodstream infections. It can also cause insidious clinical presentation accompanied by fever. Common clinical presentation is fever and clinical deterioration that do not respond to antibiotic treatments in patients with risk factors for candidemia (5). It is still difficult to diagnose candidemia in our current clinical practice because clinical symptoms are not specific, blood culture tests are missing in 50% patients, biomarkers indicating *Candida* infections (β-D-glucan, T2 magnetic resonance, etc.) are not widely used and there is still insufficient data in polymerase chain reaction-based tests (5, 6). Although the distribution of isolates in candidemia may vary according to the geographic region and patient groups, the dominant isolate is mostly *C. albicans*. Studies conducted in recent years show that infections related to non-*albicans* species (NAC) are also increasing (7).

The main objective of this study is to evaluate epidemiological features, risk factors, distribution of *Candida* species, antifungal resistance, response to antifungal therapy and mortality of candidemia cases followed in our centre.

MATERIAL AND METHOD

Forty-four patients with isolated candidemia, ranging in age from 0 to 87 years and who were hospitalized between January 2013 and October 2019, were enrolled in the study. Candidemia was defined as the isolation of the *Candida* strain in at least one vial of blood culture with clinical illness. The diagnosis of catheter-related bloodstream infection was defined according to the American Centers for Disease Control and Prevention criteria (8). Identical *Candida* species of one patient on different days was considered as a single infection. Clinical response to antifungal treatment was determined as fever regression with a decrease in inflammatory markers (C-reactive protein [CRP], procalcitonin [PCT] and leukocyte count). Patients' demographic information, existing comorbidities, duration of hospitalization, hospitalization ward, surgical interventions in the last month, neutropenia, TPN, systemic steroid administration and invasive device use (central venous catheter [CVC], mechanical ventilation, urinary catheter), antibiotic and antifungal therapies within the last month, antibiotherapies applied during candidemia and the source of candidemia were recorded. In addition, acute phase indicators (leukocyte count, CRP and PCT), *Candida* species and antifungal susceptibilities, antifungal therapy and duration, clinical response to therapy and mortality were evaluated retrospectively from the hospital and clinical microbiology laboratory records.

A BACTEC 9240 (Becton Dickinson, Sparks, MD, USA) automated blood culture system was used for blood cultures. Cultures were incubated for 7 days. Gram stain and subculture on plate media (5% sheep blood agar, MacConkey agar, chocolate agar, Sabouraud dextrose agar) were applied to positive blood cultures. Plates were incubated at 37°C for 48–72 hours. A VITEK 2 Compact® (bioMérieux, Marcy l'Etoile, France) automated system was used for identification and antifungal susceptibility tests of all *Candida* species. All data analysis was conducted *using* the Statistical Package for Social Sciences (*SPSS*, Chicago, IL, USA), version 11.0.

RESULTS

The median age of 44 patients was 57 (0–87 years); 27 (61.3%) were male and 10 were in the paediatric age group (<16 years). The median length of stay was 33.5 (2–125) days. Average number of leukocytes was 12052±9840.3/mm^3 and CRP was 137.1±105 mg/L; PCT was determined as 11.6±22.2 ng/ml. During candidemia, 54.5% cases were receiving in-patient services and the remaining 45.5% were in intensive care units (34.1% medical intensive care, 6.8% paediatric intensive care and 4.5% surgical intensive care). Candidemia was diagnosed after a mean duration of 23.5±24.7 (2–107) days after hospital admission. Multiple comorbidities accompanied 42 (95.4%) of the cases. These comorbidities, in order of frequency, were malignancy (26 patients; 24 patients solid organ tumour, two patients haematological malignancy), cerebrovascular events (six patients), kidney failure (five patients), cardiovascular diseases (four patients), diabetes (three patients), hypertension (two patients), low birth weight (one patient), short bowel syndrome (one patient), Down syndrome (one patient) and severe burn (one patient). Forty-three patients (97.7%) had received broad-spectrum antibiotic therapy in the past month, and 41 (93.1%) patients were receiving broad-spectrum antibiotic therapy during candidemia. Thirty-five (79.5%) of the cases had a CVC and TPN therapy was applied in 50% of those who developed candidemia. Systemic steroid use in supraphysiological doses was applied for anti-oedema effect in six patients with the diagnosis of intracranial malignancies. The demographics, laboratory findings and risk factors of the patients are summarized in Table 1.

C. albicans (54.6%) was the predominant species followed by *Candida tropicalis* (18.2%; Figure 1a). It has been observed that non-*albicans* species have increased over the years (Figure 1b). The source of candidemia was determined as CVC in 13 (29.5%) patients (Table 2). Fluconazole resistance was not detected in any of the identified *C. albicans* strains. In NAC, fluconazole resistance was 20% (5/20). Only seven of the patients with candidemia were examined with dilated fundoscopy and none of them had ocular disease. However, in the follow-up of a patient with biliary tract candidemia (due to complaints of vision loss), fundoscopic examination revealed ocular involvement. Endocarditis was not detected in any of the 13 (29.5%) patients who underwent echocardiography. In this study, fluconazole was the most (50%) empirical antifungal therapy used for candidemia followed by echinocandins (36.4%). Patients were given antifungals for a mean duration of 16.7±10.1 (range 0–42) days. Clinical response was obtained to antifungal treatment given in 33 (75%) cases of candidemia. Crude 30-day mortality was 36.3% (16/44 patients). There was no difference in mortality between *C. albicans* and NAC patients.

Table 1: Risk factors for candidemia

Risk factors	Patients n=44 (%)
Comorbidity/malignancy	42 (95.4%)/26 (59%)
Age (≥65/≤1)	13 (29.5%)/2 (4.5%)
Central venous catheter Mechanical ventilation Urinary catheter use	35 (79.5%) 16 (36.3%) 25 (56.8%)
Total parenteral nutrition administration	22 (50%)
Surgical intervention last month / intra-abdominal surgery	15 (34.1%)/11 (25%)
Neutropenia	5 (11.4%)
Systemic steroid use	6 (13.6%)
Chronic renal failure	5 (11.4%)
Intensive care unit patients	20 (45.5%)
Broad-spectrum antibiotic use last month	43 (97.7%)
Severe burn	1 (2.2%)
Low birth weight	1 (2.2%)

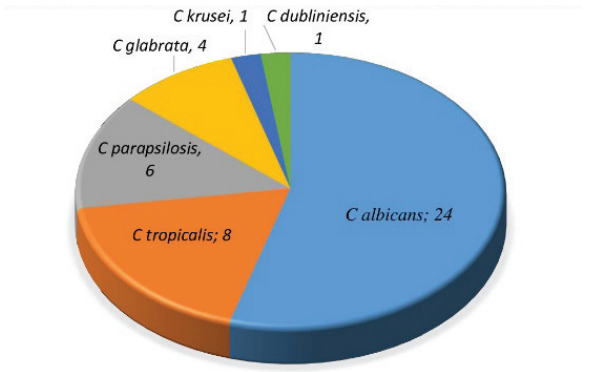

Figure 1a: Species distribution of *Candida* isolates.

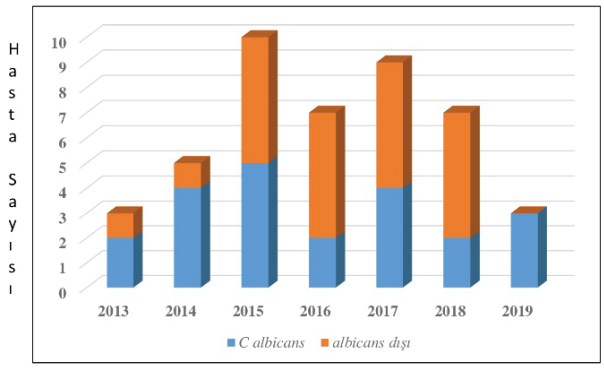

Figure 1b: Distribution of *Candida* species by years.

Table 2: Source of candidemia

Source of candidemia	Patients n=44 (%)
Unknown	15 (34%)
Central venous catheter	13 (29.5%)
Urinary tract	11 (25%)
Gastrointestinal system	5 (11.3%)

DISCUSSION

Candidemia is the most common clinical form of invasive *Candida* infection, and its incidence may differ between countries. Candidemia has been associated with features such as age group and characteristics of patients evaluated, healthcare-associated factors, blood culture techniques, unnecessary antibiotic use and antimicrobial resistance (9).

In candidemia, the rate of pathogen isolation in the blood culture is about 50%. This limitation causes difficulties in determining the epidemiology and incidence of *Candida* species (10). The incidence of candidemia was 0.47–7.07 per 1000 admissions in European hospitals and was much higher in the intensive care units of these hospitals (9,11-13). The incidence of candidemia was determined as 0.3–1.76 per 1000 admissions in studies conducted in our country (14-17). In our study, the average incidence of candidemia for 6 years and 10 months was found as 0.41 per 1000 admissions.

The incidence of candidemia is high in extremes of age. It has been determined that the most important reason for the high incidence in infants under one year of age is low birth weight (18). In older age, the incidence of candidemia is much higher due to comorbidities (19). In our study, approximately one-third (29.5%) of patients with candidemia were elderly patients. In fact, studies have shown that *Candida* infections develop due to impaired defence mechanisms of the host rather than the pathogenicity of the microorganism; existing comorbidities is the most important factor disrupting the host's defence mechanism (20).

As in our study, the most common comorbidity in patients with candidemia is malignancy, especially in those with haematological malignancy. Chemotherapy, concomitant neutropenia, the presence of mucositis in the digestive system and corticosteroid therapies used can also be considered important risk factors contributing to the development of invasive *Candida* infections. On the other hand, candidemia that developed in patients with solid organ tumours has been associated with surgical complications, intensive care hospitalization, mechanical ventilation, TPN treatment and CVC use (21). In our study, 92.3% (24/26 patient) of cases with malignancy were accompanied by a solid organ tumour.

Other risk factors determined for candidemia in the literature include corticosteroid administration, CVC use, abdominal surgery, severe burns, renal failure requiring dialysis, broad-spectrum antibiotic use and low birth weight in newborns (22). In our study, prior broad-spectrum antibiotic therapy (97.7%), CVC use (79.5%) and TPN therapy (50%) were found to be compatible with the literature (Table 1). According to the literature, 33%–55% of candidemia develops in intensive care units (23). The data we obtained from the past 7 years showed that 45.5% of candidemia developed in intensive care units.

Distribution of candidemia species may vary between geographic regions and institutions. Globally, while candidemia related to *C. albicans* is decreasing, the incidence of *C. glabrata* and *Candida krusei* remains unchanged, and the incidence due to *Candida parapsilosis* and *C. tropicalis* is increasing (10). In studies conducted in our country, while the rates related to *C. albicans* varied between 48.1% and 75%, it was determined that non-*albicans* species have increased over time, similar to the literature (14, 16, 24). Similarly, in our study, while the most common cause of candidemia was *C. albicans* (54.6%), NAC species were determined to have increased over the years (Figure 1b).

Candidemia developing with NAC depends on the underlying features of the patient. *C. parapsilosis* is mostly associated with exogenous infection, such as CVC colonization and parenteral nutrition, and the incidence is significantly higher in Mediterranean countries (21, 25). *C. glabrata* and *C. krusei* are associated with recent major abdominal surgery, solid organ tumours, advanced age, neutropenic newborns, transplant recipients and steroid-treated patients. The *C. glabrata* rate is higher in the United States (21.1%) compared to other countries in the world (7.6%–12.6%) (21). *C. tropicalis* is usually isolated in patients with solid tumours and haematological cancer, and it is the second most common species in Asia and Latin America (21). The widespread use of azoles in the last two decades has been associated with a decrease in infections related to *C. tropicalis* and *C. albicans* (26). The second most common isolate in our study was *C. tropicalis*, following *C. albicans*. Similar to the literature, the underlying disease was solid organ malignancy in the vast majority of these patients (7/8 patients). Yapar et al. reported that the most common isolate among non-*albicans* candidemia was *C. tropicalis*, similar to our study. This frequent isolation was explained by a lower ratio of patients receiving fluconazole prophylaxis applied in the study centre (15). A small number of patients (2/44 patients) were found to have fluconazole prophylaxis in our study as well. *C. parapsilosis* was identified in our study as the third highest frequency. *C. glabrata*, which does not differ between centres and is generally found between 9% and12%, was found to be 9.1%, in accordance with the literature in our centre.

Crude mortality has been reported to be 30%–60% in candidemia (27, 38). Treatment early and with an appropriate antifungal agent significantly reduces mortality. Therefore, knowing the causative agent in candidemia enables the empirical treatment selection to be directed correctly (10). Current guidelines recommend echinocandins as the first-line treatment for candidemia and recommend fluconazole treatment only in non-critical patients (29). In fact, it has been shown in a study that echinocandin therapy does not improve the outcome of non-critical care unit patients with septic shock due to candidemia (30). In the selection of empirical treatment, the patient's clinical presentation, prior use of azole, presence of neutropenia and surveillance data of the relevant centre should be taken into consideration. Considering these factors, fluconazole was the preferred treatment in 50% of patients in our study, followed by echinocandins, and our crude 30-day mortality rate was also found to be consistent with the literature.

In conclusion, candidemia should be considered in patients with risk factors such as malignancy, broad-spectrum antibiotherapy, CVC and TPN use, in intensive care units and advanced age. The recent increase in non-*albicans* candidemia cases should not be ignored. In our centre, similar to the literature, NAC has increased over the years. Our study presents the epidemiological and clinical features of retrospective candidemia cases of a single centre. The most important limiting factor is the low number of cases. Importantly, the crude mortality of candidemia is high despite advances in diagnosis, and each centre should guide the treatment knowing its own *Candida* epidemiology and antifungal resistance.

Acknowledgement: I thank Prof. Dr. Binnur Pınarbaşı Şimşek for her support.

Ethics Committee Approval: Ethics committee approval was not received due to the retrospective nature of the study.

Informed Consent: Informed consent was not received due to the retrospective nature of the study.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- Z.Ç.K.; Data Acquisition- Z.Ç.K.; Data Analysis/Interpretation- Z.Ç.K.; Drafting Manuscript- Z.Ç.K.; Critical Revision of Manuscript- Z.Ç.K.; Final Approval and Accountability- Z.Ç.K.

Conflict of Interest: Authors declared no conflict of interest.

Financial Disclosure: Authors declared no financial support.

Teşekkür: Desteklerinden ötürü Prof. Dr. Binnur Pınarbaşı Şimşek'e teşekkür ederim.

Etik Komite Onayı: Retrospektif çalışma olduğundan etik komite onayı alınmamıştır.

Bilgilendirilmiş Onam: Retrospektif bir çalışma olduğundan bilgilendirilmiş onam alınmamıştır.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Çalışma Konsepti/Tasarım- Z.Ç.K.; Veri Toplama- Z.Ç.K.; Veri Analizi/Yorumlama- Z.Ç.K.; Yazı Taslağı- Z.Ç.K.; İçeriğin Eleştirel İncelemesi- Z.Ç.K.; Son Onay ve Sorumluluk- Z.Ç.K.

Çıkar Çatışması: Yazarlar çıkar çatışması beyan etmemişlerdir.

Finansal Destek: Yazarlar finansal destek beyan etmemişlerdir.

REFERENCES

1. Horn DL, Neofytos D, Anaissie EJ, Fishman JA, Steinbach WJ, Olyaei AJ, et al. Epidemiology and outcomes of candidemia in 2019 patients: data from the prospective antifungal therapy alliance registry. Clin Infect Dis 2009;48(12):1695-703. [CrossRef]
2. Willke A. Kandidemi: nasıl değerlendirilmeli ne yapılmalı. İnfeksiyon Dergisi 2007;21:117-22.
3. Tortorano AM, Peman J, Bernhardt H, Klingspor L, Kibbler CC, Faure O, et al. Epidemiology of candidaemia in Europe: results of 28-month European Confederation of Medical Mycology (ECMM) hospital-based surveillance study. Eur J Clin Microbiol Infect Dis 2004;23(4):317-22. [CrossRef]
4. Eggimann P, Garbino J, Pittet D. Epidemiology of Candida species infections in critically ill non-immunosuppressed patients. Lancet Infect Dis 2003;3(11):685-702. [CrossRef]
5. Pilmis B. Yang ZT, Lontenier F, Lortholary O. Systemic Candidiasis. In: Cohen j, Powerly W, Opal S, editors. Infectious Diseases (Fourth Edition), Elsevier limited; 2017. p.439-45. [CrossRef]
6. Mylonakis E, Clancy CJ, Ostrosky-Zeichner L, Garey KW, Alangaden GJ, Vazquez JA, et al. T2 magnetic resonance assay for the rapid diagnosis of candidemia in whole blood: a clinical trial. Clin Infect Dis 2015;60(6):892-9. [CrossRef]
7. Pfaller MA, Diekema DJ, Turnidge JD, Castanheira M, Jones RN. Twenty years of the SENTRY antifungal surveillance program: results for *Candida* species from 1997-2016. Open Forum Infect Dis 2019;6(Suppl 1):S79-94. [CrossRef]
8. O'Grady NP, Alexander M, Burns LA, Dellinger EP, Garland J, Heard SO. Guidelines for the prevention of intravascular catheter-related infections. Clin Infect Dis 2011;52(9):e162-93. [CrossRef]
9. Bassetti M, Merelli M, Righi E, Diaz-Martin A, Rosello EM, Luzzati R, et al. Epidemiology, species distribution, antifungal susceptibility, and outcome of candidemia across five sites in Italy and Spain. J Clin Microbiol 2013;51(12):4167-72. [CrossRef]
10. Guinea J. Global trends in the distribution of Candida species causing candidemia. Clin Microbiol Infect 2014;20(S6):5-10. [CrossRef]
11. Tragiannidis A, Fegeler W, Rellensmann G, Debus V, Müller V, Hoernig-Franz I, et al. Candidaemia in a european paediatric university hospital: a 10-year observational study. Clin Microbiol Infect 2012;18(2):E27-30. [CrossRef]

12. Almirante B, Rodríguez D, Park BJ, Cuenca-Estrella M, Planes AM, Almela M, et al. ors of mortality in cases of Candida bloodstream infection: results from population-based surveillance, Barcelona, Spain, from 2002 to 2003. J Clin Microbiol 2005;43(4):1829-35. [CrossRef]
13. Bassetti M, Giacobbe DR, Vena A, Trucchi C, Ansaldi F, Antonelli M, et al. Incidence and outcome of invasive candidiasis in intensive care units (ICUs) in Europe: results of the EUCANDICU project Crit Care 2019;23(1):219. [CrossRef]
14. Tukenmez Tigen E, Bilgin H, Perk Gurun H, Dogru A, Ozben B, Cerikcioglu N, et al. Risk factors, characteristics, and outcomes of candidemia in an adult intensive care unit in Turkey. Am J Infect Control 2017;45(6):e61-e63. [CrossRef]
15. Yapar N, Pullukcu H, Avkan-Oguz V, Sayin-Kutlu S, Ertugrul B, Sacar S, et al. Evaluation of species distribution and risk factors of candidemia: a multicenter case-control study. Med Mycol 2011;49(1):26-31. [CrossRef]
16. Ulu Kilic A, Alp E, Cevahir F, Ture Z, Yozgat N. Epidemiology and cost implications of candidemia, a 6-year analysis from a developing country. Mycoses 2017;60(3):198-203. [CrossRef]
17. Yeşilkaya A, Azap Ö, Aydın M, Akçil Ok M. Epidemiology, species distribution, clinical characteristics and mortality of candidaemia in a tertiary care university hospital in Turkey, 2007-2014. Mycoses 2017;60(7):433-9. [CrossRef]
18. Greenberg RG, Benjamin DK Jr. Neonatal candidiasis: diagnosis, prevention, and treatment. J Infect 2014;69(1):S19-22. [CrossRef]
19. Wang H, Liu N, Yin M, Han H, Yue J, Zhang F, et al. The epidemiology, antifungal use and risk factors of death in elderly patients with candidemia: a multicentre retrospective study. BMC Infect Dis 2014;14:609. [CrossRef]
20. Luzzati R, Allegranzi B, Antozzi L, Masala L, Pegoraro E, Azzini A et al. Secular trends in nosocomial candidaemia in non-neutropenic patients in an Italian tertiary hospital. Clin Microbiol Infect 2005;11(11):908-13. [CrossRef]
21. Quindós G. Epidemiology of candidaemia and invasive candidiasis. A changing face. Rev Iberoam Micol 2014;31(1):42-8. [CrossRef]
22. Kotthoff-Burrell E. Candidemia (Blood Infection) and Other Candida Infections. Am J Respir Crit Care Med 2019;200(5):9-10. [CrossRef]
23. Bouza E, Muñoz P. Epidemiology of candidemia in intensive care units. Int J Antimicrob Agents 2008;32(2):S87-91. [CrossRef]
24. Yapar N, Akan M, Avkan-Oguz V, Ergon CM, Hancer M, Doluca M. Risk factors, incidence and outcome of candidemia in a Turkish intensive care unit: a five-year retrospective cohort study. APIC 2014;18(3):265-71.
25. Yapar N. Epidemiology and risk factors for invasive candidiasis. Ther Clin Risk Manag 2014;10:95-105. [CrossRef]
26. Chen PY, Chuang YC, Wu UI, Sun HY, Wang JT, Sheng WH, et al. Clonality of fluconazole-nonsusceptable Candida tropicalis in bloodstream infections, Taiwan, 2011-2017. Emerg Infect Dis 2019;25(9):1660-7. [CrossRef]
27. Flevari A, Theodorakopoulou M, Velegraki A, Armaganidis A, Dimopoulos G. Treatment of invasive candidiasis in the elderly: a review. Clin Interv Aging 2013;8:1199-208. [CrossRef]
28. Wisplinghoff H, Ebbers J, Geurtz L, Stefanik D, Major Y, Edmond MB, et al. Nosocomial bloodstream infections due to Candida spp. in the USA: species distribution, clinical features and antifungal susceptibilities. Int J Antimicrob Agents 2014;43(1):78-81. [CrossRef]
29. Pappas PG, Kauffman CA, Andes DR, Clancy CJ, Marr KA, Ostrosky-Zeichner L, et al. Clinical practice guideline for the management of Candidiasis: 2016 update by the Infectious Diseases Society of America. Clin Infect Dis 2016;62:e1-50. [CrossRef]
30. Falcone M, Giusy T, Gutiérrez-Gutiérrez B, Giammarco R, Paolo C, Chiara R, et al. Impact of initial antifungal therapy on the outcome of patients with candidemia and septic shock admitted to medical wards: A propensity score–adjusted analysis. Open Forum Infect Dis 2019;6(7):ofz251. [CrossRef]

İSTANBUL ÜNİVERSİTESİ YAYINEVİ

RESEARCH / ARAŞTIRMA
DOI: 10.26650/IUITFD.2019.0105
İst Tıp Fak Derg 2020 / J Ist Faculty Med 2020

BASELINE CHARACTERISTICS OF PATIENTS WITH GROWTH HORMONE DEFICIENCY

BÜYÜME HORMONU EKSİKLİĞİ OLAN HASTALARIN TEMEL ÖZELLİKLERİ

Utkucan UÇKUN[1], Firdevs BAŞ[1], Şükran POYRAZOĞLU[1], Nur Mine ŞÜKÜR[2], Fatma Feyza DARENDELİLER[1], Rüveyde BUNDAK[3]

[1]Istanbul University, Istanbul Faculty of Medicine, Department of Child Health and Diseases, Istanbul, Turkey
[2]Istanbul University, Institute of Child Health, Department of Pediatric Basic Sciences, Istanbul, Turkey
[3]University of Kyrenia, Faculty of Medicine, Department of Child Health and Diseases, Kyrenia, TRNC

ORCID IDs of the authors: U.U. 0000-0001-9731-154X; F.B. 0000-0001-9689-4464; Ş.P. 0000-0001-6806-9678; N.M.Ş. 0000-0003-2769-0526; F.F.D. 0000-0003-4786-0780; R.B. 0000-0001-5097-6448

Cite this article as: Uckun U, Bas, F, Poyrazoglu S, Sukur NM, Darendeliler FF, Bundak R. Baseline characteristics of patients with growth hormone deficiency. J Ist Faculty Med 2020;83(4):413-20. doi: 10.26650/IUITFD.2019.0105

ABSTRACT

Objective: The aim of this study was to describe the characteristics and the aetiological profile of patients with growth hormone deficiency (GHD)

Material and Method: Among randomly selected 320 cases with short stature with a height SDS<-3SD, 203 patients with diagnosis of GHD were evaluated with respect to their characteristics at diagnosis.

Results: 86 patients (42.4%) had idiopathic GHD, 79 patients (39%) had congenital GHD, 14 patients (6.9%) had defined syndromes with GHD and 10 patients (5%) had acquired GHD. Number of patients with isolated GHD was 154 (81.5%) and with multiple pituitary hormone deficiency (MPHD) was 35 (18.5%) among classified cases.

The most common accompanying hormone deficiency was TSH deficiency in GHD aetiologies with MPHD. Hypophyseal pathologies were most commonly seen in congenital and acquired GHD cases. Noonan syndrome was the most common syndrome with an accompanying GHD. The bone age delay was found to be over 2 years in congenital GHD. The mean IGF-1 SD score and the mean peak growth hormone stimulation tests' values were significantly low in congenital GHD.

Conclusions: Precise assessment of auxological, clinical and laboratory data could provide substantial value in the evaluation of severely short statured children with GHD.

Keywords: Severe short stature, children, aetiology, growth hormone deficiency

ÖZET

Amaç: Bu araştırmanın amacı büyüme hormonu eksikliği (BHE) olan hastaların karakteristik özelliklerinin ve etiyolojik profillerinin belirlenmesidir

Gereç ve Yöntem: Ağır boy kısalığı olan (boy SDS<-3SD) ve randomize seçilmiş 320 olgudan BHE tanısı olan 203 olgu ile araştırma yürütülmüştür

Bulgular: 86 hastada (%42,4) idiyopatik BHE, 79 hastada (%39) konjenital BHE, 14 hastada (%6,9) BHE eşlikli sendromlar ve 10 hastada (%5) edinsel BHE saptanmıştır. Sınıflandırılan olgular içinde izole BHE olan 154 (%81,5) ve çoklu hipofizer hormon eksikliği (ÇHHE) olan 35 (%18,5) olgu saptanmıştır.

ÇHHE olan BHE etiyoloji gruplarında en sık eşlik eden hormon eksikliği TSH eksikliğidir. Hipofizer patolojiler en sık konjenital ve edinsel BHE olgularında görülmektedir. BHE en sık Noonan sendromuna eşlik etmektedir. Konjenital BHE olgularında kemik yaşı gecikmesi 2 yıl ve üzeri saptanmıştır. Ortalama IGF-1 standart sapma skoru ve ortalama pik büyüme hormonu uyarı testi değeri konjenital BHE'de belirgin olarak düşük saptanmıştır.

Sonuç: Oksolojik, klinik ve laboratuvar verilerin titizlikle incelenmesi BHE'nin eşlik ettiği ağır boy kısalığı olan çocukların değerlendirilmesine önemli katkı sağlayabilmektedir.

Anahtar Kelimeler: Ağır boy kısalığı, çocuk, etiyoloji, büyüme hormone eksikliği

Corresponding author/İletişim kurulacak yazar: utkucan.uckun@istanbul.edu.tr

Submitted/Başvuru: 20.09.2019 • **Revision Requested/Revizyon Talebi:** 24.03.2020 •
Last Revision Received/Son Revizyon: 01.06.2020 • **Accepted/Kabul:** 07.06.2020 • **Published Online/Online Yayın:** 06.10.2020

INTRODUCTION

There are several aetiological causes of growth hormone deficiency (GHD), occurring at any level of the hypothalamic-pituitary axis causing growth hormone related growth retardation.

The prevalence of GHD differs between 1/3480 and 1/30,000 children according to the literature (1-8).

In a previous study (9), we analysed the aetiology of short stature in children with a height SDS<-3SDS. In this study, we aimed to describe the characteristics and the aetiological profile of GHD patients selected among those children. Auxological, demographic and endocrinological data are presented in relation to gender and the various aetiological diagnoses.

MATERIAL AND METHOD

This retrospective study was conducted in the Paediatric Endocrine Clinic of Istanbul University, Istanbul Faculty of Medicine, Istanbul, Turkey. Randomly selected 320 severely short statured (height SDS<-3SDS) children with at least 12 months of follow-up were included in our previous study (9). Among those, 203 patients with a diagnosis of GHD were selected for this study.

Data collection

Identity records (birth dates, addresses, phone numbers), presence of parental consanguinity, mother's age at menarche, history of short stature and/or pubertal delay in the family, other familial diseases, history of precocious puberty, the height values of both mothers and fathers, the chief complaint of the patient at admission, type of birth, gestational age, anthropometric values at birth, birth complications, nutritional history, neuromotor developmental history, additional diseases and previous medications were all recorded from patients' files.

The anthropometric measurements, physical examination and pubertal findings and bone ages at admission and at follow-up were recorded from the files.

The pubertal sign was based on breast budding in females and a testicular volume of over 4 ml in males. Puberty was considered to be delayed with no sign of puberty at the age of 14 in males, and at the age of 13 in females (10). Pubertal development was assessed as recommended by Marshall and Tanner (11, 12).

Patients were divided into 2 groups according to Tanner staging to be used in comparison Tables; Stage 1 (prepubertal) and Stage >1 (pubertal).

Anthropometric measurements

Height, weight and head circumference

Ages at admission of all patients were calculated and recorded as decimal years. Three main groups were designated according to age at admission (< 5years, 5-10 years and >10years).

All data regarding height, weight and head circumference at admission of the patients were collected from patients' files. The height measurements of children and their parents in our clinic are done according to standard measurement rules by using a Harpenden stadiometer by the same auxologist. Weight measurements are done by using a scale which is sensitive to 100 grams. Body mass index (BMI) of patients were calculated by using height and weight values of the patients (kg/m^2). Height, weight and BMI SDS scores were calculated according to Turkish standards by Neyzi et al. (*13-15)* (Büyüyorum v1.3 programme was used for calculations).

SD scores of birth weight, height and head circumference were calculated according to the revised Fenton growth chart. For calculations, the Peditools Fenton 2013 programme was used (16).

Patients were divided into three groups; appropriate for gestational age (AGA), small for gestational age (SGA) and large for gestational age (LGA). Birth weight and/or length were between -2SD and +2SD in AGA, less than -2SD in SGA and greater than +2SD in LGA groups.

Target height calculation

Target height values for patients, of whom parental height values were present, were calculated according to the Tanner method (17). SD scores of these values were calculated by using Turkish standards (13-15).

Predicted adult height calculation

Predicted adult height (PAH) for each patient was calculated with the *Bayley-Pinneau* method by using the *Greulich-Pyle* atlas (18). The predicted adult height could not be calculated in males with a bone age below 7 years and in females with a bone age below 6 years. SD scores of predicted adult height values were calculated according to Turkish standards (13-15).

Formation of aetiology groups

Congenital GHD results from genetic errors, and may be associated with structural defects of the brain or with midline facial defects such as a cleft palate or a single central incisor. Several genetic defects have been identified so far. Acquired GHD can occur as a result of a variety of different causes including cranial trauma (perinatal or postnatal), central nervous system infections, tumours of the hypothalamic or pituitary region (pituitary adenoma, craniopharyngioma, glioma, germinoma, metastases), radiation therapy, infiltrative diseases (Langerhans cell histiocytosis, sarcoidosis, tuberculosis). If any other diagnosis cannot be established, the GHD aetiology is considered idiopathic. GHD can be associated with defined syndromes such as Turner's syndrome as well.

Isolated growth hormone deficiency is a condition due to a severe shortage or absence of growth hormone, whereas MPHD is an endocrine disorder due to a combination of pituitary hormone deficiencies. The diagnosis is established in a multi-step approach for each patient based on history, signs and symptoms, hormonal and radiological findings and genetic testing.

The aetiology groups for GHD were divided into 4 main groups to be used in comparison tables (19);

Group A: Idiopathic growth hormone deficiency
Group B: Congenital growth hormone deficiency
Group C: Acquired growth hormone deficiency
Group D: Defined syndromes with growth hormone deficiency
Group E: Unclassified

Since Group E constituted unclassified cases due to insufficient data, it was not used in comparison tables.

Further classification of GHD was done according to whether being isolated or MPHD:

Isolated GHD
Multiple pituitary hormone deficiency (MPHD)

Laboratory tests and methods
Calculation of bone age and bone age delay
Calculations of both bone age and bone age delay were done by using the *Greulich-Pyle* atlas. The delay in bone age was divided into 2 main groups to be used in comparison tables; bone age delay <2 years and bone age delay ≥2 years.

Hormonal tests and methods
Serum IGF-1 values
IGF-1 values were recorded from patients' files. In our clinic, the *Liaison® IGF-1 (313231) CLIA (chemiluminescent immunoassay) test (DiaSorin, Sallugia, Italy)* is used to assess the IGF-1 values in ng/ml. The SD scores of the results were calculated using the kit's data.

Serum IGFBP-3 values
IGFBP-3 values were recorded from patients' files. In our clinic, the *IMMULITE® test (Siemens, Malvern, PA, USA)* is used to assess the IGFBP-3 values in ng/ml. SD scores of the results were calculated using the kit's data.

Growth hormone provocation tests
GHD was defined as a serum peak GH concentration <10 ng/mL on provocation at two separate stimulation tests. GH stimulation tests were performed with various stimuli, such as insulin, L-dopa, clonidine, and glucagon. In prepubertal boys over 11 years, intramuscular testosterone depot injections were performed 7–10 days before GH testing; and in prepubertal girls over 10 years, oral conjugated oestrogen was prescribed for 3 days before testing.

Statistics
For statistical analysis SPSS 21.0 programme was used. *Pearsons's chi-square* and *Fisher's exact* tests were applied to sets of categorical data. *t*-test was performed for between-pairs comparison, and comparisons among groups were performed using analysis of variance. The *Mann–Whitney U* test was used when sample sizes were small and/or when the data did not approximate a normal distribution. The *LSD test* was used to find out which group differs from the others. Differences were regarded as significant when *P* value was <0.05.

RESULTS

Two hundred three (106 M/97 F) patients with a diagnosis of GHD were selected for this study. Among these, 86 patients (42.4%) had idiopathic GHD, 79 patients (39%) had congenital GHD, 14 patients (6.9%) had defined syndromes with GHD and 10 patients (5%) had acquired GHD (Table 1).

MPHD was found in 14% of cases with idiopathic GHD. The most common accompanying hormone deficiencies in those cases were TSH deficiency (75%), LH/FSH deficiency (25%) and ACTH deficiency (8%) respectively. MPHD was found in 25.3% of cases with congenital GHD. The most common accompanying hormone deficiencies in those cases were TSH deficiency (90%), LH/FSH deficiency (45%) and ACTH deficiency (15%) respectively. Twenty percent of acquired GHD cases had MPHD, and all of them were TSH deficient. Only one patient, who was both TSH and ACTH deficient, had MPHD within defined syndromes with the GHD aetiology group.

One hundred eighty-nine classified cases with GHD were also divided into subgroups; isolated GHD (n=154, 81.5%) and MPHD (n=35, 18.5%).

There was no statistical difference in means of gender, history of parental consanguinity and birth weight for gestational age distributions between GHD aetiology groups (p>0.05).

The mean age values at admission of idiopathic and acquired GHD aetiology groups were statistically higher than those of the congenital GHD aetiology group (p<0.05) (Table 2).

The mean height SD score of the idiopathic GHD group was statistically higher than that of the congenital GHD group (p=0.001) (Table 2).

The mean bone age delay at admission of the congenital GHD aetiology group was statistically found to be higher than that of the idiopathic GHD group (p<0.05) (Table 2).

The mean SD scores gathered after extraction between target height SD scores and height SD scores at admission revealed that the mean extracted SD score of the id-

Table 1: Aetiology of growth hormone deficiency (GHD).

Groups	n	%	M/F
Idiopathic GHD	86	42.4	44/42
Congenital GHD	79	39	41/38
Genetic causes (Pit-1, GH1, HESX-1 defects)[1]	3		1/2
Pituitary hypoplasia, Ectopic neurohypophysis	75		39/36
Central malformations	46		22/24
Others[2]	29		17/12
Others[3]	1		1/0
Acquired GHD	10	5	6/4
Tumour of the pituitary/hypothalamic region[4]	5		4/1
Cranial tumours distant from the hypothalamo-pituitary area[5]	1		1/0
Treatment for tumours outside the cranium[6]	2		1/1
Others[7]	2		0/2
Defined syndromes with GHD[8]	14	6.9	7/7
Unclassified	14	6.9	8/6
Total	203		

[1]Homozygous mutation in PROP1 (n=1), homozygous p.Val153Phe mutation in PIT1(POUF1) gene (n=1), homozygous deletion of exon 1-2 in PIT1(POUF1) gene (n=1), [2]Empty Sella (n=5), PVL,HIE (n=5), Partial empty sella (n=3), Chiari malformation (n=3), Arachnoid cyst (n=3), Corpus callosum hypoplasia (n=2), Cerebellar vermian atrophy (n=2), Spina bifida and hydrocephalus (n=1), Corpus callozum agenesis and trigonocephaly (n=1), Cortical atrophy (n=1), Pars intermedia cyst (n=1), Rathke cleft cyst (n=1), Moya Moya syndrome (n=1), [3]46,XX,t(13;14;9) karyotype (n=1), [4]Pituitary microadenoma (n=3), pituitary macroadenoma (n=1) Craniopharyngioma (n=1), [5]Medulloblastoma (n=1), [6]Wilms's tumour (n=2), [7]Autoimmune hypophysitis (n=1), Thalassemia major with bone marrow transplantation (n=1), [8]Noonan syndrome (n=4), Silver Russel syndrome (n=3), Perrault syndrome (n=1), Prader Willi syndrome (n=1), Rubinow syndrome (n=1), Seckel syndrome (n=1), Stuve-Wiedeman syndrome (n=1), Worster Drought syndrome (n=1), Joubert syndrome (n=1)

iopathic GHD group was statistically higher than defined syndromes with GHD groups ($p<0.05$) (Table 2).

There was no significant statistical difference among GHD aetiology groups according to their interpreted BMI SD scores and Tanner staging at admission ($p>0.05$).

The bone age delay was divided into two main groups: bone age delay below 2 years and bone age delay of 2 years and over. Patients with a bone age delay over 2 years were statistically higher in the congenital GHD group ($p<0.05$).

There was no statistical difference in means of gender, age at admission, history of parental consanguinity and birth weight for gestational age distributions between the isolated GHD and MPHD groups ($p>0.05$).

The mean birth height SD score of isolated GHD was statistically lower than the MPHD group ($p=0.001$) (Table 3).

The mean bone age delay at admission of the MPHD group was statistically found to be higher than the others ($p<0.05$) (Table 3).

There were no significant statistical differences between the isolated GHD and MPHD groups according to their interpreted BMI SD scores, Tanner staging and bone age delay groups at admission ($p>0.05$).

Mean IGF-1 SD score of the congenital GHD group was statistically found to be lower than other groups ($p<0.05$) (Table 4).

The mean peak growth hormone stimulation test value of the idiopathic GHD group was statistically higher than that of the congenital GHD group ($p<0.05$) (Table 4).

Mean IGF-1 SD score of MPHD group was statistically found to be lower than the isolated GHD group ($p<0.05$) (Table 5).

The IGFBP-3 SD score and the mean peak growth hormone stimulation test value of the MPHD group was significantly lower than that of the isolated GHD group ($p<0.001$, Table 5).

Table 2: Comparisons of birth data and patients' data at admission between GHD aetiology groups.

	Group A	Group B	Group C	Group D	F	p	*df*
Gestational week (mean±SD)	39.3±2.1	38.7±2.7	39.0±2.5	38.8±2.7	0.619	0.603	
Birth weight SDS (mean±SD)	-1.1±1.7	-0.8±1.6	-0.7±1.2	-1.2±2.0	0.566	0.638	
Birth height SDS (mean±SD)	-1.1±1.3	-0.4±1.7	-1.6±1.0	-1.9±2.4	1.985	0.125	
Age at admission (mean±SD)	9.1±3.7	7.7±4.3	11.3±4.2	8.1±5.6	2.952	0.034	**A>B C>B**
Height SDS at admission (mean±SD)	-3.8±0.9	-4.5±1.1	-4.0±0.7	-4.1±1.1	5.905	0.001	**A>B**
Weight SDS at admission (mean±SD)	-2.7±1.3	-3.1±1.6	-3.4±0.9	-3.5±1.9	1.871	0.136	
Head circumference SDS at admission (mean±SD)	-1.9±1.4	-2.1±1.9	-1.9±1.1	-2.6±1.8	0.306	0.821	
BMI SDS at admission (mean±SD)	-0.5±1.3	-0.5±1.7	-1.1±0.9	-1.1±2.3	1.113	0.345	
Bone age delay (mean±SD)	2.3±1.3	3.2±1.7	2.4±1.4	2.4±2.1	3.955	0.010	**B>A**
Predicted adult height (PAH) SDS (mean±SD)	-1.9±1.4	-2.3±1.8	-1.9±1.9	-2.9±1.8	0.588	0.625	
Target height (TH) SDS (mean±SD)	-1.5±0.8	-1.5±1.2	-1.5±0.9	-0.8±0.6	1.687	0.173	
Target height minus height SDS (mean±SD)	-2.4±1.3	-2.7±1.4	-2.3±1.1	-3.6±1.6	2.764	0.045	**A>D**

Table 4: Mean IGF-1 SDS, IGFBP-3 SDS and peak growth hormone stimulation test value comparisons between GHD aetiology groups.

	Group A	Group B	Group C		Group D		F	p	*df*
IGF-1 SDS (mean±SD)	-0.8±1.1	-1.5±1.3	-0.550	1.302	-0.769	1.464	4.186	0.007	**A>B C>B D>B**
IGFBP-3 SDS (mean±SD)	-0.5±0.9	-0.9±1.2	-0.671	0.699	-0.357	1.175	1.426	0.237	
Peak Growth Hormone Stimulation Test Value (mean±SD)	6.5±5.3	4.2±3.7	4.781	3.694	5.294	3.060	3.501	0.017	**A>B**

Table 5: Mean IGF-1 SDS, IGFBP-3 SDS and peak growth hormone stimulation test value comparisons between isolated GHD and MPHD groups.

	Isolated GHD	MPHD	t	p
IGF-1 SDS (mean±SD)	-0.9±1.0	-1.8±1.7	2.943	**0,006**
IGFBP-3 SDS (mean±SD)	-0.4±1.1	-1.3±1.1	4.037	**0,000**
Peak Growth Hormone Stimulation Test Value (mean±SD)	6.7±5.1	2.1±2.2	8.605	**0,000**

DISCUSSION

Idiopathic GHD was the most common aetiology of GHD in our study. Thomas et al. found idiopathic GHD as the most common aetiology of growth hormone deficiency (41%); followed by acquired GHD (35%), congenital GHD (20%) and defined syndromes with GHD (4%). Desai et al. also found the leading cause of GHD as idiopathic GHD (75%). So, our findings were consistent with the literature (20, 21).

Although there was a slight predominance of male patients in all the GHD aetiology groups in a study by Thomas et al., there was no statistical difference in gender in our study population. The male/female ratio of idiopathic GHD was found to be 2/1 in a study by Desai et al. Moreover, there was no statistical difference in gender distribution between GHD aetiology groups in our study ($p>0,05$). These findings were not consistent with the literature (20, 21). This might be related to a change in the perception and awareness of short stature in the society (22).

There was no statistical distributional difference in age at admission groups between GHD aetiology groups. Idiopathic, acquired GHD and defined syndromes with GHD cases tended to present at over 10 years of age; whereas congenital GHD cases had a tendency to present between 5-10 years of age. Besides, the mean age at admission of idiopathic and acquired GHD cases were statistically higher than that of congenital GHD, which is probably due to early presentation and recognition of short stature in the disease process.

There was no statistical distributional difference in history of parental consanguinity between GHD aetiology groups. The distribution of parental consanguinity was slightly higher in the acquired GHD group among others, with regard to a higher history of parental consanguinity rate than the general Turkish population found in our previous study (9).

There was no statistical difference in means of birth weight for gestational age distributions between GHD aetiology groups. The majority of the cases were AGA in all the GHD aetiology groups. Moreover, there was no statistical difference in mean gestational age at birth, mean birth weight SD scores and mean birth height SD scores between GHD aetiology groups. However, the mean birth weight SD scores of idiopathic and congenital GHD was statistically lower than that of acquired GHD in a study by Thomas et al.; which in fact was inconsistent with our findings (21). The mean height SD score of the idiopathic GHD group was statistically higher than that of the congenital GHD group in our study. There was no statistical difference in the mean weight, head circumference, target height, predicted adult height (PAH) and body mass index SD scores and the mean weight for height percentages of GHD aetiology groups at admission. Our study population was mostly regarded as normal according to their interpreted BMI SD scores and weight for height percentages. However, the mean target height SD score was statistically lower than other two groups in a study by Thomas et al. (21). Our findings support the argument that normal intrauterine growth is mostly independent from fetal pituitary hormones–unlike the critical role of the endocrine system in postnatal growth (23, 24).

There were no significant statistical differences among GHD aetiology groups according to their Tanner staging at admission. Cases were mostly prepubertal in all aetiology groups in our study.

The mean bone age delay at admission of the congenital GHD aetiology group was statistically found to be higher than that of the idiopathic GHD group. Moreover, patients with a bone age delay over 2 years were statistically higher in the congenital GHD group, when it was divided into two main groups. These findings may be related to a higher incidence of MPHD in the congenital GHD group.

Mean IGF-1 SD score of the congenital GHD group was statistically lower than other groups in our study, and the mean peak growth hormone stimulation test value of the idiopathic GHD group was statistically higher than that of the congenital GHD group as expected. But there was no statistical difference in mean IGFBP-3 SD scores among GHD aetiology groups.

Multiple pituitary hormone deficiency (MPHD) was found in 14 % of cases with idiopathic GHD. It was also found in 25.3% of congenital GHD, in 20% of acquired GHD and in 7.1% of defined syndromes with GHD. In a study by Thomas et al., MPHD was found in 13% of idiopathic GHD, in 50% of congenital GHD and in 52% of acquired GHD. As a consistent finding with Thomas et al. MPHD was found less in the idiopathic group; but the highest likelihood of MPHD in the congenital group was inconsistent with that study. In another study by Desai et al. MPHD was found in 12% of idiopathic cases; whereas it was found in 21% of organic GHD cases (20, 21). Our findings might be related to progressions in diagnostic procedures as more abnormalities are being diagnosed by imaging or at the gene level over time.

The most common accompanying hormone deficiencies in MPHD cases were TSH deficiency (75%), LH/FSH deficiency (25%) and ACTH deficiency (8%) respectively. MPHD was found in 25.3% of cases with congenital GHD. The most common accompanying hormone deficiencies in those cases were TSH deficiency (90%), LH/FSH deficiency (45%) and ACTH deficiency (15%) respectively. 20% of acquired GHD cases had MPHD and all of them were TSH deficient. Only one patient in defined syndromes with the GHD aetiology group, who was both TSH and ACTH defi-

cient, had MPHD. As a consistent finding with the study by Thomas et al.; the most common accompanying hormone deficiency in MPHD cases was TSH deficiency (21).

There was no statistical difference in means of gender, history of parental consanguinity and birth weight for gestational age distributions between the isolated GHD and MPHD groups. There was a slight distributional male predominance in the MPHD with accompanying TSH deficiency group. Both groups had higher distributions in admissions over 5 years of age. However, there was no statistical difference in the mean age at admission between both groups. There was no statistical difference in mean gestational age at birth and mean birth weight SD scores. Cases were mostly AGA. In a study by Lo et al., there were also no statistical differences in means of gender, birth weight for gestational age and mean age at admission when it was divided and compared between isolated partial GHD, isolated severe GHD and MPHD groups. Our findings for these 3 parameters were also consistent with the literature. However, in our study, the mean birth height SD score of isolated GHD was statistically lower than the other group (25). But this finding should be interpreted cautiously due to lack of birth length record data in our study population (9).

There were no significant statistical differences between the isolated GHD and MPHD groups according to their weight, height, head circumference and weight for height percentage values together with the mean target height, predicted adult height (PAH), body mass index and extracted SD scores at admission. Besides, cases were mostly normal according to their interpreted mean weight for height percentages and interpreted BMI SD scores and there were no statistical differences in both parameters between both groups in our study. As a consistent finding, Lo et al. also did not find statistical differences in mean target height SD scores between the isolated GHD and MPHD groups. However, in that study, the mean height SD score of the MPHD group was statistically lower than the other; which in fact is inconsistent with our finding. But it should also be kept in mind that our study population comprises only cases with severe short stature (25).

No matter how we could not find a significant distributional statistical difference between the isolated GHD and MPHD groups among bone age delay groups at admission; the mean bone age delay at admission of the MPHD group was statistically found to be higher than the other. In a study by Lo et al. the mean bone age delay of MPHD with the accompanying TSH deficiency group at admission was higher than the isolated partial and severe GHD groups. So, our finding was consistent with the literature (25).

There was no significant distributional statistical difference among Tanner staging groups at admission between the isolated GHD and the MPHD group. Cases were mostly prepubertal in our study population.

The mean IGF-1 & IGFBP-3 SD scores and the mean peak growth hormone stimulation test value of the MPHD group were significantly lower than that of the isolated GHD group. Lo et al. also found out that the mean IGF-1 SD score and the mean peak growth hormone stimulation test value of the MPHD group were significantly lower than the others (25).

CONCLUSION

The most common cause of growth hormone deficiency in this group was idiopathic GHD. In all the MPHD aetiology groups; the most common accompanying hormone deficiency was TSH deficiency. Hypophyseal pathologies were most commonly seen in congenital and acquired GHD cases. Noonan syndrome was the most common syndrome with an accompanying GHD. The bone age delay was found to be over 2 years in congenital GHD. The mean IGF-1 SD score and the mean peak growth hormone stimulation test value were low in congenital GHD. Precise assessment of auxological, clinical and laboratory data could provide substantial value in the evaluation of severely short statured children with GHD.

Ethics Committee Approval: This study was approved by the Ethical Committee of the Istanbul University School of Medicine (Meeting No:12 File No:2015/1272).

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- U.U., F.B., Ş.P., F. F.D., R.B.; Data Acquisition- U.U., N.M.Ş.; Data Analysis/Interpretation- U.U., F.B., Ş.P., F.F.D., R.B.; Drafting Manuscript- U.U., F.F.D., R.B.; Critical Revision of Manuscript- U.U., F.B., Ş.P., F.F.D., R.B.; Final Approval and Accountability- U.U., F.B., Ş.P., F.F.D., R.B., N.M.Ş.; Technical or Material Support- F.B., Ş.P., N.M.Ş., F.F.D., R.B.; Supervision- F.F.D., R.B.

Conflict of Interest: Authors declared no conflict of interest.

Financial Disclosure: Authors declared no financial support.

Etik Komite Onayı: Bu çalışma için etik komite onayı İstanbul Üniversitesi Tıp Fakültesi Etik Kurulu'ndan alınmıştır (Toplantı No: 12 Dosya No: 2015/1272).

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Çalışma Konsepti/Tasarım- U.U., F.B., Ş.P., F. F.D., R.B.; Veri Toplama- U.U., N. M.Ş.; Veri Analizi/Yorumlama- U.U., F.B., Ş.P., F. F.D., R.B.; Yazı Taslağı- U.U., F. F.D., R.B.; İçeriğin Eleştirel İncelemesi- U.U., F.B., Ş.P., F.D., R.B.; Son Onay ve Sorumluluk- U.U., F.B., Ş.P., F. F.D., R.B., N.M.Ş.; Malzeme ve Teknik Destek- F.B., Ş.P., N.M.Ş., F.F.D., R.B.; Süpervizyon- F.F.D., R.B.

Çıkar Çatışması: Yazarlar çıkar çatışması beyan etmemişlerdir.

Finansal Destek: Yazarlar finansal destek beyan etmemişlerdir.

REFERENCES

1. Lindsay R, Feldkamp M, Harris D, Robertson J, Rallison M. Utah Growth Study: Growth standards and the prevalence of growth hormone deficiency. J Pediatr 1994;125(1):29-35. [CrossRef]
2. Vimpani GV, Vimpani AF, Lidgard GP, Cameron EH, Farquhar JW. Prevalence of severe growth hormone deficiency. Br Med J 1977;2(6084):427-30. [CrossRef]
3. Drent ML, Delemarre-Van de Waal HA, Wit JM. Eenmaal groeihormoon, altijd groeihormoon? De transitie van groeihormoonbehandeling van kind naar volwassenen. Ned Tijdschr Geneeskd 2002;146:154-7.
4. Werther G. Growth hormone measurements versus auxology in treatment decisions: the Australian experience. J Pediatr 1996;128(5pt 2):S47-51. [CrossRef]
5. Tani N. Epidemiological study of dwarfism in Nigata prefecture. Folia Endocrinologica Japonica Nihon Naibunpi Gakkai Zasshi 1985;61(12):1295-309. [CrossRef]
6. Bao XL, Shi YF, Du YC, Liu R, Deng JY, Gao SM. Prevalence of growth hormone deficiency of children in Beijing. Chin Med J (Eng) 1992;105(5):401-5.
7. Parkin JM. Incidence of growth hormone deficiency. Arch Dis Child 1974;49(11):904-5. [CrossRef]
8. Audi L, Gilabert A, Lloveras G, Marti-Henneberg C, Rodriguez- Hierro F, Vilardell E, et al. Long-term GH therapy: epidemiology and auxologic outcome. Horm Res 2002;57(3-4):113-9. [CrossRef]
9. Uckun U, Bas F, Poyrazoglu S, Sukur M, Darendeliler F, Bundak R. Evaluation of the etiology of severe short stature in children with additional diagnosis of growth hormone deficiency at a tertiary pediatric endocrinology centre. Çocuk Dergisi 2019;19(2):63-76. [CrossRef]
10. Neyzi O, Bundak R, Darendeliler F, Günöz H. Büyüme Gelişme ve Bozuklukları. In= Neyzi O, Ertuğrul T, eds. Pediyatri Cilt 1, 4th ed, Istanbul: Nobel Tıp Kitapevleri, 2010:89-14.
11. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. Arch Dis Child 1969;44(235):291-303. [CrossRef]
12. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in boys. Arch Dis Child 1970;45(239):13-23. [CrossRef]
13. Bundak R, Furman A, Gunoz H, Darendeliler F, Bas F, Neyzi O. Body mass index for Turkish children aged 6 to 18 years. Acta Paediatr 2006;95(2):194-8. [CrossRef]
14. Neyzi O, Furman A, Bundak R, Gunoz H, Darendeliler F, Bas F. Growth references for Turkish children aged 6 to 18 years. Acta Paediatr 2006;95(12):1635-41. [CrossRef]
15. Neyzi O, Gunoz H, Furman A. Türk çocuklarında vücut ağırlığı, boy uzunluğu, baş çevresi ve vücut kitle indeksi referans değerleri. Çocuk Sağlığı ve Hastalıkları Dergisi 2008;51:1-14.
16. Fenton TR, Kim JH. A systematic review and meta-analysis to revise the Fenton growth chart for preterm infants. BMC Pediatr 2013;13:59. [CrossRef]
17. Tanner JM, Goldstein H, Whitwhouse RH. Standards for children's height at age 2-9 years allowing for height of parents. Arch Dis Child 1970;45(244):755-62. [CrossRef]
18. Bayley N, Pinneau SR. Tables for predicting adult height from skeletal age: revised for use with the Greulich-Pyle hand standart. J Pediatr 1952;40(4):423-41. [CrossRef]
19. Ranke MB. The KIGS aetiology classification system. In= Ranke MB, Wilton P, eds. Growth Hormone Therapy in KIGS-10 Year's Experience. Leipzig: JA Barth Verlag, 1999:389-401.
20. Desai M, Colaco P, Sanghavi KP, Choksi CS, Vaz FEE, Ambedkar MC. Profile of growth hormone deficiency in Bombay. Indian J Pediatr 1991;58(Suppl 1):33-42. [CrossRef]
21. Thomas M, Massa G, Craen M, de Zegher F, Bourguignon JP, Heinrichs C, De Schepper J, Du Caju M, Thiry-Counson G, Maes M. Prevalence and demographic features of childhood growth hormone deficiency in Belgium during the period 1986-2001. Eur J Endocrinol 2004;151(1):67-72. [CrossRef]
22. Lacey KA, Parkin JM. Causes of short stature. A community study of children in Newcastle upon Tyne. Lancet 1974;1(7846):42-5. [CrossRef]
23. Saenger P, Czernichow P, Hughes I, Reiter EO. Small for gestational age: Short stature and beyond. Endocr Rev 2007;28(2):219-51. [CrossRef]
24. Chernausek SD. Mendelian genetic causes of the short child born small for gestational age. J Endocrinol Invest 2006;29(1):16-20.
25. Lo FS, Chang LY, Yang MH, Van YH, Wai YY. Auxological, clinical and MRI findings in Taiwanese children with growth hormone deficiency. J Pediatr Endocrinol Metab 2004;17(11):1519-26. [CrossRef]

İSTANBUL ÜNİVERSİTESİ YAYINEVİ

RESEARCH / ARAŞTIRMA
DOI: 10.26650/IUITFD.2020.0023
İst Tıp Fak Derg 2020 / J Ist Faculty Med 2020

EVALUATION OF WOMEN'S BELIEFS ABOUT PAP SMEAR SCREENING USING THE HEALTH BELIEF MODEL SCALE

KADINLARIN PAP SMEAR TARAMASINA İLİŞKİN İNANÇLARININ SAĞLIK İNANÇ MODELİ ÖLÇEĞİ İLE DEĞERLENDİRİLMESİ

Olgu AYGÜN[1], Özden GÖKDEMİR[2], Ülkü BULUT[3], Seval YAPRAK[1], Nilgün ÖZÇAKAR[4]

[1]The Ministry of Health, İzmit County Health Directorate, İzmit, Turkey
[2]Izmir University of Economics, Faculty of Medicine, Basic Medical Sciences, Izmir, Turkey
[3]Aksaray University, Faculty of Medicine, Department of Family Medicine, Aksaray, Turkey
[4]Dokuz Eylul University, Faculty of Medicine, Department of Family Medicine, Izmir, Turkey

ORCID IDs of the authors: O.A. 0000-0002-9767-011X; Ö.G. 0000-0002-0542-5767; Ü.B. 0000-0003-3011-0924; S.Y. 0000-0002-1454-1612; N.Ö. 0000-0003-0434-214X

Cite this article as: Aygun O, Gokdemir O, Bulut U, Yaprak S, Ozcakar N. Evaluation of women's beliefs about pap smear screening using the health belief model scale. J Ist Faculty Med 2020;83(4):421-6. doi: 10.26650/IUITFD.2020.0023

ABSTRACT

Objective: To evaluate women's beliefs about screening Pap smear test.

Material and Method: The survey has been conducted through a questionnaire prepared by researchers, based on the Health Belief Model Scale for the Pap smear test. The participants consisted of 266 women between 18-70 years of age registered at a Family Health Center in Izmir. Their beliefs about screening Pap smear test were evaluated using the Health Belief Model Scale.

Results: The mean age of first sexual intercourse was 21.67±4.5 (min:13--max:45 years), 86.5% reported a single partner and 41.0% of them (n=109) had never undergone a Pap smear test. Women who previously had a Pap smear test had high scores of sensitivity, seriousness, advantage and motivation, health motivation subscales, but the mean scores for obstacles were low. There was a difference between seriousness, health motivation subscales and taking the Pap smear test (p=0.021, p=0.006). The Mean scores of seriousness and health motivation of Health Belief Model Scale were higher. There was no difference between education level, working status and undergoing a Pap test (p>0.05).

Conclusion: Having a Pap smear test is still moderately frequent and there are obstacles. Planning the interventions to eliminate Pap smear test barriers is an important issue for family physicians.

Keywords: Health behaviour, health belief, cervical smear

ÖZET

Amaç: Bu çalışmanın amacı kadınların Pap smear test taramasına ilişkin inançlarını değerlendirmektir.

Gereç ve Yöntem: Araştırma, araştırmacı tarafından geliştirilen Pap smear testi için Sağlık İnanç Modeli Ölçeğini içeren bir anket aracılığıyla gerçekleştirilmiştir. Katılımcılar, İzmir'de bir Aile Sağlığı Merkezi'ne kayıtlı 18-70 yaş arası 266 kadındı. Pap smear testi taramasına ilişkin inançları Sağlık İnanç Modeli Ölçeği kullanılarak değerlendirildi.

Bulgular: Ortalama ilk cinsel ilişki yaşı 21,67±4,5 (min:13-maks:45 yıl) idi, %86,5'i tek partner olduğunu ve %41,0'ı (n=109) hiç Pap smear testi yaptırmadığını ifade etti. Daha önce Pap smear testi yapılmış kadınların hassasiyet, ciddiyet, avantaj ve motivasyon, sağlık motivasyonu alt ölçek puanları yüksekti, ancak engeller ortalama puanları düşüktü. Ciddilik, sağlık motivasyonu alt ölçekleri ile Pap smear testi yapılması arasında fark vardı (p=0,021, p=0,006), Sağlık İnanç Modeli Ölçeği'nin ciddiyet ve sağlık motivasyonu puanları ortalamaları daha yüksekti (p<0,05). Eğitim düzeyi, çalışma durumu ve Pap testi yaptırmak arasında fark yoktu (p>0,05).

Sonuç: Pap smear testi yaptırma durumu halen orta düzeydedir ve engeller bulunmaktadır. Aile hekimleri için Pap smear testini engelleyen nedenlerin ortadan kaldırılması için girişimlerin planlanması önemli bir konudur.

Anahtar Kelimeler: Sağlık davranışı, sağlık inanışları, servikal smear

Corresponding author/İletişim kurulacak yazar: ozden.gokdemir@ieu.edu.tr

Submitted/Başvuru: 12.03.2019 • **Revision Requested/Revizyon Talebi:** 20.04.2020 •
Last Revision Received/Son Revizyon: 11.05.2020 • **Accepted/Kabul:** 12.05.2020 • **Published Online/Online Yayın:** 19.10.2020

INTRODUCTION

Cervical cancer is one of the most common cancer types in the world and ranks fourth among the causes of death (1). In 2012, worldwide more than five hundred thousand cases of cervical cancer were newly diagnosed. According to the 2009 statistics, the cervical cancer rate is 4.5 per 100,000 women in Turkey (2).

Cervical cancer incidence increases after the age of 40. Besides age, the other risk factors in the development of such cancer types are gene mutations, family breast/cervical cancer history, extension in the interval between menarche and menopause, and obesity. Less serious risk factors are first sexual intercourse under the age of 16, sexually transmitted disease history of HIV, HSV-2, genital wart, HPV, high parity, smoking, lower socioeconomic level, and use of oral contraceptives (3, 4).

Screening is a public health intervention provided to prevent disease development in a healthy target population. In screening, the aim is not only to find and reveal those who are sick but also to identify individuals who are likely to have the disease itself or a precursor. The most important and primary definite outcome of cervical screening is the decline of cervical cancer by the detection and treatment of cases of pre-cancer. Additionally, screening can discover the existence of cervical cancer at an early stage and increases the chances of successful treatment (5). Both breast and cervical cancers can be prevented by early diagnosis and screening programs. Besides early diagnosis and screening, it is essential to create awareness and behavioral change in society by increasing the knowledge of causes, risk factors, and symptoms. Incidences of cancer can be reduced by raising awareness of risk factors such as smoking and alcohol use, inadequate physical activity, excessive weight and amount of fat, inadequate consumption of vegetables and fruits, and Human Papilloma Virus history (6, 7).

According to the National Cancer Control Program, quinquennial Pap smear test among 30-65 years olds constitutes the early detection, diagnosis and screening method for cervical cancer (8, 9). The Pap smear test, a screening method for the early diagnosis of cervical cancer, is a part of women's healthy lifestyle and lifestyle behaviors, and increases awareness of early diagnosis and practices. These behaviors have a very important role in decreasing the number of cases, late diagnosis ratios, and boosting cancer prevention (10). Similar to developing countries, national studies show that screening frequencies are not yet at the desired levels, the regular screening rate was 39.2% in accordance with the national screening standard (11-14). Negative attitudes towards health protection are caused by misguided attitudes and beliefs (such as the belief that a healthy lifestyle and diet, and no family history of cancer means low risk) and such beliefs determine whether or not the Pap smear test is conducted (11, 15-17). Creating awareness about the importance of early detection is a way of encouraging women to participate in screening, which is easily done by Pap smears. Most people, however, only apply for health care when they detect a symptom. This process relies on two factors. First, patients need to identify that they have a symptom, and conclude that health professionals will be able to help health-seeking behavior. Second, this process relies upon an illness having detectable symptoms, as cancer does. Screening programs are valuable as a mean to detect signs at a time when they may not be visible to the patient, on the premise that early detection leads to better treatment success.

Considering this, it is essential to understand individuals' perceptions regarding health needs, their obstacles, decisions processes and behaviors. The Health Belief Model is often used to serve this purpose. The model consists of five main structures: perceived sensitivity, perceived seriousness, perceived advantage/motivation, health motivation and perceived obstacles. The model not only explains the screening behaviors, but also the factors that facilitate patient behaviors, patient role behaviors, and performing health behaviors (18).

Healthcare professionals should understand how cultural values and beliefs affect screening practices, and to develop programs using culturally suitable messages and convenient strategies.

In this regard, the aim was to find out which factors encourage women to take a Pap smear, and the relation between the various attitudes and belief regarding Pap smear test of patients registered with a Family Health Center.

MATERIAL AND METHOD

This cross-sectional and descriptive research was carried out to determine the knowledge, attitudes, and behaviors of 18 to 70 years old women towards cervical cancer. The participants were 266 women registered with a Family Health Center in Izmir between June-September 2015. Demographical data were collected face-to-face using questions from Cervical and Pap Smear Test Health Belief Model Scale. Data were analyzed using the SPSS 15.0 program. The Mean, standard deviation and percentage was used to evaluate participants' sociodemographic characteristics of participants. Chi-square and student's t-test were used, $p<0.05$ was accepted as significant. Approval for the study was obtained from Izmir Public Health Directorate Approvals and Dokuz Eylül University Faculty of Medicine Ethics Committee.

Data collection tool: Champion developed this scale for breast cancer and mammography, and adapted Cervical Cancer and Pap smear test. This model was validated in various other countries (19). Guvenc et al. conducted the Turkish validity and reliability study (11). The scale consists of 35 items, and five main dimensions: sensitivity (3 items), seriousness (7 items), Pap smear advantage and motivation (8 items), health motivation (3 items), and Pap smear obstacles (14 items). This scale was assessed using a 5-Likert type scaling of "I definitely disagree" (1), "I disagree" (2), "neutral" (3), "I agree" (4), "I fully agree" (5) method ranging from 1 to 5. Each dimension of the scale was separately assessed. Higher scores indicate stronger feelings about that the scale sense. All subscales are positively related to screening behavior except for the "barriers", which have a negative association.

Table 1: Characteristics of participants (n=266)

	n	%
Age		
30-35	88	33.1
36-40	64	24.1
41-45	62	23.3
46-50	28	10.5
51 and above	24	9.0
Marital status		
Married	218	82.0
Widow/divorced	48	18.0
Employment status		
Housewives	82	30.8
Employee/retired	184	69.2
Education level		
Primary school	150	56.4
High school	62	23.3
College/university	54	20.3
Number of children		
0	36	13.5
1-3	198	74.5
More than 3	32	12.0
Monthly income		
Below 500 Lira	73	27.4
500-1000 Lira	70	26.3
1001-1500 Lira	83	31.3
Above 1500 Lira	40	15.0
Age of first sexual intercourse		
≤20 years	131	49.2
>20 years	135	50.8
Previous Pap smear test		
Yes	157	59.0
No	109	41.0

RESULTS

The participants' (n=266) mean age was 40±8.10 (min:30--max:70 years of age). The largest group (33.1%) was between 30-35 years of age and married (82.0%). Characteristics of the participants given in Table 1.

The participants' mean age of menarche was 13.24±1.2 (min:10--max:18 years). Those with menarche age above 14 were the largest group (41.1%). The Mean age of first sexual intercourse was 21.67±4.5 (min:13--max:45 years). 86.5% reported a single partner, 13.5% more than one.

Regarding Pap smear test rating and sociodemographic characteristics such as in our study, no difference was found between, education level, and employment status ($p>0.05$).

Scores obtained by the participants from the Health Belief Model Scale subscales are shown in Table 2.

Forty-one percent of the participants (n=109) had never undergone a Pap smear test. Women who previously had a Pap smear test had high mean scores for the subscales of sensitivity, seriousness, advantage and motivation, and their health motivation mean scores for obstacle was low. Significant relations were detected between seriousness and health motivation from the Health Belief Model Scale subgroups and taking Pap smear tests ($p=0.021$, $p=0.006$) (Table 3).

Table 2: Participants' health belief model scale scores.

	Mean	SD*	Min.	Max.
Sensitivity	8.59	2.65	3	15
Seriousness	23.63	7.02	7	35
Advantage and motivation	29.43	7.73	9	45
Health motivation	11.33	3.36	3	15
Obstacles	36.35	12.33	14	70

*SD: Standard deviation

Table 3: Participants' health belief model scale scores undergoing pap smear test or not

Health Belief Model Scale	Taking Pap smear test		p
	Yes Mean±SD*	No Mean±SD*	
Sensitivity	9.01±2.56	7.99±2.67	0,216
Seriousness	24.50±6.61	22.37±7.43	**0.021**
Advantage and motivation	30.46±7.44	27.94±7.92	0.229
Health motivation	11.78±3.12	10.70±3.,60	**0.006**
Obstacles	32.95±11.77	35.17±10.82	0.128

*SD: Standard deviation

DISCUSSION

In studies conducted with different groups and different provinces in Turkey, Pap smear test rates were found to be generally low (16, 17, 20-22) and slightly lower than the ratio of 59.0% detected in our study. These findings show that the percentage of applications for regular Pap smear test is below the desired levels in our country, especially when compared to rates in developed countries. One of the reasons for this case may be the obstacle caused by religious and cultural values.

In studies in literature, it was found that income and education level, health insurance and health resources, knowledge level and cultural factors have significant importance on attitudes to Pap smear test (15, 16, 22-24). In our study, no difference was found between sociodemographic characteristics such as education level, employment status frequency of undergoing Pap smear test ($p>0.05$).

Another possible cause of the high proportion of women under 45 years: the similarity between the age groups in terms of smear neglect is perhaps because due to their relative youth and lack of risk perception. In addition, most of the participants were working people and generally with a lower education level. Education level may explain the lack of information about the importance of the issue, while women in employment may have difficulty in allocating time. Our study shows, in line with previous studies, that a common reason for ignoring an invitation for screening was lack of knowledge (16, 21, 25, 26).

According to the Health Belief Model, with increases in the positive perception of women regarding screening with Pap smear test, here are corresponding increases in sensitivity, seriousness, and health motivation (11). In our study, we determined that the mean scores of all sub-dimensions were at a medium level. However, no change was found in participants' sensitivity and seriousness perception ($p>0.05$). However, it was detected that women with high education levels have higher seriousness score levels ($p<0.05$), which contrasts with the results of studies done previously (15, 17, 20).

Demirgoz determined that attitudes to Pap smear test were influenced by views on gynaecological examination, sociodemographic characteristics, Pap smear knowledge and risk perceptions related to cervical cancer, but the test's importance was not generally well-understood. A significant relationship was found between the participants' status at work, educational level and awareness of Pap smear test, on one hand, and benefit /motivation, health motivation and disability perception on the other. "Seriousness perception", however, was not affected by any variable (15).

According to the level of knowledge of regarding Pap smear, when the conditions are examined; Akyuz found that those who knows "how Pap smear test is used for gynecological cancer diagnosis and how often it should be", did the test, and the difference between them was statistically significant (17).

According to Buyukkayaci, on the subject of perceived susceptibility, however, most women expressed the belief that they were not at risk, and that cervical cancer only appeared in women older than 50 (20).

According to the Health Belief Model, as the obstacle perception increases, negative health behavior increases correspondingly. Shame, uncertainty and fear are among the reasons why women fail to take the test. The obstacle perception detected in our study is similar to other Turkish studies (11, 15, 20, 21, 25).

Male practitioners applying the Pap smear test may be a significant obstacle, as one-third of the participants indicated a preference for a female doctor. Another factor that prevents application for the screening test is a feeling of being healthy. In the literature, it was observed that healthy women with no obvious symptoms tend to avoid the test (26). Only one-third of the participants in our study repeated regular health checks. Even in good general health a very common misperception was that "the test is necessary only in the presence of changes in bleeding and discharge", due perhaps, to a lack of knowledge about the issue. After eliminating this mis-

conception, every woman may understand the risks. This research was based on finding obstacles and identified that the interaction of social and personal barriers influenced women's behavior and attendance for screening. The main barriers were found to be insufficient health education of people, absence of patient-friendly health services, different cultural and social health beliefs, also gender roles and personal factors.

CONCLUSION

Women who do not take Pap smear test due to reticence should be supported and encouraged to develop positive healthy behavior. Motivational interviews and greater patient-centeredness in the family medicine discipline are potential solutions to the obstacles for cervical cancer screening provided by women's health services (27).

Acknowledgments: The authors would like to thank the Simon Edward Mumford for language editor.

Ethics Committee Approval: This study was approved by the Dokuz Eylül University Faculty of Medicine Ethics Committee (2015 / 17-23).

Informed Consent: Written consent was obtained from the participants.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- O.A., Ö.G., N.Ö.; Data Acquisition- O.A., S.Y.; Data Analysis/Interpretation- O.A., Ö.G., Ü.B., N.Ö.; Drafting Manuscript- O.A., Ö.G., Ü.B., S.Y.; Critical Revision of Manuscript- Ö.G., N.Ö.; Final Approval and Accountability- O.A., Ö.G., Ü.B., S.Y., N.Ö.; Supervision- N.Ö.

Conflict of Interest: Authors declared no conflict of interest.

Financial Disclosure: Authors declared no financial support.

Teşekkür: Yazarlar, Simon Edward Mumford'a dil editörlüğünden dolayı teşekkür eder.

Etik Komite Onayı: Bu çalışma için etik komite onayı Dokuz Eylül Üniversitesi Tıp Fakültesi Etik Kurulu'ndan alınmıştır (2015 / 17-23).

Bilgilendirilmiş Onam: Katılımcılardan bilgilendirilmiş onam alınmıştır.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Çalışma Konsepti/Tasarım- O.A., Ö.G., N.Ö.; Veri Toplama- O.A., S.Y.; Veri Analizi/Yorumlama- O.A., Ö.G., Ü.B., N.Ö.; Yazı Taslağı- O.A., Ö.G., Ü.B., S.Y.; İçeriğin Eleştirel İncelemesi- Ö.G., N.Ö.; Son Onay ve Sorumluluk- O.A., Ö.G., Ü.B., S.Y., N.Ö.; Süpervizyon- N.Ö.

Çıkar Çatışması: Yazarlar çıkar çatışması beyan etmemişlerdir.

Finansal Destek: Yazarlar finansal destek beyan etmemişlerdir.

REFERENCES

1. Latest world cancer statistics - GLOBOCAN 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012. The International Agency for Research on Cancer (IARC). WHO 2013. Press Release No:223. https://www.iarc.fr/wp-content/uploads/2018/07/pr223_E.pdf
2. The Ministry of Health of Turkey Health Statistics Yearbook 2014. Republic of Turkey Ministry of Health, General Directorate of Health Research: Ankara, 2015. Report No: 1009.
3. Güner H, Taşkıran Ç. Epidemiology of cervical cancer and the role of Human Papilloma Virus Turk J Obstet Gynecol 2007;4(1):11-9.
4. Gökaslan H, Uyar EE. Screening Cervical Cancer with Pap Smear. Turkish Journal of Family Practice 2004;8(3):105-10.
5. Periodic Health Examinations and Screening Tests Recommended in Family Medicine Practice. Republic of Turkey Ministry of Health Turkey, Public Health Institution: Ankara, 2015. Report No: 991.
6. Erkoç Y, Yardım N. Policies for Tackling Non-Communicable Diseases and Risk Factors in Turkey. Republic of Turkey Ministry of Health, Directorate General Primary Health Care: Ankara, 2011. Report No:809 Available from: https://www.saglikaktuel.com/d/file/ulke_raporu_baski_hali_en.pdf
7. Bebis H, Reis N, Yavan T, Bayrak D, Unal A, Bodur S. Effect of health education about cervical cancer and papanicolaou testing on the behavior, knowledge, and beliefs of Turkish women. Int J Gynecol Cancer 2012;22(8):1407-12. [CrossRef]
8. National Cancer Control Plan 2013 - 2018. Republic of Turkey Ministry of Health Turkey Public Health Institution Cancer Control Department. (Online) Available from: https://www.iccp-portal.org/system/files/plans/Ulusal_Kanser_Kontrol_Plani_2013_2018.pdf
9. Denizli R, Sakin O, Angin AD, Cikman MS, Pirimoglu ZM. Colposcopic examination in cytology negative women who tested positive for non-16/18 HPV types. J Ist Faculty Med 2019;82(4):212-8. [CrossRef]
10. Turkey Cancer Control Programme. Republic of Turkey Ministry of Health Turkey Public Health Institution Cancer Control Department. (Online) Available from: https://www.iccp-portal.org/system/files/plans/Turkiye_Kanser_Kontrol_Program_English.pdf
11. Guvenc G, Akyuz A, Açikel CH. Health belief model scale for cervical cancer and Pap smear test: psychometric testing. J Adv Nurs 2011;67(2):428-37. [CrossRef]
12. Champion VL. Instrument refinement for breast cancer screening behaviors. Nurs Res 1993;42(3):139-43. [CrossRef]
13. Kesic V, Markovic M, Matejic B, Topic L. Awareness of cervical cancer screening among women in Serbia. Gynecol Oncol 2005;99(3 Suppl 1):S222-S225. [CrossRef]
14. Sirovich BE, Woloshin S, Schwartz LM. Screening for cervical cancer: will women accept less? Am J Med 2005;118(2):151-8. [CrossRef]
15. Bal MD. Evaluation of women having Pap smear test by Health Belief Model scale. Journal of Marmara University Institute of Health Sciences MÜSBED 2014;4(3):133-8. [CrossRef]
16. Karabulutlu O. Evaluation of the Pap smear test status of Turkish women and related factors. Asian Pac J Cancer Prev 2013;14(2):981-986. [CrossRef]

17. Akyüz A, Güvenç G, Yavan T, Çetintürk A, Kök G. Evaluation of the Pap smear test status of women and of the factors affecting this status. Gulhane Med J 2006;48:25-29.
18. Cenesiz E, Atak N. The evaluation of the researches in the health belief model in Turkey. TAF Preventive Medicine Bulletin 2007;6(6):427-34.
19. Burak LJ, Meyer M. Using the Health Belief Model to examine and predict college women's cervical cancer screening beliefs and behavior. Health Care Women Int 1997;18(3):251-62. [CrossRef]
20. Esin MN, Bulduk S, Ardic A. Beliefs about cervical cancer screening among Turkish married women. J Cancer Educ 2011;26(3):510-5. [CrossRef]
21. Duman NB, Koçak DY, Albayrak SA, Topuz Ş, Yılmazel G. Knowledge and practices breast and cervical cancer screening among womens over age of 40. Journal of Academic Research in Nursing 2015;1(1):30-8.
22. Gökgöz N, Aktaş D. Determination of women awareness level of cervical cancer & conducting pap-smear test. Yıldırım Beyazıt University Journal of Nursing 2015;3(2):11-23.
23. Esencan TY. The evaluation of the behavior of women on gynecological diagnostic methods. Medical Bulletin of Zeynep Kamil 2009;40(2):63-6.
24. Markovic M, Kesic V, Topic L, Matejic B. Barriers to cervical cancer screening: a qualitative study with women in Serbia. Soc Sci Med 2005;61(12):2528-35. [CrossRef]
25. Reis N, Bebis H, Kose S, Sis A, Engin R, Yavan T. Knowledge, behavior and beliefs related to cervical cancer and screening among Turkish women. Asian Pac J Cancer Prev 2012;13(4):1463-70. [CrossRef]
26. Karaca M, Palancı Y, Aksu SR. How common is pap smear test known and performed. J Clin Obstet Gynecol 2008;18(1):22-8.
27. Abadi MMM, Vakilian K, Safari V. Motivational interview on having Pap test among middle-aged women - a counseling service in primary care. Fam Med Prim Care Rev 2018;2:101-5. [CrossRef]

İSTANBUL ÜNİVERSİTESİ YAYINEVİ

RESEARCH / ARAŞTIRMA
DOI: 10.26650/IUITFD.2020.0001
İst Tıp Fak Derg 2020 / J Ist Faculty Med 2020

INCIDENCE OF HYDATIDIFORM MOLE IN SYRIAN IMMIGRANT WOMEN AND TURKISH WOMEN

SURİYELİ GÖÇMEN VE TÜRK KADINLARINDA HİDATİFORM MOL İNSİDANSI

Taşkın ERKİNÜRESİN[1], Hakan DEMİRCİ[2]

Bursa Provincial Health Directorate Health Sciences University, Bursa Higher Specialization Training and Research Hospital, [1]Pathology, [2]Family Medicine, Bursa, Turkey

ORCID IDs of the authors: T.E. 0000-0003-1725-6590; H.D. 0000-0003-0434-4807

Cite this article as: Erkinuresin T, Demirci H. Incidence of hydatidiform mole in Syrian immigrant women and Turkish women. J Ist Faculty Med 2020;83(4):427-33. doi: 10.26650/IUITFD.2020.0001

ABSTRACT

Objective: Immigration may affect the incidence of hydatidiform mole (HM). In this study, we aim to compare Syrian immigrant and Turkish pregnant women in terms of HM due to abortion and termination of pregnancy.

Method: An analysis of 907 endometrial curettage materials due to abortion or termination of pregnancy between the years 2016-2018 were performed. These curettage materials were examined with routine histopathologic methods. Examination of the curettage materials was repeated by a pathologist to confirm the diagnosis.

Results: HM was diagnosed in 56 of 768 Turkish pregnant women (7.30%) and it was diagnosed in 22 of 139 Syrian immigrant pregnant women (15.80%). HM incidence in Syrian immigrant pregnant women was significantly higher (2.06 times) than in Turkish women (p=0.001). The rate of Syrian pregnant women in the group aged 20 or younger was significantly higher than Turkish pregnant women (p<0.001). The rate of Turkish pregnant women aged between 30-34 and older was high in the group.

Conclusions: The incidence of HM is higher in Syrian pregnant women. The main causes affecting the incidence of HM in Syrian immigrants are nationality and age. Migration, with all its components, may explain the difference in HM incidence between these two neighboring communities.

Keywords: Adolescent pregnancy, dilatation and curettage, histopathology, hydatidiform mole, Syrian refugee

ÖZET

Amaç: Göç hidatiform mol (HM) insidansını etkileyebilir. Bu çalışmada, küretaj uygulanan ve gebeliği sonlandırılan Suriyeli göçmen ve Türk gebe kadınların HM açısından karşılaştırılması amaçlanmıştır.

Yöntem: 2016-2018 yılları arasında küretaj uygulanan ve gebeliği sonlandırılan 907 endometriyal küretaj materyalinin analizi yapıldı. Bu küretaj materyalleri rutin histopatolojik yöntemlerle incelendi. Küretaj materyallerinin incelenmesi, tanıyı doğrulamak için bir patolog tarafından tekrarlandı.

Bulgular: HM, 768 Türk gebe kadından 56'sında (%7.30) ve 139 Suriyeli göçmen gebe kadından 22'sinde (%15,80) teşhis edildi. Suriyeli göçmen gebe kadınlarda HM insidansı Türk gebe kadınlara göre (2,06 kat) anlamlı derecede yüksekti (p=0.001). Yirmi yaşından küçük olan gruptaki Suriyeli gebe kadınların oranı Türk gebe kadınlara göre anlamlı olarak daha yüksekti (p<0.001). Türk gebe kadınların oranı ise 30-34 yaş ve daha yaşlı olan grupta yüksekti.

Sonuç: Suriyeli göçmen gebe kadınlarda HM insidansı daha yüksektir. Suriyeli göçmenlerde HM insidansını etkileyen başlıca nedenler milliyet ve yaştır. Göçmenlik tüm bileşenleri ile bu iki komşu topluluk arasındaki HM insidansındaki farkı açıklayabilir.

Anahtar Kelimeler: Adolesan gebelik, dilatasyon ve küretaj, hidatiform mol, histopatoloji, Suriyeli göçmen

Corresponding author/İletişim kurulacak yazar: erkinuresin@hotmail.com

Submitted/Başvuru: 15.01.2020 • **Revision Requested/Revizyon Talebi:** 14.04.2020 •
Last Revision Received/Son Revizyon: 18.04.2020 • **Accepted/Kabul:** 29.06.2020 • **Published Online/Online Yayın:** 05.10.2020

INTRODUCTION

There has been intense immigration towards Turkey from Syria since 2011 due to the ongoing conflict in Syria. The Ministry of Internal Affairs Directorate General of Migration Management declared that the total population of Syrian refugees in Turkey registered by their biometric data as of 8th November 2018 is 3 million 594 thousand 232 (1). 1 million 950 thousand 182 of these refugees are male and 1 million 644 thousand 50 of them are female (1). Syrian refugees benefit from health services in our country free of charge. According to official records, 276 thousand 158 Syrian babies were born in Turkish hospitals from 2011, when Syrians first started to enter the country, until 11th December 2017 (2).

Unintended pregnancy is of high incidence among the immigrant population. Termination of unintended pregnancy is legal until the 10th week after conception. After 10 weeks of pregnancy, abortion can only be performed in case of fetal impairment. Abortion is performed in pregnant women with findings of hydatidiform mole (HM) existence based on their human Chorionic Gonadotropin (hCG) levels and ultrasonography examination (USG) during periodic pregnancy follow-ups. HM is one of the diseases caused by villous trophoblasts associated with pregnancy (3). The most benign disease among gestational trophoblastic diseases is HM (4). HM has two histological types, partial hydatidiform mole (PHM) and complete hydatidiform mole (CHM) (5). hCG levels of CHMs are usually over 100,000 mIU/mL and fetal heart sounds do not exist (6-9). hCG levels are over 100,000 mIU/mL in less than 10% of PHMs (10-12).

HM prevalence varies throughout the world and occurs between 0.3 and 2.0 in one thousand pregnancies (5, 13-15). HM prevalence in curettage materials due to abortion or termination of pregnancy varies between 2.2% and 6.9% (3, 12, 27). There is proof that the incidence of HM has been decreasing in all societies in the last 30 years (16, 17).

Two risk factors detected in CHM development are advanced or early maternal age and previous molar pregnancy (18, 19). When compared to women aged between 21-35, women older than 35 and younger than 21 have a 1.9-fold increase in risk of CHM. Women older than 40, experience a 7.5-fold increase in risk (18, 19). Risk of HM pregnancy following another HM pregnancy is more than 10-20-fold higher depending on the society (20, 21). Another risk factor for HMs is spontaneous abortion history. This situation poses a risk of HM pregnancy of more than 2-3-fold higher depending on the society (22). Moreover, the age of menarche, parity, the time between previous pregnancies, genetic factors, malnutrition, viral infections and low socio-economic level provide predisposition for gestational trophoblastic diseases (GTD) (23). It is also detected that there is a reverse relationship between HM and the existence of ß-carotene and animal-fat in the diet (24, 25).

In this study, we aim to compare Syrian immigrant and Turkish pregnant women in terms of HM incidence in endometrial curettage materials performed due to abortion and termination of pregnancy in a tertiary hospital.

METHOD

Ethics committee approval for the study was obtained from the ethics committee for clinical research of our institution. Pathology reports of 907 endometrial curettage materials performed due to abortion or termination of pregnancy between the years 2016-2018 were digitally analyzed. Cases were evaluated retrospectively in light of the information obtained from pathology reports. Cases were classified as Syrian or Turkish. Cases with HM were compared in terms of age and incidence of the disease.

Curettage materials submitted to our pathology laboratory due to abortion or termination of pregnancy were examined with routine histopathologic methods (Hematoxylin & Eosin staining). Examination of the curettage materials was repeated by a pathologist to confirm the diagnosis.

In light of the histopathological findings described below, we diagnosed HM (partial or complete).

Microscopic examination shows a mixture of two villus populations consisting of small, fibrotic and normal-looking villi with large, irregularly shaped, slightly synsityotrophoblastic hyperplasia followed by hydropic villi in partial HM (Image 1). In some large villi, cisternae (cavitation) can be seen, while other large villi appear dysmorphic with their irregular scalloped complex contours and their

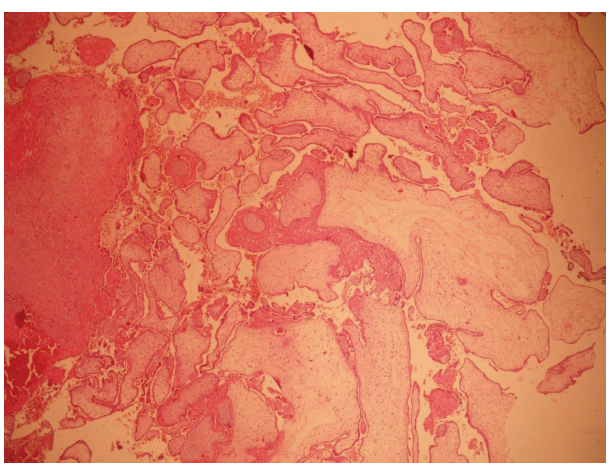

Image 1: Microscopic examination shows a mixture of two villus populations consisting of small, fibrotic and normal-looking villi in partial HM and large, irregularly shaped, slightly synsityotrophoblastic hyperplasia followed by hydropic villi. Cisterns (cavitation) can be seen in some large villi, while other large villi appear dysmorphic with their irregular scalloped complex contours and their invaginations and inclusions paved with trophoblasts. 10X H&E.

invaginations and inclusions paved with trophoblasts (Image 1 and 2). Generally, mild villous and chorionic plate trophoblast (predominantly synsityotrophoblastic) hyperplasia is seen. There is usually no apparent cytological atypia in trophoblasts. They also often contain fetal erythrocytes with villous blood vessels nucleus. Other evidence of fetal development (embryonic or fetal tissue, chorionic membrane, amnion, yolk sac, and umbilical cord) can also be seen.

In complete HM, the lesion consists entirely of large, hydropic and often cisternal villi. In addition, the common trophoblast layers that combine one or more villi and encircle the villi are also a distinct finding. The concentric distribution of this villous trophoblastic proliferation to some villi and the formation of both cytotrophoblasts and syncytiotrophoblasts is also a remarkable finding. Usually, syncytiotrophoblasts may appear immature and form Medusa-like festoons that emerge from molar villi (Image 3). There is also a marked trophoblastic atypical especially in the decidual implantation area (Image 4). Non-villous gestational tissues (embryonic or fetal tissue, chorionic membrane, amniotic, yolk sac, and umbilical cord) and fetal erythrocytes with nucleus are typically absent. Despite all these histopathological findings, in some early cases, the complete HM may be confused with partial HM. P57 immunohistochemical examination may be useful in this distinction. Nuclear staining is seen in trophoblasts that lay villi in partial HM, while no staining is seen in complete HM (Image 5).

Normal distribution suitability of variables was analyzed with the Shapiro Wilk test. Age as a result of the normality test is expressed as mean ± standard deviation and

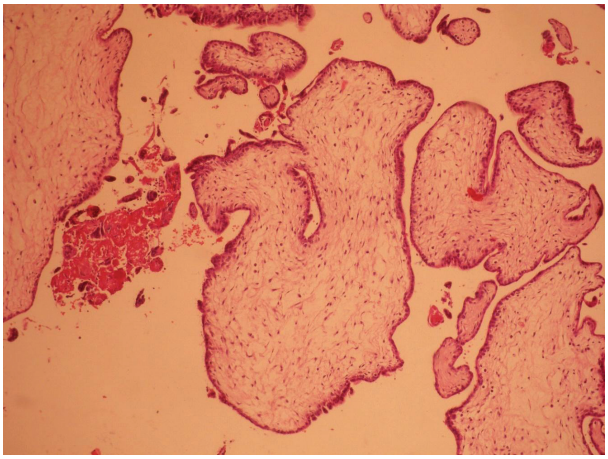

Image 2: Partial HM, large scalloped contoured villi with trophoblasts with paved invaginations and one inclusion. 10X H&E.

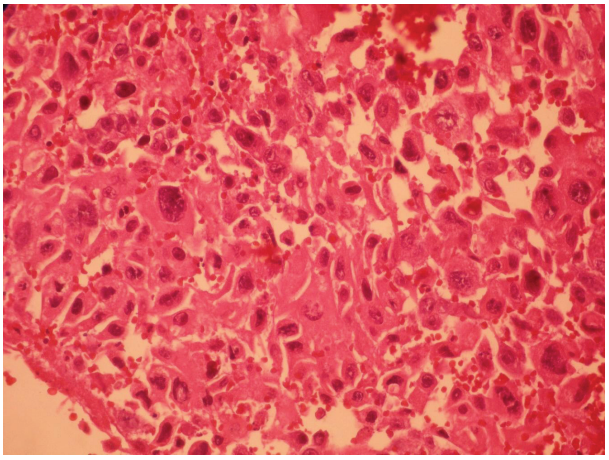

Image 4: Prominent trophoblastic atypical histology in complete hydatidiform mole. 40X H&E.

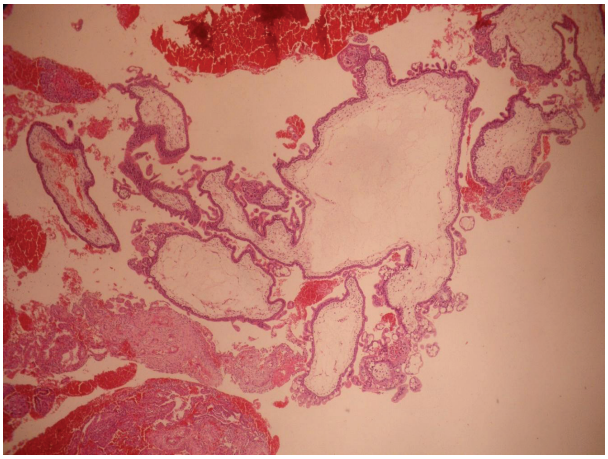

Image 3: Complete HM, completely large, cavitation and circumferential trophoblastic hyperplasia of villi consisting of lesions. 10X H&E.

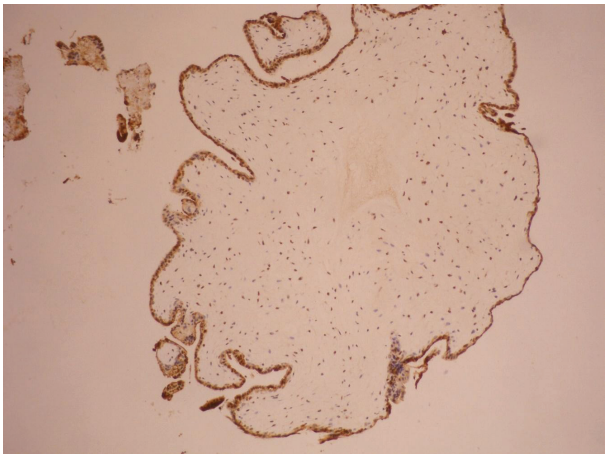

Image 5: Widespread and strong nuclear staining with p57KIP-2 is observed in trophoblasts in the villus containing a small cavitation with a large scalloped contour in the partial hydatidiform mole. 10X p57KIP-2.

(minimum: maximum) values. Categorical variables are expressed with n (%). The unpaired t-test was used in the comparison of age between HM groups. The Pearson chi-square test was used for comparisons of categorical variables among groups. Independent risk factors considered to be effective in HM detection were analyzed with binary logistic regression analysis. SPSS (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.) The program was used for statistical analysis and $p<0.05$ was accepted as statistically significant.

RESULTS

The arithmetic mean age of the 907 pregnant women who underwent curettage due to abortion or termination of pregnancy was 29.64 (ranging between 13-54).

HM was diagnosed in 78 of 907 pregnant women (8.59%) who underwent curettage due to abortion or termination of pregnancy. 768 of these 907 pregnant women were Turkish (84.67%) and 139 of them were Syrian immigrants (15.33%) (Table 1). HM was diagnosed in 56 of 768 Turkish pregnant women (7.30%) and in 22 of 139 Syrian immigrant pregnant women (15.80%). There was a significant difference between Turkish women and Syrian immigrant women in terms of HM incidence (p=0.001).

Between the years 2016-2018, 33,108 live births took place in our hospital. 25,764 of these (77.82%) were Turkish and 7, 344 were Syrian (22.18%). HM was diagnosed in curettage of 56 Turkish women out of 768 and in 22 Syrian women out of 139. According to this result, the total HM incidence between the years 2016-2018 among all pregnant women was 2.29 per thousand (78/34 015), HM incidence in Turkish pregnant women was 2.11 per thousand (56/26 532) and in Syrian pregnant women it was 2.93 per thousand (22/7 483) and the difference between groups was not statistically significant (p=0.185).

While the arithmetic mean age of the 768 Turkish pregnant women was 30.41±6.83, the arithmetic mean age of 139 Syrian immigrant pregnant women was 25.40±7.22. The arithmetic mean age of the HM diagnosed 78 pregnant women was calculated as 27.94±8.07 (Table 1). HM frequency was higher in the group with age less than 20 compared to the group with age 35-39 (Figure 1). The arithmetic mean age of the HM diagnosed 56 Turkish pregnant women was 29.61±7.87 and the arithmetic mean age of the HM diagnosed 22 Syrian immigrant pregnant women was 23.18±6.71. The rate of Syrian pregnant women in the group aged younger than 20 was significantly higher (p<0.001). Similarly, the rate of Syrian pregnant women was also high in the group aged 20-24 (p=0.001). There was no significant difference in the group aged 25-29 (p=0.399). The rate of Turkish pregnant women was high in the group aged 30-34 (p<0.001). The rate for Turkish women was also high in the group aged 35-39 (p=0.032). The rate for Turkish women was also high in the group aged ≥40 years (p=0.035). The age dis-

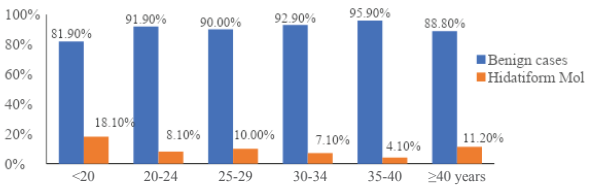

Figure 1: Age distribution of hydatidiform mole cases.

Table 1: Comparisons between hydatidiform mole and benign groups.

	Benign cases (n=829)	Hydatidiform mole (n=78)	p-value
Nationality			
Turkish	712 (92.70%)	56 (7.30%)	**0.001**[a]
Syrian	117 (84.20%)	22 (15.80%)	
Age	29.81±7.01 (13:54)	27.94±8.07 (14:47)	**0.017**[b]
Age group			
<20 years	59 (81.90%)	13 (18.10%)	**0.013**[a]
20-24 years	158 (91.90%)	14 (8.10%)	
25-29 years	181 (90%)	20 (10%)	
30-34 years	197 (92.90%)	15 (7.10%)	
35-39 years	163 (95.90%)	7 (4.10%)	
≥40 years	71 (88.80%)	9 (11.30%)	

Data is given as n (%) and mean ± standard deviation (minimum: maximum).
a: Pearson chi-square test, b: Independent samples t test.
Percentages given in the table are reported according to the variables in the rows.

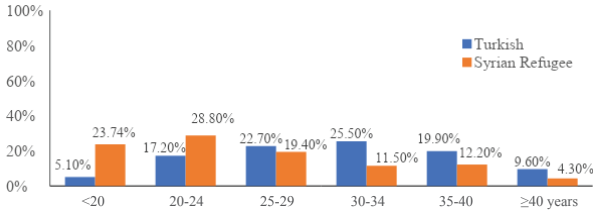

Figure 2: Age distribution of Turkish and Syrian immigrant pregnant women who underwent curettage.

tribution of Turkish and Syrian immigrant pregnant women who underwent curettage is shown in Figure 2.

Risk factors affecting HM occurrence is shown in Table 2. Nationality was found to be a risk factor in HM determination and the risk was 2.06 times higher in Syrian than Turkish women. Presence in the group aged 35-39 years was detected as a protective factor with respect to the group aged <20 years. Presence in the group aged 35-39 years reduced the risk of HM detection at a rate of 74%.

Table 2: Risk factors affecting hydatidiform mole occurrence.

Risk Factor	Wald	OR (95%CI)	p-value
Age Group			
<20 (Ref. Cat)	-	-	-
20-24	3.14	0.47 (0.21:1.08)	0.076
25-29	1.20	0.64 (0.29:1.42)	0.274
30-34	3.15	0.47 (0.20:1.08)	0.076
35-39	7.16	0.26 (0.10:0.70)	**0.007**
≥40	0.26	0.78 (0.30:2.03)	0.609
Nationality			
Turkish (Ref. Cat.)	-	-	-
Syrian	6.14	2.06 (1.16:3.64)	**0.013**

Logistics regression model was significant (p=0.004)
OR: Odds ratio, CI: Confidence interval, Ref. Cat: Reference category

DISCUSSION

At the end of this study, the incidence of HM in curettage materials performed due to abortion or termination of pregnancy in 768 Turkish pregnant women was 7.30%. This value is in conformity with previous studies performed in Turkey. The incidence of HM in curettage materials performed due to abortion or termination of pregnancy in 139 immigrant pregnant women was 15.80%. Statistically, the HM incidence in Syrian immigrant pregnant women was significantly higher (2.06 times) than Turkish women. The arithmetic mean age of Syrian pregnant women was lower than that of Turkish pregnant women. Age and nationality were detected as risk factors for HM.

In the literature, the incidence of HM is usually given over the number of pregnancies, but the incidence in curettage materials due to abortion or termination of pregnancy has been analyzed less. We could find only three research studies in the literature that examined HM incidence in curettage materials due to abortion or termination of pregnancy. Biscaro et al. found the HM incidence in Brazilian pregnant women who underwent curettage due to abortion or termination of pregnancy to be 2.24% (10 HM in 446 curettage materials) (12). In our study, the incidence of HM in Turkish pregnant women in our country who underwent curettage due to abortion or termination of pregnancy was 3.3 times higher than Brazilian women. However, in Germany, in the study performed by Horn et al. (27), the incidence of HM in curettage materials due to abortion or termination of pregnancy in German pregnant women was 5.1%. In the study performed by Mulisya et al. in Uganda (25), the incidence of HM in Ugandan (sub-Saharan Africa) pregnant women was 6.1% (11 HM in 118 curettage materials) and in the study performed by Adalı et al. in Turkey (3), the incidence of HM in Turkish pregnant women in the Kars province was 6.9% (19 HM in 277 curettage materials).

The aim of this study was to compare the HM incidence in curettage materials due to abortion or termination of pregnancy in Syrian immigrant pregnant women and Turkish pregnant women. At the end of the study, it was found that the incidence of HM in curettage materials due to abortion or termination of pregnancy in Syrian immigrant pregnant women was significantly higher than Turkish pregnant women. The fact that pregnancy in adolescence is approximately 5 times higher in Syrian immigrant women explains the high HM incidence. Besides this, stress (war and migration), low socio-economic level and probable infections that may occur due to these factors may have contributed to this increase in HM incidence. The effect of these variables on the incidence of HM in immigrants should be analyzed in future studies.

The likelihood of many more induced abortions in Turkish pregnant women should not be overlooked during the assessment of the data. Despite the fact that both societies are Muslim, induced termination of pregnancy in curettages under 10 weeks may cause a decrease in the HM rate in Turkish pregnant women.

An important limitation of the study is that this was a mono-centre study. What is the situation in other hospitals in the province of Bursa? What is the situation in private hospitals? Further research studies are necessary on these issues. Another limitation is that this was a retrospective study. For this reason, probable causes that may affect HM could not be interrogated.

In conclusion, this is the first study that investigated the incidence of HM in Syrian immigrant pregnant women. In our

region, the incidence of HM in Syrian immigrant pregnant women was significantly higher than Turkish pregnant women. Major probable causes that may affect HM incidence in Syrian immigrant pregnant women are nationality and age. The effect of factors such as socio-economic level, nutrition, infection, stress and exposure to conditions of warfare on this situation should be analyzed in further studies.

Ethics Committee Approval: Bursa Higher Specialization Training and Research Hospital, Clinic Researches Ethical Committee Number: 2011-KAEK-25 2018/11-25.

Informed Consent: Written consent was obtained from the participants.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- T.E., H.D..; Drafting Manuscript- T.E., H.D..; Final Approval and Accountability- T.E., H.D.

Conflict of Interest: Authors declared no conflict of interest.

Financial Disclosure: Authors declared no financial support.

Etik Komite Onayı: Bursa Yüksek İhtisas Eğitim ve Araştırma Hastanesi Klinik Araştırmalar Etik Kurulu Numara: 2011-KAEK-25 2018/11-25.

Bilgilendirilmiş Onam: Katılımcılardan bilgilendirilmiş onam alınmıştır.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Çalışma Konsepti/Tasarım- T.E., H.D.; Yazı Taslağı- T.E., H.D..; Son Onay ve Sorumluluk- T.E., H.D.

Çıkar Çatışması: Yazarlar çıkar çatışması beyan etmemişlerdir.

Finansal Destek: Yazarlar finansal destek beyan etmemişlerdir.

REFERENCES

1. Mülteciler Derneği. Türkiyedeki Suriyeli Sayısı. Available from: URL: https://multeciler.org.tr/turkiyedeki-suriyeli-sayisi/ Access Date: 22.05.2019.
2. Anadolu Ajansı. Türkiye'de doğan Suriyeli bebek sayısı açıklandı. Available from: https://www.aa.com.tr/tr/turkiye/turkiyede-dogan-suriyeli-bebek-sayisi-aciklandi/1144564. Access Date: 22.05.2019.
3. Seckl MJ, Sebire NJ, Berkowitz RS. Gestational trophoblastic disease. Lancet 2010;376(9742):717-29. [CrossRef]
4. Adalı Y, Eroğlu HA, Güvendi GF, Deniz R, Baykuş Y. Frequency of hydatidiform mole in curettage material between 2014 and 2016 in a university hospital. Med Sci 2018;13(3):66-71. [CrossRef]
5. Lurain JR. Gestational trophoblastic disease I: epidemiology, pathology, clinical presentation and diagnosis of gestational trophoblastic disease, and management of hydatidiform mole. Am J Obstet Gynecol 2010;203(6):531-9. [CrossRef]
6. Curry SL, Hammond CB, Tyrey L, Creasman WT, Parker RT. Hydatidiform mole: diagnosis, management, and long-term follow up of 347 patients. Obstet Gynecol 1975;45(1):1-8. [CrossRef]
7. Kohorn EI. Molar pregnancy: presentation and diagnosis. Clin Obstet Gynecol 1984;27:181-9. [CrossRef]
8. Soto-Wright V, Bernstein M, Goldstein DP, Berkowitz RS. The changing clinical presentation of complete molar pregnancy. Obstet Gynecol 1995;86(5):775-9. [CrossRef]
9. Hou JL, Wan XR, Xiang Y, Qi QW, Yang XY. Changes of clinical features in hydatidiform mole: analysis of 113 cases. J Reprod Med 2008;53(8):629-33.
10. Czernobilsky B, Barash A, Lancet M. Partial moles: a clinicopathologic study of 25 cases. Obstet Gynecol 1982;59:75-7.
11. Szulman AE, Surti U. The clinicopathologic profile of the partial hydatidiform mole. Obstet Gynecol 1982;59:597-602.
12. Berkowitz RS, Goldstein DP, Bernstein MR. Natural history of partial molar pregnancy. Obstet Gynecol 1985;66:677-81.
13. Biscaro A, Silveira SK, Locks GD, Mileo LR, Silva Júnior JP, Pretto P. Frequency of hydatidiform mole in tissue obtained by curettage. Rev Bras de Ginecol Obstet 2012;34(6):254-8.
14. Mbamara SU, Obiechina NJ, Eleje GU, Akabuike CJ, Umeononihu OS. Gestational trophoblastic disease in a tertiary hospital in Nnewi, southeast Nigeria. Niger Med J 2009;50(4):87.
15. Moodley M, Tunkyi K, Moodley J. Gestational trophoblastic syndrome: an audit of 112 patients. A South African experience. Int J Gynecol Cancer 2003;13(2):234-9. [CrossRef]
16. Brinton LA, Bracken MB, Connelly RR. Choriocarcinoma incidence in the United States. Am J Epidemiol 1986;123:1094-100. [CrossRef]
17. Smith HO, Qualls CR, Prairie BA, Padilla LA, Rayburn WF, Key CR. Trends in gestational choriocarcinoma: a 27-year perspective. Obstet Gynecol 2003;102(5):978-87. [CrossRef]
18. Parazzini F, LaVecchia C, Pampallona S. Parental age and risk of complete and partial Hydatidiform mole. Br J Obstet Gynecol 1986;93:582-5. [CrossRef]
19. Sebire NJ, Foskett M, Fisher RA, Rees H, Seckl M, Newlands E. Risk of partial and complete hydatidiform molar pregnancy in relation to maternal age. BJOG 2002;109(1):99-102. [CrossRef]
20. Sand PK, Lurain JR, Brewer JI. Repeat gestational trophoblastic disease. Obstet Gynecol 1984;63(2):140-4.
21. Berkowitz RS, Im SS, Bernstein MR, Goldstein DP. Gestational trophoblastic disease: Subsequent pregnancy outcome, including repeat molar pregnancy. J Reprod Med 1998; 43:81-6.
22. Parazzini F, Mangili GI, La CV, Negri E, Bocciolone L, Fasoli M. Risk factors for gestational trophoblastic disease: a separate analysis of complete and partial hydatidiform moles. Obstet Gynecol 1991;78(6):1039-45.
23. Ghaemmaghami F, Ashraf-Ganjooie T. Gestational trophoblastic neoplasia. Asia Pac J Clin Oncol 2006;2(1):9-21. [CrossRef]
24. Berkowitz RS, Cramer DW, Bernstein MR, Cassells S, Driscoll SG, Goldstein DP. Risk factors for complete molar pregnancy from a case-control study. Am J Obstet Gynecol 1985;152(8):1016-20. [CrossRef]

25. Parazzini F, La Vecchia C, Mangili G, Caminiti C, Negri E, Cecchetti G, Fasoli M. Dietary factors and risk of trophoblastic disease. Am J Obstet Gynecol 1988;158(1):93-9. [CrossRef]
26. Horn LC, Rosenkranz M, Bilek K. The value of placental histology for the detection of genetically-induced abortions. Z Geburtshilfe Perinatol 1991;195(2):47-53.
27. Mulisya O, Roberts DJ, Sengupta ES, Agaba E, Laffita D, Tobias T, Mpiima DP, Henry L, Augustine S, Abraham M, Hillary T. Prevalence and Factors Associated with Hydatidiform Mole among Patients Undergoing Uterine Evacuation at Mbarara Regional Referral Hospital. Obstet Gynecol Int 2018;2018:9561413. [CrossRef]

İSTANBUL ÜNİVERSİTESİ YAYINEVİ

RESEARCH / ARAŞTIRMA
DOI: 10.26650/IUITFD.2020.0018
İst Tıp Fak Derg 2020 / J Ist Faculty Med 2020

STANDARDIZATION OF WORK ACCIDENTS AND OCCUPATIONAL DISEASES INDICATORS OF SOCIAL SECURITY INSTITUTION BETWEEN 2008-2017 YEARS

2008-2017 YILLARI ARASINDAKİ SOSYAL GÜVENLİK KURUMUNUN İŞ KAZALARI VE MESLEK HASTALIKLARI GÖSTERGELERİNİN STANDARDİZASYONU

Halim İŞSEVER[1], Elif EZİRMİK[1], Gözde ÖZTAN[2], Tuğçe İŞSEVER[3]

Istanbul University, Istanbul Faculty of Medicine, [1]Department of Public Health, [2]Department of Medical Biology, Istanbul, Turkey
[3]TUSEB- Health Institutes of Turkey, Istanbul, Turkey

ORCID IDs of the authors: H.İ. 0000-0002-5435-706X; E.E. 0000-0001-6828-4378; G.Ö. 0000-0002-2970-1834; T.İ. 0000-0002-9059-8709

Cite this article as: Issever H, Ezirmik E, Oztan G, Issever T. Standardization of work accidents and occupational diseases indicators of social security institution between 2008-2017 years. J Ist Faculty Med 2020;83(4):434-45. doi: 10.26650/IUITFD.2020.0018

ABSTRACT

Objective: The work accidents are one of the biggest issues of today's occupational lives regarding the human losts and economical losts. The humanity lost of this daily issue is pretty huge.

Material and Method: At this study, it is aimed to compare the EU's work accidents resulted with death and SSI Statistical Yearbook datas standardized number of the workers with insurances according to their work accidents, occupational diseases, and death rates from different occupational branches.

Results: At the data which are achieved by indirect standardization method of SSI 2008-2017 Statistical Yearbook, it is seen that the mining industry is leading regarding work accidents, occupational diseases and death rates. Although the highest standardization rate belonged to "Activities of Domestic Employees" in 2008, between 2009-2017 years, the "Mining coal and lignite" placed always at the first place. Regarding the occupatinal disease standardization between 2008-2017 the "Mining coal and lignite" and "Metal mining" industries are placed at the top.

Conclusion: It is expected to increase the feed back quantity by applying 6331th Occupational Health and Security law in the future. Hence, with the state occupational accidental feedbacks, rates would change and corrective considerations would be more efficient.

Keywords: SSI, work accident, occupational disease, death rate, standardization, worker with insurance

ÖZET

Amaç: İş kazaları, günümüz mesleki yaşamlarının insan kayıpları ve ekonomik kayıplarla ilgili en büyük sorunlarından biridir. Bu sorundan kaynaklı insan kaybı oldukça yüksek sayıdadır.

Gereç ve Yöntem: Bu çalışmada, Ülkemizde SGK İstatistik Yıllığı standardize edilmiş iş kazaları, meslek hastalıkları verilerinin, AB ülkelerinin ölümlü olan ve olmayan iş kazası sonuçlarının karşılaştırılması amaçlanmıştır.

Bulgular: SGK 2008-2017 İstatistik Yıllığı'ndan dolaylı standardizasyon yöntemi ile elde edilen veriler değerlendirildiğinde, maden endüstrisinin iş kazaları, iş kazası ölüm oranları ve meslek hastalıkları yönünden ilk sıralarda yer aldığı görülmektedir. En yüksek standardize oran 2008 yılında " Ev içi çalışanların faaliyetleri" ne ait olsa da, 2009-2017 yılları arasında "Kömür ve Linyit Çıkartılması" her zaman ilk sırada yer almaktadır. 2008-2017 yılları standardize edilmiş meslek hastalıkları hızlarında "Kömür ve Linyit Çıkartılması" ve "Metal Cevheri Madenciliği" endüstrileri en üst sıradadır.

Tartışma: 6331'inci İş Sağlığı ve Güvenliği yasasının uygulanması ile geri bildirim miktarının artırılması beklenmektedir. Bu nedenle, devletin iş kazasıyla ilgili geri bildirimleriyle oranlar değişecek ve düzeltici hususlar daha etkili olacaktır.

Anahtar Kelimeler: SGK, iş kazası, meslek hastalığı, ölüm oranı, standardizasyon, sigortalı işçi

Corresponding author/İletişim kurulacak yazar: hissever@istanbul.edu.tr

Submitted/Başvuru: 21.02.2020 • **Accepted/Kabul:** 20.03.2020 • **Published Online/Online Yayın:** 29.09.2020

INTRODUCTION

The work accidents, occupational diseases and related deaths are now more important than past at the all around the world. The workers are losing their lives related to the work accidents or diseases every day. Every day, people die as a result of work accidents or work-related diseases more than 2.78 million deaths per year. Additionally, there are some 374 million non-fatal work-related injuries each year, resulting in more than 4 days of absences from work. The human cost of this daily adversity is vast and the economic burden of poor occupational safety and health practices is estimated at 3.94 per cent of global Gross Domestic Product each year (1). The increase of the competition and production and the development of the technology increases the risks against the workers' health and occupational safety. Especially at the XXth century which we faced industrialization and new production methods, the death and loss of organs events increased related to the mechanical causes (2). Thus, the term called "Occupational Health and Safety" (OHS) term became more important accordingly development of the industry and technology by protecting the workers against the negative factors at their workplaces, keeping the production continously and increasing the efficiency.

Work accident; occurs because of the motion and untrustable conditions and it puts the workers's lives in threat, mostly causes injuries, equipment break downs and interrups the production and they are unplanned events (3). Today, almost in every business branch, the work accidents may happen. This situation creates a rate of risk either the developed or undeveloped countries. Despite implementing the safety strategies in workplaces, work accidents and incidents have been increasing in parallel to developing industries and consequently their consequences can be unpleasant. The socio-economic impacts and human costs of occupational and industrial accidents are tremendous around the world (4). Thus, this problem is needed to be handled globally by the worldwide countries.

The occupational diseases are temporarily or permanently loss of health, disability or physchological injury situation according to the insured worker's work type, repeated conditions or working condition (5). Alike the work accidents, the occupational diseases are handicap for our country. Unfortunately most of the people are unaware of they have occupational disease. The occupational diseases may appear after leaving the work too, so most of the occupational diseases can not defined as occupational diseases by physicians and patients.

In addition to job losses of employees as a result of work accidents and occupational diseases in businesses, the effects of employees' lives on both the economy of the country and the efficiency of the company are not negligible. In a country where work accidents and occupational diseases are seen as high, considering the losses suffered by the workers, employers and national economy, it would be a better approach to give the necessary importance to occupational safety services (6). Unfortunately, regarding our national death events related work accidents and occupational diseases keep their high rate especially for some particular business branches. "Occupational Health and Safety" became more important for our country by applying to European Union. The exertion for European Union (EU) effected the worker's health and safety in a positive way and pushed our standards to take to the developed countries standards (7, 8).

By taking action on the occupational health and occupational safety cautions, decreasing the work accidents and occupational diseases cause important consequences for workers, employers and finally social safety systems (9). In order to put the happening work accidents and occupational diseases and their consequences in minimum level, first of all the datas should be recorded healthily. Inefficacy of the records is the biggest problem for defination of the real bigness of the troubles.

In our country, the only one resource of the work accidents and occupational diseases is the datas in yearbooks of Social Safety Institute. In this study, it is aimed to be standardized and evaluated according to the number of insured workers and to be compared with the EU results by the data from SSI 2008-2017 statistical yearbook for work accidents, occupational diseases and death rates in "business branches".

MATERIAL AND METHOD

In our study, the number of work accidents, occupational diseases and deaths caused by these reasons were taken from the 2008-2017 SSI statistical yearbook (10) and given in tables by standardized according to the number of 4/1a compulsorily insured workers. In order to control the effects of the mixing variables, a kind of statistical process of indirect method of standardization is used (11). The following formulations are used during the work accident standardization:

Incidence rate = Number of accidents (fatal or non-fatal) / Number of employed persons in the covered population

- **Turkish General Work Accident Rate** = Work Accident Number/Compulsorily Insured Workers Number (4-1/a)
- **Expected Number of Popullation Examined** = Examined Popullation (Number of workers) x Turkish General Work Accident Rate
- **Standardised Incidence Ratio (SIR)** = (Number of observed/Expected Number) x 100

As we calculate the confidence interval;
To calculate a 95% confidence interval (CI) for a standardized incidence ratio (SIR), use the following formula: CI=SIR±(1.96xSE) (SE: Standard Error)

where: SE=SIR/square root of d

d=Number of observed events

$$\text{Standard Error} = \frac{\text{Standardized Incidence Ratio}}{\sqrt{\text{Number of observed events}}}$$

Confidence Interval (CI 95%)=Standardized Incidence Ratio±(1.96xStandard Error)

RESULTS

Work Accident and Occupational Disease indicators in our country between 2008-2017 are given in Table 1.

When Table 1 is examined, there are 1200-1500 deaths in work accidents that occur every year, and there is continuous incapacity between 1800-2000. When occupational diseases are examined; between 500 and 1000 occupational diseases appear to be registered in the system. When we compare our country statistics with other EU countries, a picture in our country that there is almost no occupational disease and deaths from work accidents is very high. When we look at the statistics annuals, it is seen that the rates of work accidents have decreased from 13 to 6 per thousand, and the work accident death rates have changed between 10 and 20 per one hundred thousandth. The number of occupational diseases in the statistics is also very thought-provoking. Approximately 1000 occupational diseases result in the system in our country annually (10). According to the Social Security Institution (SSI) data, it is seen that especially work accidents are in significant dimensions and the number of occupational diseases is much lower than expected. In addition, losses that are not reflected in SSI statistics and that result from unrecorded and unregistered work accidents and occupational diseases should also be taken into consideration. It should also be noted that data on occupational diseases are only based on cases that have been decided. These statistics show that there is a need to reduce work accidents and that there are problems in the detection and reporting of occupational diseases, in this direction, a result-oriented preventive and preventive study is required.

Table 2 shows the standardized results of occupational accident rates between 2008 and 2017.

When Standardized Accident Rates were examined between 2008-2017; Coal mining is in the first place and Main Metal Industry is in the second place. While the first two branches did not show any change between 2009 and 2017, it is observed that Airways Transporting took the second place in 2013.

When we compare the results of standardization in Turkey 2008-2017 year, we see Table 2 as one of the three big tables (work accident standardization- work field); "Coal and Lignite Mining" with a rate of 900-2877% at the peak of all years. Although their rates are decreasing,

Table 1: Work Accidents and occupational disease rates between 2008-2017.

Years	Number of the insured	Work accident	Work accident-fatal	Work accident continuous loss of work	Occupational disease	Work accident rate (per 1.000)	Work accident death rate-(per 100.000)	Occupational disease rate-(per 100.000)
2008	8.802.989.00	72963	865	1452	539	8.29	9.8	6.12
2009	9.030.202.00	64316	1171	1668	429	7.12	12.9	4.75
2010	10.030.810.00	62903	1444	1976	533	6.27	14.4	5.31
2011	11.030.939.00	69227	1700	2093	697	6.28	15.4	6.32
2012	11.939.620.00	74871	744	2036	395	6.27	6.2	3.31
2013	12.484.113.00	191389*	1360	1660	371	15.33	10.8	4.68
2014	13.240.122.00	221366*	1626	1421	494	16.72	12.2	3.73
2015	13.999.398.00	241 547*	1252	3433	510	17.25	8.9	3.64
2016	13.775.188.00	286.068*	1405	4447	597	20.77	10.2	4.33
2017	14.477.817.00	359.653*	1633	3987	691	24.84	11.3	4.77

*As of 2013, with the introduction of the work accident notification form electronically, the data of all insured numbers who have had a work accident started to be given by taking into account the European Union Standards (ESAW-European Statistics on Accidents at Work).

Table 2: Standardization of work accident numbers between 2008-2017.

Business code	Business branches	2017	2016	2015	2014	2013	2012	2011	2010	2009	2008
5	Coal and lignite mining	900	1.048	1.078	1.462	1.514	2.794	2.877	2.592	2.220	1.411
24	Main metal industry	372	428	493	489	493	483	537	508	574	
9	Mining supporting service						313	509			
3	Fishing and seafood growing		327								
23	Non metallic products	248	256			286		352	345		
25	Factory metal products	248		287	280		317	327	340	317	
6	Crude oil and natural gas industry				294						
7	Metal mineral mining					286					
51	Airways transporting		280	395	370	602					
27	Electric equipment manufacturing						306		300		
29	Motor vehicles and trailer manufacturing			275						407	
30	Manufacture of other transport vehicles	264									
31	Furniture manufacturing									434	744
58	Publishing activities										2.018
91	Library. Archive and museums										1.376
97	Activities of domestic employees										2.607

we should signify that their rates are higher than 1000% level. Beside this, when we evaluate the 2008 year, the "Activities of Domestic Employees "activities are at the first place. The second place belonged to the work accidents during the publishing activities.

At the second place, "Main Metal Industry" is existing whish has various rates between 372-574%. At the second place in 2013 year, "Airway Transportation" is existing with the rate of 602%. Despite of the same business branch existed with the rate 395% in 2015, and 370% in 2014 as the third place; it is significant to observe their change where they were not at top 5 in the older years.

"Non-Metallic Products Production" in the 2017, 2016, 2013, 2011, 2010 years, they were among top 5. Although the 2012 rate of this business branch is more than the rate of its last three years, it is not among the top five in ranking; in 2012, business branches are valuable in terms of showing indirect increase of standardization rates. In the 2016 and 2017 years, in order 1048% and 900% rates of "Coal and Lignite Mining" business branch still defending their first place at work accident standardization.

Table 3 shows the standardized results of occupational disease rates between 2008 and 2017.

When we see the Table 3 (the standardization of occupational diseases at the business branches); we realize that the results vary depending the years. Generally, the first place belongs to (with a high rate of 13739% in 2012 year) ''Coal and Lignite Mining" business branch.

The "Non-Metallic Products Production" business branch did not be among at top 5 between 2008-2013, they increased their rates in the following years and placed at top 5 with the rates of 1049% and 611% in they years 2014-2017.

Another significant situation at the list is the rate of the "Metal Mineral Mining" in 2011 year. The same sector

Table 3: Occupational diseases standardization between 2008-2017.

Business code	Business branches	2017	2016	2015	2014	2013	2012	2011	2010	2009	2008
5	Coal and lignite mining	1.994	4.579	5.760	1.250	3.079	13.739	5.223	3.461	8.056	10.865
7	Metal mineral mining	1.051					538	18.378			
9	Mining supporting services		612			501					
23	Non metallic products manufacturing	1.049	897	611	765						
24	Main metal industry	607		539		245				440	
27	Electric equipment manufacturing		714						645	861	257
28	Machine and equipment manufacturing								514		278
29	Motor vehicles and trailer manufacturing			513	385						
30	Manufacture of other transport vehicles	893	423	521	764	894		308	1.732	1.877	
31	Furniture manufacturing										238
32	Other products						507			575	
33	Machine and equipment installation and repair							858	652		
36	Water collection treatment and distribution					247					
38	Evaluation of wastes						238				
58	Publishing activities										1.213
72	Scientfic research and development activities							219			
98	Activities of domestic employees				1.453		1.345				

ranked second in 2017 and third in 2012. It ranked second in " Manufacture of other Transport Vehicles" in 2009 and 2010. It ranks in the top five between 2013 and 2017, but did not rank in the top 5 in 2008 and 2012. It is significant that this business branch did not exist at top 5 in 2008 and 2012 rankings.

"Machine and Equipment Installation and Maintanence" in order had 652-858% rates and took third place in the years 2010-2011; in the following years this rate significantly decreased and in the year of 2015 this business brand did not exist at the top 5 ranking. This situation can be taken as an improvement for this business branch. "Coal and Lignite Mining" business branch with rates of in the years of 2016 and 2017 in order 4579% and 1994%, still defend their place at the top regarding the occupational diseases standardization.

Table 4 shows the standardized results of occupational accident mortality rates between 2008 and 2017.

When we check the Table 4 (standardization of deaths in business branches), the business branches existing with 235-7724% rates at the top varies depending the years.

In the year 2010, the "Telecommunication" business branch took fifth place with the rate 854%. In the years of 2010-2011, "Creative Arts and Entertainment Activities" took first place with pretty high rate of 7400%; "International Organization and Representative Activities" business branch had 1016-1329% rates and took third place;

Table 4: Standardization of the fatal work accidents rate between 2008-2017.

Business code	Business branches	2017	2016	2015	2014	2013	2012	2011	2010	2009	2008
5	Coal and lignite mining	749		713	6.799	739	654	748	1.265		
6	Crude oil and natural gas removal					885					
7	Metal mineral mining	524		645		665	518	540			
8	Other mining and quarries	529	1.040	742	562	459	480			235	
9	Mining supporting service					561				417	
11	Beverage manufacturing						395				
12	Tobacco products manufacturing		525								
39	Improvement and other waste management services		860	535						828	
42	Construction of non-building buildings		352	377	351					416	904
43	Special construction activities		392								
50	Waterway transport	1.034			478						
58	Publishing activities									1.049	4.442
61	The telecommunication								854		
68	Real estate activities										870
90	Creative arts. entertainment activities							7.724	7.479		
91	Library archive and museums							1.667	905		3.351
92	Gambling and betting activities						738				
97	Activities of domestic employees										6.896
98	Own needs activities by households	631									
99	International organization and representation activities				448			1.329	1.016		

"Library, Archive and Museums" business branch with the rates in order 905-1667% and took fourth place and second place. The same business branch ranked third in 2008. "Gambling and Betting Activities" took first place in the year of 2012 with 738% rate. It is surprising that these business branches took top places with these rates in the indicated particular years only.

In 2013, "Crude Oil and Natural Gas Removal" business branch took first place with 885% rate. "Coal and Lignite Mining" business branch which took also first place regarding the standardization of work accidents and occupational diseas, leaded at top with 6799% rate in 2014. In this year, mining work accidents happened and resulted with deaths in Soma/Manisa (301 deaths), in Ermenek/

Karaman (18 deaths). Additionally, the death resulted mining accidents explain this year's high rates in Dagkonak-Kemerli/Sirnak, Amasra/Bartin, Gelik/Zonguldak, Alacakaya/Elazig, Genc/Bingol, Elbistan/Kahramanmaras. This business branch is also the only business branch which took always first place in 6 years of rankings.

In the year of 2015, "Other Mining and Quarrying"business branch took first place with the rate of 742%. It is very important that they were in top ten in the former years for their first place explanation for this year. "Metal Mining" business branch could be evaluated with the same logic that they were at top 10 for the past 10 years and most of them they were among top 5. The second place filled by "Coal and Lignite Mining" business branch in 2015 which never came out of top 5 in all past years. Beside this, the first three places took by mining industry, significally. "Construction of Non-Building Buildings" is another significant business branch in this table with regularly increasing rates in 2015 with 377% rate.

"Other Mining and Quarrying"business branch took first place in 2016 with 1040% rate, "Improvement and Other Disposable Management Systems" business branch took second place. May be the most significant point at the standardization of the work accidents resulted with death rates in 2016, is the situation of "Coal and Lignite Mining" and "Metal Mining" business branches this year did not be among top five although they had significant death rates in the past years.

In 2017, "Waterway Transportation" took first place with the rate 1034%. This business branch as different than evaluated other business branches, had high standardization rate as a first time. The highest death rate belonged to "Coal and Lignite Mining" business branch almost each of the past years and took second place this year with 749% rate.

Table 5 shows the Standardized Work Accident Rates in the Top Ten in 2017.

In the Table 5, the data for 2017 were standardized by work accidents by number of the insured workers on the basis of business branches, ranked from small to large and the first five business branches were listed in order as

Table 5: The values of the standardized work accident rates in top ten in 2017.

Business branches	Number of work accidents	Number of Insured	Expected Number	SIR	SE	CI 95% Lower	CI 95% Upper
05-Coal and lignite mining	8.468	37.596	939.90	900.95	9.79	881.35	920.14
24- Main metal industry	15.670	168.084	4202.10	372.91	2.98	353.31	378.75
30- Manufacture of other transport vehicles	3.397	51.278	1281.95	264.99	4.55	245.39	273.90
25-Manufacture of fabricated metal products except machinery and equipment	23.627	379.581	9489.52	248.98	1.62	229.38	252.15
23-Manufacture of other non-metallic mineral products	14.183	228.354	5708.85	248.44	2.09	228.84	252.53
38-Waste collection rehabilitation and disposal activities. recovery of substances	6.106	98.399	2459.97	248.21	3.18	228.61	254.44
07- Metal mineral mining	1.622	27.746	693.65	233.84	5.81	214.24	245.22
29-Manufacture of motor vehicle land vehicles (trailers) and semi trailers	11.475	202.365	5059.12	226.82	2.12	207.22	230.97
51- Airways transporting	1.420	25.244	631.10	225.00	5.97	205.40	236.71
17-Manufacture of paper and paper products	3.078	55.194	1379.85	223.07	4.02	203.47	230.95

SIR: Standardized Incidence Ratios, SE: Standard Error

Table 6: The values of the standardized work accident resulted with death rates in top ten in 2017.

Business branches	Death	Exp. death rate	SMR	SE	Lower	Upper
50-Waterway transportation	17	1.64	1034.72	250.96	542.84	1526.59
05-Coal and lignite mining	31	4.14	749.60	134.63	485.72	1013.47
08-Other mining and quarrying	38	7.17	529.99	85.98	361.48	698.51
07- Metal mineral mining	16	3.05	524.24	131.06	267.36	781.11
49-Land transport and pipeline transport	211	60.54	348.51	23.99	301.49	395.54
42-Civil engineering	158	45.89	344.28	27.39	290.60	397.97
43-Special construction activities	89	36.80	241.87	25.64	191.62	292.12
41-Building construction	340	146.49	232.10	12.59	207.43	256.77
23-Manufacture of other non-metallic mineral products	58	25.12	230.90	30.32	171.48	290.33
35-Electricity, gas, steam and ventilation system production and distribution	25	11.65	214.54	42.91	130.44	298.64

SMR: Standardized Mortality Ratio. SE: Standard Error

"Coal and Lignite Mining", Metal Mining", "Other Transportation Vehicle Production", "Machinery and Fabrication of Metal Products Excluding Equipment", "Other Non-Metallic Mineral Manufacturing".

Table 6 shows the Standardized Work Accident Death Rates in the Top Ten in 2017.

In Table 6, the data for 2017 were standardized according to the number of insured workers on the basis of business branches and ranked from small to large, and the first five business branches were "Water Transportation", "Coal and Lignite Mining", "Mining and Quarrying", "Metal Mineral Mining", "Land Transport and Pipeline Transport".

When we evaluate the EU countries;

Work accidents resulted with death in 2010 result from the six regions where WHO separated the world (including Russia, Georgia, Tajikistan, Kyrgyzstan, Uzbekistan, Ukraine,

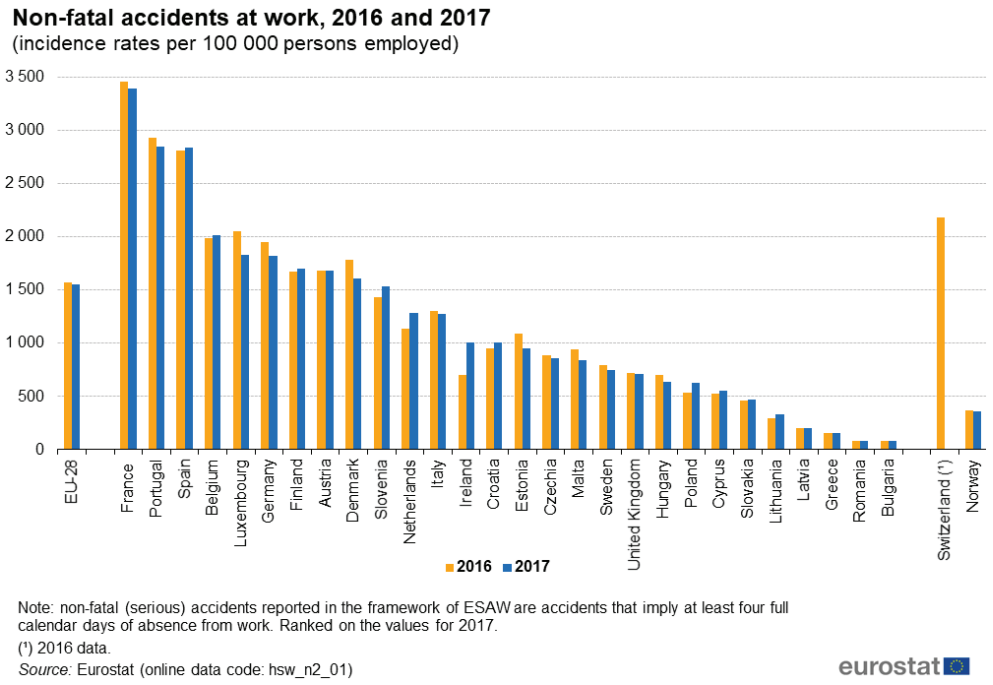

Figure 1: EU-28 countries 2016-2017 non-fatal work accident counts and incidence rates (per 100.000 workers)

Moldova, Tajikistan, Turkmenistan, Kazakhstan, Israel, Armenia, Kosovo in addition to EU countries) its rate is 2.9 (per 100.000 workers) (12). This ratio is 14.4 in the same year in which the source of Turkey's statistical yearbook SSI data (10). The rate of EU countries in 2014 is 1.27 (per 100,000 workers) (12). This ratio is 12.2 in the same year, Turkey's Statistical Yearbook SSI data source. Similar to 2010, most of the Work accidents in 2014 had occurred in the SEARO and WPRO regions as shown in Table 10. About two-thirds of the work accidents fell almost equally under these two regions. Compared to the 2010 figures, there was a rise in the number of work accidents for all the WHO regions except for the HIGH (High Income countries) and EURO regions.

Figure 1 shows the 2016-2017 Non-Fatal Occupational Accident Numbers and Frequency Rates in EU-28 countries.

Across the EU-28, there were 1558 non-fatal accidents per 100 000 persons employed in 2017. In 2017, the range for incidence rates among the EU Member States was from less than 100 non-fatal accidents per 100.000 persons employed in Bulgaria and Romania to more than 2800 per 100.000 persons employed in Spain and Portugal, while a considerably higher rate was recorded in France (3396 accidents per 100.000 persons employed; see Figure 2). Particularly low incidence rates for non-fatal accidents may reflect an under-reporting problem caused by a poorly-established reporting system, little financial incentive for victims to report, non-binding legal obligation for the employers, etc. In the same way, the well-established reporting/recognition systems may often explain the high incidence rate in some countries. The phenomenon of low non-fatal incidence rates can be considered to reflect under-reporting following the assumption that many accidents remain unreported. The situation for incidence rates of fatal accidents is different as it is much more difficult to avoid reporting fatal accidents (13).

Beside this, considering that the dimensions of the workforces are different, the incidence rate gives the results more clearly. Therefore, considering the insidence rates; in 2017, the incidence of non-fatal workplace accidents in the EU-28 was highest in "Construction", with 2876 accidents per 100.000 employees. This is followed by the "Transport and Storage" (2663 per 100.000), "Administrative and Support Service Activities" (2365 per 100.000), "Agriculture" (2099 per 100.000) business branches (13).

EU-28 countries 2016-2017 Fatal Work Accident Counts and Incidence rates are presented in Figure 2.

In 2017, there were just over 3.3 million non-fatal accidents that resulted in at least four calendar days of absence from work and 3.552 fatal accidents in the EU-28 , a ratio of approximately 942 non-fatal accidents for every fatal accident. There was an increase in the total number of non-fatal accidents at work in the EU-28 between 2016 and 2017, some 4.574 more (equivalent to growth of 0.1%). By contrast, there were 36 fewer fatal accidents at work in the EU-28 during 2017 when compared with a year before (equivalent to a decrease of 1%) (13).

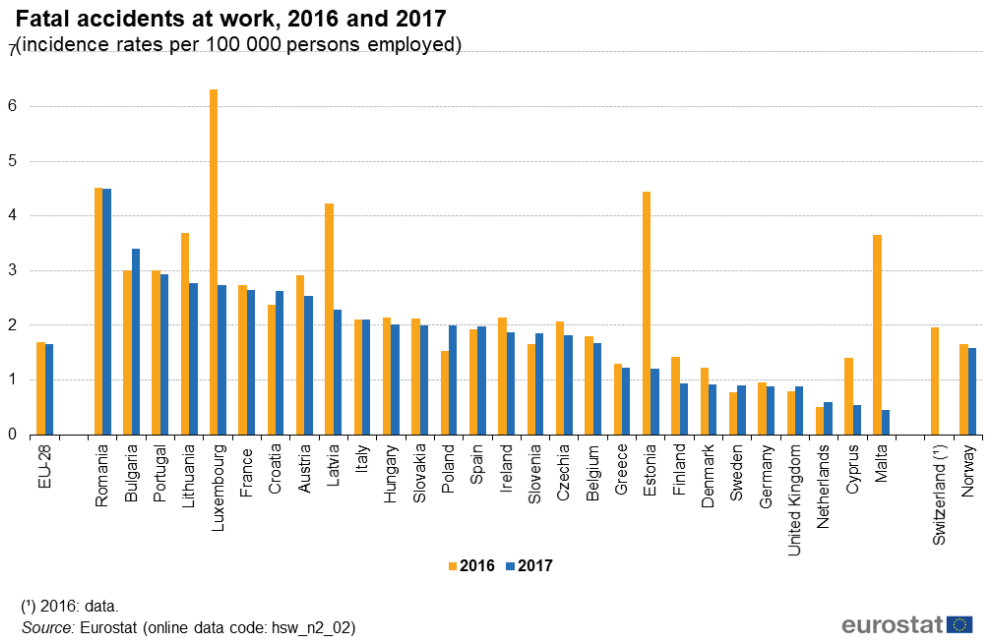

Figure 2. EU-28 countries 2016-2017 fatal work accident counts and incidence rates in business branches (per 100.000 workers)

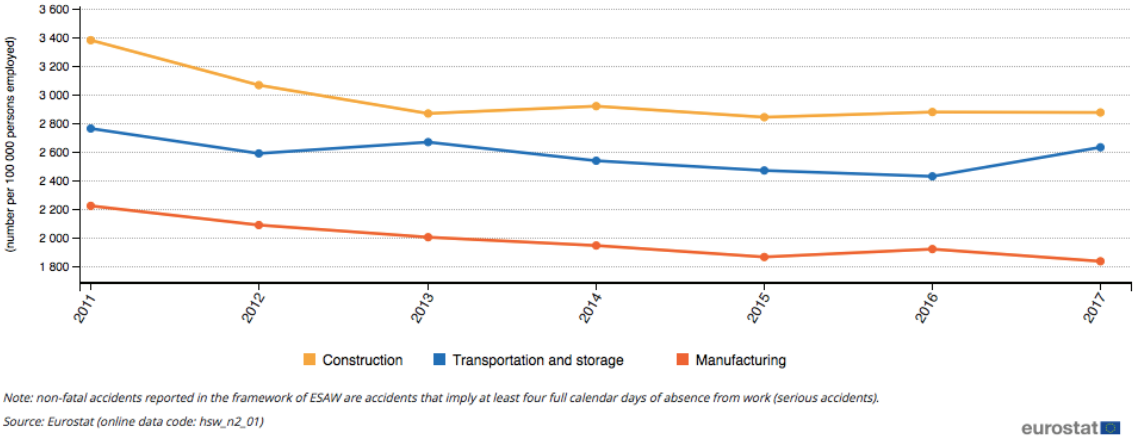

Figure 3: EU-28 countries 2011-2017 non-fatal work accidents incidence rates (per 100.000 workers)

Non-Fatal Occupational Accident Rates by business lines in EU-28 countries between 2011-2017 are shown in Figure 3.

In 2017, more than 3.3 million of non-fatal work accidents occured in the EU-28, resulting at least four calendar days of absenteeism, between 2011 and 2017, 2.1% of the total number of non-fatal accidents in the workplace in the EU-28 there was a decrease (14).

The Fatal and Non-Fatal Work accidents in EU countries in 2017 according to NACE section are shown in Figure 4.

Within the EU-28, the construction, transportation and storage, manufacturing, and agriculture, forestry and fishing sectors together accounted for around two thirds (65.2%) of all fatal accidents at work and more than two fifths (43.6%) of all non-fatal accidents at work in 2017. In 2017, one fifth (20.6%) of all fatal accidents at work in the EU-28 took place within the construction sector, while the transportation and storage sector (17.8%) had the next highest share; manufacturing (14.0%) and agriculture, forestry and fishing (12.8%) were the only other NACE sections to record double-digit shares of the total number of fatal accidents. Non-fatal accidents were rel-

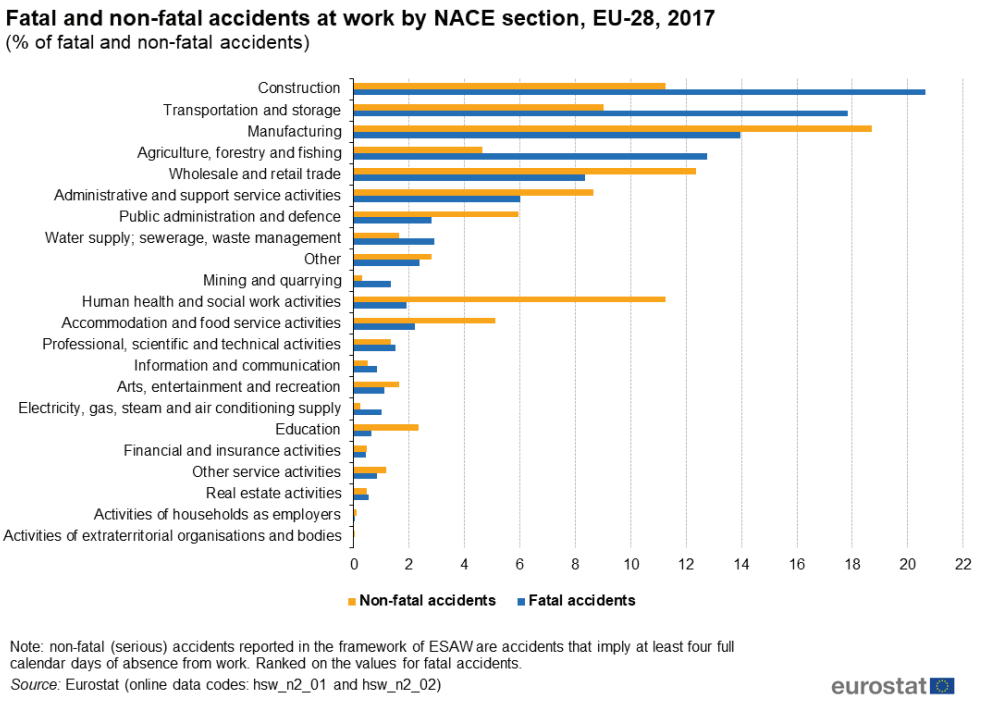

Figure 4: The fatal and non-fatal work accidents in EU countries in 2017 (per 100.000 workers)

atively high within manufacturing (18.7%), wholesale and retail trade (12.5% of the total in the EU-28 in 2017), construction (11.3%), human health and social work activities (11.3%). Administrative and support service activities as well as public administration and defence accounted for 8.6% and respectively 6% (13).

CONCLUSION

The most common tendency for occupational health and safety issues is the examination of work accident and occupational disease reports and records. The available data is held by Social Security Institution in Turkey. There is no information regarding work accidents and occupational diseases for areas not covered by law. There is also no information regarding unregistered employees. An important point that very little occupational diseases related to Turkey and this is not reflected enoughly to the records.

Statistical information about work accidents and occupational diseases are reported after the incident. As for preventive practices, clearer and premise indicators should be developed. By using these indicators, a system can be established on how to prevent diseases and accidents. İt may be difficult to create such indicators, however, these indicators can accurately and clearly illustrate the subject. Global exertion is needed to improve the issue of indicators.

Work accidents, occupational diseases, the number of deaths occured in Turkey "business branches" have been examined and standardized, examined changes compared to the past years; then EU rates are given. There is no major changes in the published annuals other than those known on the business branches (except for the unexpected branches of business from time to time).

Work accident death rate when compared to the EU countries and Turkey in terms of the way it is noteworthy that there is a high difference. On the basis of statistics for 2014 work accident death-rate care from the EU-28 average of 1.83, while this ratio was realized as 12.2 in Turkey. According to work accident fatality rate in Turkey has realized more than approximately 6.7 times higher than the EU average (15).

When we evaluate all these datas, the results obtained from Turkey shows a bad scene compared to EU countries. Turkey has taken a very important step by adopting the proactive and adopting participatory approach and spread this to the also all around the state by taking to the scope of the installation law No. 6331 Occupational Health and Safety Prevention. A protective and preventive approach has been adopted in terms of occupational health and safety with this law prepared on the basis of the ILO and EU acquis. This development by building modern occupational health and safety system based on the principle of protection, Turkey has fulfilled the legislative infrastructure for review at regular intervals. It is now in an effort to ensure effective implementation of this system. However, the available data indicate that a desired level of gain has not yet been achieved in work accidents.

When the data is analyzed, work accidents and occupational diseases are predominantly concentrated in the branches of "Coal Mining", "Metal Industry", "Construction", "Motor Vehicle Manufactoring". These business branches where serious deaths ocur, require more precautions. However, firstly, work accident and occupational disease data should be recorded officially. Having rooted culture of safety in Turkey, it is seen a major problem in terms of occupational health and safety. At this point, it is necessary to create a long-term action plan for the development of Turkey's safety culture. In this context, it is necessary to contribute more to the social dialogue-based work carried out by institutions and organizations related to occupational health and safety (16).

In business branches where fatal work accidents occur, increasing occupational safety performance and reducing work accidents and negative consequences will enable more effective measures to be taken in the field of occupational health and safety, ensure that employees comply with occupational safety rules, and work in a more controlled manner in the related field. The registration system regarding work accidents and occupational diseases should be developed in a way to reach real numbers in this field. Sensitivity should be increased especially in occupational diseases and real numbers should be reflected in national statistics. A modern approach to occupational health and safety has been adopted by reviewing national policies and practices in line with the ILO Conventions 155 and 187 and Recommendation 197.

Occupational injuries remain an important issue worldwide particularly after the economic globalization and industrialization. High risk nature of certain occupations and concentration of migrant workers and ethnic minorities in these high risk occupations contribute to the increased rate of fatal occupational injuries. Impacts at individual, community, societal and organizational levels warrant development and implementation of effective prevention programs and enforcement of laws to assure safe workplaces (17).

The insufficiency of records is the biggest obstacle to the determination of the real dimensions of the occupational safety problem in our country. However, with the enforcement of the Occupational Health and Safety Law No. 6331, it is expected that the notifications will increase and reach the expected figures. Thus, different rates will be observed in the coming years with work accidents to be announced from the public and effects of protective measures can be evaluated.

Ethics Committee Approval: This study was approved by the Istanbul Faculty of Medicine Clinical Investigations Ethics Committee. (Number: 20, Date: 06/01/2020)

Peer Review: Externally peer-reviewed.

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

Conflict of Interest: Authors declared no conflict of interest.

Financial Disclosure: Authors declared no financial support.

Etik Komite Onayı: Bu çalışma için etik komite onayı İstanbul Tıp Fakültesi Klinik Araştırmalar Etik Kurulu'ndan alınmıştır. (Sayı: 20, Tarih: 06/01/2020)

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Çalışma Konsepti/Tasarım- H.İ. E.E., G.Ö. T.İ.; Veri Toplama- H.İ. E.E., T.İ.; Veri Analizi/Yorumlama- H.İ., G.Ö., E.E.; Yazı Taslağı- H.İ. E.E., G.Ö. T.İ.; İçeriğin Eleştirel İncelemesi- H.İ., T.İ., G.Ö.; Son Onay ve Sorumluluk- H.İ. E.E., G.Ö. T.İ.

Çıkar Çatışması: Yazarlar çıkar çatışması beyan etmemişlerdir.

Finansal Destek: Yazarlar finansal destek beyan etmemişlerdir.

REFERENCES

1. International Labour Organization (ILO). Safety and health at work. Available from: URL: https://www.ilo.org/global/topics/safety-and-health-at-work/lang--en/index.htm.
2. Mitchel P. The Impact of Globalization on Health and Safety at Work. Jul, 1999 Jul. Available from: URL: https://www.wsws.org/en/articles/1999/07/who-j23.html.
3. Rahmani A, Khadem M, Madreseh E, Aghaei HA, Raei M, Karchani M. Descriptive Study of Occupational Accidents and their Causes among Electricity Distribution Company Workers at an Eight-year Period in Iran. Saf Health Work 2013;4(3):160-5. [CrossRef]
4. Ceylan H. Türkiye'deki İş Kazalarinin Genel Görünümü ve Gelişmiş Ülkelerle Kıyaslanması. International Journal of Engineering Research and Development 2011;3(2):18-24.
5. Sosyal Sigortalar ve Genel Sağlık Sigortası Kanunu. Mevzuat Bölüm 2, Madde 11. Available from: URL: https://www.mevzuat.gov.tr/MevzuatMetin/1.5.5510.pdf
6. Bekar İ, Oruç D, Bekar E. İş Kazası ve Meslek Hastalıklarının Maliyeti (2005-2014). Uluslararası Ekonomik Araştırmalar Dergisi 2017:3(3):479-89.
7. Baradan S. Türkiye İnşaat Sektöründe İş Güvenliğinin Yeri ve Gelişmiş Ülkelerle Kıyaslanması. DEÜ Mühendislik Fakültesi Fen ve Mühendislik Dergisi 2006;8(1):87-100.
8. Yılmaz F. Avrupa Birliği ülkeleri ve Türkiye'de iş sağlığı ve güvenliği kurulları: Türkiye'de kurulların etkinliği konusunda bir araştırma. Uluslararası İnsan Bilimleri Dergisi 2010;7(1):149-92.
9. Korkmaz A, Avsallı H. Çalışma Hayatında Yeni Bir Dönem:6331 Sayılı İş Sağlığı ve Güvenliği Yasası, SDÜ Fen Edebiyat Fakültesi Sosyal Bilimler Dergisi 2012;26:153-67.
10. Sosyal Güvenlik Kurumu (SGK). SGK 2007-2017 İstatistik Yıllıkları. Available from: URL: http://www.sgk.gov.tr/wps/portal/sgk/tr/kurumsal/istatistik/sgk_istatistik_yilliklari.
11. Nicholl J, Jacques RM and Campbell MJ. Direct risk standardisation: a new method for comparing casemix adjusted event rates using complex models. BMC Medical Research Methodology 2013;13(9):133. [CrossRef]
12. Nenonen N, Saarela K. Global Estimates of Occupational Accidents And Fatal Work-Related Diseases in 2014. Tampere University of Technology Department of Industrial Management, Finland 2014. Available from: URL: https://www.wsh-institute.sg/-/media/wshi/past-publications/2014/global-estimates-of-occupational-accidents-and-work-related-illness-2014.pdf?la=en&hash=CC0F4E20BDDEB6189A8B1BB4786D7973
13. Eurostat. Accidents At Work Statistics. 2016 May. Available from: URL: https://ec.europa.eu/eurostat/statistics-explained/index.php/Accidents_at_work_statistics .
14. Eurostat Statistics Explained. Accidents at work - statistics by economic activity. Available from: URL: https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Accidents_at_work_-_statistics_by_economic_activity
15. Şen M, Dursun S, Güven M. Türkiye'de İş Kazalari: Avrupa Birliği Ülkeleri Bağlaminda Bir Değerlendirme. OPUS–Uluslararası Toplum Araştırmaları Dergisi 2018;9(16):1167-90. [CrossRef]
16. Bilir, N: İş Sağlığı ve Güvenliği Profili: Türkiye. Yayın No: 62. Çalışma ve Sosyal Güvenlik Bakanlığı: Ankara; 2016
17. Mekkodathil A, El-Menyar A, Thani HA. Occupational injuries in workers from different ethnicities. Int J Crit Illn Inj Sci 2016;6(1):25-32. [CrossRef]

İSTANBUL ÜNİVERSİTESİ YAYINEVİ

OLGU SUNUMU / CASE REPORT
DOI: 10.26650/IUITFD.2020.0048
İst Tıp Fak Derg 2020 / J Ist Faculty Med 2020

A RARE PRESENTATION OF MUSCULOSKELETAL TUBERCULOSIS: TENOSYNOVITIS OF THE FLEXOR TENDONS OF THE WRIST AND DIGITS

MUSKULOSKELETAL TÜBERKÜLOZUN NADİR BİR FORMU: EL BİLEĞİ VE PARMAK FLEKSÖR TENOSİNOVİTİ

Alper Şükrü KENDİRCİ[1], Helin AKAY[1], Mustafa Sencer TOROMAN[1], Mehmet DEMİREL[1], Taha KIZILKURT[1], Ömer Naci ERGİN[1]

[1]Istanbul University, Istanbul Faculty of Medicine, Departments of Orthopedicis and Traumatology, Istanbul Turkey

ORCID IDs of the authors: A.Ş.K. 0000-0001-6250-2469; H.A. 0000-0002-3385-3443; M.S.T. 0000-0003-2977-3624; M.D. 0000-0003-1131-7719; T.K. 0000-0001-9832-8140; Ö.N.E. 0000-0001-6848-6930

Cite this article as: Kendirci AS, Akay H, Toroman MS, Demirel M, Kizilkurt T, Ergin ON. A Rare presentation of musculoskeletal tuberculosis: tenosynovitis of the flexor tendons of the wrist and digits. J Ist Faculty Med 2020;83(4):446-50. doi: 10.26650/IUITFD.2020.0048

ABSTRACT

One of the extrapulmonary *mycobacterium tuberculosis* infection is wrist tenosynovitis and treatment of the wrist tenosynovitis includes antibiotics and surgical debridement. We report the diagnosis and treatment of a patient with tuberculosis tenosynovitis of the flexor tendons of the wrist and digits. Our patient was 65 years old. In his physical examination, swelling, redness, and warming along the wrist and draining open wounds on the palmar surfaces of the first and fifth fingers at the same hand were detected. In the laboratory tests, his acute-phase reactants had increased and in the T2A series, MRI findings depicted millimetric nodular images on the sheath of the flexor tendons of the wrist and first and fifth digits, which were consistent with synovitis. Debridement and synovectomy of flexor tendons of the wrist and D1, D5 of the left side was performed. A total of 6 months of a rifampicin-based anti-tuberculosis treatment was administered. In the second year after the operation, there was no problem with previous wound, and range of motion and muscle strength of the fingers and wrist joint were complete.

Keywords: Tuberculosis tenosynovitis, musculoskeletal, flexor, wrist

ÖZET

Ekstrapulmoner *Mycobacterium Tuberculosis* enfeksiyonlarından biri el bileği fleksör tenosinovitidir. Tedavisi antibiyoterapi ve cerrahi debridmandır. Bu vaka sunumunda; el bileği ve parmak fleksör tendon tüberkülozu olan bir hastanın tanı ve tedavi süreci anlatılmaktadır. Hastamız 65 yaşındaydı, fizik muayenesinde şişlik, kızarıklık ve el bileğinde ısı artışı ile birlikte birinci ve beşinci parmakların palmar yüzlerinde drene açık yaralar mevcuttu. Laboratuvar testlerinde akut faz reaktanları yüksekti ve MRG bulguları T2A serisinde el bileği ve D1, D5 parmak fleksör tendonlarının kılıflarında sinovit ile uyumlu milimetrik nodüler görüntüler mevcuttu. Sol el bileği ve D1, D5 fleksör tendonlarının debridmanı ve sinovektomi işlemi yapıldı. Ameliyattan sonra 6 ay süre ile rifampisin bazlı anti-tüberküloz tedavi uygulandı. Ameliyat sonrası 2. yılda hastanın yara yeri problemi yoktu, el ve parmak eklem hareket açıklıkları ve kas gücü tamdı.

Anahtar Kelimeler: Tüberküloz tenosinovit, muskuloskeletal, fleksör, el bileği

Corresponding author/İletişim kurulacak yazar: alpersukru@gmail.com

Submitted/Başvuru: 03.05.2020 • **Revision Requested/Revizyon Talebi:** 28.05.2020 •
Last Revision Received/Son Revizyon: 07.07.2020 • **Accepted/Kabul:** 09.07.2020 • **Published Online/Online Yayın:** 02.10.2020

INTRODUCTION

Tuberculosis infection, mainly caused by *Mycobacterium Tuberculosis*, continues to be a significant health problem worldwide. Although the infection primarily affects the lungs, it can affect extrapulmonary tissues such as the musculoskeletal system (1). In 2018, there were an estimated 7 million new and relapse tuberculosis (TB) cases worldwide. The rate of extrapulmonary tuberculosis is around 15% in the 7.0 million incident cases (2). Musculoskeletal tuberculosis accounts for 10% of extrapulmonary tuberculosis cases in the USA, and the most commonly affected musculoskeletal areas are the spine, pelvis, and long bones of the lower limb (3). However, tuberculosis tenosynovitis of the flexor tendons of the wrist and digits constitutes an uncommon condition with only a limited number of cases reported in the literature.

This case report aimed to describe the clinical and radiological features of a rare case of tuberculous tenosynovitis of the digits and wrist flexors and to illustrate the medical and surgical treatment planning in the management of this rare manifestation of extrapulmonary tuberculosis.

CASE REPORT

A male aged 65 years was admitted to our department with pain, redness, and swelling in the volar side of his left wrist. His medical history revealed that the symptoms started 1 year ago. Once the pain became too intense, the patient applied to the rheumatology department. After a thorough investigation in terms of rheumatologic diseases was carried out, the patient was referred to our department.

In his physical examination; swelling, redness, and warming along the wrist and draining open wounds on his palmar faces of the first and fifth fingers at the same hand were determined (Figure 1-A). Laboratory tests revealed 19.76 mg/L of C-reactive protein (CRP), 21 mm/h of eryth-

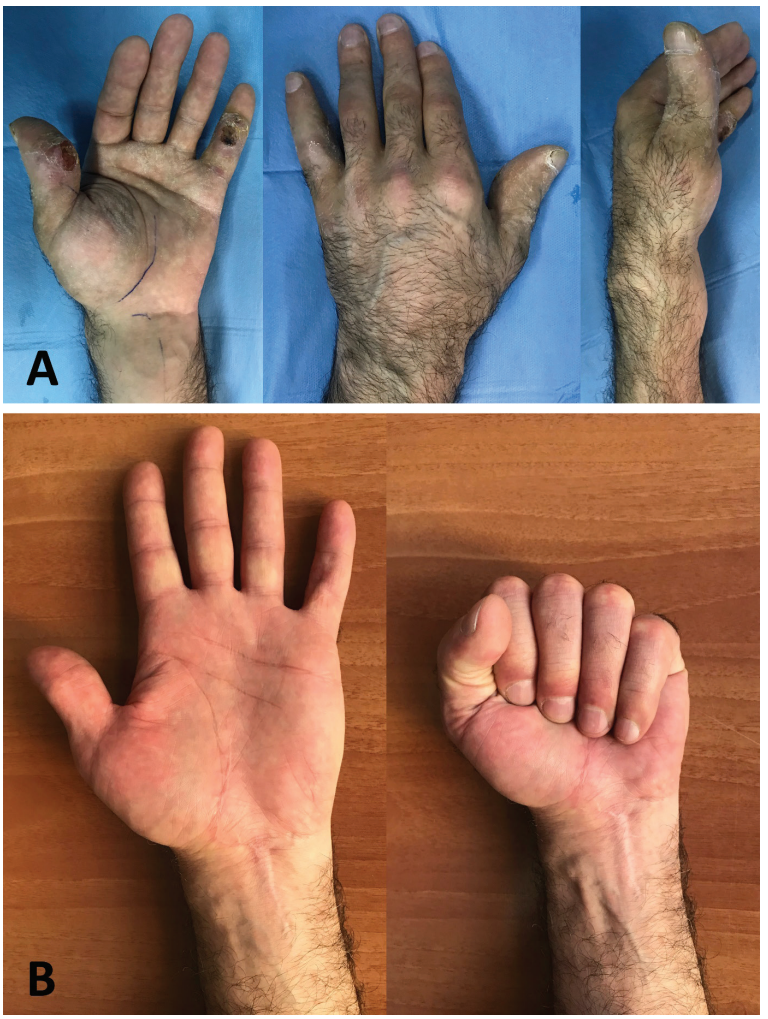

Figure 1-A: Swelling and redness in the wrist along with draining open wounds on palmar faces of the first and fifth fingers, **B:** Postoperative second year, no wound problem and full range of motion of wrist and digits.

rocyte sedimentation rate (ESR), and 7.5×10^3 mm^3 of white blood cells (WBC). In the radiological examination, a wrist X-ray was undertaken, which displayed a remarkable increase in the soft tissue density with periarticular osteoporosis (Figure 2-A). Additionally, magnetic resonance imaging (MRI) depicted millimetric nodular images on sheaths of flexor tendons of the wrist and D1, D5 digits in the T2A series, which were consistent with synovitis (Figure 3-A). Then, the decision for biopsy was made, additional workup including PPD test and chest radiography was undertaken. PPD was 18 mm and chest radiography showed no clinically significant finding. Pathologic examination revealed necrotizing granulomatous inflammation with central abscess. Additionally, in the microbiologic examination, m. tuberculosis was derived from the Lowenstein Jensen medium.

In light of the aforementioned findings, the diagnosis of tuberculosis tenosynovitis was established, and the surgical debridement was planned. Informed consent about the surgery was achieved from the patient. At the operating room, left wrist flexor tendon synovectomy and D1, D5 flexor tendon synovectomy were performed under an axillary block. During the operation, the transverse carpal ligament was released, and the median nerve was protected. Synovial hypertrophy was seen in the flexor tendons' sheath at the wrist and first-fifth digital (Figure 4). All synovia was debrided, and tissue examples were sent to the laboratory for culture and pathological diagnosis. After the operation, oral rifampicin-based anti-tuberculosis treatment was continued for six months. Extension exercises for wrist and digitals were applied to the patient for musculoskeletal rehabilitation.

Six months later, there was no synovitis in the MRI (Figure 3-B) and there was no periarticular osteoporosis in the x-ray examination (Figure 2-B), and the second year after the operation, there was no problem with the previous wound and, range of motion and muscle strength of the fingers and wrist joint were complete (Figure 1-B).

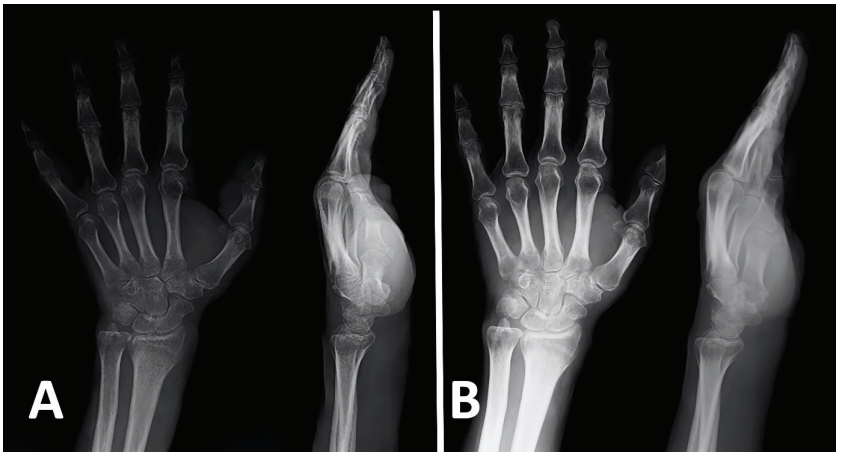

Figure 2-A: Preoperative wrist x-ray, increase in the soft tissue density with periarticular osteoporosis, B: Postoperative sixth month wrist x-ray.

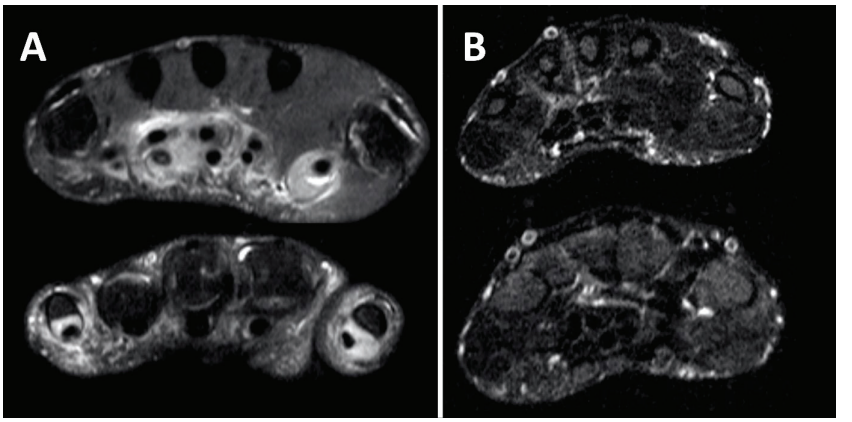

Figure 3-A: Preoperative MRI, T2A sequence axial view, millimetric nodular images on sheaths of flexor tendons of the wrist and D1, D5 digits, **B:** Postoperative sixth month MRI, T2A sequence axial view.

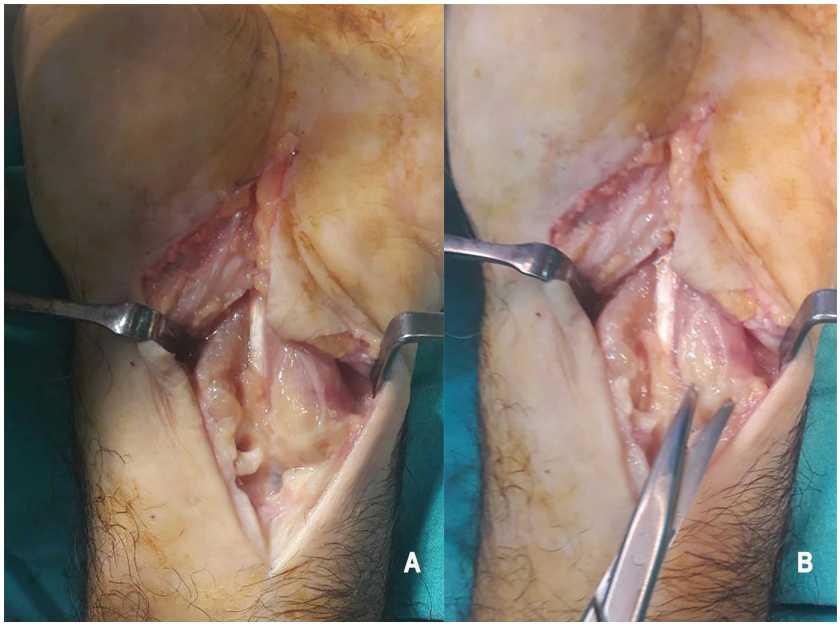

Figure 4-A: Intraoperative view, **B:** Rice bodies around the wrist flexor tendons.

DISCUSSION

Musculoskeletal tuberculosis infection can affect the vertebra, pelvis, and wrist, as well. Although rare, the involvement of the tendon sheath is possible and generally secondary to hematogenous spread (4).

Following the invasion of the tuberculosis bacteria into the tissue, tissue exudation and granulation may occur. As a result of the progression of inflammation, rice bodies can be seen. Woon et al. stated that during the surgery, the appearance of Rice stems, millet seeds or melon seeds suggest a high rate of tuberculosis tenosynovitis (5). In individuals with the involvement of tendon sheath, symptoms are typically non-specific such as swelling and pain; hence, it can be diagnosed late because of the dearth of systemic findings. Furthermore, some patients may manifest with the findings of carpal tunnel syndrome (6). The diagnosis of musculoskeletal tuberculosis can be overlooked, with the average time from onset of symptoms to diagnosis is 16-19 months (7). In our patient, the diagnosis was established 12 months later, and the first clinical and radiographic findings led to the diagnosis of seronegative arthritis. In the presence of tenosynovitis, wrist X-ray may have an ability to display soft tissue swelling and osteoporotic bony changes around the wrist joint. However, T2-weighted sequences on MRI support the diagnosis better. In T2-weighted sequences, central erosion and peripheral abscesses, along with hypointense focus and synovia, distinguish tuberculosis from other types of inflammatory arthritis (8,9). In our case, we have observed similar changes in soft tissue and carpal bones.

According to Kanavel, involvement of the tendon sheath in tuberculosis consists of three stages. In the first stage, severe serous exudation occurs after sheath thickening. The second stage is the proliferative phase of granulomatous tissues leading to the rice bodies, and in the last stage, necrosis occurs (10). In our patient, the diagnosis was established in the second stage of the disorder.

In the management of tuberculosis, antituberculosis drugs are a treatment of choice. Surgical treatment only confers symptomatic relief. However, surgical treatment can be effective in people diagnosed at stage 2 or 3. Benchakroum et al gave antituberculosis drug treatment to 11 patients with wrist tuberculosis, but medical treatment failed in patients with abscess and applied surgery to them (11). Treatment becomes difficult in patients with rice bodies, because debridement is required to complete treatment and prevent recurrence. It is also recommended to remove all bursas in the appearance of the rice body.

CONCLUSION

Tuberculosis tenosynovitis is a rare disease. However, it can be diagnosed with detailed physical examination and imaging. Although surgical debridement is required after the diagnosis, anti-tuberculosis oral therapy is necessary for the recovery and prevention of recurrence.

Ethics Committee Approval: This study was not approved by an ethical committee. (Case Report).

Informed Consent: Written consent was obtained from the participants.

Peer Review: Externally peer-reviewed.

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

Conflict of Interest: Authors declared no conflict of interest.

Financial Disclosure: Authors declared no financial support.

Etik Komite Onayı: Etik komite onayı alınmamıştır. (Olgu sunumu).

Bilgilendirilmiş Onam: Katılımcılardan bilgilendirilmiş onam alınmıştır.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Çalışma Konsepti/Tasarım- A.Ş.K., M.D.; Veri Toplama- A.Ş.K., H.A., M.S.T.; Veri Analizi/Yorumlama- A.Ş.K., M.D.; Yazı Taslağı- A.Ş.K., H.A., M.S.T.; İçeriğin Eleştirel İncelemesi- M.D., T.K.; Son Onay ve Sorumluluk- A.Ş.K, H.A., M.S.T., M.D., T.K., Ö.N.E.

Çıkar Çatışması: Yazarlar çıkar çatışması beyan etmemişlerdir.

Finansal Destek: Yazarlar finansal destek beyan etmemişlerdir.

REFERENCES

1. Sandgren A, Hollo V, Van der Werf MJ. Extrapulmonary tuberculosis in the European Union and European economic area, 2002 to 2011. Eurosurveillance 2013;18(12):20431.
2. Annabel B, Anna D, Hannah M. Global tuberculosis report 2019. Geneva: World Health Organization; 2019.p.7-9.
3. Leonard MK, Blumberg HM. Musculoskeletal Tuberculosis. In: Tuberculosis and Nontuberculous Mycobacterial Infections, D. Schlossberg (Ed.). 2017.p.371-392. [CrossRef]
4. Bayram S, Erşen A, Altan M, Durmaz H. Tuberculosis tenosynovitis with multiple rice bodies of the flexor tendons in the wrist: a case report. Int J Surg Case Rep 2016;27:129-32. [CrossRef]
5. Woon CY-L, Phoon E-S, Lee JY-L, Puhaindran ME, Peng Y-P, Teoh L-C. Rice bodies, millet seeds, and melon seeds in tuberculous tenosynovitis of the hand and wrist. Ann Plast Surg 2011;66(6):610-7. [CrossRef]
6. Kang HJ, Park SY, Shin SJ, Kang ES,Hahn SB. Tuberculous tenosynovitis presenting as carpal tunnel syndrome. J Korean Soc Surg Hand 2000;5:137-141.
7. Lee SH, Abramson SB. Infections of the musculoskeletal system by M. tuberculosis. In: Rom WN, Garay SM, eds. Tuberculosis. New York, NY: Little, Brown and Company,1996. p.635-644.
8. Hsu C-Y, Lu H-C, Shih TT-F. Tuberculous infection of the wrist: MRI features. Am J Roentgenol 2004;183(3):623-8. [CrossRef]
9. Sawlani V, Chandra T, Mishra RN, Aggarwal A, Jain UK, Gujral RB. MRI features of tuberculosis of peripheral joints. Clin Radiol 2003;58(10):755-62. [CrossRef]
10. Kanavel AB. Tuberculous tenosynovitis of the hand: a report of fourteen cases of tuberculous tenosynovitis. Surg Gynecol Obstet 1923;37:635-47.
11. Benchakroun M, Bardouni A El, Zaddoug O, Kharmaz M, Yaacoubi M El, Ouadghiri M, et al. Tuberculosis of the wrist. Symptoms and outcome in eleven cases. Rev Chir Orthop Reparatrice Appar Mot 2004;90(4):337-45. [CrossRef]

İSTANBUL ÜNİVERSİTESİ YAYINEVİ

CASE REPORT / OLGU SUNUMU
DOI: 10.26650/IUITFD.2019.0079
İst Tıp Fak Derg 2020 / J Ist Faculty Med 2020

HYPONATREMIA AS A FIRST SIGN OF SMALL CELL LUNG CANCER: A CASE REPORT

İLK BULGUSU HİPONATREMİ OLAN AKCİĞERİN KÜÇÜK HÜCRELİ KARSİNOMU: OLGU SUNUMU

Emel BAYRAK[1], Kayser ÇAĞLAR[2]

Losev Losante Child and Adult Hospital, [1]Department of Internal Medicine, [2]Department of Nephrology, Ankara, Turkey

ORCID IDs of the authors: E.B. 0000-0001-8003-9391; K.Ç. 0000-0002-1428-8880

Cite this article as: Bayrak E, Caglar K. Hyponatremia as a first sign of small cell lung cancer: a case report. J Ist Faculty Med 2020;83(4):451-3. doi: 10.26650/IUITFD.2019.0079

ABSTRACT

A 62-year- old woman with a history of hypertension and diabetes mellitus was admitted to the emergency department because of headache, weakness, insomnia and impaired balance. Severe hyponatremia was detected and patient was admitted to the hospital. The drugs that could lead to hyponatremia were stopped. Additional investigations were made because of the persistence of hyponatremia. After the diagnosis of inappropriate ADH syndrome computed tomography of the chest was performed which revealed a pulmonary mass. The histopathological test revealed small-cell carcinoma of the lung.

Keywords: Hyponatremia, inappropriate ADH syndrome, lung neoplasms

ÖZET

Hipertansiyon ve diyabetes mellitus tanıları olan altmış iki yaşındaki kadın hasta; halsizlik, uykusuzluk, dengesizlik ve baş ağrısı yakınmaları ile acil servise başvurdu. Yapılan tetkiklerinde ciddi hiponatremi saptanan ve hastaneye yatırılan hasta hiponatremiye yol açabilecek ilaçları kesilerek takip edildi. Hiponatremisi devam eden hastada yapılan incelemelerde uygunsuz antidiüretik hormon (ADH) sendromu saptanarak çekilen torax tomografisinde akciğerde kitle tespit edildi ve histopatolojik inceleme akciğerin küçük hücreli karsinomu olarak rapor edildi.

Anahtar Kelimeler: Hiponatremi, uygunsuz ADH sendromu, akciğer kanseri

INTRODUCTION

Hyponatremia is the most common electrolyte disorder in clinical practice and may be asymptomatic or lead to life-threatening clinical conditions. One of the factors causing hyponatremia is inappropriate ADH syndrome and it may develop as a paraneoplastic syndrome. The case of a patient with lung cancer who first presented with hyponatremia is discussed in this case report.

CASE PRESENTATION

A 62-year-old female patient was admitted to the emergency department with complaints of high blood pressure, increasing fatigue in recent days, difficulty in maintaining balance during walking, headache and insomnia. She did not complain of hemoptysis, cough, weight loss, fever, chest pain or dyspnea. Her chest was clear on oscultation. Cardiac examination revealed a regular heart rate. Pretibial edema and lymphadenopathy were not detected. Main laboratory tests performed in the emergency department of the patient are glucose: 102 mg/dl, sodium: 115 mEq/L, potassium: 4.67 mEq/L, creatinine: 0.60 mg/dl, calcium: 8.60 mg/dl, hemoglobin: 13.4 g/dl.

Her medical history included hypertension, type II diabetes mellitus and history of smoking. Medications included telmisartan / hydrochlorothiazide, metformin, seratonin reuptake inhibitor (SSRI) and metoprolol.

Corresponding author/İletişim kurulacak yazar: emel.bayrak@gmail.com

Submitted/Başvuru: 18.09.2019 • **Revision Requested/Revizyon Talebi:** 07.01.2020 •
Last Revision Received/Son Revizyon: 08.01.2020 • **Accepted/Kabul:** 11.02.2020 • **Published Online/Online Yayın:** 14.05.2020

At first the patient was given 3% NaCl infusion due to severe low levels of serum sodium as well as complaints of restlessness and headache. Antihypertensives were switched to calcium channel blockers, and thiazide and SSRI inhibitor were stopped. No significant mass lesion or active infiltration were detected on chest radiography. The patient did not agree to having the recommended Torax CT scan. She was discharged from the hospital when the sodium level increased to 128 meq/L. 10 days after her discharge she was seen again at outpatient clinic and found to have hyponatremia and admitted to the hospital. The laboratory values during the hospitalization period were as follows: serum sodium: 118 meq/L, serum potassium: 5.04 meq/L, creatinine: 0.69 mg/dl, uric acid: 1.60 mg/dl, urine sodium: 48 meq/L, urine osmolality: 351 mosm/kg, serum osmolality: 243 mosm/kg.

Upon finding low serum osmolality, high urine osmolality, high urine sodium levels, and clinical euvolemia, and in the absence of renal, pituitary, adrenal and thyroid diseases, inappropriate ADH syndrome was diagnosed and fluid restriction and 3% NaCl were applied to the patient. Although she did not complain of any of the pulmonary symptoms and despite the anterior-posterior chest plain film not having any evidence of active pulmonary disease, CT of the thorax was performed for the aetiology consideration of inappropriate ADH syndrome (Figure 1). CT of the thorax revealed a peripheral lobulated contoured pulmonary nodule in the lower lobe of superior segment and lesions compatible with enlarged lymph nodes in the mediastinal area suggesting local advanced stage of primary malignancy. PET CT was performed and revealed hypermetabolism areas defined in the superior segment of the lower lobe of the right lung and mediastinum were found to be compatible with malignant metastatic processes. Histopathological sampling was recommended for primary lung malignancies. Biopsy of the mass revealed small cell carcinoma of the lung. There was no metastasis. The patient provided written informed consent for publication.

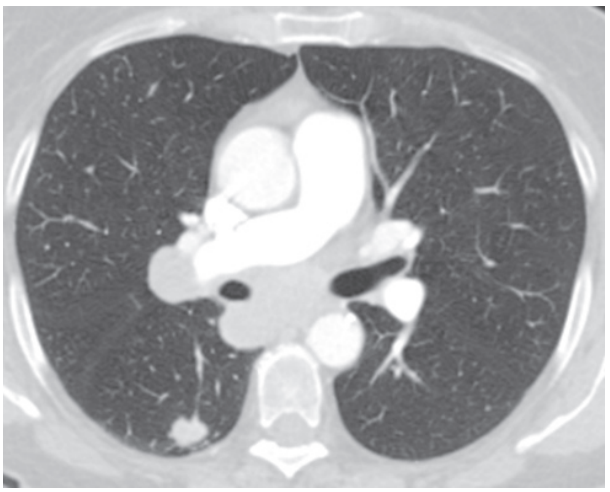

Figure 1: Thorax CT image.

DISCUSSION

We report here a case of small cell carcinoma of the lung whose first presentation was compatible with inappropriate ADH syndrome. Hyponatremia, defined as serum sodium concentration below 135 mEq/L, is the most common electrolyte disorder in clinical practice. Hyponatraemia is primarily a disorder of water balance, with a relative excess of body water compared to total body sodium and potassium content. It is usually associated with a disturbance in the antidiuretic hormone (1). Detailed history and physical examination were required to evaluate hyponatremia; electrolite-rich fluid loss due to diuretic use or gastrointesinal looses and the medication that may cause hyponatremia should be looked for. In the physical examination, the presence of edema might be an indicatation of diseases that may lead to hyponatremia such as heart failure, chronic liver disease and renal failure. The presence of signs of thyroid disease or adrenal insufficiency, pulmonary disease, central nervous system disease, and malignancy should be also investigated (2).

In the present case, because the use of certain drugs can cause hyponatremia such as thiazide and SSRI, these agents were immediately discontinued. Although hyponatremia usually occurs in the first weeks, some patients may develop it months later. SSRI inhibitors may also cause hyponatremia by causing inappropriate ADH syndrome (3). In our case, these agents were thought to be aetiologic factors, but the lack of improvement in hyponatremia after termination of these agents required further investigation. Laboratory examinations revealed that the serum osmolality of the patient was low and urine osmolality was found to be high, but adrenal and thyroid functions were normal. When evaluated together with other findings, it meets the criteria of inappropriate ADH syndrome (1). Although the chest X-ray was found to be normal, lung malignancy was detected after tomographic examination. The most common cancers associated with hyponatremia are various forms of lung cancer. The most common causes of hyponatremia in cancer patients are both the syndrome of inappropriate antidiuretic hormone secretion [syndrome of inappropriate antidiuretic hormone (ADH)] and volume depletion (4). In this study , we report on a case presenting with hyponatremia as the first sign of small cell lung cancer. Because of the aggressive nature of the disease, the most common manifestation of small cell carcinoma of the lung is a metastatic one. Approximatelly 70% of patients present with metastatic disease (5).

Therefore, identifying the disease in earlier stages is very important. Because of the limitations of the screening guidelines, physicians should be suspicious of possible early symptoms or abnormal laboratory values.

CONCLUSION

Hyponatremia due to inappropriate ADH syndrome is rare in the onset of malignancy. In our case, by following hyponatremia, we detected a small cell lung cancer when the chest X-ray was normal and neither pulmonary symptoms nor other organ metastasis had developed. The malignancy factor should not be forgotten in the diagnostic evaluation of hyponatremia.

CT of the thorax revealed a peripheral lobulated contoured pulmonary nodüle in the lower lobe of superior segment and lesions compatible with enlarged lymph nodes in the mediastinal area suggesting primary malignancy.

Etik Komite Onayı: Etik komite onayı alınmamıştır. (Olgu sunumu).

Bilgilendirilmiş Onam: Katılımcılardan bilgilendirilmiş onam alınmıştır.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Çalışma Konsepti/Tasarım- E.B., K.Ç.; Yazı Taslağı- E.B., K.Ç.; Son Onay ve Sorumluluk- E.B., K.Ç.; Süpervizyon- K.Ç.; Author Contributions: Conception/Design of Study- E.B., K.Ç.; Drafting Manuscript- E.B., K.Ç.; Final Approval and Accountability- E.B., K.Ç.; Supervision- K.Ç.

Çıkar Çatışması: Yazarlar çıkar çatışması beyan etmemişlerdir.

Finansal Destek: Yazarlar finansal destek beyan etmemişlerdir.

Ethics Committee Approval: This study was not approved by an ethical committee. (Case Report).

Informed Consent: Written consent was obtained from the participants.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- E.B., K.Ç.; Drafting Manuscript- E.B., K.Ç.; Final Approval and Accountability- E.B., K.Ç.; Supervision- K.Ç.

Conflict of Interest: Authors declared no conflict of interest.

Financial Disclosure: Authors declared no financial support.

REFERENCES

1. Spasovski G, Vanholder R, Allolio B, Annane D, Ball S, Bichet D, et al.; on behalf of the Hyponatraemia Guideline Development Group. Clinical practice guideline on diagnosis and treatment of hyponatraemia. Nephrol Dial Transplant 2014:29(Suppl.2):ii1-ii39. [CrossRef]
2. Sterns RH, Diagnostic evaluation of adults with hyponatremia. In: UpToDate, Emmett M(Ed), UpToDate, Waltham, MA. (Accessed on July 19, 2019.)
3. Rawal G, Kumar R, Yadav S. Severe hyponatremia associated with escitalopram, J Family Med Prim Care 2017;6(2):453-4. [CrossRef]
4. Kitchlu A, Rosner M Hyponatremia in patients with cancer. Current Opinion in Nephrology and Hypertension 2019;8(5):433-40. [CrossRef]
5. Molina JR, Yang P, Cassivi SD, Schild SE, Adjei AA. Non-small cell lung cancer: epidemiology, risk factors, treatment, and survivorship. Mayo Clin Proc 2008;83(5):584-94. [CrossRef]