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Türkiye'deki iki referans yanık merkezinin üç yıllık veriler eşliğinde değerlendirilmesi

Evaluation of two reference burn centers in Turkey with three-year data

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ÖZ

Amaç: Bu çalışmada, iki referans yanık merkezinin üç yıllık verileri değerlendirilerek epidemiyolojik bilgilerin analiz edilmesi ve yanık travmalarını önlemeye yönelik seçenekler sunulması amaçlandı.

Gereç ve Yöntem: Ankara Bilkent Şehir Hastanesi Erişkin Yanık Merkezi ve İzmir Bozyaka Eğitim ve Araştırma Hastanesi Yanık Merkezi'nde 2019, 2020 ve 2021 yıllarında yatarak tedavi gören toplam 1839 hastanın verileri (cinsiyet, yatarak tedavi gören hasta sayısı, %20 üzeri toplam vücut yanık alanı olan hasta sayısı**,** yanık derecesi, yanık nedeni, mortalite oranı ve yabancı uyruklu hasta sayısı) retrospektif olarak incelendi.

Bulgular: Toplam 1839 hastanın %62,1'i erkek, %37,9'u kadındı. Erkekler kadınlara oranla daha sık majör yanık travması geçirmiş idi. En fazla haşlanma yanığı (%54,5) görüldü. Ankara Bilkent Şehir Hastanesi Erişkin Yanık Merkezi'nde 2021'de 2019'a göre 2.derece yanık vakalarının arttığı, haşlanma nedenli yanıkların azaldığı, alev yanıklarının arttığı görüldü. İzmir Bozyaka Eğitim ve Araştırma Hastanesi Yanık Merkezi'nde 2019'da %2,8 olan 2.derece yanıklı hasta oranının 2020'de %3,5'e ve 2021'de ise %30,1'e yükseldiği tespit edildi. Kaba mortalite oranının İzmir Bozyaka Eğitim ve Araştırma Hastanesi Yanık Merkezi'nde yaklaşık 5 kat daha yüksek olduğu görüldü.

Sonuç: Yanık merkezleri ciddi yanık vakalarının tedavi ve takip edildiği en donanımlı yanık tedavi birimleridir. Mevcutta olan yanık yaralanmalarını önlemeye yönelik tedbirlerin etkisini değerlendirmek için yanık tedavi birimlerinde kayıtların eksiksiz ve düzenli bir şekilde tutulması hayati önem taşımaktadır.

Anahtar Sözcükler: Yanık, yanık epidemiyolojisi, mortalite, yanık merkezi.

ABSTRACT

Aim: *The aim of this study was to analyze epidemiological information by evaluating three-years of data from two reference burn centers and to present options to prevent burn trauma.*

Materials and Methods: *Data (gender, number of inpatients, number of patients with total body burn area over 20%, burn grade, cause of burn, mortality rate, and number of foreign patients) of a total of 1839 patients admitted to Ankara Bilkent City Hospital Adult Burn Centre and Izmir Bozyaka Training and Research Hospital Burn Centre in 2019, 2020, and 2021 were retrospectively analyzed.*

Results: *Of the total 1839 patients 62.1% were male and 37.9% were female. Men had major burn trauma more frequently than women. Scald burns were the most common (54.5%). In Ankara Bilkent City Hospital Adult Burn Center 2nd degree burn cases increased, scald burns decreased, and flame burns increased in 2021 compared to 2019.In Izmir Bozyaka Training and Research Hospital Burn Centre the rate of patients with 2nd degree burns increased from 2.8% in 2019 to 3.5% in 2020 and 30.1% in 2021. It was observed that the crude mortality rate was approximately 5 times higher in Izmir Bozyaka Training and Research Hospital Burn Centre.*

Sorumlu yazar: Babür Uygar Çiçek
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Conclusion: *Burn centers are the most equipped burn treatment units where severe burn cases are treated and followed up. It is vital that complete and organized records are kept in burn treatment units in order to impact of measures to prevent burn injuries.*

Keywords: *Burn, burn epidemiology, mortality, burn center.*

GİRİŞ

Yanık, çok eski zamanlardan beri insanlığı etkileyen, biyolojik, kimyasal, elektriksel ve fiziksel ajanların neden olduğu, lokal ve sistemik etkileri olan termal bir yaralanmadır (1). Ciddi bir yanık yaralanması, bir kişinin karşılaşabileceği en önemli travmatik olaylardan biridir ve bu yaralanmanın uzun süreli morbiditesi diğer yaralanmalardan farklı olup genellikle yaşam kalitesinin kaybıyla ilişkilidir (2-4). Dünya Sağlık Örgütü, yaşam boyu majör yanık görülme sıklığının %1 (5) olduğunu ve dünya çapında her yıl 300.000'den fazla kişinin yangına bağlı yanıklardan öldüğünü tahmin etmektedir (6). Amerika Birleşik Devletleri'nde yanık yaralanması geçirenlerin yıllık insidansının 1,2 milyon olduğu tahmin edilmekte olup, rapor edilen yangına bağlı kaza sayısı 2 milyondur (7). Yanık yaralanmaları gelişmiş ülkelerde azalıyor olsa da yanık travmalarının sıklığı yanıkların ~%90'ının meydana geldiği düşük ve orta gelirli ülkelerde artmaktadır (8, 9). Gelişmekte olan ülkelerde, 5 yaşın altındaki çocuklar, yaşlılar ve kontrol altına alınamayan epilepsi ve diyabet gibi önceden kronik hastalıkları bulunan kişiler yanık travmasına karşı en savunmasız gruplardır. Yoksul ülkelerin kırsal kesimlerinde işsizlik, yoksulluk, aşırı kalabalık, düşük eğitim ve yeterli elektrik enerjisi olmaması yanıklar için en önemli risk faktörleridir (10).

Fizyopatolojisi nedeniyle yanık yaralanması özellikli bir yaralanmadır (11). Yanık fizyopatolojisi yanığın neden olduğu hemodinamik değişikliklerin ve ödem oluşumunun lokal ve sistemik etkileri olarak anlaşılsa da (12) bu etkilerin ortaya çıkması karmaşık inflamatuar yanıta bağlı olarak gelişir (13). Toplam Vücut Yanık Alanı (TVYA) %30 ve üzerine çıktığında yanık alanından salgılanan sitokinler ve diğer inflamatuar mediyatörler vücutta sistemik yanıt oluşturacak düzeye erişirler (1, 14).

Yanık travmasının ciddi bir travma olması ve sonrasında oluşabilecek ağır sonuçları birçok yazarı yanık epidemiyolojisini araştırmaya yönlendirmiştir (15). Epidemiyolojik veriler yanık travmalarını engelleme amaçlı stratejilerin oluşturulması ve yanık tedavisinde etkili seçeneklerin belirlenmesi için yanıkla ilgilenen uzmanlara faydalı bilgiler sağlamaktadır (16,17). Ülkemizde yanık tedavi birimlerinin kurulması ve işleyişi 2019 yılı Ekim ayında Türkiye Cumhuriyeti Sağlık Bakanlığı'nca yayınlanan "Yanık Tedavi Birimleri Hakkında Yönetmelik" hükümlerine göre yürütülmektedir ve bu yönetmelikte Yanık Merkezi; diğer birimlerden ayrı bir yerde yapılandırılmış, tüm yanık hastalarının en ileri tıbbi imkanlarla tedavi edilebildiği, sterilizasyon şartlarının gerçekleştirilmiş olduğu, giriş ve çıkışların kontrollü yapıldığı en donanımlı yanık tedavi birimi olarak tanımlanmıştır (18).

Yanık travmasına yatkınlık yaratan faktörler şehirler ve bölgeler arasında farklılık gösterebilmekte olup, bu yaralanmaların önlenebilmesi ve bu hastalara verilebilecek olan yanık bakım ve tedavi hizmeti ile ilgili seçenekler üretebilmek için yanık epidemiyolojisi her bölge için ayrı ayrı incelenmelidir (19).

Bu çalışmanın amacı ülkemizin en yoğun nüfuslu ikinci ve üçüncü şehrinde faaliyet gösteren iki referans yanık merkezinde 2019, 2020 ve 2021 yıllarında yatarak tedavi görmüş olan hastaların bazı demografik özelliklerini, etiyolojik faktörleri, yanık merkezlerini hem kendi içindeki hem de üç yıllık toplam verileri karşılaştırmalı olarak değerlendirerek bu iki yanık merkezi ile ilgili epidemiyolojik bilgileri analiz etmek ve yanık travmalarını önleme amaçlı seçenekler sunmaktır.

GEREÇ ve YÖNTEM

Ankara Bilkent Şehir Hastanesi Erişkin Yanık Merkezi (ABŞHEYM) ve İzmir Bozyaka Eğitim ve Araştırma Hastanesi Yanık Merkezi (İBEAHYM)'nde 2019, 2020 ve 2021 yıllarında yatarak tedavi görmüş olan toplam 1839 hastanın yanık merkezlerinden elde edilen veriler retrospektif olarak incelendi. Cinsiyet dağılımı, yatarak tedavi gören hasta sayısı, %20 üzeri t TVYA olan hasta sayısı**,** yanık derecesi ve nedeni, mortalite oranı ve yatarak tedavi gören yabancı uyruklu hasta sayısını gösteren verilerin yanında yanık merkezleriyle ilgili olan başka bir yanık merkezine sevk edilen hasta sayısı ve yanık yoğun bakım yatak doluluk oranı gibi kayıtlı

veriler de değerlendirilmiştir. Çalışma için gerekli olan etik kurul onayı Yıldırım Beyazıt Üniversitesi Yenimahalle Eğitim ve Araştırma Hastanesi Klinik Araştırmalar Etik Kurulu'nca verilmiştir (25.10.2023 Karar No:E-2023-45).

Literatür araştırmalarımıza göre, çalışmamız iki yanık merkezinin verilerinin karşılaştırıldığı ülkemizde yapılan ilk çalışma olma özelliğini taşımaktadır.

ABŞHEYM 6'sı yanık yoğun bakım yatağı 14'ü yanık servis yatağı olmak üzere toplam 20 yatak ve İBEAHYM 4'ü yanık yoğun bakım yatağı 8'i yanık servis yatağı olmak üzere toplam 12 yatak kapasitesi ile hizmet veren ülkemizin referans yanık merkezleridir. Her iki yanık merkezinde de sorumlu hekim olarak genel cerrahi uzmanları, yanık yoğun bakım ve yanık ameliyathanelerinde anesteziyoloji ve reanimasyon hekimleri, yanık hemşireleri, psikologlar, diyetisyenler ve fizyoterapistler multidisipliner bir ekip olarak hizmet vermektedir. Yanık Hastaları genel olarak Ankara, İzmir ve çevre illerden 112 Acil Servis ambulanslarıyla (kara ve hava ambulansları) yanık merkezlerine sevk edilmiş olup, majör yanıklı hastaların bakımı konusunda her iki merkez de benzer deneyime sahiptir. Ülkemizde yanık tedavi birimleri ile ilgili bir yönetmeliğin bulunması yanık tedavisinde bir standart sağlamış olup yanık hastalarının koordinasyonu ve tedavisi konusunda örnek alınabilecek bir organizasyonun hayata geçirilmesinde önemli bir kılavuz olmuştur.

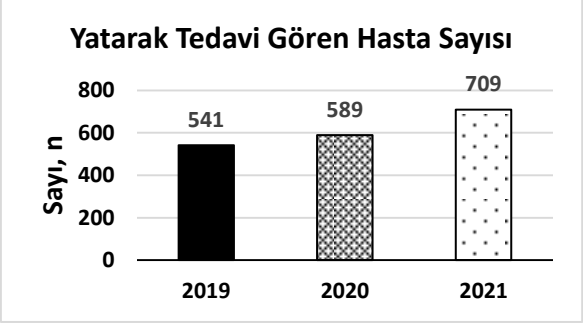
İstatistiksel analiz

Verilerin analizinde SPSS (Statistical Package for Social Sciences) 11.5 programından faydalanıldı. Tanımlayıcı olarak nitel değişkenler için hasta sayısı (yüzde) kullanıldı. İki nitel değişken arasındaki ilişki incelenmek istendiğinde ki-kare testi kullanıldı. İstatistiksel anlamlılık düzeyi $p<0,05$ olarak alındı.

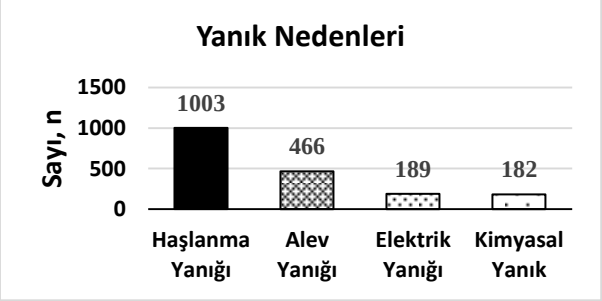
BULGULAR

İncelenen 3 yılda ABŞHEYM ve İBEAHYM'de yatarak tedavi gören toplam 1839 hastanın 1143'ünün (%62,1) erkek ve 696'sının (%37,9) kadın olduğu görülmüştür. Yıllara göre yatarak tedavi gören hasta sayısına bakıldığında 2019 yılında toplam 541 hasta, 2020 yılında toplam 589 hasta ve 2021 yılında ise toplam 709 hasta yatarak tedavi görmüştür (Şekil-1). Her iki yanık merkezinde 430'u (%60,4) erkek ve 283'ü (%39,6) kadın olmak üzere TVYA ≥%20 olan toplam 713 hasta yatarak tedavi görmüştür. 1019 (%55,4) hasta 2.derece yanık ve 820 (%44,6) hasta ise 3.derece yanık tanısı ile yatırılarak tedavi edilmiştir.

Yanık merkezlerinde 3 yıllık süre incelendiğinde yanık nedeni olarak haşlanma (n=1003, %54,5), alev (n=466, %25,4), elektrik (n=189, %10,2), kimyasal (n=182, %9,9) yanık olduğu görülmüştür (Şekil-2). İncelenen 3 yılda toplam 223 yabancı uyruklu hasta tedavi edilmiş olup, yatarak tedavi görmüş olan hastalar içindeki oranının %12,1 olduğu anlaşılmıştır. ABŞHEYM ve İBEAHYM'den söz konusu yıllarda başka bir yanık merkezine sevk edilen hiçbir hasta kaydı olmadığı görülmüştür. Üç yılda yanık yoğun bakım yatak doluluk oranları sırasıyla ABŞHEYM'de % 56,5, %52,7 ve % 50,3 olurken İBEAHYM'de % 88,1, % 87,9, %89 olarak hesaplanmıştır.



Şekil-1. Yıllara göre yatarak tedavi görmüş olan toplam hasta sayısı



Şekil-2. Üç yıllık süre içinde görülen yanık nedenleri

Tablo-1'de ABŞHEYM'de yıllara göre değişim verilmiştir. Yanık derecesi için 2019-2021 yılı arası fark anlamlı bulunmuştur (p=0,050). 2019 yılında 2. derece yanık tanısı alan hasta oranı %93,8 iken bu oran 2021 yılında %96,9 olarak bulundu. Yanık nedeni bakımından 2019-2021 yılı arası fark anlamlı bulunmuştur (p=0,013). 2019 haşlanma nedenli yanık oranı %71,9, alev nedenli yanık oranı %10,9, elektrik nedenli yanık oranı %3,1 ve kimyasal nedenli yanık oranı %

14,1 iken bu oranlar 2021 yılında sırasıyla %68,4, %19,3, %3,3 ve %9,0 olarak bulunmuştur. Mortalite oranı bakımından 2019-2020 yılı arası fark anlamlı bulunmuştur (p=0,048). 2019 yılında mortalite oranı %2,3 iken bu oran 2020 yılında %5,8'di. 2019-2020 ve 2019-2021 yılları arasında yatarak tedavi gören yabancı uyruklu hasta sayısı bakımından anlamlı fark bulunmuştur (sırasıyla p=0,014 ve p=0,001). 2019 yılında yatarak tedavi gören yabancı uyruklu hasta oranı %7,0 iken bu oran 2020 yılında %2,5 ve 2021 yılında %1,9 olarak bulunmuştur.

Tablo-2'de İBEAHYM'de yıllara göre değişim incelenmiştir. Yanık derecesi için 2019-2021 ve 2020-2021 yılları arası farklar anlamlı bulunmuştur (sırasıyla p<0,001 ve p<0,001). 2019 yılında 2. derece yanık tanısı alan hasta oranı 2,8 iken bu oran 2020 yılında %3,5 ve 2021 yılında %30,1 olarak bulundu.

Tablo-3'de iki yanık merkezinde üç yıllık toplam olarak elde edilen veriler bakımından fark olup olmadığına bakılmıştır. İki yanık merkezi arasında yanık derecesi, yanık nedeni, mortalite oranı ve yatarak tedavi gören yabancı uyruklu hasta sayısı bakımından anlamlı fark bulunmuştur (p<0,05). ABŞHEYM'de 2. derece yanığa sahip hasta oranı %95,8 iken bu oran İBEAHYM'de %12,0 olarak bulunmuştur. ABŞHEYM'de haşlanma nedenli yanık oranı %70,6, alev nedenli yanık oranı %15,1, elektrik nedenli yanık oranı %4,4 ve kimyasal nedenli yanık oranı %9,9 iken bu oranlar İBEAHYM'nde sırasıyla %37,1, %36,3, %16,8 ve %9,8 olarak bulunmuştur. ABŞHEYM'de mortalite oranı %4,8 iken bu oran İBEAHYM'de %25,4 olarak bulunmuştur. ABŞHEYM'de yatarak tedavi gören yabancı uyruklu hasta oranı %3,5 iken bu oran İBEAHYM'de %21,4 olarak bulunmuştur.

Tablo-1. Yıllara göre ABŞHEYM'nin kendi içindeki değişimi.

Değişkenler		**2019**	**2020**	**2021**	**p değeri**[a]
Yatarak tedavi		n (%)	n (%)	n (%)	
	Kadın	96 (37,5)	89 (32,0)	160 (38,1)	AD
	Erkek	160 (62,5)	189 (68,0)	259 (61,9)	
TVYA≥%20					
	Kadın	14 (48,3)	8 (25,8)	10 (26,3)	AD
	Erkek	15 (51,7)	23 (74,2)	28 (73,7)	
Yanık şiddeti					
	2.derece	240 (93,8)	267 (96,0)	407 (96,9)	0,05[y]
	3.derece	16 (6,2)	11 (4,0)	13 (3,1)	
Yanık nedeni					
	Haşlanma	184 (71,9)	203 (73,0)	287 (68,4)	
	Alev	28 (10,9)	35 (12,6)	81 (19,3)	0,013[y]
	Elektrik	8 (3,1)	19 (6,8)	14 (3,3)	
	Kimyasal	36 (14,1)	21 (7,6)	38 (9,0)	
Mortalite					
	Yok	250 (97,7)	262 (94,2)	397 (94,5)	0,048[x]
	Var	6 (2,3)	16 (5,8)	23 (5,5)	
Uyruk					
	TC	238 (93,0)	271 (97,5)	412 (98,1)	0,014[x], 0,001[y]
	Yabancı	18 (7,0)	7 (2,5)	8 (1,9)	

Kısaltmalar: TYVA, toplam yanık vücut alanı; **AD,** anlamlı değil

a: Ki-kare testi, x: 2019 vs 2020, y: 2019 vs 2021, z: 2020 vs 2021.

Tablo-2. Yıllara göre İBEAHYM 'nin kendi içindeki değişimi.

Değişkenler		2019	2020	2021	p değeri[a]
Yatarak tedavi		n (%)	n (%)	n (%)	
	Kadın	106 (37,0)	128 (41,0)	118 (40,8)	AD
	Erkek	179 (63,0)	184 (59,0)	171 (59,2)	
TVYA≥%20					
	Kadın	75 (40,3)	94 (41,2)	82 (40,8)	AD
	Erkek	111 (59,7)	134 (58,8)	119 (59,2)	
Yanık şiddeti					
	2.derece	8 (2,8)	11 (3,5)	86 (30,1)	$0,001^{y,z}$
	3.derece	277 (97,2)	301 (96,5)	202 (69,9)	
Yanık nedeni					
	Haşlanma	118 (41,4)	102 (32,7)	109 (37,7)	AD
	Alev	90 (31,6)	123 (39,4)	109 (37,7)	
	Elektrik	47 (16,5)	55 (17,6)	46 (15,9)	
	Kimyasal	30 (10,5)	32 (10,3)	25 (9,7)	
Mortalite					
	Yok	207 (72,6)	235 (75,3)	219 (75,8)	AD
	Var	78 (27,4)	77 (24,7)	70 (24,2)	
Uyruk					
	TC	228 (80,0)	246 (78,8)	222 (76,8)	$0,014^{x}$ $0,001^{y}$
	Yabancı	57 (20,0)	66 (21,2)	67 (23,2)	

Kısaltmalar: TYVA, toplam yanık vücut alanı; **AD,** anlamlı değil

a: Ki-kare testi, x: 2019 vs 2020, y: 2019 vs 2021, z: 2020 vs 2021.

Tablo-3. İki yanık merkezinin 3 yıllık toplu verilerle karşılaştırılması.

Değişkenler		ABŞHEYM	İBEAHYM	p değeri[a]
Yatarak tedavi		n (%)	n (%)	
	Kadın	345 (36,2)	351 (39,7)	AD
	Erkek	609 (63,8)	534 (60,3)	
TVYA≥%20				
	Kadın	32 (32,7)	251 (40,8)	AD
	Erkek	66 (67,3)	364 (59,2)	
Yanık şiddeti				
	2.derece	914 (95,8)	105 (12,0)	0,001
	3.derece	40 (4,2)	780 (88,0)	
Yanık nedeni				
	Haşlanma	674 (70,6)	329 (37,1)	0,001
	Alev	144 (15,1)	322 (36,3)	
	Elektrik	41 (4,4)	148 (16,8)	
	Kimyasal	95 (9,9)	87 (9,8)	
Mortalite				
	Yok	909 (95,2)	661 (74,6)	0,001
	Var	45 (4,8)	225 (25,4)	
Uyruk				
	TC	921 (96,5)	696 (78,6)	0,001
	Yabancı	33 (3,5)	190 (21,4)	

Kısaltmalar: TYVA, toplam yanık vücut alanı; **ABŞHEYM**, Ankara Bilkent Şehir Hastanesi Erişkin Yanık Merkezi; **İBEAHYM,** İzmir Bozyaka Eğitim ve Araştırma Hastanesi Yanık Merkezi; **AD,** anlamlı değil

a: Ki-kare testi

TARTIŞMA

Yanık Bakım Üniteleri ilk olarak 1950'lerde ABD ve İngiltere'de yanık hastalarına özel tedaviyi geliştirmek ve epidemiyolojik katılım indekslerini iyileştirmek amacıyla kurulmuştur (20). Ülkemizde de 2012 yılından sonra yanık tedavi birimlerinin sayısında artış olmuş ve yanık tedavi hizmetlerinde hızlı bir gelişme yaşanmıştır. ABŞHEYM İç Anadolu bölgesinin, İBEAHYM ise Ege Bölgesinin en gelişmiş tedavi olanakları bulunan iki referans yanık merkezidir.

Bu çalışmamızda iki referans yanık merkezinin 2019, 2020 ve 2021 yılı kayıtları incelendi ve bazı demografik ve epidemiyolojik veriler analiz edilerek sunuldu.

Verileri analiz edilen 3 yılda yatarak tedavi gören hasta sayısının her yıl arttığı ve yatarak tedavi gören hasta sayısı bakımından erkek cinsiyetin baskın olduğu tespit edilmiştir. Yatarak tedavi gören toplam 1839 yanık hastasının 1143'ü (%62,1) erkek ve 696'sı (%37,9) kadındı. Erkek/Kadın oranı 1,6/1 idi. Değerlendirilen yanık merkezleri özelinde de yatarak tedavi gören erkek hasta sayısı kadınlara oranla daha fazla idi. ABŞHEYM'de yatarak tedavi gören hastaların 609'u (%63,8) erkek 345'i (%36,2) kadın, İBEAHYM'de de 534'ü (%60,3) erkek 351'i (%39,7) kadındı. Bu bulguyla paralellik gösteren çeşitli çalışmalar mevcuttur (21-23). Farklı olarak, literatürde kadın baskınlığının olduğu sonuçlar da bulunmaktadır (24, 25). Erkek prevalansının daha yüksek olmasının nedeninin sanayi tesisleri, endüstriyel işletmeler ve elektrik dağıtım şirketleri gibi daha ağır çalışma koşulları olan iş kollarında hizmet vermeleri olduğunu düşünüyoruz.

%20 ve üstünde TVYA olan yanık vakaları majör (ciddi) yanık olarak sınıflandırılmaktadır (26, 27). Çalışmamızda her iki yanık merkezi toplam hasta sayısı göz önüne alındığında erkeklerin (%60,4) kadınlara (%39,6) oranla daha sık majör yanık travmasına maruz kaldığı görülmüştür. Bu bulgu erkeklerin kadınlara oranla daha aktif bir çalışma hayatları olduğu ve buna bağlı olarak yanık risk faktörlerine maruz kalma olasılıklarının daha yüksek olduğu düşüncesini desteklemektedir (28).

İki yanık merkezinin 3 yıllık toplam verilerine bakıldığında 2.derece yanıkların (%55,4) 3.derece yanıklardan daha fazla olduğu saptanmıştır ve ülkemizde yapılan iki çalışmanın sonucu ile uyumludur (29, 30).

Yanık merkezlerinde 3 yıllık toplam süreç incelendiğinde hastaların en fazla haşlanma yanığına, sonra sırasıyla alev yanığına, elektrik yanığına ve kimyasal yanığa maruz kaldıkları anlaşılmıştır (%54,5, %25,4, %10,2 ve %9,9).Bulgularımız, ülkemizde Marmara bölgesinde yapılan bir çalışmanın sonuçlarıyla benzerlik göstermektedir (31).

Her iki yanık merkezinden söz konusu yıllarda başka bir yanık merkezine sevk edilen hiçbir hasta olmamıştır. Bu bulgu sevindirici bir bulgudur çünkü diğer yanık merkezlerine gereksiz sevkler yapılmamış, böylece yatak işgali ve işgücü bölünmesi engellenmiştir.

ABŞHEYM yanık yoğun bakım yatak doluluk oranları sırayla üç yıllık zaman süresince yaklaşık olarak %50'ler civarında seyrederken İBEAHYM'de ise yaklaşık %85'ler civarında kaydedilmiştir. Türkiye'de yapılan bir çalışmada İBEAHYM yanık yoğun bakım yatak doluluk oranlarına yakın bir oran (%74,7) raporlanmıştır (32).

Yıllara göre ABŞHEYM'nin verileri kendi içinde değerlendirildiğinde ABŞHEYM'nin verileri kendi içinde yıllara göre değerlendirildiğinde; 2021 yılında 2019 yılına göre 2.derece yanık vakalarının arttığı, 2021 yılında 2019 yılına göre haşlanma nedenli yanıkların azaldığı buna karşın alev yanıklarının arttığı, kaba mortalite oranının 2019'da %2,3 ken 2020'de %5,8' e yükseldiği ve yatarak tedavi gören yabancı uyruklu hasta sayısının her yıl azaldığı görülmüştür (Tablo-1).

Yıllara göre İBEAHYM'nin verileri kendi içinde değerlendirildiğinde; verilerin birçoğunun yıllar içinde anlamlı bir farklılığa uğramadığı, sadece yanık derecesi için 2019-2021 ve 2020- 2021 yılları için anlamlı bir fark olduğu gözlenmiştir.2019'da %2,8 olan 2.derece yanık teşhisi olan hasta oranı 2020'de %3,5'a ve 2021'de ise %30,1'e yükselmiştir (Tablo-2).

İki yanık merkezinin üç yıllık toplam verileri karşılaştırıldığında; ABŞHEYM'de 2. derece yanığa sahip hasta oranının anlamlı derecede yüksek olduğu, ABŞHEYM'de haşlanma nedenli yanık oranının, buna karşılık İBEAHYM'de ise alev nedenli yanık oranının anlamlı şekilde yüksek olduğu, kaba mortalite oranının İBEHYM'de (%25,4) ABŞHEYM'ye (%4,8) göre yaklaşık 5 kat daha yüksek olduğu ve İBEAHYM'de yatarak tedavi gören yabancı uyruklu hastanın anlamlı derecede yüksek olduğu bulunmuştur. (Tablo-3). Bu bulgular yanık travmalarını önlenmesi ve tedavisinde daha etkili yöntemlere ihtiyaç duyulduğunu göstermektedir.

İBEAHYM'de kaba mortalite oranının (%25,4) anlamlı derecede yüksek olmasının bu yanık merkezinde yatarak tedavi gören hastaların ağırlıklı olarak 3.derece yanık (%88) tanısıyla tedaviye alınmış olması ve yatarak tedavi gören hastaların yanık nedeninin %36,1 oranında alev yanığı olmasından kaynaklandığını tahmin ediyoruz. Türkiye'nin en kalabalık şehri olan İstanbul'da faaliyet gösteren bir yanık merkezinde yapılan çalışmada, ölüm oranının en fazla olduğu yanık nedeninin alev yanıkları olduğu sonucu bildirilmiştir (32).

Kısıtlılıklar

ABŞHEYM'ye, aynı hastanede pediyatrik yanık vakalarına hizmet veren başka bir yanık tedavi birimi bulunduğu için sadece erişkin hasta alınması, buna karşılık İBEAHYM'de her yaş grubundan yanık hastasının takip edilmesinden dolayı yaş parametresi çalışmamıza dahil edilememiştir. Bunun yanında ortalama yatış süresi verilerinin eksik tutulması ve sağlıklı bir şekilde elde edilememesi yüzünden ortalama yatış süresi ile ilgili analizler de yapılamamıştır.

SONUÇ

Yanık travması bir canlının hayatında yaşayabileceği en ciddi travmalardan biridir. Yanık yaralanmalarının fiziki, psikososyal ve maddi birçok olumsuz sonuçları olabilmektedir. Ülkemizde yanık tedavi birimlerinin sayısı 2012 yılından itibaren yüz güldürücü şekilde artmıştır. Yanık Merkezleri ciddi yanık vakalarının güncel tedavi yöntemleri uygulanarak takip edildiği en donanımlı birimlerdir. Yanık epidemiyolojisine yönelik çalışmalar, mevcutta olan yanık yaralanmalarını önlemeye yönelik tedbirlerin etkisini değerlendirmek için çok önemlidir.

Epidemiyolojik veriler yanık travmalarının sıklığını azaltmak amacıyla bir önleme programı planlamak, yanık tedavisinde etkili bir klinik süreç belirlemek ve yeni seçenekler tasarlamak için değerli bilgiler sağlar (31). Bu epidemiyolojik verilere dayanarak yanıkla ilgilenen uzmanlar risk altındaki nüfusa yanık güvenliği ile ilgili bilgiler verebilir.

İnsanlar günlük hayatta dikkatli olur ve uygun şekilde eğitilirse yanık travmalarının çoğu önlenebilir. Bu nedenle, mevcut yanık önleme programları güncel durum göz önüne alınarak yenilenmeli ve gelecekteki önlemler ebeveynlerin, küçük çocukların ve yaşlıların yanık yaralanmaları konusundaki farkındalığını artırmalıdır.

Bu çalışmamızda amacımız ülkemizin ikinci ve üçüncü büyük şehrinde faaliyet gösteren iki referans yanık merkezinde 2019, 2020 ve 2021 yıllarındaki verileri analiz edilerek epidemiyolojik bazı bilgilere ulaşmaktı. Türkiye'de hizmet veren yanık tedavi birimlerinde bütün verilerin sağlıklı ve düzenli bir şekilde kayıt altına alınması gelecekte yapılacak araştırmalarda yol gösterici olacaktır. Çalışmamızda ortaya çıkan bulgularla gelişmekte olan ulusal yanık bilgi havuzuna katkıda bulunmayı umuyoruz.

Çıkar çatışması: Yazarlar herhangi bir çıkar çatışması beyan etmemektedir.

Teşekkür: Çalışmanın istatistiki verilerinin analizinde yaptığı katkılardan dolayı Batuhan Bakırarar'a teşekkür ederiz

Kaynaklar

1. Hettiaratchy S, Dziewulski P. ABC of burns: pathophysiology and types of burns. BMJ 2004;328:1427-9.
2. Barry Press. Grabb and Smith's plastic surgery, 5th ed. Philadelphia: Lippincott-Raven Publishers; 1997
3. Salvador-Sanza JF, Sanchez-Paya J, Rodriguez-Marin J. Quality of life of the Spanish burn patient. Burns 1999;25(7):593–8.
4. Ehde DM, Patterson DR, Wiechman SA, Wilson LG. Post-traumatic stress symptoms and distress 1 year after burn injury. J Burn Care Rehabil 2000;21(2):105–11.
5. Murray CJL, Lopez AD. The global burden of disease. a comprehensive assessment of mortality and disability from diseases injuries and risk factors in 1990 and projected to 2020 (Harvard University School of Public Health, 1996).
6. WHO. A WHO plan for burn prevention and care (World Health Organization, 2008).
7. Church D, Elsayed S, Reid O, Winston B, Lindsay R. Burn wound infections. Clin Microbiol Rev 2006;19:403–34.
8. Smolle C, et al. Recent trends in burn epidemiology worldwide: a systematic review. Burns. 2017;43:249–57.
9. Greenhalgh DG. Management of burns. N. Engl. J. Med 2019;380:2349–59.

10. Rybarczyk MM, Schafer JM, Elm CM, Sarvepalli S, Vaswani PA, Balhara KS, Jacquet GA. A systematic review of burn injuries in low-and middle-income countries: epidemiology in the WHO-defined African Region. African Journal of Emergency Medicine, 2017;7(1):30-7.
11. Türkiye'de Özellikli Planlama Gerektiren Sağlık Hizmetleri (2011-2023), Sayfa 244.
12. Koçman AE, Özgül M. Yanık patofizyolojisinde güncel gelişmeler. Turkiye Klinikleri Plastic Surgery-Special Topics 2016;5(2):7-12.
13. Arturson G. Forty years in burns research—the postburn inflammatory response. Burns 2000;26(7):599-604.
14. Gueugniaud PY, Carsin H, Bertin-Maghit M, Petit P. Current advances in the initial management of major thermal burns. Intensive Care Medicine 2000;26(7):848.
15. Zayakova Y, Vajarov I, Stanev A, Nenkova N, Hristov H. Epidemiological analysis of burn patients in East Bulgaria. Burns 2014;40(4):683-8.
16. Arslan H, Kul B, Derebaşınlıoğlu H, Çetinkale O. Epidemiology of pediatric burn injuries in Istanbul, Turkey. Turkish Journal of Trauma and Emergency Surgery 2013;19(2):123-6.
17. Kao CC, Garner WL. Acute burns. Plastic and Reconstructive Surgery 2000;105(7):2482-92.
18. Yanık Tedavi Birimleri Hakkında Yönetmelik, Resmi Gazete 30912 (8Ekim2019), md 4/1
19. Özçetin, B., Tihan, D., Demirci, H., Altıntaş, M. M., Arayıcı, V., & Taha, A. (2012). Yeni kurulan bir yanık merkezinde 2.5 yıllık deneyim. *Turkish Journal of Surgery*, *28*(3), 146-8.
20. Al-Mousawi AM, Mecott-Rivera GA, Jeschke MG, Herndon DN. Burn teams and burn centers: the importance of a comprehensive team approach to burn care. Clinics Plastic Surgery 2009;36(4):547.
21. Lindblad BE, Mikkelsen SS, Larsen TK, Steinke MS. A comparative analysis of burn injuries at two burns centres in Denmark. Burns 1994;20(2):173-5.
22. Chien WC, Pai L, Lin CC, Chen HC. Epidemiology of hospitalized burns patients in Taiwan. Burns, 2003;29(6):582-8.
23. Jie X, Baoren C. Mortality rates among 5321 patients with burns admitted to a burn unit in China: 1980–1998. Burns 2003;29(3):239-45.
24. Fernández-Morales E, Gálvez-Alcaraz L, Fernández-Crehuet-Navajas J, Gómez-Gracia E, Salinas-Martínez JM. Epidemiology of burns in Malaga, Spain. Burns, 1997;23(4):323-32.
25. Liu EH, Khatri B, Shakya YM, Richard BM. A 3-year prospective audit of burns patients treated at the Western Regional Hospital of Nepal. Burns 1998;24(2):129-33.
26. Toppi J, Cleland H, Gabbe B. Severe burns in Australian and New Zealand adults: Epidemiology and burn centre care. Burns 2019;45(6):1456-61.
27. Greenwood JE, Tee R, Jackson WL. Increasing numbers of admissions to the adult burns service at the Royal Adelaide Hospital 2001–2004. ANZ J Surg 2007;77(5):358-63.
28. Li H, Yao Z, Tan J, Zhou J, Li Y, Wu J, Luo G. Epidemiology and outcome analysis of 6325 burn patients: a five-year retrospective study in a major burn center in Southwest China. Scientific Reports 2017;7(1):46066.
29. Al B, Yıldırım C, Çoban S, Aldemir M, Güloğlu C. Mortality factors in flame and scalds burns: our experience in 816 patients. Ulus Travma Acil Cerrahi Derg 2009;15(6):599-606.
30. Kurtoğlu M, Alimoğlu O, Ertekin C, Güloğlu R, Taviloğlu K. Evaluation of severe burns managed in intensive care unit. Ulus Travma Acil Cerrahi Derg 2003;9(1):34-6.
31. Esen O, Güven M, Yıldırım A, Turgut HT, Tiryaki Ç, Yazicioglu MB, Esen HK. Epidemiology of burn injuries in the burn center. Southern Clinics of Istanbul Eurasia 2021;32(4):360-5.
32. Yüce Y, Kilavuz O. Profile of moderate and severe burns: Turkish experience in a tertiary care burn unit. The Ulutas Medical Journal 2018;4(1):25-31.

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Evaluating biomarkers for diagnosis and treatment monitoring in Gaucher Disease

Gaucher Hastalığında tanı ve tedavi izleminde biyobelirteçlerin değerlendirilmesi

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ABSTRACT

Aim: The primary goal of this study is to explore the impact of consistent treatment on key disease marker, Lyso-Gb1. Additionally, this research aims to evaluate the influence of splenectomy on Lyso-gb1 concentrations within the patient group.

Materials and Methods: 37 patients diagnosed with GD were categorized based on treatment compliance into three groups: 28 in the regular treatment group, who consistently followed their treatment; 6 in the irregular treatment group, with inconsistent treatment adherence; and 3 in the untreated group. A control group of 33 healthy individuals without GD was also included. Enzyme replacement therapy was utilized as the treatment regimen. The analysis of Lyso-Gb1 levels was performed using liquid chromatography coupled with tandem mass spectrometry, ensuring high precision in measurement.

Results: Lyso-Gb1 levels were significantly higher in GD patients compared to the healthy control group ($p < 0.05$), affirming its potential as a specific biomarker. Treatment was associated with a reduction in Lyso-Gb1 levels ($p < 0.05$). No significant difference in Lyso-Gb1 levels was observed between treated patients with Type 1 and Type 3 GD ($p > 0.05$). Notably, patients who underwent splenectomy exhibited significantly higher Lyso-Gb1 levels than those who did not ($p < 0.05$).

Conclusion: Our findings support the utility of Lyso-Gb1 as a specific biomarker for GD. While pre-treatment Lyso-Gb1 levels in the treated group remain unknown, our results underscore the need for larger, longitudinal studies to further elucidate Lyso-Gb1's role in monitoring disease progression and treatment efficacy in GD.

Keywords: Biomarker, Gaucher disease, glucosylsphingosine, Lyso-Gb1, lysosomal storage disorders.

ÖZ

Amaç: *Bu çalışmanın temel amacı düzenli tedavinin anahtar hastalık belirteci Lyso-Gb1 üzerindeki etkisini araştırmaktır. Ayrıca bu araştırma, hasta grubunda splenektominin Lyso-Gb1 konsantrasyonları üzerindeki etkisini değerlendirmeyi amaçlamaktadır.*

Gereç ve Yöntem: *Gaucher Hastalığı (GH) tanısı alan 37 hasta tedavi durumuna göre üç gruba ayrıldı: Tedavilerini tutarlı bir şekilde takip eden düzenli tedavi grubunda 28; düzensiz tedavi grubunda 6 ve henüz tedavi almayan grupta 3 hasta mevcuttu. GH olmayan 33 sağlıklı bireyden oluşan bir kontrol grubu da dahil edildi. Tedavi rejimi olarak enzim replasman tedavisi kullanıldı. Lyso-Gb1 seviyelerinin analizi, ölçümde yüksek hassasiyet sağlayan tandem kütle spektrometresi ile birleştirilmiş sıvı kromatografisi kullanılarak gerçekleştirildi.*

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Bulgular: *Lyso-Gb1 seviyeleri Gaucher hastalarında sağlıklı kontrol grubuyla karşılaştırıldığında anlamlı derecede yüksekti (p<0.05), bu da bunun spesifik bir biyobelirteç olma potansiyelini doğruluyor. Tedavi, Lyso-Gb1 seviyelerinde bir azalma ile ilişkilendirildi (p<0.05). Tedavi edilen Tip 1 ve Tip 3 GH arasında Lyso-Gb1 düzeyleri açısından anlamlı bir fark gözlenmedi (p>0,05). Özellikle splenektomi yapılan hastalarda, yapılmayanlara göre anlamlı derecede daha yüksek Lyso-Gb1 seviyeleri sergilendi (p<0.05).*

Sonuç: *Bulgularımız Lyso-Gb1'in GH için spesifik bir biyobelirteç olarak kullanımını desteklemektedir. Tedavi edilen grupta tedavi öncesi Lyso-Gb1 seviyeleri bilinmemekle birlikte, sonuçlarımız Lyso-Gb1'in GD'de hastalığın ilerlemesini ve tedavi etkinliğini izlemedeki rolünü daha fazla aydınlatmak için daha büyük, boylamsal çalışmalara olan ihtiyacın altını çiziyor.*

Anahtar Sözcükler: *Biyobelirteç, Gaucher hastalığı, glukozilsfingozin, Lyso-Gb1, lizozomal depo hastalıkları.*

INTRODUCTION

Gaucher Disease (GD) stands as the most common lysosomal storage disorder, attributable to biallelic mutations in the GBA gene. These mutations disrupt the normal function of the β-glucocerebrosidase enzyme, essential for cleaving glucosylceramide into glucose and ceramide in unaffected individuals (1). Classified based on neurological involvement, GD manifests in three distinct forms: Type I (T1GD), Type II (T2GD), and Type III (T3GD) (2). Although the majority of Turkish patients have been reported to have T1GD, there are regional differences in Turkey (3-5). As the burden of GD is profound, the patients often experience severe symptoms across multiple somatic organs. However, these effects can be mitigated through treatments such as enzyme replacement therapy (ERT) or substrate reduction therapy (SRT), which have shown efficacy, particularly in the management of T1GD and T3GD (6).

Diagnosis typically follows clinical suspicion, with initial assessments focusing on reduced acid β-glucocerebrosidase activity in blood and/or tissues, and is confirmed through genetic analysis identifying pathogenic variants in the GBA gene (2). Traditional biomarkers for GD, including acid phosphatase, angiotensin-converting enzyme, ferritin, chitotriosidase, and chemokine ligand 18, have been employed for assessment and follow-up. However, their lack of disease specificity and sensitivity has been a significant limitation, often resulting in diagnostic uncertainty. The discovery of glucosylsphingosine (Lyso-Gb1), a deacylated derivative of glucosylceramide, has advanced the search for a definitive biomarker. Lyso-gb1 has been consistently elevated in individuals with GD, positioning it as a potential key indicator for diagnosing the disorder. Nonetheless, there is still a need for studies focusing on the specificity of Lyso-Gb1 in reflecting the presence of GD and its fluctuation in response to therapeutic interventions (7).

Given the necessity for tracking disease progression and gauging response to treatments, our research is dedicated to affirming the biomarker's clinical significance. Our investigation presents a thorough comparative study of the levels of several biomarkers in GD patients relative to healthy individuals. By employing both longitudinal and cross-sectional methods, we investigated the effects of treatment regularity on a variety of essential disease indicators, with an emphasis on Lyso-Gb1. The treatment regimen consisted of ERT. The indicators included measurements of Lyso-Gb1, platelet counts, hemoglobin, ferritin, and chitotriosidase levels. Furthermore, we assessed the effect of splenectomy on Lyso-GHb1 concentrations among the patient cohort.

Exploring the complexities of GD through the lens of our dataset reveals intriguing avenues for investigation, particularly in understanding how genotypic variations influence the clinical severity and treatment outcomes of this condition. One area of focus is the relationship between specific genotypes and the clinical manifestations of GD. It is hypothesized that patients exhibiting certain genotypic classifications, such as being homozygous for allele N370S, may present milder clinical symptoms and show more significant improvements in blood biomarkers following treatment compared to those with other genetic backgrounds. Furthermore, the timing of diagnosis poses another critical factor in the management and prognosis of the disease. It is posited that an early diagnosis could lead to

more effective disease management and better clinical outcomes, as evidenced by improvements in key blood biomarkers. Moreover, this dataset prompts an examination of potential gender-specific differences in both the clinical presentation of GD and the response to treatment, suggesting that male and female patients may experience and respond to the disease in distinct ways. Additionally, the study aims to delve into how specific genotypes correlate with baseline levels of certain biomarkers, such as chitotriosidase and Lyso-Gb1, and how these levels change in response to treatment across different genotypes. This multifaceted research approach not only seeks to unravel the genetic underpinnings of GD but also aims to enhance our understanding of its clinical implications and inform more tailored treatment strategies.

MATERIALS and METHODS

Categorization of Study Groups

The present case-control study encompassed seventy participants (Figure-1). The participants were divided into a control group (n=33) and a case group (n=37). The latter, comprising GD patients, was subdivided into four categories based on treatment adherence. These included: patients with Type 1 GD (T1GD) and Type 3 GD (T3GD) who received continuous treatment (termed the regular T1GD and regular T3GD groups, respectively), patients who had discontinued treatment for six months or longer, constituting the irregular T1GD group; and untreated patients, referred to hereafter as 'native' patients. This segmentation facilitated a detailed analysis aimed at identifying variations in clinical and biochemical parameters among different treatment statuses within the GD cohort. Of note, the case group included 32 patients with T1GD and five with T3GD.

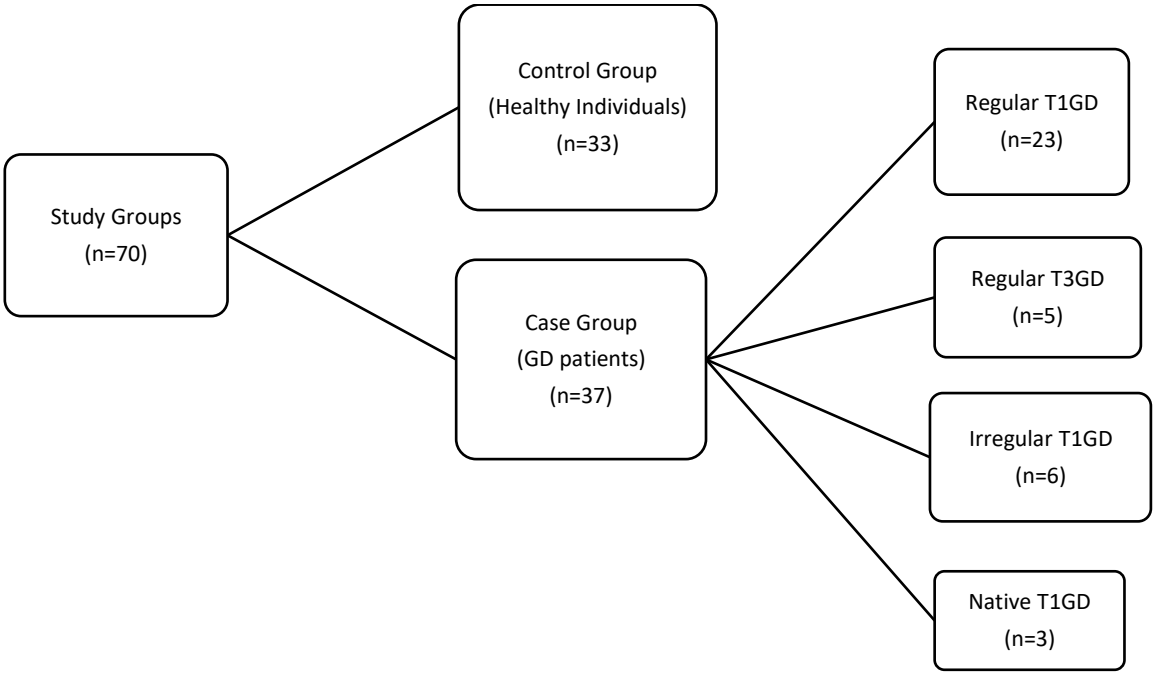

Figure-1. Categorization of study groups. *N: Number of individuals; GD: Gaucher's Disease; T1GD: Type1 Gaucher's Disease; T3GD: Type3 Gaucher's Disease.*

Demographic, Clinical and Laboratory Characteristics

The study examined various demographic, clinical, and laboratory parameters, including type of GD (T1GD or T3GD), gender, age at diagnosis age at treatment, genotypic classification based on alleles 1 and 2, type of treatment (imiglucerase or taliglucerase-α) and dosage of treatment (30 or 60 U/kg/q2wks; low or high dose, respectively), the occurrence of surgical intervention (splenectomy), the presence of comorbid diseases (none vs. one or more), and blood ferritin (F) level-1 and -2, blood hemoglobin (HB) level-1 and -2, white blood cells (WBCs)

count-1 and -2, blood platelets (PLT) count-1 and -2, blood chitotriosidase (C) level-1 and -2, blood lyso-Gb1 (L) level-1 and -2.

These parameters were systematically assessed to understand their interrelations and correlations with several outcomes. The second measurements were reported six months later than the first measurements.

Measurement of Plasma Lyso-Gb1 Levels by LC-MS/MS

In the study, high-purity glucosylsphingosine and lyso-lactosylceramide were used as primary and internal standards, respectively, sourced from reputable suppliers. The analytical process involved ultra-performance liquid chromatography (UPLC) using a Waters ACQUITY system with a specific methanol and formic acid mobile phase protocol. Mass spectrometry was performed using a Waters XEVO TQD system, employing enhanced ionization conditions and gas flows for precise quantification. The method developed by Ouyang et al. Has been modified and used (8). 100 µL of Lyso GB-2 (IS) (50 ng/mL, prepared in methanol) and 1 mL of methanol/acetone (V/V, 1/1) were added to 50 µL of plasma and vortexed for 30 seconds. The mixture is then centrifuged at 14000 g for 10 minutes at +4 °C. The supernatant is transferred to an LC-MS MS plate and evaporated under nitrogen gas and then reconstituted with 100 µL of methanol. After having vortex-mixed thoroughly for 30 seconds, a volume of 2 µL was injected into the LC-MS/MS system.

Statistical Analysis

Descriptive statistics such as mean, standard deviation, median, minimum and maximum values, as well as frequency and percentages, were calculated. The Kolmogorov-Smirnov and Shapiro-Wilk tests were applied to assess the normal distribution of the variables. Comparative analyses were performed using the Mann-Whitney U test for quantitative data, the Wilcoxon test for repeated measures, and the Chi-square test for qualitative data. Statistical analyses were conducted using SPSS version 25.0. Results with $p<0.05$ were considered as statistically significant.

RESULTS

Comparative Analysis of Lyso-Gb1 Levels Between Multiple Groups

The difference in Lyso-gb1 results between the healthy individuals (control) (n=33) and GD patients (case) (n=37) is statistically significant (Mann-Whitney U test, $p<0.001$) (Figure-2, Figure-3).

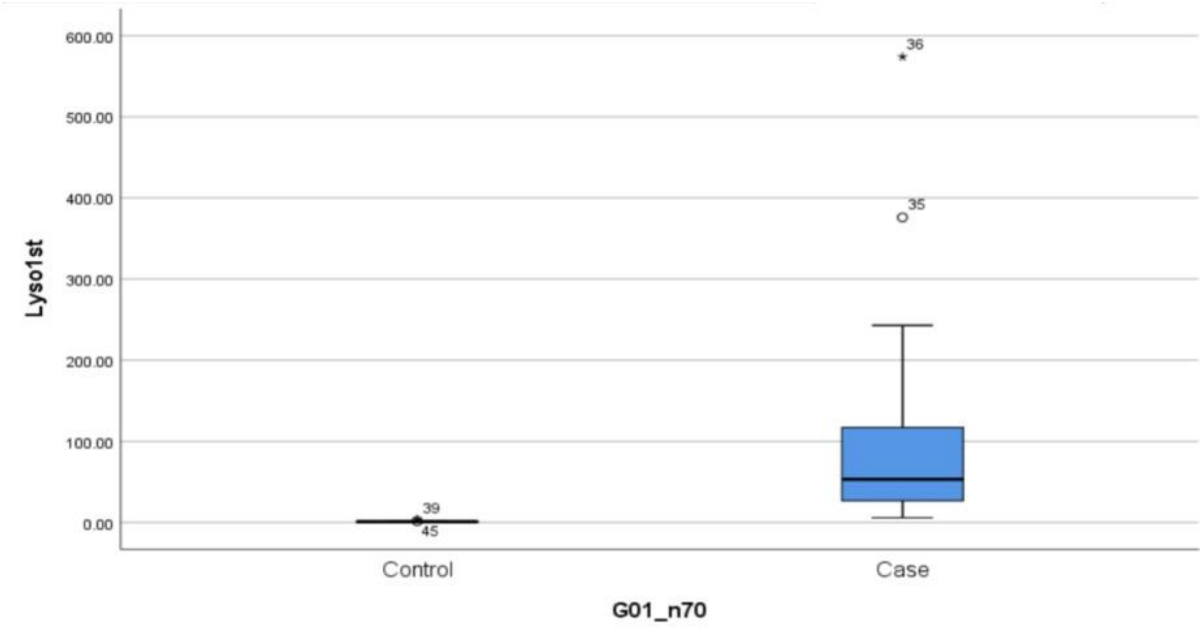

Figure-2. Comparison of Lyso-Gb1 levels between healthy individuals (control) and GD patients (case).

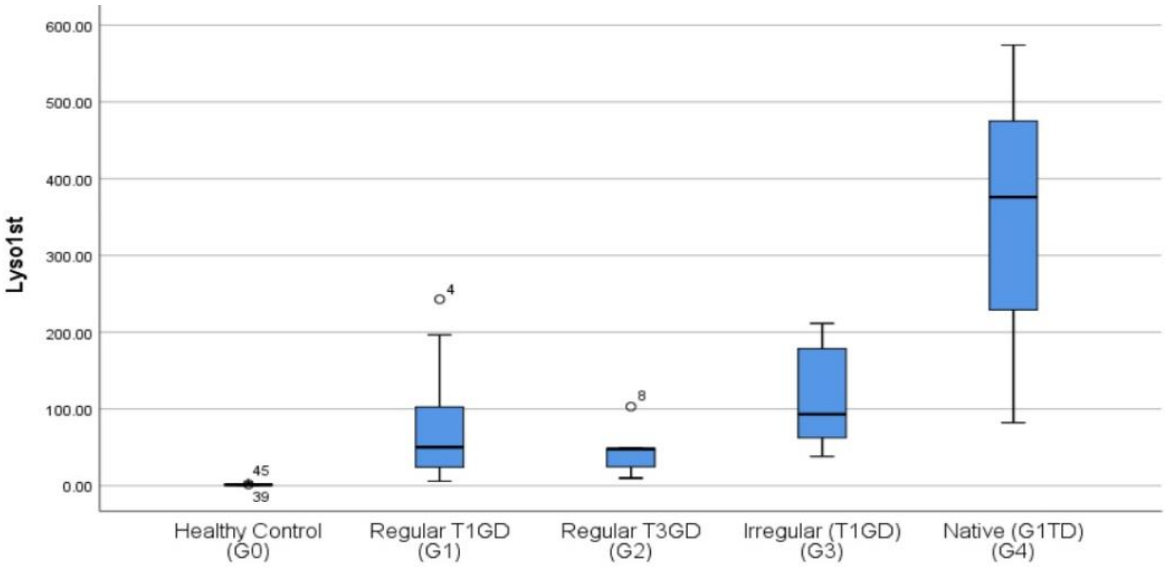

Figure-3. Comparative analysis of Lyso-Gb1 levels between multiple groups.

Comparative Analysis of Demographic and Clinical Features

Table-1 presents a comparative analysis of demographic and clinical features across study groups concerning treatment adherence. The groups are categorized by disease type, gender, surgical intervention, comorbid diseases, and genotype classification. The treatment adherence is divided into regular and irregular.

Table-1 shows that 71.4% of girls (20 out of 28) adhered regularly to treatment, whereas 28.6% of boys (8 out of 28) did so. In contrast, a higher proportion of boys showed irregular adherence (66.7%, 4 out of 6) compared to girls (33.3%, 2 out of 6). The p-value for gender is 0.076, which is above the conventional threshold of 0.05 for statistical significance. This suggests that there is no statistically significant difference in treatment adherence between boys and girls in this study.

In terms of disease types, T1GD and T3GD are compared. Of the patients with T1GD, 82.1% (23 out of 28) had regular adherence, and all patients with T1GD (6 out of 6) who were non-adherent were irregular. There were no patients with T3GD who were irregular in their adherence. The p-value here is 0.559, indicating no statistically significant difference between the disease types T1GD and T3GD in terms of treatment adherence.

When looking at surgical intervention, 25% of splenectomized patients (7 out of 28) were regular in their adherence, compared to 75% of non-splenectomized patients (21 out of 28). For irregular adherence, 16.7% were splenectomized (1 out of 6), and 83.3% were non-splenectomized (5 out of 6). The p-value is 1, signifying no statistical significance in adherence to treatment between splenectomized and non-splenectomized groups.

In brief, the data from Table-1 suggests no statistically significant association between treatment adherence and the demographic or clinical features studied, given the p-values are all above the standard threshold of 0.05. This means that the differences observed in adherence rates across gender, disease type, and surgical intervention are not statistically significant and could be due to chance.

The first measurement Lyso-Gb1 value in the splenectomy group was significantly ($p < 0.05$) higher than the non-splenectomy group. In the splenectomy group, the second measurement Lyso-Gb1 value did not show a significant change ($p>0.05$) compared to the first measurement. In the group without splenectomy, the second measurement Lyso-Gb1 value did not show a significant change ($p>0.05$) compared to the first measurement. The amount of Lyso-Gb1 change in the second measurement did not differ significantly ($p > 0.05$) in the groups with and without splenectomy (Table-2).

Table-1. Comparison of demographic, clinical, and genotype characteristics of GD patients.

		Gender		SI		CoM		Genotype Classification		
		Female	Male	NS	S	Unknown	One or more	A N370S +/+	B N370S +/- or -/+	C Non-N370S
Regular T1GD	Count	18	5	16	7	17	6	8_a	$14_{a, b}$	1_b
	Expected Count	16.1	6.9	18.0	5.0	16.2	6.8	5.6	13.7	3.7
	% within Group	78	22	70	30	74	26	35	61	4
Regular T3GD	Count	2	3	5	0	3	2	0_a	0_a	5_b
	Expected Count	3.5	1.5	3.9	1.1	3.5	1.5	1.2	3.0	0.8
	% within Group	40	60	100	0	60	40	0	0	100
Irregular T1GD	Count	2	4	5	1	3	3	0	6	0
	Expected Count	4.2	1.8	4.7	1.3	4.2	1.8	1.5	3.6	1.0
	% within Group	33	67	83	17	50	50	0	100	0
Native T1GD	Count	2	1	3	0	3	0	1	2	0
	Expected Count	2.1	0.9	2.4	0.6	2.1	0.9	0.7	1.8	0.5
	% within Group	67	33	100	0	100	0	33	67	0

*SI: Surgical Intervention, NS: Non-splenectomized, S: Splenectomized, CoM: Comorbidity. *Chi-square (X^2) test (Fisher's Exact: Gender p=0.103; Surgical Intervention p=0.468; Comorbidity p=0.488; Genotype Classification: p<0.001)*

Table-2. Comparison of laboratory test levels according to splenectomy.

Variable	Splenectomy (-)			Splenectomy (+)			p	
	Mean±sd		Median	Mean±sd		Median		
Lyso-Gb1								
First	43.8 ± 36.9		30.9	120.3 ± 77.0		117.1	***0.008***	m
Second	33.0 ± 36.6		21.8	111.3 ± 43.8		129.1	***0.001***	m
Variation	-5.3 ± 22.8		-2.6	-9.0 ± 50.5		2.0	0.545	m
Variation in groups p	0.145		w	1.000		w		

m Mann-Whitney u test / w Wilcoxon test

Pharmacological Treatment Distribution in Gaucher's Disease Patients

In the treatment cohort for GD, a total of thirty individuals received imiglucerase, while another four were administered taliglucerase-alfa. ERT was dosed between 30 and 60 units per kilogram biweekly (U/kg/q2wk) (Table-3).

The therapy was continued for an average duration of 26.8 months, with a standard deviation of 13.5 months, ranging from 6 to 36 months in length. Throughout the treatment period, no serious adverse effects were reported.

Table-3. Distribution of GD patients according to treatment regimens.

Treatment Regimen		Regular		Irregular		Total (N)
Agent Name	**Dose***	**N**	**Ratio (%)**	**N**	**Ratio (%)**	
Imiglucerase	30	12	71	5	23	17
	60	13	100	0	0	13
Taliglucerase-α	30	1	50	1	50	2
	60	2	100	0	0	2

**U/kg/q2wk*

Table-4. Results of clinical and laboratory features across GD patient groups according to treatment adherence.

Treatment adherence		Regular		Irregular		p	
Variable		**Mean±sd/n-%**	**Median**	**Mean±sd/n-%**	**Median**		
Age of diagnosis		17.4 ± 17.7	11.5	23.7 ± 15.9	20.5	0.222	[m]
Gender	Girl	20 71.4%		2 33.3%		0.076	[X²]
	Boy	8 28.6%		4 66.7%			
Gaucher	Type I	23 82.1%		6 100%			[X²]
	Type III	5 17.9%		0 0.0%			
Splenectomy	(-)	21 75.0%		5 83.3%		1.000	[X²]
	(+)	7 25.0%		1 16.7%			
Lyso-Gb1		63.7 ± 58.9	48.7	112.7 ± 67.7	93.0	**0.031**	[m]
WBC		6680 ± 2512	6010	8200 ± 2247	7020	0.140	[m]
PLT (x10³)		222.7 ± 61.0	231.5	171.0 ± 25.2	158.0	**0.026**	[m]
Hemoglobin		13.0 ± 1.4	12.7	14.2 ± 0.8	14.1	0.072	[m]
Ferritin		230.8 ± 333.3	92.5	386.8 ± 342.4	274.0	0.118	[m]
Chitoriosidase		49.4 ± 38.7	51.0	97.0 ± 10.4	91.0	**0.041**	[m]

[m] Mann-whitney u test/ [X²] Chi-square test

Tablo-5. Comparison of laboratory test levels according to *g*enotype classification.

	Genotype A N370S +/+ (n=9)		Genotype B N370S +/- or -/+ (n=19)		Genotype C Non-N370S (n=6)		p
	Mean±sd	**Median**	**Mean±sd**	**Median**	**Mean±sd**	**Median**	
Lyso-Gb1	56.66 ± 73.57	28.35	85.75 ± 63.66	28.35	48.93 ± 32.16	48.17	0.2485 [k]
WBC	6421 ± 2675	5290	7033 ± 2212	6730	7340 ± 3578	6590	0.6515 [k]
PLT (x10³)	214.113 ± 73.28	227.500	199.89 ± 50.92	189.00	266.80 ± 43.30	251.00	0.0701 [k]
Hemoglobin	13.46 ± 1.38	13.70	13.02 ± 1.552	12.95	13.30 ± 0.79	13.00	0.7773 [k]
Ferritin	235.0 ± 214.5	169.5	302.1 ± 405.7	133.0	71.33 ± 37.87	83.0	0.3616 [k]
Chitoriosidase	24.53 ± 34.92	9.5	67.35 ± 38.19	71.00	53.30 ± 27.67	62.00	**0.0484** [k]

[k] Kruskal Wallis test

Comparison of GD patient groups according to treatment adherence

There was no significant difference ($p > 0.05$) between the study groups that received regular treatment and those that did not receive regular treatment in terms of patients' age, gender characteristics, GD type, and splenectomy (Table-4).

Lyso-Gb1 in the group receiving regular treatment was significantly ($p < 0.05$) lower than the group not receiving regular treatment. WBC, hemoglobin, and ferritin did not show any significant difference ($p > 0.05$) between the groups receiving regular treatment and those not receiving regular treatment. PLT in the group receiving regular treatment was significantly ($p < 0.05$) higher than the group not receiving treatment. In the regular treated group, chitotriosidase was significantly ($p < 0.05$) lower (Table-4).

When the data obtained were compared according to genotypes, a significant difference was found between the groups only in chitotriosidase levels (Table-5). While chitotriosidase enzyme activity in genotype 1 was found to be significantly lower than that in genotype 2, no significant difference was found between genotypes A and C and genotypes B and C.

Comparison of Laboratory Test Levels Between Regular Treated T1GD and T3GD Patients

The Mann-Whitney U test outcomes offer a compelling narrative about how Type 1 and Type 3 Gaucher Disease patients differ, especially in terms of diagnostic and treatment timelines. Notably, the age at which patients are diagnosed is markedly different, as evidenced by a significant p-value of. 001. This significant variation extends to the initiation of treatment, with an equally significant p-value of. 001, suggesting a divergence in the onset of therapeutic intervention between the two disease types. Doses for both initial and subsequent therapies also diverge between patient groups, indicated by p-values below the. 05 significance level, although these differences are not as pronounced as those observed in age-related factors. In contrast, for Ferritin and Hemoglobin levels, and for White Blood Cells and Chitotriosidase levels, the analysis does not reveal any notable differences, with p-values not meeting the threshold for statistical significance. However, the Platelet Count stands at the cusp of significance with a p-value of. 044, hinting at a potentially noteworthy difference in platelet counts that may require additional scrutiny. Lastly, the p-value of. 569 for Lyso-Gb1 levels points to no significant difference in this biomarker among the two groups of GD patients (Table-6).

In the group receiving regular treatment, WBC, PLT, hemoglobin, ferritin, and chitotriosidase did not change significantly ($p > 0.05$) 6 months after treatment compared to first values, while Lyso Gb1 levels were found to be significantly lower ($p = 0.0074$) (Table-7).

Table-6. Comparison of laboratory test levels between regular treated T1GD and T3GD patients.

Disease type	**Gaucher Type I**				**Gaucher Type III**				**p**	
Variable	**Mean±sd**			**Median**	**Mean±sd**			**Median**		
Lyso-Gb1	67.4	±	62.8	50.12	46.72	±	35.45	47.57	0.600	[m]
WBC	6522	±	2278	5990	7340	±	3578	6590	0.603	[m]
PLT (x10³)	212.2	±	60.6	222.0	266.8	±	43.9	251.0	0.064	[m]
Hemoglobin	12.9	±	1.5	12.6	13.3	±	0.8	13.0	0.415	[m]
Ferritin	256.0	±	352.9	110.0	71.3	±	37.9	83.0	0.315	[m]
Chitoriosidase	48.4	±	41.5	44.5	53.3	±	27.7	62.0	0.634	[m]

[m] Mann-whitney u test

Table-7. Comparison of laboratory levels 6 months later in groups receiving regular treatment.

Variable	First Lyso-Gb1				Second Lyso-Gb1				p	
	Mean±sd			Median	Mean±sd			Median		
Lyso-Gb1	58.22	±	61.42	39.25	45.51	±	43.38	27.17	**0.0074**	w
WBC	6728	±	2547	6500	6966	±	2475	6520	0.585	w
PLT (x10³)	239.6	±	48.6	240.0	237.2	±	49.7	238.0	0.985	w
Hemoglobin	12.8	±	1.4	12.7	12.9	±	1.4	12.9	0.192	w
Ferritin	161.5	±	201.9	77.0	150.9	±	184.2	72.0	0.415	w
Chitoriosidase	47.8	±	35.4	51.0	47.1	±	37.4	50.0	0.583	w

Demographic and Clinical Features of Native Patients

Table-8 presents data on patients diagnosed with GD (T1GD) that received no treatment (G4, native patients). The list includes criteria such as age at diagnosis, age at treatment commencement, genotypic information, surgical history, and lyso-gb1 levels, a biomarker for GD.

The first patient was diagnosed at the age of 2 months but has not yet started treatment. Their genotype shows a combination of N370S and R463H alleles. They have not undergone splenectomy, and their lyso-gb1 level is recorded at 375.92 ng/mL.

The second patient was diagnosed at 19 years of age and began treatment at the same age. Their genotype is N370S/R159M. Similar to the first patient, they have not been splenectomized, and their lyso-gb1 level is considerably higher, at 574.00 ng/mL.

The third patient received their diagnosis at 62 years old, which is also when they started treatment. Their genotype is a homozygous N370S mutation. They have not undergone a splenectomy, and their lyso-gb1 level is 82.01 ng/mL, which is markedly lower than the levels observed in the younger patients.

All the data pertains to individuals with T1GD and none have had a splenectomy. The data also indicates variability in the age of diagnosis, treatment initiation, and lyso-gb1 levels among the patients. However, there is a considerable range in both age at diagnosis and Lyso-gb1 levels, with the youngest having not yet started treatment. The mutations vary across the patients, potentially correlating with the differences in Lyso-Gb1 levels.

Table-8. Demographic and clinical features of native patients.

Variable	**Patient-1**	**Patient-2**	**Patient-3**
Gender	Male	Female	Female
Disease Type	T1GD	T1GD	T1GD
Age at diagnosis	2 months	19 years	62 years
Ferritin Level	81	201	1624
Hemoglobin Level	12.5	11.5	11.3
WBC Count	8090	4570	4370
PLT Count (Nx10³)	170	63	131
Chitoriosidase Level	121	104	53
Lyso-Gb1 Level	375.92	574.00	82.01
Allel I	N370S	N370S	N370S
Allel II	R463H	R159M	N370S
SI	NS	NS	NS

GD: Gaucher Disease; WBC: White blood cells; PLT: Platelet; SI: Surgical Intervention.

DISCUSSION

In this study, we investigated whether Lyso-Gb1 has a role only in the diagnosis of GD or also has a role in disease monitoring that reflects treatment consistency. Our results show that in GD, Lyso-Gb1 is a reliable biomarker for diagnosis and treatment monitoring.

Our first remarkable finding is that the Lyso-Gb 1 level shows a correlation to treatment adherence. In the patient group compatible with treatment chitotriosidase and Lyso-Gb1 were found to be significantly lower than the group that did not comply with treatment. While there was no significant change in the WBC, PLT, hemoglobin, ferritin, and chitotriosidase levels in the patient group receiving treatment in our cohort, which was measured for the second time after 6 months, we found a significant change in only Lyso-Gb1 levels. Our second finding is Lyso-Gb1 levels appear to correlate according to splenectomy supported by our data from the comparison of laboratory test levels according to splenectomy.

Previous studies have found an association between Lyso-Gb1 levels and treatment adherence. While treatment interruptions have been known from case reports and a small case series (9-12) Cozma et al showed that Lyso-Gb1 increased in the group that had 'treatment holidays'. Continuing treatment with SRT may be an option in this patient group whose non-compliance with ERT. While ERT is a treatment method that can only be applied in hospitals under the supervision of a physician in our country, SRT is a per-oral treatment option. Dinur et al proved the important role of Lyso-Gb1 levels in the treatment decisions of patients with GD (13).

We presented in detail the laboratory and clinical features of three native patients from our study cohort. They have a considerable range in both age at diagnosis and Lyso-Gb1 levels. Although Lyso-Gb1 is not included among the criteria for starting treatment for GD within the legal rules in our country, it is legally among the criteria for starting treatment in Israel, which is the country where GD is most common (13, 14). We also believe that Lyso-Gb1 should be among the treatment initiation criteria based on the results we obtained from our study.

Genotype-phenotype correlation is well well-recognized in GD (3, 15). In our cohort, numerically higher plasma Lyso-Gb1 concentrations were observed in patients with non-N370S genotype; no statistically significant difference in Lyso-Gb1 concentration was observed between patients with different disease types or mutation types. Although there is a publication in the literature stating that Lyso-Gb1 level is related to genotype (16), there are also publications showing that there is no significant statistical relationship (17-19). In our cohort of limited size, genotype did not seem to be correlated with the disease course. Further studies that monitor more frequently and in large groups are needed to provide more clear information.

In line with most previous studies, Lyso-Gb1 levels were also correlated with splenectomy in our study population (7, 20). Tylki-Szymanska et al. Reported an interesting study data obtained from 64 GD. Lyso-Gb1 was not dependent on splenectomy status (19). It would be worth a further biochemical investigation of whether the levels of Lyso-Gb1 correlated non-correlated in splenectomized patients in prospective studies.

We have some limitations as follows. First, we did not have pre-treatment Lyso-Gb1 concentrations in the treated cohort. Second, the number of patients in our cohort was a small group of total GD patients in the world. Third, we did not have more measurements in a long follow-up period.

CONCLUSION

In summary, our study shows that Lyso-Gb1 is a promising marker in diagnosis and in evaluating the treatment periods. Since its association with the pathogenesis of the disease, Lyso-Gb1 must be considered when developing new strategies for treatment.

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

References

1. Brady R, Kanfer J, Bradley R, Shapiro D. Demonstration of a deficiency of glucocerebroside-cleaving enzyme in Gaucher's disease. The Journal of clinical investigation. 1966;45(7):1112-5.
2. Beutler E. Gaucher disease. The metabolic and molecular bases of inherited disease. 2001:3635-68.
3. Karaca E, Kalkan S, Onay H, Aykut A, Coker M, Ozkinay F. Analysis of the β-glucocerebrosidase gene in Turkish Gaucher disease patients: mutation profile and description of a novel mutant allele. Journal of Pediatric Endocrinology and Metabolism. 2012;25(9-10):957-62.
4. Gumus E, Karhan AN, Hizarcioglu-Gulsen H, Demir H, Ozen H, Temizel INS, et al. Clinical-genetic characteristics and treatment outcomes of Turkish children with Gaucher disease type 1 and type 3: A sixteen year single-center experience. European Journal of Medical Genetics. 2021;64(11):104339.
5. Bulut FD, Kör D, Şeker-Yılmaz B, Hergüner Ö, Ceylaner S, Özkınay F, et al. Four Gaucher disease type II patients with three novel mutations: a single centre experience from Turkey. Metabolic Brain Disease. 2018;33:1223-7.
6. Biegstraaten M, Cox T, Belmatoug N, Berger M, Collin-Histed T, Vom Dahl S, et al. Management goals for type 1 Gaucher disease: An expert consensus document from the European working group on Gaucher disease. Blood Cells, Molecules, and Diseases. 2018;68:203-8.
7. Murugesan V, Chuang WL, Liu J, Lischuk A, Kacena K, Lin H, et al. Glucosylsphingosine is a key biomarker of Gaucher disease. American journal of hematology. 2016;91(11):1082-9.
8. Ouyang Y, Chen B, Pan X, Wang Z, Ren H, Xu Y, et al. Clinical significance of plasma globotriaosylsphingosine levels in Chinese patients with Fabry disease. Experimental and therapeutic medicine. 2018;15(4):3733-42.
9. Elstein D, Abrahamov A, Hadas-Halpern I, Zimran A. Withdrawal of enzyme replacement therapy in Gaucher's disease. British journal of haematology. 2000;110(2):488-92.
10. Vom Dahl S, Poll LW, Häussinger D. Clinical monitoring after cessation of enzyme replacement therapy in M. Gaucher. British Journal of Haematology. 2001;113(4):1084-5.
11. Czartoryska B, Tylki-Szymańska A, Ługowska A. Changes in serum chitotriosidase activity with cessation of replacement enzyme (cerebrosidase) administration in Gaucher disease. Clinical Biochemistry. 2000;33(2):147-9.
12. Schwartz IVD, Karam S, Ashton-Prolla P, Michelin K, Coelho J, Pires RF, et al. Effects of imilglucerase withdrawal on an adult with Gaucher disease. British Journal of Haematology. 2001;113(4).
13. Dinur T, Bauer P, Beetz C, Cozma C, Becker-Cohen M, Istaiti M, et al. Contribution of Glucosylsphingosine (Lyso-Gb1) to Treatment Decisions in Patients with Gaucher Disease. International Journal of Molecular Sciences. 2023;24(4):3945.
14. Elstein D, Abrahamov A, Hadas-Halpern I, Meyer A, Zimran A. Low-dose low-frequency imiglucerase as a starting regimen of enzyme replacement therapy for patients with type I Gaucher disease. QJM: monthly journal of the Association of Physicians. 1998;91(7):483-8.
15. Alfonso P, Aznarez S, Giralt M, Pocovi M, Giraldo P. Mutation analysis and genotype/phenotype relationships of Gaucher disease patients in Spain. Journal of human genetics. 2007;52(5):391-6.
16. Mao X-Y, Burgunder J-M, Zhang Z-J, An X-K, Zhang J-H, Yang Y, et al. Association between GBA L444P mutation and sporadic Parkinson's disease from Mainland China. Neuroscience letters. 2010;469(2):256-9.
17. Ida H, Watanabe Y, Sagara R, Inoue Y, Fernandez J. An observational study to investigate the relationship between plasma glucosylsphingosine (lyso-Gb1) concentration and treatment outcomes of patients with Gaucher disease in Japan. Orphanet Journal of Rare Diseases. 2022;17(1):401.
18. Saville JT, McDermott BK, Chin SJ, Fletcher JM, Fuller M. Expanding the clinical utility of glucosylsphingosine for Gaucher disease. Journal of Inherited Metabolic Disease. 2020;43(3):558-63.
19. Tylki-Szymańska A, Szymańska-Rożek P, Hasiński P, Ługowska A. Plasma chitotriosidase activity versus plasma glucosylsphingosine in wide spectrum of Gaucher disease phenotypes–A statistical insight. Molecular Genetics and Metabolism. 2018;123(4):495-500.
20. Chipeaux C, de Person M, Burguet N, de Villemeur TB, Rose C, Belmatoug N, et al. Optimization of ultra-high pressure liquid chromatography–tandem mass spectrometry determination in plasma and red blood cells of four sphingolipids and their evaluation as biomarker candidates of Gaucher's disease. Journal of Chromatography a. 2017;1525:116-25.

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Mid-term outcomes of Osgood-Schlatter patients undergoing arthroscopic excision

Artroskopik eksizyon uygulanan Osgood-Schlatter hastalarının orta dönem sonuçları

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ABSTARCT

Aim: If surgical intervention is necessary for Osgood-Schlatter patients, a number of surgical techniques including open surgical, arthroscopic and bursoscopic procedures are available. The aim of this study was to evaluate the mid-term clinical results of patients who underwent arthroscopic excision with the diagnosis of OSD.

Materials and Methods: This study was modeled with a retrospective design.16 patients who underwent arthroscopic ossicle excision were included in this study. The Visual Analog Scale (VAS) Score, Tegner Activity Scale and Lysholm Knee Score forms were administered to the patients in order to compare their pre-operative and post-operative condition. In addition, complications such as infection, residual bone fragments, re-hospitalization or recurrence were evaluated and recorded.

Results: A total of 16 patients were included in the study, and of these patients, 11 (68.75%) were male and 5 (31.25%) were female. The mean age of the patients is 28.8 (20–41 ± 7) years. The mean follow-up period was 82.9 (61–108 ± 15) months. The mean time for return to sports-related training activities for all of the patients was 9.2 (8–11) weeks. The mean VAS decreased from 6.8 ± 1.1 points preoperatively, to 5.7 ± 1.3 at the final follow-up ($P < 0.001$). In addition, the mean Tegner Activity Level score improved from 5.7 ± 0.6 preoperatively to 7.8 ± 0.9 at the final follow-up ($P < 0.001$). The mean Lysholm Knee Scale score was 77.4 ± 4.6 points in the preoperative period, increasing to 97.7 ± 5.8 points at the final follow-up ($P < 0.001$). In one patient, recurrence occurred at the 105th postoperative month and revision surgery was performed.

Conclusion: Arthroscopic ossicle excision for OSD can be considered an adequate technique when the mid-term results are evaluated. Although rare, recurrence may occur after arthroscopic surgery.In order to demonstrate the superiority of the arthroscopic method over open surgical procedures, comparative studies containing long-term results are required.

Keywords: Osgood-Schlatter, knee, arthroscopy, arthroscopic excision.

ÖZ

Amaç: *Osgood-Schlatter hastalarında cerrahi müdahale gerekiyorsa açık cerrahi, artroskopik ve bursoskopik işlemler de dahil olmak üzere çok sayıda cerrahi teknik mevcuttur. Bu çalışmanın amacı Osgood-Schlatter tanısıyla artroskopik eksizyon uygulanan hastaların orta dönem klinik sonuçlarını değerlendirmektir.*

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***Gereç ve Yöntem**: Bu çalışma retrospektif olarak modellenmiştir. Artroskopik kemikçik eksizyonu yapılan 16 hasta çalışmaya dahil edilmiştir. Hastaların ameliyat öncesi ve ameliyat sonrası durumlarını karşılaştırmak amacıyla Görsel Analog Skala (VAS) Skoru, Tegner Aktivite Skalası ve Lysholm Diz Skoru formları uygulanmıştır. Ayrıca enfeksiyon, kalıntı kemik parçaları, yeniden hastaneye yatış veya nüks gibi komplikasyonlar da değerlendirilerek kaydedildi.*

***Bulgular:** Çalışmaya toplam 16 hasta dahil edildi ve bu hastaların 11'i (%68,75) erkek, 5'i (%31,25) kadındı. Hastaların ortalama yaşı 28,8 (20-41±7) yıldı. Ortalama takip süresi 82,9 (61-108 ± 15) aydı. Tüm hastaların sporla ilgili antrenman faaliyetlerine dönüş süresi ortalama 9,2 (8-11) haftaydı. Ortalama VAS ameliyat öncesi 6,8 ± 1,1 puandan son takipte 5,7 ± 1,3'e düştü (p<0,001). Ek olarak, ortalama Tegner Aktivite Düzeyi skoru ameliyat öncesi 5,7 ± 0,6'dan son takipte 7,8 ± 0,9'a yükseldi (p<0,001). Ortalama Lysholm Diz Skalası skoru ameliyat öncesi dönemde 77,4 ± 4,6 puan iken, son takipte 97,7 ± 5,8 puana yükseldi (p<0,001). Bir hastada postoperatif 105. ayda nüks gelişti ve revizyon ameliyatı uygulandı.*

***Sonuç:** Osgood-Schlatter hastalığı için artroskopik kemikçik eksizyonu, orta dönem sonuçları değerlendirildiğinde yeterli bir teknik olarak düşünülebilir. Artroskopik cerrahi sonrası nadir de olsa nüks meydana gelebilir. Artroskopik yöntemin açık cerrahi işlemlere üstünlüğünü ortaya koymak amacıyla uzun dönemli karşılaştırmalı çalışmalar yapılabilir.*

***Anahtar Sözcükler:** Osgood-Schlatter, diz, artroskopi, artroskopik eksizyon.*

INTRODUCTION

Osgood-Schlatter disease (OSD) is a traction apophysitis of the tibial insertion of the patellar tendon (1). It occurs as a result of repeated mechanical strain of the quadriceps femoris muscle on the tibial tubercle. OSD is manifested by painful inflammation at the point of attachment of the patellar tendon on the tibial tubercle (2).OSD patients are mostly asymptomatic, but roughly 25% of patients experience pain that occurs especially during and after physical activity and swelling around the tibial tubercle (3, 4). The aforementioned symptoms most often occur in males between the ages of 10–15, and in females between the ages of 8–13 (5). Although symptoms improve in most patients after the completion of skeletal maturation, these symptoms may persist and/or appear in a number of patients (6, 7).

Conservative therapy is carried out as the first step when symptoms appear. Conservative therapy for OSD consists of cold application, immobilization, rest, and adjunctive use of non-steroidal anti-inflammatory drugs (2, 4). In some patients, conservative treatment does not lead to adequate relief and a return to a normal level of activity. In these patients, surgical intervention may help to relieve pain and rehabilitate patients to a normal level of activity (8, 9). If surgical intervention is necessary, various surgical techniques have been suggested ranging from ossicle resection to fusion (10). There are a limited number of publications in the literature on the results of arthroscopic ossicle excision, which is one of these techniques. In most studies on this subject, open surgical procedures have been preferred for ossicle excision. Open surgical procedures cause irritation, especially in squatting and kneeling situations, due to the scar tissue located in the anterior part of the knee (11). Due to these negative effects, arthroscopic ossicle excision has become popular, especially in the last decade. It was thought that the arthroscopic excision technique would have advantages over the open technique in accelerating both cosmetic and functional recovery. In addition to these positive aspects, the fact that the arthroscopic technique enables ossicle excision and provides a limited opportunity for tubercleplasty that has been stated as one of the disadvantages of this technique (11). Apart from this basic information, there are a limited number of publications in the literature on the results of arthroscopic ossicle excision, which is one of these techniques.

The aim of this study was to evaluate the mid-term clinical results of patients who underwent arthroscopic excision with the diagnosis of OSD.

MATERIALS and METHODS

Patient Selection

This study was modeled with a retrospective design. The necessary permissions for the study were obtained from the ethics committee of our university. All patients signed an informed consent form for participation in this study.

Patients who underwent arthroscopic excision with a diagnosis of OSD between May 2011 and August 2014 were evaluated retrospectively. Patients who had knee surgery for another reason and had neurovascular pathology in the extremity that underwent surgery were not included in the study. A total of 17 patients met the inclusion criteria, and of these 17 patients, 1 did not volunteer to participate in the study.

All of the patients in the study diagnosed with OSD were primarily treated with conservative therapy, including cold application, elevation, rest and non-steroidal anti-inflammatory drug therapy. After providing the necessary information, arthroscopic excision treatment was recommended to patients whose pain persisted and activities were restricted despite 6 months of conservative treatment. Arthroscopic excision was performed on patients who accepted the operation in order to treat the OSD.

Surgical Procedure

All of the patients were operated on by the same surgeon (MU). The operations were performed under anesthesia after applying a pneumatic lower extremity tourniquet. Anteromedial portals, opened close to the patellar tendon, and anterolateral portals were used for imaging and operation. Patients were first checked for any additional intraarticular pathologies. No intraarticular pathology was present in any of the patients included in the study. After checking the intraarticular area, the retro-patellar and infrapatellar fat pads were debrided away with the help of a shaver to get a clearer image and expand the operation area. After the bone structure was reached, it was freed from soft tissue with the help of a shaver, punch, and radiofrequency. Then, the bone structure was exposed using a grasper. Complete exposure of the bone structure was confirmed intra-operatively via C-arm imaging. After the bone structure was exposed, debridement was applied to the anomalous parts of the retro-patellar surface of the patellar tendon. The disordered parts of the tibial tubercle were rearranged with the help of a burr. Then, the operation was finalized Figure-1.

Rehabilitation

All of the patients were given weight-bearing, joint range of motion, and quadriceps exercises, as tolerated, on the 1st day post-op. The patients were allowed to return to both daily activities and sports without any restrictions after 6 weeks.

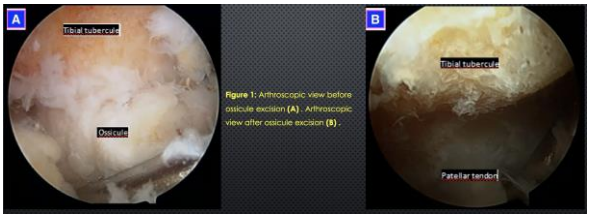

Figure-1. Arthroscopic view before ossicle excision (A). Arthroscopic view after ossicle excision (B).

Clinical Assessment

The Visual Analog Scale (VAS) Score (12), Tegner Activity Scale (13), and Lysholm Knee Score (14) forms were administered to the patients in order to compare their pre-operative and post-operative condition. In addition, complications such as infection, residual bone fragments, re-hospitalization or recurrence were evaluated and recorded Figure-2.

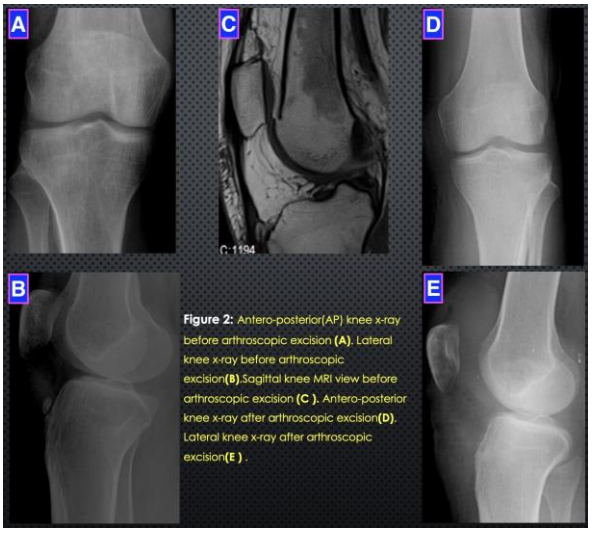

Figure-2. Antero-posterior (AP) knee X-ray before arthroscopic excision (A). Lateral knee X-ray before arthroscopic excision (B). Sagittal knee MRI section before arthroscopic excision (C). Antero-posterior (AP) knee X-ray after arthroscopic excision (D). Lateral knee X-ray after arthroscopic excision (E).

Statistical Analysis

Statistical analysis was performed with IBM SPSS Statistics for Windows 18.0 (IBM Corp., Armonk, NY, USA). The VAS score, Tegner Activity Level score, and Lysholm Knee Scale score preoperative and postoperative outcomes were compared and statistical parameters were calculated (mean, standard deviation, minimum, and maximum value). The t test was applied to determine statistical significance between the pre and postoperative scores.

RESULTS

A total of 16 patients were included in the study, and of these patients, 11 (68.75%) were male and 5 (31.25%) were female. The mean age of the patients is 28.8 (20–41 ± 7) years. The mean follow-up period was 82.9 (61–108 ± 15) months. None of the patients had any infection or residual ossicles. The patients' complaints were improved after surgery. Kneeling and squatting were possible after surgery in all of the patients. The mean time for return to sports-related training activities for all of the patients was 9.2 (8–11) weeks. The preoperative and postoperative mean scores of VAS, Tegner Activity Level Score and Lysholm Knee Scale score are shown in Table-1.

After all of the evaluations were completed, the patient who was not included in the study was admitted to our clinic stating that his preoperative complaints had reappeared. This patient was admitted to our clinic 105 months after the surgery was performed. Patients have some symptoms, pain that occurs during physical activity and swelling around the tibial tubercle. Direct radiography and MRI indicated that the patient may have a recurrence of OSD or heterotopic ossification that developed due to surgery. The symptoms (of the patient were not relieved after 6 weeks of conservative treatment. Then, the patient underwent excision through an open surgical procedure. The excised ossicle was submitted for pathological examination and it was concluded that the fragment was compatible with OSD. The patient's VAS Score was 7, Tegner Activity Scale score was 3, and Lysholm Knee Scale score was 61 before the second surgery. The patient returned to both daily and sports activities without any restrictions at 6 weeks postoperatively. The patient's VAS Score was 2, Tegner Activity Scale score was 5, and Lysholm Knee Scale score was 82 at 8 weeks postoperatively. Positive progress was made on all of the scores Figure-3.

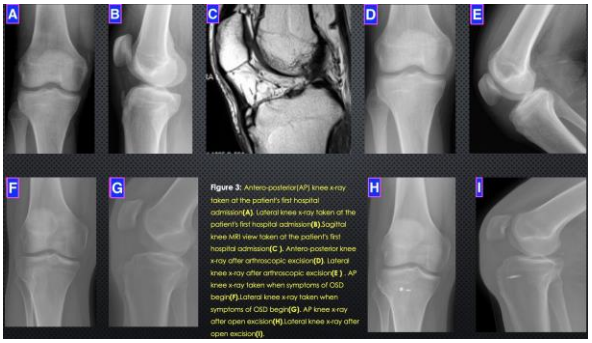

Figure-3. Antero-posterior (AP) knee X-ray taken at the patients first hospital admission (A). Lateral knee X-ray taken at the patients first hospital admission (B). Sagittal knee MRI section taken at the patients first hospital admission (C). Antero-posterior (AP) knee X-ray after arthroscopic excision (D). Lateral knee X-ray after arthroscopic excision (E). AP knee X-ray taken when symptoms of OSD begin (F). Lateral knee X-ray taken when symptoms of OSD begin (G). AP knee X-ray after open excision(H). Lateral knee X-ray after open excision (I).

DISCUSSION

OSD is a relatively common disease that especially affects young and adolescent populations (9). Symptoms mostly appear between the ages of 8–15 years, and the symptoms disappear with the completion of skeletal maturation (15). In patients whose symptoms do not improve and who do not respond to conservative treatment, surgical treatment methods can be utilized in order to restore knee function (15, 16). The 1990 study of Krause et al. showed that OSD does not cause a significant loss in activity in 76% of patients if left untreated, but only 60% of these patients can kneel painlessly (17). This may cause OSD to follow a worse symptomatic course and conservative treatment options to fail, especially in patients who need long-term knee hyperflexion or kneeling due to religious and sports activities.

The treatment options for OSD include different surgical procedures such as open surgical, arthroscopic and bursoscopic procedures (10).

Table-1. Patients preoperative and postoperative mean scores

Parameter	Preoperative (Mean±SD)	Postoperative (Mean±SD)	*p*
Visual Analog Pain Score	6,8±1,1	5,7±1,3	<0,005*
Tegner Activity Scale Score	5,7±0,6	7,8±0,9	<0,005*
Lysholm Knee Score	77,4±4,6	97,7±5,8	<0,005*

The most common approaches of open surgery are the excision of the ossicle and the excision of the prominence in the tibial tubercle (9, 16). In his article, which discussed the results of 35 patients who underwent ossicle excision and tibial Tubercleplasty using the open method, Flowers reported 95% pain relief (16). Similarly, In their study discussing the results of patients who underwent an open surgical procedure, Weiss et al. reported that 2 out of 15 of their total patients could not fully return to sports and daily activities, and 1 of them could not reach the pre-operative levels of activity[9]. El-Husseini et al. also achieved similar clinical results in 37 patients' knees that underwent excision with the open surgical technique that El-Husseini et al. defined themselves (18). Although clinically successful results were achieved, it was reported that sensitivity occurred in 10% of these patients, especially in the surgical incision area, during the postoperative period (9, 18, 19). Although the clinical scores improved, iatrogenic patellar tendon injury and surgical incision-related problems may occur during open surgery (20).

Arthroscopy is a less invasive surgical procedure that causes less intra-articular pathologies and thus it has also been used in the treatment of OSD (15, 21, 22). In their study discussing the results of arthroscopic treatment applied to 11 professional athletes, Circi et al. reported that there was a statistically significant improvement in the patients' Lysholm Knee Scale scores and Tegner Activity Level scores when compared to the pre-operative period, and that they returned to sports activities in a short period of 6 to 7 weeks postoperatively.(20). Circi et al. did not report any complications in the patients who experienced a positive progress in their clinical scores (20). There are case reports in the literature about OSD patients who have achieved successful results through arthroscopic excision using different arthroscopic surgical techniques (15, 22–25). In some of these case reports, giant ossicles were excised. Similar to the aforementioned studies, significant improvement in the clinical scores and complete return to daily/sports activities were achieved after surgery in all of the patients who underwent arthroscopic excision in the current study. It is thought that the full return to daily activities and sports being achieved at different times in different studies is related to the rehabilitation programs that were followed. This study and the data on this subject in the literature showed that successful results can be achieved with arthroscopic excision in OSD with less invasion and a lower complication rate.

In the current study, only one patient had recurrent symptoms, at 105 months postoperatively. No ossicles were observed in the radiographs of this patient taken after arthroscopic excision. No similar complication(recurrence) has been observed in the literature in any patient who underwent excision using arthroscopic technique (15, 20–25). It was shown in this study that recurrent ossicles may develop, albeit rarely, after arthroscopic excision. This complication, which is rarely encountered, was treated by excision with an open surgical procedure. However, this issue has been made open for discussion through this study. Should revision arthroscopy or open surgical procedures be preferred in the case of recurrence? In order to be able to answer this question, more informative entries into the literature are required.

This study had several limitations. The first of these was the fact that a small number of patients were included in the study. The second limitation was the lack of a control group for comparison with open surgical treatment. The other limitation is retrospective design of this study.

CONCLUSION

Arthroscopic ossicle excision for OSD can be considered an adequate technique when the mid-term results are evaluated. Although rare, recurrence may occur after arthroscopic surgery. In order to demonstrate the superiority of the arthroscopic method over open surgical procedures, comparative studies containing long-term results are required.

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

References

1. Lucenti L, Sapienza M, Caldaci A, De Cristo C, Testa G, Pavone V, et al. The Etiology and Risk Factors of Osgood-Schlatter Disease: A Systematic Review 2022. https://doi.org/10.3390/children9060826.
2. Cohen B, Wilkinson RW. The Osgood-Schlatter lesion; a radiological and histological study. Am J Surg 1958;95:731–42. https://doi.org/10.1016/0002-9610(58)90622-6.

3. Nkaoui M, el Alouani EM. Osgood-schlatter disease: risk of a disease deemed banal. Pan Afr Med J 2017;28. https://doi.org/10.11604/PAMJ.2017.28.56.13185.
4. Indiran V, Jagannathan D. Osgood-Schlatter Disease. N Engl J Med 2018;378:e15. https://doi.org/10.1056/NEJMICM1711831.
5. Gholve PA, Scher DM, Khakharia S, Widmann RF, Green DW. Osgood Schlatter syndrome. Curr Opin Pediatr 2007;19:44–50. https://doi.org/10.1097/MOP.0B013E328013DBEA.
6. Ladenhauf HN, Seitlinger G, Green DW. Osgood-Schlatter disease: a 2020 update of a common knee condition in children. Curr Opin Pediatr 2020;32:107–12. https://doi.org/10.1097/MOP.0000000000000842.
7. Vaishya R, Azizi AT, Agarwal AK, Vijay V. Apophysitis of the Tibial Tuberosity (Osgood-Schlatter Disease): A Review. Cureus 2016;8. https://doi.org/10.7759/CUREUS.780.
8. Glynn MK, Regan BF. Surgical treatment of Osgood-Schlatter's disease. J Pediatr Orthop 1983;3:216–9. https://doi.org/10.1097/01241398-198305000-00012.
9. Weiss JM, Jordan SS, Andersen JS, Lee BM, Kocher M. Surgical treatment of unresolved Osgood-Schlatter disease: ossicle resection with tibial tubercleplasty. J Pediatr Orthop 2007;27:844–7. https://doi.org/10.1097/BPO.0B013E318155849B.
10. Circi E, Atalay Y, Beyzadeoglu T. Treatment of Osgood–Schlatter disease: review of the literature. Musculoskelet Surg 2017;101:195–200. https://doi.org/10.1007/S12306-017-0479-7.
11. Eun SS, Lee SA, Kumar R, Sul EJ, Lee SH, Ahn JH, et al. Direct bursoscopic ossicle resection in young and active patients with unresolved Osgood-Schlatter disease. Arthroscopy 2015;31:416–21. https://doi.org/10.1016/J.ARTHRO.2014.08.031.
12. Yaray O, Akesen B, Ocaklioğlu G, Aydinli U. Validation of the Turkish version of the visual analog scale spine score in patients with spinal fractures. Acta Orthop Traumatol Turc 2011;45:353–8. https://doi.org/10.3944/AOTT.2011.2528.
13. Briggs KK, Kocher MS, Rodkey WG, Steadman JR. Reliability, validity, and responsiveness of the Lysholm knee score and Tegner activity scale for patients with meniscal injury of the knee. J Bone Joint Surg Am 2006;88:698–705. https://doi.org/10.2106/JBJS.E.00339.
14. Celik D, Coşkunsu D, Kılıçoğlu Ö. Translation and cultural adaptation of the Turkish Lysholm knee scale: ease of use, validity, and reliability. Clin Orthop Relat Res 2013;471:2602–10. https://doi.org/10.1007/S11999-013-3046-Z.
15. DeBerardino TM, Branstetter JG, Owens BD. Arthroscopic treatment of unresolved Osgood-Schlatter lesions. Arthroscopy 2007;23:1127.e1-1127.e3. https://doi.org/10.1016/J.ARTHRO.2006.12.004.
16. Flowers MJ, Bhadreshwar DR. Tibial tuberosity excision for symptomatic Osgood-Schlatter disease. J Pediatr Orthop 1995;15:292–7. https://doi.org/10.1097/01241398-199505000-00005.
17. Krause BL, Williams JP, Catterall A. Natural history of Osgood-Schlatter disease. Undefined 1990;10:65–8. https://doi.org/10.1097/01241398-199001000-00012.
18. El-Husseini TF, Abdelgawad AA. Results of surgical treatment of unresolved Osgood-Schlatter disease in adults. J Knee Surg 2010;23:103–7. https://doi.org/10.1055/S-0030-1267474.
19. Results of surgical treatment of unresolved Osgood-Schlatter lesion | Read by QxMD n.d. https://read.qxmd.com/read/11204962/results-of-surgical-treatment-of-unresolved-osgood-schlatter-lesion (accessed November 1, 2022).
20. Circi E, Beyzadeoglu T. Results of arthroscopic treatment in unresolved Osgood-Schlatter disease in athletes. Int Orthop 2017;41:351–6. https://doi.org/10.1007/S00264-016-3374-1.
21. Lee YS, Ahn JH, Chun D il, Yoo JH. A case of arthroscopic removal of symptomatic ossicle associated with Osgood-Schlatter disease in an athletic. European Journal of Orthopaedic Surgery and Traumatology 2011;21:301–4. https://doi.org/10.1007/S00590-010-0697-2.
22. Beyzadeoglu T, Inan M, Bekler H, Altintas F. Arthroscopic excision of an ununited ossicle due to Osgood-Schlatter disease. Arthroscopy 2008;24:1081–3. https://doi.org/10.1016/J.ARTHRO.2007.03.010.
23. McDonough GR, Rossi MJ. Arthroscopic Resection of Symptomatic Tibial Tubercle Ossicles for Recalcitrant Osgood-Schlatter Disease Using a 2-Portal Technique. Arthrosc Tech 2022;11:e813–8. https://doi.org/10.1016/J.EATS.2021.12.041.
24. Tsakotos G, Flevas DA, Sasalos GG, Benakis L, Tokis A v. Osgood-Schlatter Lesion Removed Arthroscopically in an Adult Patient. Cureus 2020;12. https://doi.org/10.7759/CUREUS.7362.
25. Zhi-Yao LI. Arthroscopic Excision of a Huge Ununited Ossicle Due to Osgood-Schlatter Disease in an Adult Patient. J Orthop Case Rep 2013;3:4–7. https://doi.org/10.13107/JOCR.2250-0685.092.

Research Article / *Araştırma Makalesi*

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Results of patients who were followed up with special dressings without the use of drains after primary total knee arthroplasty

Primer total diz artroplastisi sonrası dren kullanılmadan özel pansumanla takip edilen hastaların sonuçları

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ABSTRACT

Aim: This study aimed to compare the clinical and functional outcomes of patients who used drains with those who used compressive dressings without drains after tourniquetless total knee arthroplasty (TKA) for primary gonarthrosis.

Materials and Methods: Between January 2019 and June 2023, 316 patients who had undergone total knee replacement were retrospectively evaluated. After excluding patients who used tourniquets, 120 patients who met the inclusion criteria were included in the study. Postoperative hemoglobin changes, early–late postoperative edema, pain, range of motion (ROM), clinical scores, and functional outcomes for 68 patients with drain (18 males and 50 females) were evaluated and compared with those of 52 patients without drain (11 males and 41 females).

Results: No significant difference in preoperative and postoperative hemoglobin values, 6th week Knee Society Score (KSS)–knee, and KSS-functional scores was observed between patients with and without drains ($p > 0.05$). Although a statistically significant difference in ROM was noted on the first postoperative day, no statistically significant difference in the postoperative second-week ROM was observed. Moreover, a significant difference in day 1 and 3 visual analog scale scores was observed between patients with and without drains ($p < 0.001$).

Conclusion: The findings of this study revealed that using a drain after primary TKA is not necessary. Although the clinical results of patients without a drain after TKA are similar to those of patients with a drain, patients can be treated and followed up with compressive dressings without a drain.

Keywords: Complication, compressive bandage, drain, total knee replacement.

ÖZ

Amaç*: Çalışmamızda primer gonartroz nedeniyle, turnikesiz total diz artroplastisi (TDA) yapılan hastalarda, dren kullanılan hasta grubuyla dren kullanılmayıp kompresif pansuman yapılan hasta grubunu klinik ve fonksiyonel olarak karşılaştırmayı amaçladık.*

Gereç ve Yöntem: *2019-Ocak ile 2023-Haziran tarihleri arasında total diz protezi gerçekleştirilen 316 hasta retospektif olarak değerlendirildi. Turnike kullanımı olan hastaların çalışma dışı bırakılması sonrasında dahil edilme kriterlerini içeren 120 hasta çalışmaya dahil edildi. Dren kullanılan 68 hasta (18 erkek,50 kadın) ile dren kullanılmayan 52 hastanın (11 erkek, 41 kadın) post-operatif hemoglobin değişiklikleri, cerrahi sonrası erken dönem ödemleri, ağrı durumları, hareket açıklıkları, klinik skorları ve fonskiyonel sonuçları değerlendirilerek birbiri ile karşılaştırıldı.*

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Bulgular: *Dren kullanımı olmayan hastalar dren kullanılan hastalarla karşılaştırıldığında hastaların pre-operatif ve post-operatif dönem hemoglobin değerlerinde, 6. hafta Knee Society Score (KSS)-diz ve KSS-fonksiyonel skorlarında anlamlı bir fark olmadığı görüldü. (p>0.05) Post-operatif 1. gün range of motion (ROM)'larında istatistiksel olarak anlamlı fark olmakla beraber post op 2. hafta ROM'ları arasında istatistiksel olarak bir fark gözlenmedi. Dren kullanılmayan hastaların 1. gün ve 3. gün Visual Analogue Scale (VAS) skorlarında dren kullanılanlara göre anlamlı fark olduğu izlendi (p <0.001).*

Sonuç: *Bu çalışmayla, primer TDA sonrası dren kullanımının mutlak gereklilik olmadığı sonucuna varılmıştır, Total diz artroplastisi sonrası dren kullanılmayan hastaların klinik sonuçları dren kullanılanlarla benzer olmakla beraber dren kullanılmadan kompresif pansuman yapılarak, hastaların tedavisi ve takibi mümkündür.*

Anahtar Sözcükler: *Dren, kompresif bandaj, komplikasyon, total diz protezi.*

INTRODUCTION

Total knee replacement surgery is one of the most common orthopedic surgeries and is associated with a significant risk of bleeding as it involves a soft tissue procedure and a surgical procedure on the bone. Severe bleeding after major surgery disrupts patients' hemodynamics and may worsen their vital signs and general condition. Approximately 10%–38% of patients undergoing total knee arthroplasty (TKA) require postoperative transfusion and show an average blood loss of 1,450–1,790 mL (1, 2).

Several studies have explored strategies for controlling bleeding during TKA to facilitate the surgical process and reduce postoperative complications, such as hematoma, circulatory disorders, and wound problems due to the lack of circulation in the skin. These strategies include preoperative use of erythropoietin and iron supplements, intraoperative use of tourniquet, tranexamic acid, hypotensive anesthesia, bleeding control, fiberglass adhesive, and femoral intramedullary canal occlusion with plugs (3). In addition, several precautions have been taken to reduce the risk of hematoma after surgery. These precautions mainly include the use of postoperative drains and tranexamic acid. The effect of tourniquets and drains on bleeding control has been evaluated previously (4). While some studies have suggested that tourniquet and drain are required, others have reported that the use of tourniquet and drain is not necessary (5). In the present study, patients who received compressive dressing without using a tourniquet and drain were compared with those who used a drain.

The use of drains has advantages and disadvantages. The main concerns of using drains are as follows: patients are subjected to an additional invasive procedure, the fixation material may cause an allergic reaction, a superficial skin infection may develop at the drain site, retrograde contamination may occur due to the contact of the closed blood flow route with air with each drain discharge, and additional surgical costs may incur (5, 6).

This study aimed to determine whether there is a difference in clinical and functional outcomes after TKA when a drain is not used and followed up with a compressive dressing versus when a drain is used.

The main hypothesis of this study is that using drains is not a necessity. We believe that using a compressive dressing with appropriate wound closure will have similar results to using a drain and that not using a drain will positively impact pain and range of motion (ROM) even in the early postoperative period.

MATERIALS AND METHODS

Patients

Between January 2019 and June 2023, 316 patients who had undergone TKA were retrospectively evaluated. After applying the exclusion and inclusion criteria, 120 patients who met the inclusion criteria were included in this study (Figure-1). The included patients were divided into two groups: those who used a drain (group 1) and those who did not use a drain but were followed up with a compressive dressing (group 2). Both groups did not use tourniquets. Group 1 included 68 patients (18 males and 50 females) and group 2 included 52 patients (11 males and 41 females). The mean body mass index (BMI) of group 1 was 28.6 ± 0.76 kg/m^2, whereas that of group 2 was 28.9 ± 0.6 kg/m^2, with no statistically significant difference between the two groups ($p > 0.05$). Early postoperative visual analog scale (VAS) scores, edema amounts, ROM on the first day, ROM at week 2,

Knee Society Score (KSS)–knee and KSS-functional scores at week 6, preoperative hemoglobin values, postoperative hemoglobin values, hemoglobin change amounts, and surgical times were evaluated and compared between the two groups.

The study protocol was approved by the regional ethical committee, and all patients provided informed consent.

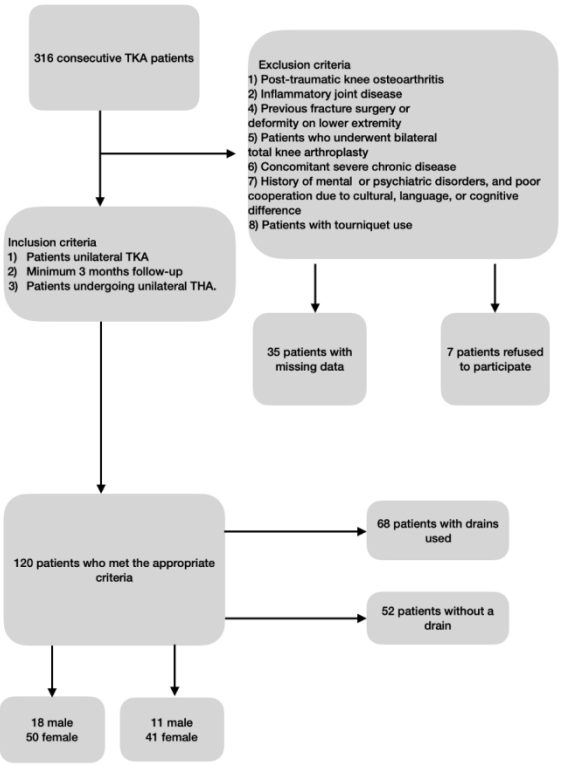

Figure-1. Inclusion and exclusion criteria for the patients.

Surgical method and dressing after surgery

All arthroplasty surgeries were performed under spinal or general anesthesia. All patients had undergone knee arthroplasty surgery using the standard medial parapatellar approach. There was no use of a tourniquet. A drain was used at the surgeon's preference. In patients in group 1, the drain was removed after 24 h and a standard dressing was applied. In patients in group 2, no drain was used, but a compressive dressing was applied. After the sponges were placed in the compressive dressing, tape was used to fix them. Afterward, one or two abdominal compresses were used around the knee, depending on the patient's leg circumference, followed by wrapping of an elastic bandage containing plaster cotton (Figure-2). The patients were dressed on the second postoperative day, and the sutures were removed 1 week after the standard dressing follow-up. In group 1, the elastic bandage was applied after standard dressing, and the dressing was renewed on the second postoperative day. Routine dressing follow-up was continued, and sutures were removed in the second week.

All patients received cruciate ligament-cutting implants. Preoperative and postoperative hemogram values, duration of surgery, and change in hemoglobin values were recorded.

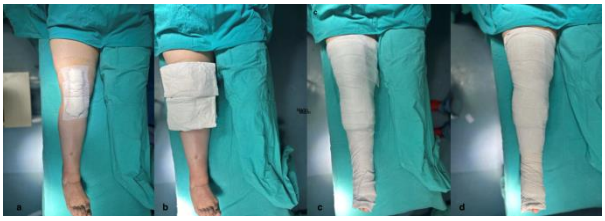

Figure-2. a, taping of dressing sponges; b, placing abdominal compresses suitable for the patient's leg size; c, cotton wrapping; d, wrapping elastic bandage.

Postoperative follow-up

In the group without a drain, knee joint motion began on the first day. In the group with a drain, knee joint motion began after the drain was removed. Patients in both groups received the same analgesic treatment for pain management: 2*1 diclofenac sodium (75 mg), 3*1 Contramal (100 mg), and 2*1 Parol (1 g). Patients whose preoperative bleeding parameters were evaluated received 0.4 clexane for deep vein thrombosis (DVT) prophylaxis in the postoperative period, and DVT prophylaxis was continued for 3 weeks. Infection prophylaxis was performed 1 h preoperatively, and antibiotic prophylaxis was given until 48 h postoperatively. Blood loss was calculated based on the changes in the hemogram on days 1 and 3.

Patients were followed up for hematoma, ecchymosis, bullae, and superficial skin infection in the early postoperative period. Clinically, the ROM, tension, and excessive swelling of the crus were evaluated in terms of DVT. Suprapatellar circumference was measured to assess hematoma. The preoperative suprapatellar circumferences of the patients were measured on day 2 and at week 2 after surgery, and the difference between the preoperative and postoperative values was recorded.

Patients were discharged when their ROM exceeded 90° and their general condition was stable. Patients were examined at 2 weeks, 6

weeks, and 3 months through outpatient clinic visits.

Statistical analysis

The data were analyzed using the Statistical Package for Social Sciences (version 21.0; SPSS Inc, Chicago, Illinois, USA). All quantitative variables were calculated using central location measurements (mean and median) and dispersion measures (standard deviation and standard error). The normality of data was determined using skewness measurements and the Kolmogorov–Smirnov tests. Student's *t* test was used to compare normally distributed data between the two groups. All statistical tests were conducted two-sided with a significance level of 0.05.

RESULTS

Group 1 included 18 male and 50 female patients, whereas group 2 included 11 male and 41 female patients. The mean age of the patients in group 1 was 65.4 ± 8.1 (range 48–85) years, whereas that of the patients in group 2 was 65.9 ± 7.2 (range 47–78) years ($p > 0.05$). The BMI of the patients in group 1 was 28.6 ± 0.76, whereas that of the patients in group 2 was 28.9 ± 0.6, with no statistically significant difference between the two groups ($p > 0.05$). All patients included in the study were followed up by the same team until week 6; group 1 had a mean follow-up period of 23.04 ± 7.9 months, whereas group 2 had a mean follow-up period of 11.5 ± 9.2 months. During the follow-up period, no complications were observed in both groups. The demographic data and clinical results of the patients are shown in Table-1.

The VAS scores of the patients in groups 1 and 2 were 5.7 ± 0.79 and 6.8 ± 0.86, respectively, on the first day and 5.9 ± 0.82 and 3.8 ± 0.69, respectively, on the third day. A statistically significant difference in pain scores was observed on days 1 and 3 ($p < 0.001$). While patients without drains had better ROM on day 1, there was no statistically significant difference in ROM between the two groups at week 2 ($p > 0.05$). The mean total operative time was 64.9 ± 3.3 min in group 1 and 62.9 ± 3.2 min in group 2. As a result of not using a drain, the average surgical time was reduced by approximately 2 min. A statistically significant difference in the duration of surgery was observed between the two groups ($p < 0.001$).

Table-1. Demographic and Clinical Characteristics.

	Group 1 (With Drain)	Group 2 (Without Drain)	P value
Age (y)	65.4 ± 8.1 (range 48–85)	65.9 ± 7.2 (range 47–78)	
Sex (female/male)	50/18	41/11	
Side (right/left)	41/27	36/26	
BMI (kg/m^2)	28.6 ± 0.76	28.9 ± 0.6	p > 0.05
Postoperative day 1 VAS	6.8 ± 0.86	5.7 ± 0.79	p < 0.001
Postoperative day 3 VAS	5.9 ± 0.82	3.8 ± 0.69	p < 0.001
Postoperative day 1 ROM	92.1° ± 8.8°	94.9° ± 7.3°	p < 0.001
Postoperative ROM at week 2	101° ± 12.8°	106.7° ± 12.5°	p > 0.05
Preoperative Hb (g/dL), mean (SD)	13.4 ± 1.2	13.4 ± 1.4	p > 0.05
Postoperative Hb (g/dL), mean (SD)	13.2 ± 10.1	12.9 ± 1.4	p > 0.05
Hemoglobin decrease Hb (g/dL), mean (SD)	1.2 ± 0.7	0.5 ± 0.16	p < 0.001
Postoperative early edema (cm): day1	2.5 ± 1.1 cm	1.7 ± 0.84 cm	p < 0.001
Postoperative late edema (cm): week 2	2.4 ± 0.8	1.4 ± 0.4 cm	p < 0.001
KSS–knee score at week 6	80.6 ± 9.2	79.6 ± 9.6	p > 0.05
KSS-functional score at week 6	80.8 ± 10.4	81.6 ± 10.9	p > 0.05
Surgery time (min)	64.9 ± 3.3	62.9 ± 3.2	p < 0.001

VAS, visual analog scale; KSS, Knee Society Score; ROM, range of motion

While there was no statistically significant difference in preoperative and postoperative hemoglobin levels between the two groups (p> 0.05), a statistically significant difference in terms of a decrease in hemoglobin level was observed (p < 0.001). No patient was transfused in the postoperative period unless the hemoglobin level dropped below 8 mg/dL and their clinical status deteriorated. Patients in both groups did not require blood transfusions. Moreover, no statistically significant difference in the sixth week KSS–knee score and KSS-functional score was observed between the two groups (Table-1).

Peripatellar ecchymosis was observed in 15 patients in group 1 and 8 patients in group 2. In both groups, no bullae formation was observed. Two patients in group 1 had superficial redness and heat on their skin, but none in group 2. The cause of the superficial skin infection and redness around the drain was assumed to be a reaction to the drain and Vicryl. These two patients were treated without using antibiotics. There was no periprosthetic infection in any of the patients. None of the patients developed DVT or pulmonary embolism.

The difference in diameter of the suprapatellar region before and after surgery was used to diagnose early and late postoperative edema. A statistically significant difference in early (postoperative day 2) and late (postoperative week 2) edema (p < 0.001) was observed between the two groups.

DISCUSSION

Several studies on drain have focused on postoperative blood loss, reduced hemoglobin, and the need for transfusion. (4) Studies on postoperative pain and the need for analgesics also exist. Our study evaluated both postoperative blood loss and hemoglobin change and early and late postoperative clinical–functional well-being.

Wound healing problems are frequently encountered after TKA. The occurrence of circulatory failure due to severe hematoma formation and the need for revision surgeries is one of the predisposing factors in wound healing problems. The use of drains is an important approach for preventing hematoma formation (7, 8). Drains are believed to reduce bleeding into soft tissue, prevent hematoma formation, and reduce wound site discharge (9, 10). However, using a drain eliminates the tamponade effect of the hematoma and may increase blood loss (11). In our study, although there was no statistically significant difference in postoperative hemoglobin values between patients with and without drains, there was a statistically significant difference in the amount of bleeding between them.

In our study, we benefited from the tamponade effect of intra-articular hemorrhage using a compressive dressing we made in the absence of a drain. We prevented bleeding that would require transfusion using the compressive effect of the special dressing. Patients with hemoglobin levels less than 8 g/dL and symptoms such as hypotension and tachycardia mostly undergo transfusion (12). None of our patients developed this condition, and no blood transfusion was necessary. Thus, we were able to prevent allergic reactions, immune hemolytic reactions, transfusion-related acute lung injury, graft versus host disease, hepatitis, and viral infections, including acquired immune deficiency syndrome (13).

The use of drains does not increase postoperative complications, prolong surgical time, and affect postoperative functional scores (14). In our study, the duration of surgery for patients in group 1 was 64.9 ± 3.3 min, whereas that for patients in group 2 was 62.9 ± 3.2 min (p < 0.001). Using a drain that disrupts skin integrity and deep tissue continuity causes peripheral sensitization and decreases the nociceptor threshold (15). When skin continuity is disrupted, the concentration of local inflammatory mediators increases, causing secondary central sensitization. This two-level effect causes pain, hypersensitivity, and persistent pain at the injury site. In addition, drain extraction after a major surgical intervention causes pain and discomfort in the patient (16). This condition also increases postoperative stress. Our study found that these patients' VAS scores on days 1 and 3 were higher than those of patients who used drains (p < 0.001).

Several studies have focused on the use of drains, tourniquets, and various other methods to prevent hematoma formation and reduce bleeding after total knee replacement. In our study, we evaluated the effect of compressive dressing, which we developed using our methods, on surgical outcomes, in addition to not using drains. The transfusion needs and the change in hemoglobin values of patients who did

not use drains and instead used compressive dressings were comparable to those of patients who used drains; the clinical well-being of the patients in the early postoperative period was higher; and the need for additional invasive procedures due to drain removal was eliminated.

The strengths of this study include the presence of homogeneous study groups, follow-up with special dressings without the use of drains, the fact that the surgeons who performed surgery in both groups were single surgeons, and the fact that all patients were followed up by the same surgical team. The limitations of this study were that only a small number of patients were evaluated and the surgeons in both groups were different. Bleeding control and surgeon-dependent factors may change depending on the surgeon's preference.

Future studies with large sample sizes are needed to assess the impact of the use of drainless closure with specific dressing on the occurrence of rare complications.

CONCLUSION

Compressive dressing without the use of drains is an effective and simple method that does not increase complications in TKA surgery. Although the patients who did not use drains had better ROM and pain scores in the early period, their clinical and functional outcomes in the midterm were comparable to those of patients who used drains.

Conflict of interest: The authors declared no conflict of interest.

References

1. Sehat KR. Hidden blood loss following hip and knee arthroplasty: Correct management of blood loss should take hidden loss into account. J Bone Joint Surg 2004;86-B(4):561-5.
2. Goodnough LT, Verbrugge D, Marcus RE. The relationship between hematocrit, blood lost, and blood transfused in total knee replacement. Implications for postoperative blood salvage and reinfusion. Am J Knee Surg 1995;8(3):83-7.
3. Themistoklis T, Theodosia V, Konstantinos K, Georgios DI. Perioperative blood management strategies for patients undergoing total knee replacement: Where do we stand now? World J Orthop 2017;8(6):441-54.
4. Hui S, Peng Y, Tao L, Wang S, Yang Y, Du Y, vd. Tranexamic acid given into wound reduces postoperative drainage, blood loss, and hospital stay in spinal surgeries: a meta-analysis. J Orthop Surg 2021;16:401.
5. Titley-Diaz WH, De Cicco FL. Suture hypersensitivity. Treasure Island (FL): StatPearls Publishing; 2023.
6. Brunner W, Härle A. [Risks of wound infection caused by drainage]. Z Orthop Ihre Grenzgeb 1989;127(4):510-2.
7. Bullocks J, Basu CB, Hsu P, Singer R. Prevention of hematomas and seromas. Semin Plast Surg 2006;20(4):233-40.
8. Guo H, Wang B, Ji Z, Gao X, Zhang Y, Yuan L, vd. Closed drainage versus non-drainage for single-level lumbar discectomy: a prospective randomized controlled study. BMC Musculoskelet Disord 2020;21:484.
9. Jeon YS, Park JS, Kim MK. Optimal release timing of temporary drain clamping after total knee arthroplasty. J Orthop Surg 2017;12:47.
10. Maniar RN, Pradhan P, Bhatnagar N, Maniar A, Bidwai R, Bindal P. Role of suction drain after knee arthroplasty in the tranexamic acid era: A randomized controlled study. Clin Orthop Surg 2019;11(1):73-81.
11. Zhou XD, Li J, Xiong Y, Jiang LF, Li WJ, Wu LD. Do we really need closed-suction drainage in total hip arthroplasty? A meta-analysis. Int Orthop 2013;37(11):2109-18.
12. Yaddanapudi S, Yaddanapudi L. Indications for blood and blood product transfusion. Indian J Anaesth 2014;58(5):538-42.
13. Wick MR, Moore S, Taswell HF. Non-A, non-B hepatitis associated with blood transfusion. Transfusion (Paris) 1985;25(2):93-101.
14. Si HB, Yang TM, Zeng Y, Shen B. No clear benefit or drawback to the use of closed drainage after primary total knee arthroplasty: a systematic review and meta-analysis. BMC Musculoskelet Disord 2016;17:183.
15. Gold MS, Gebhart GF. Nociceptor sensitization in pain pathogenesis. Nat Med 2010;16(11):1248-57.

Research Article / *Araştırma Makalesi*

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Effect of preoperative C-reactive protein/albumin ratio on postoperative survial in gastric adenocarcinomas

Gastrik adenokarsinomlarda ameliyat öncesi C-reaktif protein/albümin oranının ameliyat sonrası sağkalıma etkisi

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ABSTRACT

Aim: Since survival time is still low in end-stage gastric cancers, additional treatment and prognostic factors are being investigated. This study aimed to evaluate the significance of the preoperatively measured C-reactive protein (CRP)/ Albumin ratio in gastric adenocarcinomas and its effect on postoperative survival.

Materials and Methods: A total of 258 patients who underwent elective gastric adenocarcinoma surgery were investigated retrospectively. Disease-free and overall survival were evaluated according to the last CT, MRI, and PET/CT scans performed during their follow-up. Demographic data, operation reports, pathology reports, and imaging results of the patients were collected. The preoperative values of CRP and albumin were recorded, and the CRP/ Albumin ratio was calculated. After exclusion criteria, 208 patients were included in the study.

Results: There was a significant relationship between the preoperatively measured CRP/Albumin ratio and postoperative survival time. The predictive power of the CRP/Albumin ratio on the exit was 4.7%. Together with the other parameters affecting survival, the predictive power of the CRP/albumin ratio on the exit increased to 42.5%.

Conclusion: A high CRP/Albumin ratio measured preoperatively was associated with low postoperative survival in patients with gastric adenocarcinomas who underwent curative surgery. Considering that the elevation of CRP may not be kept down due to tumoral tissue, fixing the albumin level by healing the nutritional status of the patients in the preoperative period is the most important way to manage this rate.

Keywords: CRP/ albumin ratio, gastric adenocarcinoma, survival.

ÖZ

Amaç: *Son evre mide kanserlerinde sağ kalım süresi hala düşük olduğundan, ek tedavi ve prognostik faktörler araştırılmaktadır. Bu çalışmada, mide adenokarsinomlarında ameliyat öncesi ölçülen C-reaktif protein (CRP)/Albümin oranının önemi ve ameliyat sonrası sağ kalım üzerindeki etkisinin değerlendirilmesi amaçlanmıştır.*

Gereç ve Yöntem*: Elektif mide adenokarsinom ameliyatı geçiren toplam 258 hasta retrospektif olarak incelendi. Hastalıksız, genel sağ kalım, takipleri sırasında yapılan son BT, MRI ve PET/BT taramalarına göre değerlendirildi.*

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Hastaların demografik verileri, ameliyat raporları, patoloji raporları ve görüntüleme sonuçları toplandı. Ameliyat öncesi CRP ve albümin değerleri kaydedildi ve CRP/Albümin oranı hesaplandı. Dışlama kriterlerinden sonra, toplam 208 hasta çalışmaya dahil edildi.

Bulgular: *Ameliyat öncesi ölçülen CRP/Albümin oranı ile ameliyat sonrası sağ kalım süresi arasında anlamlı bir ilişki vardı. CRP/Albümin oranının ölümü belirmedeki öngörü gücü %4,7 idi. CRP/Albümin oranının ölümü öngörü gücü, sağkalımı etkileyen diğer parametrelerle birlikte %42,5'e kadar yükseldi.*

Sonuç: *Ameliyat öncesi ölçülen yüksek CRP/Albümin oranı, küratif cerrahi geçiren gastrik adenokarsinomlu hastalarda postoperatif dönemde düşük sağkalımla ilişkiliydi. CRP yüksekliğinin tümör dokusu nedeniyle düşük tutulamayabileceği gerçeği göz önüne alındığında, ameliyat öncesi dönemde hastaların beslenme durumunun iyileşmesiyle albümin düzeyinin sabitlenmesi bu oranı yönetmenin en önemli yoludur.*

Anahtar Sözcükler: *CRP/albümin oranı, gastrik adenokarsinom, sağkalım.*

INTRODUCTION

Gastric cancer is the third leading cause of cancer-related death diagnosed cancer types. According to GLOBOCAN 2020 data, the number of newly diagnosed cancers worldwide in 2020 is 19.3 million, and cancer-related deaths are 10 million. Gastric cancer includes 5.6% of patients diagnosed with new cancer and 7.7% of patients who died of cancer (1). Surgery is the only curative treatment for gastric cancer (2). Surgery can be performed after neoadjuvant therapy or before adjuvant therapy, depending on several factors such as tumor localization, metastatic lymph node, and size of the tumor. Despite all this, the survival time is still low in end-stage gastric cancers, and additional management methods are required to be carried out. For this reason, many prognostic values that may predict survival in gastric cancers are being investigated.

The 5-year survival rates for resected gastric adenocarcinoma stages I, II, and III in the United States are approximately 75%, 50%, and 25%, respectively. Adjuvant chemotherapy alone has not proven effective, at least in studies from Europe and the United States (3). A high neutrophil/lymphocyte ratio (NLR) is associated with a worse prognosis (4). R0 resection must be done for a better survival ratio. However, there is a recurrence of gastric cancer even after R0 resection in many cases, reflecting the current limits of this parameter (5, 6). The C-reactive protein (CRP) /Albumin ratio is a newly defined marker of inflammation and is associated with lower survival time in different diseases, such as sepsis in patients with burn injuries (7) and pancreatic and hepatocellular cancers (8). Few studies also demonstrate the impact of CRP and albumin values in patients with gastric cancer (9, 10).

We aimed to calculate the preoperative CRP/Albumin ratio in the patients with gastric adenocancer applied to our clinic and to investigate the effect on postoperative survival. Additionally, we aimed to reveal the predictive power of the CRP/Albumin ratio so that it may be considered a prognostic value measured preoperatively.

MATERIALS and METHODS

The study was conducted in the Ege University Department of General Surgery, and the ethics committee approval (No: 22-10.1T/13) was obtained. A total of 258 patients who underwent elective gastric adenocarcinoma surgery between May 2013- December 2020 were investigated. Both laparoscopic and laparotomy cases were included in the study. Elective patients who did not have perioperative distant metastasis and underwent D2 lymph node dissection were included. Due to the effects on the overall survival period, patients who underwent urgent and semi-urgent surgeries because of bleeding or obstruction and the patients who required reoperation due to postoperative complications were excluded. A total of 50 patients were excluded. The study target included the patients who underwent total, subtotal, and distal gastrectomy + D2 lymph node dissection.

Disease-free and overall survival durations were calculated according to the last CT, MRI, and PET/CT scans. Two hundred-eight patients matching the inclusion criteria were counted. Past medical records, including demographic data (age, gender), operation reports(operation data and operation technique: distal, subtotal or total gastrectomy + D2 lymph node dissection), pathology reports (tumor location, tumor size, postoperative histological type, and subtype,

surgical margins, lymphovascular invasion, perineural invasion, venous invasion, number of the metastatic lymph nodes and number of the removed lymph nodes, tumors T, N, and M stage according to the AJCC 8th edition), postoperative imaging results (local recurrence and distance organ metastasis during the follow-up periods) and the exitus data were evaluated.

The preoperative values of CRP and albumin were recorded, and CRP/ Albumin ratio was calculated for each patient. Tumor stages are classified according to pTNM values in the 8th edition of AJCC and UICC classification. The relationship between CRP/albumin ratio, gender distribution, neoadjuvant treatment status, tumor location, tumor size, histological subtype, surgical margin positivity, lymphovascular invasion, perineural invasion, venous invasion, and pTNM stages was evaluated between the groups.

Statistical Analysis

Statistical analyses were calculated with SPSS v25.0. Mean ± standard deviation was used for numerical measurements, and percentages and numbers were used for qualitative measurements from descriptive statistics. The normality of the cross-group distribution was tested with the Shapiro-Wilk test. Normally distributed independent data by Student's T test, dependent data were evaluated by Paired T and ANOVA tests, and non-normally distributed data by Mann Whitney U and Kruskal Wallis tests according to the number of groups—survival analysis performed by the Kaplan-Meier test. The Cox Regression model determined factors affecting survival. ROC-curve analysis and the Yuoden index were used to evaluate the CRP/Albumin ratio as a predictive value. A logistic regression test evaluated the survival effect of the parameters. The confidence interval was determined as 95%, and a p-value of <0.05 was significant.

RESULTS

137 of 208 patients were male (65.86%), while 71 were female (34.14%). The mean age was 66.48 (± 10.36 standard deviation), and the median was 67 (min. 40- max. 93).

Two hundred-eight patients were divided into three groups according to the type of operation. Total gastrectomy was performed in 115 patients (55.29%), subtotal gastrectomy in 48 patients (23.07%), and distal gastrectomy in 45 patients (21.64%). In the compared data, only tumor location ($p<0.01$) was significantly correlated according to the operation type. At the same time, there was no statistically significant difference between the groups according to the operation type in other comparisons.

There was a statistically significant correlation between tumor size *($p<0.001$),* histological subtype *($p=0.001$),* surgical margin positivity *($p=0.01$),* lymphovascular invasion *($p<0.001$),* perineural invasion *($p<0.001$)* and venous invasion *($p<0.001$)* according to the pTNM stages.

Survival analysis

The mean survival time of those who underwent total gastrectomy, subtotal gastrectomy, and distal gastrectomy was found to be 48.21 months (41.04 – 55.37), 45.24 months (34.73 – 55.74), and 50.54 months (39.26 – 61-82), respectively (Figure-1).

There was a statistically significant difference between stages regarding survival times *($p<0,001$).* The mean survival of the patients with stage 1A was 87.71 months (79.4 – 95.9). It was 65.2 months for stage 1B (43.8-86.7), 66.9 months for stage 2A (55.77-78.1), 65.9 months for stage 2B (49.6 – 82.3), 51.1 months for stage 3A (37.0 – 65.3), 34.5 months for stage 3B (17, 6 – 51.4), 34.08 months for stage 3C (26.04 – 41.7) **(**Figure-2**).**

There was an inverse correlation between the CRP/Albumin ratio and survival, and a 1 unit increase in the cut-off value of the CRP/ Albumin ratio reduced survival 1.68 times (1.19 – 2.38) (Figure-3). Neoadjuvant therapy increased survival 2.69 times (0.55 – 13.06); lymphovascular invasion 0.63 times (0.41 – 0.98), perineural invasion 0.47 times (0.27 – 0.83), and distant organ metastasis 0.49 times (0.30 – 0.79) decreased survival.

The age parameter affected the survival 1.03 times (1.01 – 1.05) according to the stages, while the CRP/Albumin ratio affected the survival 1.73 times (1.12 – 2.66) (Figure-4). Considering the factors affecting survival in terms of stages, it was determined that neoadjuvant treatment status, surgical margin, lymphovascular invasion, perineural invasion, venous invasion, and local recurrence did not affect survival. However, distant organ metastasis decreased survival by 0.6 times (0,36 – 0,98) (Table-1).

Predictive analysis of CRP/Albumin ratio

Using the CRP/Albumin ratio as a predictive test was statistically significant(*$p=0.009$)*.

Considering the CRP/Albumin ratio as a predictive test, the true positivity of this parameter was determined as 68%. When true negativity, true positivity, false negativity, and false positivity were taken together, an average of 59.1% of results were statistically correct. It was determined that a 1 unit increase in the CRP/Albumin ratio could increase the death rate 21.7 times *(p=0,007*).

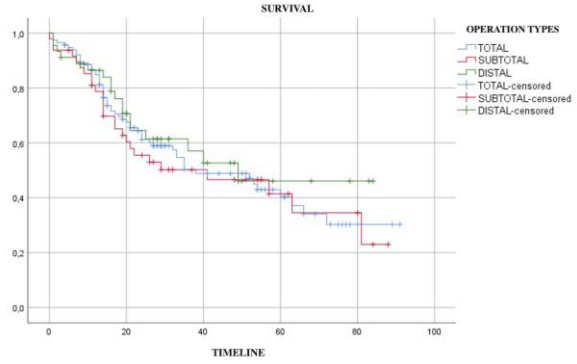

Figure-1. Overall Survival analysis regarding to operation types.

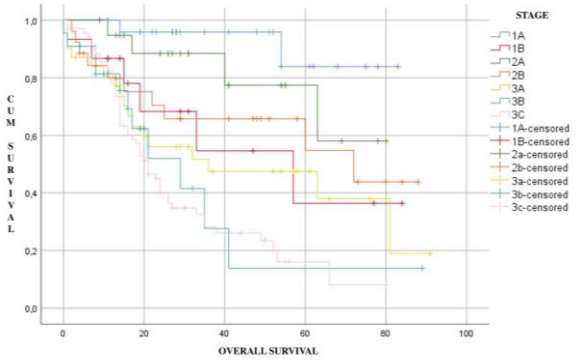

Figure-2. Overall Survival differences between pTNM stages.

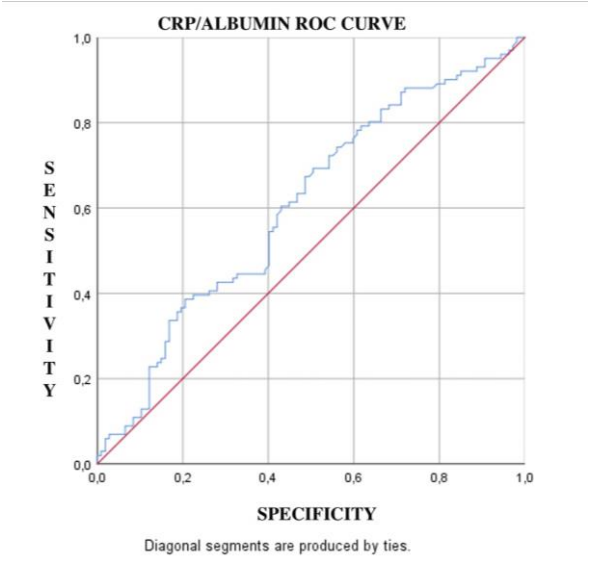

Figure-3. ROC curve of CRP/ Albumin ratio.

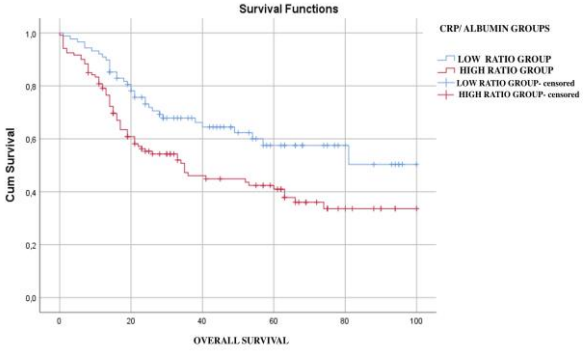

Figure-4. Overall survival regarding CRP/Albumin groups.

Table-1. Effect of the parameters included in the study on survival.

	p Value	Hazard ratio	95% CI	
			Lower Limit	Upper Limit
AGE	0.005*	1.032	1.01	1.054
CRP/ALBUMIN RATIO	0.003*	1.684	1.191	2.38
NEOADJUVANT THERAPY	0.22	2.69	0.554	13.068
SURGICAL MARGIN	0.002*	2.457	1.383	4.363
LYMPHOVASCULAR INVASION	0.044*	0.636	0.409	0.987
PERINEURAL INVASION	0.009*	0.477	0.273	0.834
VENOUS INVASION	0.905	0.967	0.554	1.687
LOCAL RECURRENCE	0.139	0.283	0.053	1.507
DISTANT METASTASIS	0.004*	0.494	0.305	0.798

The mean overall survival with a low CRP/Albumin ratio was 66.4 months, while it was 49.61 months in the patients with a high CRP/Albumin ratio. There was a statistically significant difference between the survival rates of these two groups *(p=0,005).*

Excluding the parameters of lymphovascular invasion, perineural invasion, and venous invasion from the analysis, which were determined to have no statistically significant contribution, the power of CRP/Albumin ratio, neoadjuvant treatment, surgical margin, tumor stage, and distant organ metastasis status together was found to determine the exitus with 42.5% probability. When the CRP/Albumin ratio was used alone to predict the exitus rate, a 1-unit CRP/Albumin ratio increased the exitus risk 21 times. The predictive power of the CRP/Albumin ratio on exitus was 4.7%.

DISCUSSION

The main goal of this study was to consider the preoperatively measured CRP/Albumin ratio as a predictive value in evaluating postoperative survival in patients with gastric adenocarcinoma. According to the cut-off value, the CRP/Albumin ratio had an effect on survival, and survival was lower in the patients with a higher CRP/Albumin ratio.

Age significantly impacts both overall survival and cancer-related survival [7, 8]. In younger patients, there is a higher prevalence of the diffuse histo-type, and tumors may be diagnosed at more advanced stages. However, when considering the tumor stage, young age alone does not independently affect prognosis. Gender does not have an independent prognostic value for cancer-related survival in most studies (7, 8). Additionally, geographic location and ethnicity are other patient-related prognostic factors for gastric cancer. Recent studies conducted have shown that Asian Americans have better outcomes compared to other ethnicities (9). In our study, age was found to affect survival 1.03 times. It was also revealed that the CRP/Albumin ratio affected the survival 1.73 times. With increasing age and comorbidities, the value of albumin decreases, so the correlation between increasing age and albumin can also be clarified.

The median tumor size value was 5 cm (4.81-5.87) in patients who underwent total gastrectomy, 4 cm (3.69-4.98) who underwent subtotal gastrectomy, and 3,5 cm (3,21-4,25) in the group of distal gastrectomy. As expected in the compared data, only the tumor location *(p<0.01)* was significantly correlated according to the operation type. Since the type of operation is determined according to the tumor location and size, such a statistical result was predicted. The other parameters were the results frequently obtained after the operation, and they did not affect the type of operation.

Several studies conducted the advantages of chemotherapy after D2 gastrectomy (4, 5). A study showed a 69% overall 5-year survival rate in locally advanced patients treated with D2 gastrectomy (6). Our study found that the advantage of neoadjuvant chemotherapy is that it increases survival by 2.69 times. Considering that tumor size, lymph node metastasis, and distant organ metastasis determine the pTNM stage of the disease, the statistical results obtained from our study were as expected. The reason why histological subtype and surgical margin positivity were associated with the stage of the disease is related to the unequal distribution of the 208 patients included in the study. Considering that 58% of the entire patient group included in our study consisted of stage 3 patients, it is natural to detect such a statistical relationship.

As in the 2016 study by Jin Qi et al. comparing total gastrectomy and distal gastrectomy in terms of survival (11) and as in the 1999 study by Bozzetti et al. in terms of survival(12), there was no significant difference between all 3 surgical procedures and the patient's survival. There was no significant relationship between the operation types and survival *(p=0,52).* In this respect, our results are substantially consistent with the literature.

Liu et al. reported that parameters of CRP and albumin might have predictive value in determining survival; however, they did not reveal a cut-off value (10-13). Besides, we obtained additional results by deciding that the predictive power of this rate alone was 4.7%. In all of the stages, CRP/Albumin ratio *(p=0.012),* neoadjuvant treatment status *(p=0.025),* pTNM stage *(p=0.022),* and distant organ metastasis were effective on survival. The predictive power was determined to increase to 42.5% when the parameters were used together.

In another study by Minjie Mao et al., consisting of 337 patients in 2017, there was a relationship

between the CRP/Albumin ratio and survival. Still, the neutrophil/lymphocyte ratio (NLR) parameter was used to increase this predictive power (14). Similarly, the effects of parameters, systemic immune-inflammatory index (SII), and Glasgow Prognostic Score (GPS) on preoperative survival in gastric adenocarcinomas have been investigated in the literature for predictive value purposes (10). However, they all have low predictive power and increase the predictive power when used together.

Considering that CRP elevation is related to the characteristics of the cancer (15), the most important way to change the CRP/Albumin ratio is to change the albumin value (16). Low albumin levels due to nutritional disorders are common in gastric cancers. So, increasing the value of albumin by good nutrition preoperatively may impact survival in the postoperative period.

CONCLUSION

This study evaluated the CRP/albumin ratio as a parameter that measured preoperatively in gastric adenocarcinomas and can guide us in predicting survival.

CRP/Albumin ratio alone was predictive in predicting survival *(p=0,006).* A 1-unit increase in the CRP/Albumin ratio increased the risk of death 21 times. The predictive power of the CRP/Albumin ratio alone on the exitus was 4.7%. When other parameters affecting survival (neoadjuvant treatment, surgical margin, pTNM stage, and distant organ metastasis status) were evaluated with the predictive effect of CRP/Albumin, the predictive power on the exitus increased to 42.5%.

Considering the effect of the CRP/Albumin ratio on survival in gastric cancers, it is essential to correct patients' low albumin values in the preoperative period and thus improve their nutritional status.

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References

1. H. Sung *et al.*, “Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries,” *CA Cancer J Clin*, vol. 71, no. 3, pp. 209–249, May 2021, doi: 10.3322/CAAC.21660.
2. B. J. Dicken, D. L. Bigam, C. Cass, J. R. Mackey, A. A. Joy, and S. M. Hamilton, “Gastric Adenocarcinoma: Review and Considerations for Future Directions,” *Ann Surg*, vol. 241, no. 1, p. 27, Jan. 2005, doi: 10.1097/01.SLA.0000149300.28588.23.
3. I. H. Kim, “Current status of adjuvant chemotherapy for gastric cancer,” *World J Gastrointest Oncol*, vol. 11, no. 9, p. 679, Sep. 2019, doi: 10.4251/WJGO.V11.I9.679.
4. H. Shimada *et al.*, “High preoperative neutrophil-lymphocyte ratio predicts poor survival in patients with gastric cancer,” *Gastric Cancer*, vol. 13, no. 3, pp. 170–176, Aug. 2010, doi: 10.1007/S10120-010-0554-3.
5. M. W. Kattan, M. S. Karpeh, M. Mazumdar, and M. F. Brennan, “Postoperative nomogram for disease-specific survival after an R0 resection for gastric carcinoma,” *J Clin Oncol*, vol. 21, no. 19, pp. 3647–3650, Oct. 2003, doi: 10.1200/JCO.2003.01.240.
6. V. E. Strong *et al.*, “Comparison of gastric cancer survival following R0 resection in the United States and Korea using an internationally validated nomogram,” *Ann Surg*, vol. 251, no. 4, pp. 640–646, Apr. 2010, doi: 10.1097/SLA.0B013E3181D3D29B.
7. Y. Yu, W. Wu, Y. Dong, and J. Li, “C-Reactive Protein-to-Albumin Ratio Predicts Sepsis and Prognosis in Patients with Severe Burn Injury,” *Mediators Inflamm*, vol. 2021, 2021, doi: 10.1155/2021/6621101.
8. Y. J. Fu, K. Z. Li, J. H. Bai, and Z. Q. Liang, “C-reactive protein/albumin ratio is a prognostic indicator in Asians with pancreatic cancers: A meta-analysis,” *Medicine*, vol. 98, no. 48, Nov. 2019, doi: 10.1097/MD.0000000000018219.
9. Q. Yu *et al.*, “Clinical significance and prognostic value of C-reactive protein/albumin ratio in gastric cancer,” *Ann Surg Treat Res*, vol. 100, no. 6, pp. 338–346, Jun. 2021, doi: 10.4174/ASTR.2021.100.6.338.
10. X. Liu *et al.*, “Preoperative C-Reactive Protein/Albumin Ratio Predicts Prognosis of Patients after Curative Resection for Gastric Cancer,” *Transl Oncol*, vol. 8, no. 4, pp. 339–345, Aug. 2015, doi: 10.1016/J.TRANON.2015.06.006.

11. J. Qi, P. Zhang, Y. Wang, H. Chen, and Y. Li, “Does Total Gastrectomy Provide Better Outcomes than Distal Subtotal Gastrectomy for Distal Gastric Cancer? A Systematic Review and Meta-Analysis,” 2016, doi: 10.1371/journal.pone.0165179.
12. F. Bozzetti, E. Marubini, G. Bonfanti, R. Miceli, C. Piano, and L. Gennari, “Subtotal versus total gastrectomy for gastric cancer: five-year survival rates in a multicenter randomized Italian trial. Italian Gastrointestinal Tumor Study Group,” *Ann Surg*, vol. 230, no. 2, pp. 170–178, Aug. 1999, doi: 10.1097/00000658-199908000-00006.
13. H. Saito *et al.*, “Prognostic Significance of the Preoperative Ratio of C-Reactive Protein to Albumin and Neutrophil–Lymphocyte Ratio in Gastric Cancer Patients,” *World J Surg*, vol. 42, no. 6, pp. 1819–1825, Jun. 2018, doi: 10.1007/S00268-017-4400-1/TABLES/2.
14. M. Mao *et al.*, “C-reactive protein/albumin and neutrophil/lymphocyte ratios and their combination predict overall survival in patients with gastric cancer,” *Oncol Lett*, vol. 14, no. 6, pp. 7417–7424, Dec. 2017, doi: 10.3892/OL.2017.7179/HTML.
15. S. Lee, J. W. Choe, H. K. Kim, and J. Sung, “High-Sensitivity C-Reactive Protein and Cancer,” *J Epidemiol*, vol. 21, no. 3, pp. 161–168, May 2011, doi: 10.2188/JEA.JE20100128.
16. D. Gupta and C. G. Lis, “Pretreatment serum albumin as a predictor of cancer survival: A systematic review of the epidemiological literature,” *Nutr J*, vol. 9, no. 1, pp. 1–16, Dec. 2010, doi: 10.1186/1475-2891-9-69/TABLES/5.

Araştırma Makalesi / *Research Article*

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Hipertansiyon prevalansı ve ilişkili uç organ hasarı; retrospektif tek merkez deneyimi

Hypertension prevalence and connected end organ damage: a retrospective single center experience

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ÖZ

Amaç: Hipertansiyon, inme, miyokard enfarktüsü, konjestif kalp yetmezliği, periferik vasküler hastalık ve son dönem böbrek hastalığı için en yaygın görülen değiştirilebilen risk faktörüdür. Hipertansiyon prevalansı, sayısız epidemiyolojik çalışmada tutarlı bir şekilde bildirilmiştir. Çoğu sanayileşmiş ülkede yetişkin nüfus örneklerinde %25-55 olarak tespit edilmiştir. İç Hastalıkları polikliniğimizde yaptığımız çalışmada polikliniğimize başvuran hastalarda hipertansiyon prevalansının ve uç organ hasarı oranlarının saptanması ve rutin biyokimya ile hemogram değerleri ile uç organ hasarı gelişimi arasındaki ilişkinin değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntem: Ege Üniversitesi Tıp Fakültesi Hastanesi İç Hastalıkları Polikliniğine Ağustos 2018-Mart 2019 tarihleri arasında başvuran hastaların biyokimyasal testleri ve konsültasyon notları retrospektif olarak Elektronik Hasta Dosyası üzerinden incelendi. Yaş, cinsiyet, sigara kullanımı, yaşadığı şehir, kullandığı antihipertasif ilaçlar, ek hastalıkları, biyokimyasal verileri, spot idrar ve tam idrar tetkiki, 12 derivasyonlu Elektrokardiyografi (EKG), fundoskopik bakı gibi hipertansiyon ilişkili uç organ hasarı ile ilişkili verileri incelendi. İstatistiksel analiz için SPSS 25.0 programı kullanıldı.

Bulgular: Çalışmaya bu tarihler arasında polikliniğimize başvuran toplam 1267 hasta dahil edildi. Bunlardan 332 kişinin hipertansif 935 kişinin normotansif olduğu belirlendi ve iki grup oluşturuldu. Hipertansiyon (HT) prevalansı %26,2, altı aylık HT insidansı %12,5 saptandı. Olguların %40'ı poliklinikte kan basıncı ölçümü sırasında tanı alan hastalardı. EKG'lerde Sokolow Lyon kriterlerine göre hastaların %12,3 ünde sol ventrikül hipertrofisi mevcuttu. Spot idrar verilerine göre proteinürik hasta oranı %23 bulundu. Hipertansif retinopatili hasta oranı %32,85 bulundu. Hipertansif retinopati ile serebrovasküler olay sıklığı arasında yakın korelasyon gösteren bir ilişki saptandı (p.0.002). Serum total kolesterol düzeyi yüksek olan hipertansif hastalarda belirgin oranda hipertansif retinopati sıklığının arttığı saptandı.

Sonuç: Hipertansif retinopati ve serebrovasküler olay (SVO) sıklığı arasında pozitif korelasyon gösteren bir ilişki saptadık. HT ilişkili sol ventrikül hipertrofisi (LVH) ile hipertansif retinopati arasında pozitif korelasyon gösteren bir ilişki saptadık. Serum total kolesterol yüksekliği ile retinopati gelişimi arasında yakın bir ilişki saptadık. Tüm hipertansif hastaların lipid düzeylerinin görülerek gerekli tedaviye erken dönemde başlanması retinopati gelişimini önleyebilir.

Anahtar Sözcükler: Hipertansiyon prevalansı, nefropati, retinopati, sol ventrikül hipertrofisi.

ABSTRACT

***Aim**: Hypertension is the most commonly altered risk factor for stroke, myocardial infarction, congestive heart failure, peripheral vascular disease and end-stage kidney disease.*

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The prevalence of hypertension has been consistently reported in numerous epidemiological studies. It has been determined as 25-55% in adult population samples in most industrialized countries. In our study conducted in our Internal Medicine outpatient clinic, we aimed to determine the prevalence of hypertension and end organ damage rates in patients applying to our outpatient clinic and to evaluate the relationship between routine biochemistry and hemogram values and the development of end organ damage.

***Materials and Methods**: Biochemical tests and consultation notes of patients who applied to the Ege University Medical Faculty Hospital Internal Diseases Policlinic between August 2018 and March 2019 were analyzed retrospectively through the Electronic Patient File. Data related to hypertension related end organ damage such as age, gender, smoking, city of residence, antihypertensive drugs used, additional diseases, biochemical data, spot urine and full urine examination, ECG, fundoscopic examination were examined. SPSS 25.0 program was used for statistical analysis.*

***Results**: A total of 1267 patients who applied to our outpatient clinic between these dates were included in the study. Of these, 332 people were hypertensive, 935 were normotensive, and two groups were formed. The prevalence of HT was 26.2%, and the incidence of six months HT was 12.5%. 40% of the cases were patients diagnosed in the outpatient clinic during blood pressure measurement. According to Sokolow Lyon criteria, 12.3% of patients had LVH in the ECG s that was examined regarding HT related end organ injury. Proteinuria rate was 23% according to spot urine data. The rate of hypertensive retinopathy was 32.85%. A close correlation was found between hypertensive retinopathy and the frequency of cerebrovascular events (p:0002). It was found that the frequency of hypertensive retinopathy increased significantly in hypertensive patients with high serum total cholesterol level.*

***Conclusions**: We found a positive correlation between hypertensive retinopathy and SVO frequency. We found a positive correlation between hypertension-associated LVH and hypertensive retinopathy and a close relationship between serum total cholesterol elevation and the development of retinopathy. Starting the necessary treatment early by monitoring the lipid levels of all hypertensive patients may prevent the development of retinopathy.*

***Keywords**: Hypertension prevalence, left ventricular hypertrophy, nephropathy, retinopathy.*

GİRİŞ

Hipertansiyon (HT); kalp hastalıkları, serebrovasküler hastalıklar, renal hastalık ve erken ölüme sebep olan önemli bir sağlık sorunudur. Türkiye'de erişkinlerde HT prevalansı %31,8 (Patent 2 çalışmasında), 4 yıllık insidans hızı ise %21,4 (>65 yaşta %43,3) olarak belirlenmiştir. Patent 1 çalışmasında ise prevalans %30.3 bulunmuştur (1). Sırasıyla kadın ile erkek oranlaması %20 ve %24 şeklindedir. 60 yaş üstü popülasyonda prevalans artmakta ve yüzde %60 üzerine çıkmaktadır. Yaş gruplarına göre bakıldığında HT prevalansı en yüksek 70-79 yaş arasındakilerde %85,2 olarak bulunmuştur (2). Bugüne dek yayınlanan çoğu HT kılavuzunun ortak amacı yüksek kan basıncının önlenmesini, farkındalığını, tedavisini ve kontrolünü iyileştirmek ve kardiyovasküler kötü sonlanımları engellemektir. Ege Üniversitesi Tıp Fakültesi Hastanesi İç Hastalıkları Polikliniği'nde yapılan bu çalışmada toplumda HT sıklığı, hastaların farkındalıkları ve HT ilişkili uç organ hasarının değerlendirilmesi amaçlanmıştır.

2018 yılı Türkiye Nefroloji Diyaliz Transplantasyon güncel verilerine göre hemodiyalize (HD) giren olgularda son dönem böbrek yetmezliği (SDBY) etyolojisine göre dağılıma bakılırsa, diabetes mellitus (DM) (%35,8) sonrası en sık etyolojik sebep %27,38 oranla HT'dur. Periton diyalizine (PD) giren ve 2018 yılı içinde ilk kez PD'ye başlanan olgularda ise HT %33,4'lük oran ile SDBY etyolojisinde ilk sırada bulunmuştur. Bunu %20,89 oran ile DM takip etmektedir. Ancak renal replasman tedavisi ihtiyacı olan olgularda hipertansiyonun primer değil de kronik böbrek yetmezliğine bağlı oluşabilecek sekonder HT olduğuna dair kuvvetli şüpheler olduğundan bahsedilmektedir (3).

HT İlişkili Organ Hasarı (HMOD), artmış kan basıncının neden olduğu arterlerde veya uç organlarda (kalp, kan damarları, beyin, gözler ve böbrek) yapısal veya fonksiyonel değişiklikleri ifade eder ve preklinik, asemptomatik kardiyovasküler hastalığın (KVH) bir belirtecidir. HMOD, şiddetli veya uzun süreli hipertansiyonda yaygındır, ancak daha az şiddetli hipertansiyonda da bulunabilir. Görüntülemenin daha yaygın kullanılmasıyla, HMOD asemptomatik hastalarda giderek daha belirgin hale gelmektedir. KVH riski,

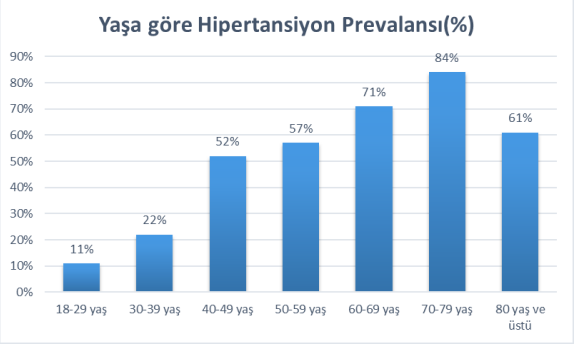
HMOD varlığı ile artar ve hasar çok sayıda organı etkilediğinde daha da fazla olur. Bazı HMOD türleri, özellikle erken kullanıldığında antihipertansif tedavi ile tersine çevrilebilir, ancak uzun süredir devam eden hipertansiyonda, HMOD, kan basıncı kontrolüne rağmen geri dönüşümsüz hale gelebilir. Her ne kadar zayıf teknik tedarik ve maliyet bazı ülkelerde HMOD araştırmasını sınırlayabilse de, tüm hipertansif hastalarda HMOD için temel taramanın yapılması ve HMOD varlığının tedavi kararlarını etkileyebileceği durumlarda daha ayrıntılı değerlendirme yapılması European Society of Cardiology (ESC) 2018 hipertansiyon kılavuzunda da önerilmektedir (4). Biz de çalışmamızda bölgenin en büyük hastanesi olan kliniğimizdeki HT prevalansını saptayarak Ege bölgesinin prevalansını görmek ve HT ile uç organ hasarlarının ilişkisini araştırmak istedik.

GEREÇ ve YÖNTEM

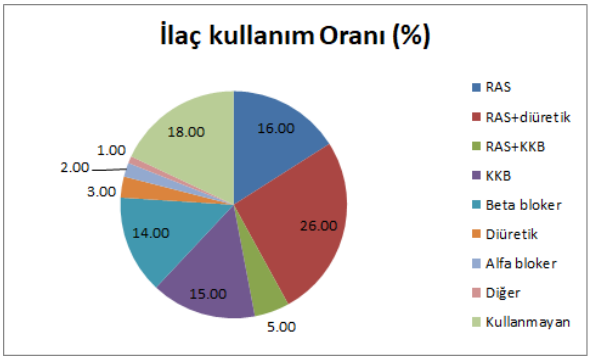
Ege Üniversitesi Tıp Fakültesi Hastanesi İç Hastalıkları polikliniğine Ağustos 2018-Mart 2019 tarihleri arasında başvuran 1267 hasta dahil edildi. Hipertansif olanlar (yeni veya eski tanılı) ve olmayanlar olarak kontrol grubu olacak şekilde iki grup oluşturuldu. Yaş, cinsiyet, sigara kullanımı, yaşadığı şehir, kullandığı antihipertansif ilaçlar, komorbid hastalıkları, Vücut kitle indeksi (kilo/boy^2) (VKİ), biyokimyasal verileri, spot idrar ve tam idrar tetkiki, 12 derivasyonlu EKG, fundoskopik bakı gibi hipertansiyona ikincil uç organ hasarı ile ilişkili verileri incelendi. Kan basıncı ölçümlerinde en az 2 kez usulüne uygun olarak 140 / 90 mmHg ve üzeri ölçülen kişiler hipertansif gruba dahil edildi. Hipertansif hasta grubunun glomerüler filtrasyon hızı (GFR) 2021 CKD Epidemiology Collaboration (CKD-EPI) formülüne göre hesaplandı. LVH, Sokolow Lyon kriterlerine göre belirlendi. Hipertansif grupta spot idrar protein/ kreatinin oranı 150 mg/mg ve üzerinde ola kişiler nefropatili kabul edildi ve hipertansif nefropatili gruba dahil edildi. Hipertansif retinopati durumu ise Göz Hastalıkları uzmanı tarafından değerlendirilen konsültasyon notlarına ulaşılarak elde edildi. Keith- Wagner-Barker Sınıflamasına göre evre 1 ve üzeri olan olgular retinopatili olarak değerlendirildi. Elde edilen bulguların istatistiksel analizleri için SPSS (Statistical Package for Social Sciences) for Windows 25 programı kullanıldı. Çalışmanın verilerini değerlendirmek için tanımlayıcı istatistik yöntemleri (ortalama, ortanca, sayı, yüzde); eşleştirilmiş örneklem t testi, bağımsız örneklem t testi, ANOVA testi kullanılmıştır. Normal dağılıma uyan değişkenler için ortalama ve standart sapma kullanılarak verilecektir. Bağımsız grupların karşılaştırılmasında normal dağılmayan veriler ve ordinal ve nominal veriler için ki-kare ve Mann-Whitney U testi, normal dağılım gösteren verilerin karşılaştırılmasında student t testi uygulandı. Sonuçlar %95 güven aralığında, anlamlılık ise $p<0.05$ altında kabul edildi.

BULGULAR

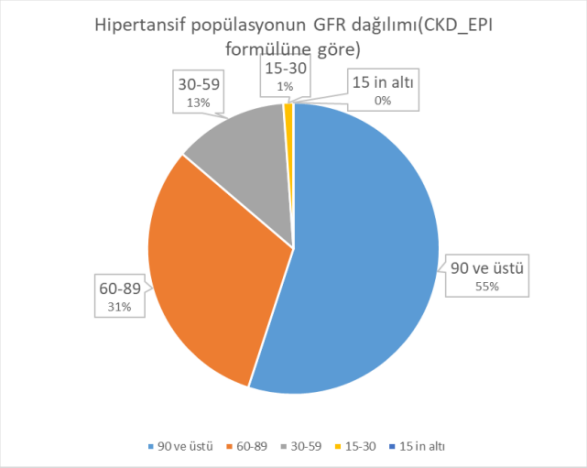
Çalışmaya dahil edilen bu tarihler arasında polikliniğimize başvuran toplam 1267 hastadan 332 kişinin hipertansif 935 kişinin normotansif olduğu belirlendi ve iki grup oluşturuldu. Gruplara ait demografik veriler ve kan basıncı ortalama değerleri Tablo-1'de özetlenmiştir. Yaşa göre hipertansiyon prevalansı değerlendirilmiş ve sonuçlar Şekil-1'de sunulmuştur. Toplam 332 hipertansif hastanın %40'ı poliklinikteki tansiyon ölçümleri ile yeni tanı alan hastalardı. Altı aylık HT insidans hızı %12,5, prevalans %26,2 olarak bulundu. Hipertansif hastaların %32,4'ü sigara kullanmaktaydı. Ortalama sigara maruziyeti 8 paket yılı olarak saptandı. Toplam 274 hipertansif hastanın boy kilo verilerine ulaşılmış olup; VKİ ortalaması hipertansif grupta 29,8±5,5 kg/m^2 olarak hesaplandı.



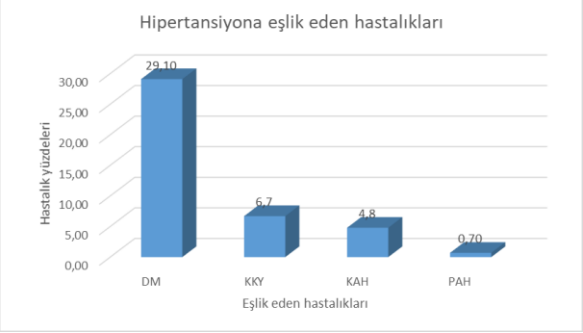
Şekil-1. Yaşa göre hipertansiyon prevalansı verileri (%)



Şekil-2. Hipertansiyon tanılı olguların kullandığı ilaç grupları dağılım grafiği *(1 RAS blokeri,2 RAS+ diüretik kombinasyonu, 3 RAS + Kalsiyum Kanal Blokeri (KKB) kombinasyonu, 4 KKB, 5 Beta bloker, 6 diüretik, 7 alfa bloker, 8 diğer ajanlar).*



Şekil-3. Hipertansif popülasyonun GFR (CKD-EPI formülüne göre) değerine göre dağılım grafiği *(ml/dk/1.73 m^2)*



Şekil-4. Hasta popülasyonunda hipertansiyona eşlik eden diğer hastalıklar ve sıklıkları *(1 Diabetes Mellitus, 2 Koroner Arter Hastalığı, 3 Konjestif Kalp Yetmezliği, 4 Periferik Arter Hastalığı)*

HT tanılı olguların ilaç kullanım verileri incelendiğinde 198 bilinen HT tanılı hastanın 160'ı antihipertansif ilaç kullanmaktaydı. İlaç kullanım oranı bilinen HT tanılı hastalarda %80,8 olarak saptandı. Tüm hipertansif gruba bakılırsa bu oran %57,6'da kaldı. Kullanılan ilaç grupları incelendiğinde hipertansif popülasyonun en sık kullandığı ilaç grubu; Renin-Anjiotensin Sistemi (RAS) blokeri+diüretik kombinasyonu idi, ilaç gruplarına göre yüzde dağılımı Şekil-2'de gösterilmiştir.

Hipertansiyon ilişkili uç organ hasarı ile ilgili olarak bakılan EKG'lerde toplam 322 hastanın 201 inin EKG'sine ulaşıldı. Sokolow Lyon kriterlerine göre bu hastaları %12,3'ünde LVH mevcuttu. Popülasyonun %55'inin GFR'si >90 ml/dk/1.73 m^2 bulundu (Şekil-3). Hastaların tam idrar tetkikine göre proteinüri %17,7 iken spot idrar verilerine göre bu oran %23 bulundu.

Retinopati ilişkili olarak toplam 71 hastanın göz dibi bakısı verisine ulaşıldı. Hipertansif retinopati oranı %32,85 olarak saptandı. Hipertansif hastaların %45'inin birinci derece akrabalarında da hipertansiyon öyküsü mevcuttu. Hipertansiyon ilişkili kardiyovasküler sonlanımlar ve inme verileri incelendiğinde; hastaların %2,9'unun miyokard infarktüsü geçirdiği, %1,4'ünün serebrovasküler olay öyküsü olduğu, %6,3'ünün de konjestif kalp yetmezliği olduğu görüldü. Hipertansif hasta grubunda eşlik eden kronik hastalık verileri Şekil-4'te özetlenmiştir.

Hipertansif hasta grubu ile normotansif kontrol grubunun biyokimyasal verileri ve hemogram değerleri incelenmiş ve ortalama değerler, standart sapma ve p değerleri Tablo-2 ve 3'te sunulmuştur. Çalışmamızda hipertansif popülasyonda Nötrofil/Lenfosit Oranı (NLR) (2.57±1.81) ve Platelet/Lenfosit Oranı (PLR) (147.55±110.77) değerlerini daha yüksek saptadık (p:0.011, p:0.003) (Tablo-4). NLR ve PLR ortalama değerleri incelendiğinde nefropati durumu ile bir ilişki saptamadık (Tablo-5).

Tablo-1. Demografik veriler ve ortalama kan basıncı değerleri.

Demografik Veriler	Hipertansif	Normotansif	P değeri
Sayı	332	935	
Yaş	57.5±14.7	40.7±16.4	<0.001
Cinsiyet (%kadın)	65	70	0.121
Sistolik Kan Basıncı (mmHg)	149.80±17.8	116.35±12.5	<0.001
Diyastolik Kan Basıncı (mmHg)	90.35±12.3	77.31±8.0	<0.001
Nabız	79±14	79±12	0.49

Tablo-2. Hipertansif ve normotansif popülasyonda biyokimyasal veriler *(ortalama, standart sapma ve p değeri) (Birimler: AST, ALT, alkalen fosfataz, GGT için U/L; total protein, albümin için g/dl; total kolesterol, trigliserit, glukoz, üre, kreatinin, ürik asit, kalsiyum, fosfor, CRP için mg/dl; TSH için mIU/L; sodyum, potasyum, klorür için mEq/L olarak verilmiştir.)*

Biyokimyasal Veriler	Hipertansif Grup Ort.	Kontrol Grubu Ort.	p
SGOT (AST)	20.09±11.09	19.24±15.50	0.475
SGPT (ALT)	21.51±17.99	19.00±18.17	0.116
Alkalen Fosfataz	79.63±25.88	74.19±28.08	0.032
GGT	28.49±34.41	20.05±19.43	0.001
Total Protein	7.32±0.47	7.30±0.51	0.789
Albümin	4.58±0.39	4.68±0.43	0.014
Total Kolesterol	202.14±45.65	192.67±44.77	0.046
Trigliserid	164.66±104.75	123.10±68.18	<0.001
Glukoz (Açlık)	121.29±6355	96.73±26.85	<0.001
Üre	32.51±14.99	26.79±11.88	<0.001
Kreatinin	0.83±0.26	074. ±0.21	<0.001
GFR CKD ERI	88.31±22.13	106.03±21.53	<0.001
Ürik Asid	5.36±1.57	4.65±1.36	<0.001
Sodyum	140.01±3.19	139.45±2.55	0.037
Potasyum	4.57±0.49	4.51±0.35	0.112
Klorür	100.66±3.62	100.96±2.64	0.323
Kalsiyum	9.62±0.44	9.60±0.40	0.628
Fosfor	3.51±0.61	3.51±0.58	0.966
CRP	083±1.76	0.75±2.50	0.742
TSH	2.35±	2.18±1.82	0.417

Tablo-3. Hipertansif ve normotansif popülasyonda hemogram verileri *(ortalama, standart sapma ve p değeri).*

Hemogram Verileri	Hipertansif Grup			Normotansif Grup			p değeri
Lökosit(x10^3/mm^3)	7.99	±	2.85	7.43	±	2.36	**0.01**
Nötrofil %	59.70	±	9.99	57.75	±	9.84	**0.02**
Lenfosit %	29.55	±	8.69	31.83	±	8.91	**0.00**
Monosit %	7.66	±	2.55	7.69	±	2.18	0.87
Eozinofil %	2.37	±	2.86	2.17	±	1.90	0.34
Bazofil Nabız (dk)%	0.58	±	0.28	0.55	±	0.26	0.32
İmmatür Granülosit %	0.61	±	0.98	0.48	±	1.47	0.21
Nötrofil (x10^3/mm^3)	4.69	±	1.90	4.41	±	2.06	0.11
Lenfosit (x10^3/mm^3)	2.18	±	0.84	2.25	±	0.71	0.25
Monosit (x10^3/mm^3)	0.59	±	0.33	0.56	±	0.20	0.17
Eozinofil (x10^3/mm^3)	0.16	±	0.14	0.16	±	0.14	0.50
Bazofil (x10^3/mm^3)	0.04	±	0.02	0.04	±	0.02	**0.01**
İmmatür Granülosit (x10^3/mm^3)	0.03	±	0.02	0.04	±	0.14	0.52
Eritrosit(x10^6/mm^3)	4.68	±	0.61	4.72	±	0.60	0.49
Hemoglobin(g/dl)	13.12	±	1.98	13.20	±	1.83	0.63
Hematokrit%	39.70	±	5.16	39.86	±	4.63	0.69
MCV (fL)	85.12	±	7.01	84.88	±	7.31	0.70
MCH (pg)	28.12	±	2.99	28.12	±	3.21	0.99
MCHC (g/dl)	32.98	±	1.47	33.04	±	1.44	0.60
RDW%	13.96	±	1.88	13.78	±	1.87	0.26
Trombosit (x10^3/mm^3)	269.57	±	95.06	265.02	±	69.50	0.53
MPV (fL)	10.36	±	1.22	10.46	±	0.95	0.33
PCT%	0.28	±	0.09	0.27	±	0.07	0.45

Tablo-4. Gruplar arasında ortalama NLR ve PLR değerleri arasındaki ilişki.

	Hipertansif Grup	**Normotansif Grup**	**p değeri**
NLR(Nötrofil-Lenfosit Oranı)	2.57±1.81	2.18±1.65	**0.011**
PLR(Trombosit-Lenfosit Oranı)	147.55±110.77	123.24±74.38	**0.003**

Tablo-5. Hipertansif popülasyonun nefropati durumuna göre karşılaştırmalı verileri *(ortalama±standart sapma, yüzde şeklinde).*

Nefropati	**Var**	**Yok**	**p değeri**
Yaş	60±13	56±14	**0.04**
Cinsiyet (%kadın)	57	70	**0.05**
Sistolik Kan Basıncı (mmHg)	150±20	148±17	0.56
Diyastolik Kan Basıncı (mmHg)	89±15	90±12	0.55
Nabız (dk)	80±12	78±14	0.58
BMI (kg/m^2)	29±5	30±6	0.26
Antihipertansif ilaç kullanım yüzdesi (%)	59	57	0.86
EKG de Sol Ventrikül Hipertrofisi (%)	16	12	0.53
Hipertansif Retinopati Yüzdesi (%)	45	28	0.17
Kardiyovasküler Olay Öyküsü Yüzdesi (%)	4	3	0.86
Serebrovasküler Olay Öyküsü Yüzdesi (%)	5	1	**0.01**
Diyabetik Olgu Yüzdesi (%)	38	27	0.11
ALT (SGPT) (U/L)	26±28	20±13	**0.04**
Alkalen Fosfataz (U/L)	88±29	77±24	**0.01**
GGT (U/L)	34±22	27±38	0.23
Albumin (g/dl)	4.5±0,5	4.6±0,3	0.17
Total Kolesterol (mg/dl)	194±38	205±47	0.13
Trigliserid (mg/dl)	172±121	161±100	0.53
Glukoz (mg/dl)	139±87	115±51	**0.01**
Üre (mg/dl)	36.7±23	31.3±22	**0.02**
Kreatinin (mg/dl)	0.9±0.3	0.8±0.2	**0.01**
GFR (CKD-EPI formülüne göre)	81.90±25	90.46±21	**0.01**
Ürik Asit (mg/dl)	5.83±1.7	5.25±1.5	**0.03**
Lökosit (WBC) ($x10^3/mm^{3)}$	8.18±2.40	7.89±3.05	0.50
Nötrofil Yüzdesi (%)	63	59	**0.01**
Hematokrit (%)	38±6	40±5	**0.03**
PLR (Trombosit/Lenfosit Oranı)	163±127	145.56±109	0.31
NLR (Nötrofil Lenfosit Oranı)	2.92±1.84	2.48±1.78	0.09

Tablo-6. Hipertansif popülasyonun retinopati durumuna göre karşılaştırmalı verileri *(ortalama±standart sapma ve yüzde şeklinde).*

Retinopati	Var	Yok	p değeri
Yaş	60±11	59±13	0.87
Cinsiyet (%kadın)	65	66	1
Sistolik Kan Basıncı (mmHg)	149±16	146±16	0.4
Diyastolik Kan Basıncı (mmHg)	89±12	89±11	0.86
Nabız (dakika)	77±11	80±18	0.53
BMI ($kg/m^{2)}$	28.6±4.7	29.5±5.8	0.55
Antihipertansif ilaç kullanım yüzdesi (%)	70	63	0.6
EKG de Sol Ventrikül Hipertrofisi (%)	28	3	**0.002**
Hipertansif Nefropati Yüzdesi (%)	41	24	0.17
Kardiyovasküler Olay Öyküsü Yüzdesi (%)	9	2	0.24
Serebrovasküler Olay Öyküsü Yüzdesi (%)	9	0	**0.05**
Diyabetik Olgu Yüzdesi (%)	43	26	0.17
ALT(SGPT) (U/L)	17±6	24±24	0.19
Alkalen Fosfataz (U/L)	92±25	83±29	0.41
GGT (U/L)	23±11	30±23	0.21
Albümin (g/dl)	4.53±0.28	4.53±0.4	0.94
Total Kolesterol (mg/dl)	223±48	185±41	**0.004**
Trigliserit (mg/dl)	202±163	157±88	0.21
Glukoz (mg/dl)	127±80	119±67	0.67
Üre (mg/dl)	34±17	33±13	0.94
Kreatinin (mg/dl)	0.82±0.38	0.84±0.27	0.84
GFR (CKD-EPI formülüne göre)	89±21	87±20	0.72
Ürik Asit (mg/dl)	5.37±1	5.32±1	0.89
Lökosit (WBC)($x10^3/mm^3$)	7.19±2	9.26±5	0.09
Nötrofil Yüzdesi (%)	58	60	0.61
Hematokrit (%)	39±4	39±4.6	0.9
PLR (Platelet/Lenfosit Oranı)	125±47	151±113	0.3
NLR (Nötrofil Lenfosit Oranı)	2.16±1.29	3.23±2.65	0.08

Hipertansif popülasyonda nefropati durumuna göre verileri kıyaslandı ve Tablo-5'te özetlendi. Nefropatisi olan grubun SVO yüzdesi anlamlı olarak daha yüksek saptandı (%5, p:0.01). Laboratuvar verileri incelendiğinde ALT, ALP, açlık kan şekeri, üre, kreatinin, ürik asit, nötrofil yüzdesi ve hematokrit değerlerinin ortalaması nefropatisi olan grupta anlamlı olarak daha yüksek saptanırken; nefropatisi olan grupta GFR ortalaması daha düşük saptandı. Hemogram verileri incelendiğinde nefropatisi olan grupta nötrofil yüzdesi diğer gruba oranla yüksek saptanırken, hematokrit ortalamaları daha düşük saptandı. Hematokritteki bu düşüklüğün hipertansif nefropati ve kronik böbrek hastalığı ilişkili anemi nedenli olabileceği düşünüldü.

Hipertansiyonu olan hastalar retinopati durumuna göre de kıyaslandı ve veriler Tablo-6'da sunuldu. Hipertansif retinopatisi olan olgularda anlamlı şekilde LVH'de artış olduğu görüldü. Retinopatisi

olan hastalarda LVH sıklığı %28 iken olmayan grupta %3 saptandı (p:0.002). Serebrovasküler olay sıklığı %9 oranı ile retinopatisi olanlarda daha fazla saptandı (p:0.05). Total kolesterol düzeyi ile retinopati arasında pozitif korelasyon gösteren bir ilişki saptandı. Retinopatisi olan grupta ortalama total kolesterol 223,83±48,30 mg/dl iken retinopati olmayan grupta bu değer 185.47±41.38 mg/dl de kaldı (p:0.004).

TARTIŞMA

HT çağımızın ciddi mobidite ve mortaliteye neden olan sık görülen bir hastalığıdır. Bizim çalışmamızda HT prevalansı Türkiye verilerinin %5,6 altındadır. Antihipertansif ilaç kullanım oranı da PatenT 2 çalışmasına göre %10,1 yüksek saptanmıştır (1, 2). Bu farklılıkların sebebi Akdeniz tarzı beslenme yapısı ve Ege Bölgesindeki sosyoekonomik düzeyin Türkiye ortalamasına göre yüksek olması ile açıklanabilir. İlaç kullanım verileri incelendiğinde bilinen HT tanılı hastalarda belirgin olmak üzere tüm grupta PatenT 2 çalışmasına göre yüksek bulunmuştur (1, 2). Bizim popülasyonumuzda ilaç kullanım oranı daha iyi olması kadın hasta popülasyonundaki yükseklik nedeniyle ilaç kullanım oranlarındaki artışa bağlı olabilir. Hipertansif popülasyonun yaş dağılımına bakılırsa PatenT 2 verileri ve grafiği ile örtüştüğü görülmektedir. Çalışmamızda kombinasyon antihipertansif tedavi kullanan olgularda PatenT 2 çalışmasıyla benzer olarak en sık tercih edilen grup ARB+diüretik ve ACEi+diüretik kombinasyonuydu (1, 2).

Rakotovao ve arkadaşları tarafından yapılan 151 hipertansif hastanın hemogram verilerinin incelendiği bir çalışmada hastaların %60,3'ünde patolojik kan sayımı gözlenmiş. Hastaların %33,9'unda anemi, %33'ünde lökositoz saptanmış (5). Bizim çalışmamızda da hemoglobin değerleri hasta popülasyonda daha düşük gözlendi; ancak anlamlı değerlendirilmedi. Bu çalışma ile benzer şekilde hipertansif grupta lökosit sayısı anlamlı olarak daha yüksekti (p:0.01).

Bozduman ve ark tarafından yapılan 409 hastanın dahil edildiği bir çalışmada; non-dipper hipertansif, prehipertansif ve normotansif hasta popülasyonları arasında GGT, NLR ve PLR değerlerini kıyaslanmış ve her üç değer de non-dipper hipertansiflerde daha yüksek bulunmuş (6). Non-dipper hipertansiyonda yüksek gözlenen bu değerleri biz kendi çalışmamızda hipertansif popülasyonda anlamlı olarak yüksek bulduk. Ancak ambulatuvar kan basıncı izlemi yapmadığımızdan dipper, non-dipper durum ile ilgili yorum yapamadık. Trombosit aktivasyonu, MPV (mean platelet volume), PCT (platekrit) değerleri ile non-dipper tansiyon arasındaki ilişkiyi gösteren çok sayıda yayın mevcuttur. Cetin N ve ark.'na ait çalışmada 153 çocukta ambulatuvar kan basıncı ölçümü verileri ve hemogram verileri incelenmiş; non-dipper kolda nötrofil sayısı, MPV, PCT değeri anlamlı olarak daha yüksek bulunmuş. Yine aynı çalışmada gruplar arasında CRP, NLR ortalama değerleri arasında anlamlı fark bulunmamıştı (7). Erdoğan D ve ark'a ait bir çalışmada daha MPV değerinin non-dipper hipertansif grupta hipertansif ve normotansiflere göre anlamlı olarak yüksek olduğu saptanmıştır (8). Bu sonuçlar bizim bulgularımız ile tam örtüşmemektedir. Çalışmamızda MPV, PCT değerleri ile hipertansif durum arasında anlamlı bir korelasyon saptamadık.

Sunbul M ve ark.'na ait olan bir başka çalışmada non-dipper hipertansif hastalarda NLR ve PLR değerleri anlamlı olarak dipper hipertansif gruba göre yüksek bulunmuş (9). Sun X ve ark 80 yaş ve üzeri hipertansif hastalarda NLR yüksekliğinin tüm sebeplere bağlı ölüm için iyi bir ön gördürücü olduğunu saptamışlardır (10). Biz çalışmamızda hipertansif popülasyonda NLR ve PLR değerlerini anlamlı olarak daha yüksek saptadık. Bu parametreler HT gelişimi açısından ön gördürücü olarak kullanılabilir.

HT ile uç organ tutulumları arasındaki ilişkiyi saptamak için retinopatili ve nefropatili olguların karşılaştırmalı verilerini inceledik. Çalışmamızda hipertansif nefropati prevalansını %23 saptadık. Hipertansif nefropati prevalansı İtalya ve Fransa verilerine göre sırasıyla %25 ve %17 olarak bildirilmiştir (11, 12). Bizim nefropati prevalans verilerimiz İtalya ve Fransa verileri ile örtüşmektedir. Hipertansif retinopati prevalansını %32,8 olarak saptadık. Literatürdeki retinopati insidans, prevalans çalışmaları değerlendirildiğinde bizim çalışmamızda bulunan hipertansif retinopati prevalans verilerimiz diğer çalışmaların oldukça altındadır (13, 14). Bu farklılık çalışmamızda 71 hastaya ait gözdibi bakısı verisine ulaşılabilmesinden kaynaklanabilir. Ayrıca literatürle olan bu farklılık bazı ülkelerde ve çalışmalarda retinopati evresine göre farklı değerlendirme yapılmasından kaynaklanabilir.

Julian Segura ve ark tarafından yapılan bir hipertansiyon ilişkili kardiyovasküler ve renal sonlanımların incelendiği çalışmada; SDBY gelişimi ile yaş, sistolik kan basıncı, serum kreatinin, ürik asit, açlık şekeri ve total kolesterol, trigliserit, LDL-K değeri arasında pozitif korelasyon saptanmış (15). Biz de çalışmamızda yaşlılarda ürik asit, açlık kan şekeri ve kreatinin yüksekliği olanlarda nefropati gelişme sıklığını yüksek saptadık, ancak lipid düzeyleri ile hipertansif nefropati arasında bir ilişki saptayamadık. Serum ürik asit düzeyinin hipertansif nefropati açısından ön gördürücü olarak kullanılabileceğini düşünüyoruz. Wang A. ve arkadaşlarının yaptığı 24300 kişinin dahil edildiği bir kohortta hipertansiyon ilişkili proteinüri ve serebrovasküler olaylar arasında anlamlı bir ilişki saptanmıştır. Proteinürinin SVO için güçlü bir ön gördürücü olduğu belirtilmiştir (16). Bizim çalışmamızda da hipertansif nefropatisi olan grupta anlamlı olarak SVO öyküsü nefropatisi olmayanlara göre daha fazlaydı. Verilerimiz bu çalışma ile örtüşmektedir. Bu durum hipertansiyona sekonder gelişen endotel hasarı ve ateroskleroza bağlı olabilir.

Shirafkan A ve ark.'na ait bir çalışmada hipertansif 102 hastada LVH ve hipertansif retinopati arasındaki ilişki incelenmiş. Bu hastalarda ortalama sistolik ve diyastolik kan basıncı ve LVH arasında pozitif korelasyon saptanmış. Ancak hipertrofi ciddiyeti ile retinopati arasında anlamlı ilişki saptanmamış (17). Ancak bizim bulgularımıza göre hipertansif retinopati ile LVH arasında pozitif korelasyon gösteren bir ilişki mevcuttur.

Literatürde birçok çalışmada retinopati ile serum lipid değerleri arasında pozitif korelasyon gösteren bir ilişki olduğu saptanmıştır (18, 19). Biz de yaptığımız bu çalışmada retinopatili olgularda serum total kolesterol düzeyini anlamlı olarak daha yüksek saptadık. Serum total kolesterol düzeyi yüksekliği retinopatinin bir ön gördürücüsü olabilir. Ancak bizim çalışmamızda hastalara ait retinopati evrelemesi ve serum LDL kolesterol verisi mevcut olmadığından kolesterol düzeyinin yüksekliği ile retinopati evresi ve LDL ilişkisi hakkında yorum yapamadık.

Çalışmanın kısıtlılıklarına bakacak olursak; çalışma grubumuz Ege Üniversitesi Tıp Fakültesi Hastanesi İç Hastalıkları polikliniğine başvuran hastalardan oluşmakta olup örneklem evreni temsil etmeyebilir, tüme genellenemeyebilir. Hipertansif grup içinde sekonder hipertansiyon tanılı olgular da mevcut olabileceğinden, bulgular esansiyel hipertansiyonlu popülasyonlarda yapılan araştırmalar ile örtüşmeyebilir. Ambulatuvar kan basıncı gözlemi yapamadığımızdan, non–dipper, dipper hipertansif durum ile ilgili veri elde edemedik.

Sonuç olarak hipertansif retinopati ve SVO sıklığı arasında, hipertansif LVH ile retinopati arasında ve total kolesterol yüksekliği ile retinopati gelişimi arasında pozitif korelasyon gösteren bir ilişki saptadık. Tüm hipertansif hastaların lipid düzeylerinin görülerek gerekli tedaviye erken dönemde başlanması retinopati gelişmini engelleyebilir. Fundoskopik bakı, 12 derivasyonlu EKG, spot idrar protein/kreatinin oranı tetkiklerinin tüm hipertansif hastalarda düzenli olarak yapılması gerektiğini düşünüyoruz. Bu sayede hipertansiyonun doğuracağı sekonder hastalıklar önlenebilir.

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Kaynaklar

1. Sengul S, Akpolat T, Erdem Y, Derici U, Arici M, Sindel S, et al. Changes in hypertension prevalence, awareness, treatment, and control rates in Turkey from 2003 to 2012. Journal of hypertension. 2016;34(6):1208-17.
2. Aydoğdu S, Güler K, Bayram F, Altun B, Derici Ü, Abacı A, et al. Türk hipertansiyon uzlaşı raporu 2019. Turk Kardiyol Dern Ars. 2019;47(6):535-46.
3. Seyahi N, Ateş K, Süleymanlar G. Current status of renal replacement therapy in Turkey: A summary of the Turkish society of nephrology registry report. Current Status of Renal Replacement Therapy in Turkey. 2020.
4. Bergler-Klein J. What's new in the ESC 2018 guidelines for arterial hypertension: The ten most important messages. Wiener Klinische Wochenschrift. 2019;131(7-8):180-5.

5. Rakotovao-Ravahatra ZD, Randriatsarafara FM, Razafimanantsoa F, Rabetokotany FR, Rakotovao AL. Blood count results from hypertensive patients seen in laboratory of CHU-HJRB Antananarivo in 2013. The Pan African Medical Journal. 2016;23:49-.
6. Bozduman F, Yildirim E, Cicek G. Biomarkers of nondipper hypertension in prehypertensive and hypertensive patients. Biomarkers in Medicine. 2019;13(05):371-8.
7. Cetin N, Tufan AK. Platelet activation and inflammation in hypertensive children with non-dipper and dipper status. Iranian journal of kidney diseases. 2019;13(2):105.
8. Erdogan D, Icli A, Aksoy F, Akcay S, Ozaydin M, Ersoy I, et al. Relationships of different blood pressure categories to indices of inflammation and platelet activity in sustained hypertensive patients with uncontrolled office blood pressure. Chronobiology International. 2013;30(8):973-80.
9. Sunbul M, Gerin F, Durmus E, Kivrak T, Sari I, Tigen K, et al. Neutrophil to lymphocyte and platelet to lymphocyte ratio in patients with dipper versus non-dipper hypertension. Clinical and Experimental Hypertension. 2014;36(4):217-21.
10. Sun X, Luo L, Zhao X, Ye P, Du R. The neutrophil-to-lymphocyte ratio on admission is a good predictor for all-cause mortality in hypertensive patients over 80 years of age. BMC Cardiovascular Disorders. 2017;17:1-9.
11. Thilly N, Boini S, Kessler M, Briançon S, Frimat L. Management and control of hypertension and proteinuria in patients with advanced chronic kidney disease under nephrologist care or not: data from the AVENIR study (AVantagE de la Néphroprotection dans l′ Insuffisance Rénale). Nephrology Dialysis Transplantation. 2009;24(3):934-9.
12. Galassi A, Brancaccio D, Cozzolino M, Bellinghieri G, Buoncristiani U, Cavatorta F, et al. Awareness of hypertension and proteinuria in randomly selected patients in 11 Italian cities. A 2005 report of the National Kidney Foundation of Italy. The Journal of Clinical Hypertension. 2009;11(3):138-43.
13. Erden S, Bicakci E. Hypertensive retinopathy: incidence, risk factors, and comorbidities. Clinical and Experimental Hypertension. 2012;34(6):397-401.
14. Del Brutto OH, Mera RM, Viteri EM, Pólit J, Ledesma EA, Cano JA, et al. Hypertensive retinopathy and cerebral small vessel disease in Amerindians living in rural Ecuador: The Atahualpa Project. International Journal of Cardiology. 2016;218:65-8.
15. de la Sierra A, Segura J, Banegas JR, Gorostidi M, de la Cruz JJ, Armario P, et al. Clinical features of 8295 patients with resistant hypertension classified on the basis of ambulatory blood pressure monitoring. Hypertension. 2011;57(5):898-902.
16. Wang A, Dai L, Su Z, Chen S, Li J, Wu S, et al. Proteinuria and risk of stroke in patients with hypertension: the Kailuan cohort study. The Journal of Clinical Hypertension. 2018;20(4):765-74.
17. Shirafkan A, Motahari M, Mojerlou M, Rezghi Z, Behnampour N, Gholamrezanezhad A. Association between left ventricular hypertrophy with retinopathy and renal dysfunction in patients with essential hypertension. Singapore Med J. 2009;50(12):1177-83.
18. Hicks PM, Melendez SAC, Vitale A, Self W, Hartnett ME, Bernstein P, et al. Genetic epidemiologic analysis of hypertensive retinopathy in an underrepresented and rare federally recognized native American population of the intermountain west. Journal of community medicine & public health. 2019;3(1).
19. Gupta RP, Gupta S, Gahlot A, Sukharamwala D, Vashi J. Evaluation of hypertensive retinopathy in patients of essential hypertension with high serum lipids. Medical Journal of Dr DY Patil University. 2013;6(2):165-9.

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İdiyopatik pulmoner fibroziste seri solunum fonksiyon testlerinin prognozu belirlemedeki önemi: "retrospektif analiz"

The importance of serial pulmonary function tests in determining prognosis in idiopathic pulmonary fibrosis: "retrospective analysis"

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ÖZ

Amaç: Çalışmamızın amacı; idiyopatik pulmoner fibrozisli (IPF) hastaların seri solunum fonksiyon testi (SFT) ölçüm parametrelerinin prognozu öngörmedeki değerini retrospektif olarak gözlemlemektir. İkincil olarak ise yaş, cinsiyet, sigara öyküsü, sistolik pulmoner arter basıncı (SPAB) yüksekliği gibi diğer değişkenlerle prognoz ve SFT parametreleri arasındaki ilişkiyi saptamaktır.

Gereç ve Yöntem: Çalışmaya dahil edilen 94 olgunun demografik verileri yanı sıra ilk başvurudaki ve izlemdeki (6.ay, 12.ay, 24.ay, 36.ay, 48.ay ve 60.ay) SFT ölçümleri ve SPAB değerleri kaydedilmiştir.

Bulgular: Çalışma grubunun yaş ortalaması 62,8±10,3 olup %71,3'ü (67 olgu) erkektir. Ortalama izlem süreleri 41,03±27,02 aydır. Hastaların %63,8'i (60 hasta) çalışma izleminde hayatını kaybetmiştir. Otuz iki olgunun (%34) tanısı cerrahi akciğer biyopsisi ile doğrulanmıştır. Yaşın genel sağkalımla veya izlemdeki SFT kayıplarıyla ilişkisiz olduğu saptanmıştır. Ancak 50 yaş altı olgu grupta ortalama sağkalımın belirgin düşük olduğu gözlenmiştir (p=0,039). SPAB düzeyi yüksek olan olguların gerek genel sağkalım gerekse ekokardiyografi sonrası sağkalım sürelerinin anlamlı düşük olduğu saptanmış (p=0,006 ve p<0,001) ve SPAB yüksekliğinin karbonmonoksit difüzyon kapasitesinin alveoler volüme oranı (DLCO/VA) ile ilişkili olduğu gözlenmiştir (p=0,05). Bazal zorlu vital kapasite ve DLCO değerinin sırasıyla %10 ve %15'ini ilk 6 ve 12 ay içerisinde kaybeden olguların sağkalımlarının bu kaybı yaşamayanlarla farklı olmadığı görülmüştür.

Sonuç: Çalışmamızda IPF'de prognozu belirleyen parametreler; 50 yaşın altında tanı almak ve SPAB'nın yüksek saptanması olarak belirlenmiştir. Elli yaş altı grubun prognozu SFT'deki değişimlerle ilişkisiz, SPAB yüksekliği ise DLCO/VA'daki değişim ile ilişkili saptanmıştır. IPF'de etkili tedavilerin kullanılmaya başlanmasıyla birlikte tedavi izleminin yanı sıra hastaların uygun zamanda transplantasyona yönlendirilmesi açısından prognostik çalışmalar önem taşımaktadır.

Anahtar Sözcükler: İdiyopatik pulmoner fibrozis, solunum fonksiyon testi, sistolik pulmoner arter basıncı.

ABSTRACT

Aim: *Our study aimed to retrospectively observe the value of serial pulmonary function test (PFT) parameters in predicting prognosis in patients with idiopathic pulmonary fibrosis (IPF). Secondarily, to determine the relationship between PFT parameters and other variables such as age, gender, smoking, and systolic pulmonary artery pressure (SPAP).*

Materials and Methods: *Demographic data and PFT's at initial and follow-up (6,12,24,36,48, and 60, months) and SPAP values were recorded in 94 patients.*

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Results: *The mean age of the population was 62.8±10.3 years, and 71.3% (67 patients) were male. The mean follow-up period was 41.03±27.02 months. Of the patients, 63.8% (60 patients) died during follow-up. Thirty-two patients (34%) had their diagnosis confirmed by surgical lung biopsy. Age was not associated with survival or PFT losses. However, patients under the age of 50 and with high SPAP had significantly lower survival (p=0.039, p<0.001). High SPAP was associated with the ratio of carbon monoxide diffusion capacity to alveolar volume (DLCO/VA,p=0.05). The survival of patients who lost 10% and 15% of their baseline forced vital capacity and DLCO, respectively, within the first 6 and 12 months, was not different from those who didn't experience this loss.*

Conclusion: *In this study, the parameters determining the prognosis in IPF were diagnosed under the age of 50 and with high SPAP. In the under-50 group, prognosis was not associated with changes in PFT, whereas high SPAP was associated with DLCO/VA. With the introduction of effective treatments in IPF, prognostic studies are essential in monitoring and also directing patients to transplantation at appropriate time.*

Keywords: *Idiopathic pulmonary fibrosis, pulmonary function test, systolic pulmonary artery pressure.*

GİRİŞ

İdiyopatik pulmoner fibrozis (IPF); bilinmeyen bir nedenle meydana gelen, kronik, ilerleyici bir interstisyel pnömonidir. Tanı; klinik ve radyolojik bulgulara ek olarak, biyopside karakteristik olağan interstisyel pnömoni (UIP) bulgularının olması ile konur. Ancak yaygın bal peteği bulguları gözlenen ileri fibrozisi olan hastalarda biyopsinin zorluğu nedeniyle genellikle tanı, rehberlere dayanan klinik ve radyolojik bulgularla konmaktadır (1). Prevalans çalışmalarında 100,000'de 2 ile 29 arasında değişen oranlar belirtilmiştir (2-4).

İdiyopatik pulmoner fibrozis, yaygın fibrozise yol açan hastalıklar arasında en sık görüleni ve en ölümcül olanıdır. IPF'de tanı konduktan sonra tedavisiz yaşam beklentisi, çeşitli kaynaklarda 2,4 ile 4,2 yıl arasında bildirilmektedir (5, 6). Antifibrotik tedavilerin yaygın kullanıma girdiği son dekatta ise öngörülen yaşam beklentisinin arttığı gözlemlenmektedir (7).

İdiyopatik pulmoner fibrozisli hastaların prognozu belirleyen parametrelerle düzenli izlenmesi önemlidir. Hastalığın progresyon hızının saptanması, semptomlardaki kötüleşmenin ek değerlendirilmelerle objektif olarak gösterilmesi, tedavi etkinliğinin gözlenmesi ve transplantasyon için doğru zamanlama hastaların düzenli izlemleri ile sağlanabilir (8, 9).

Prognozu gösteren bağımsız değişkenler literatürde gözden geçirildiğinde; sigara kullanımı, amfizem varlığı ve pulmoner arteryel hipertansiyon gelişimi ile hastalık prognozu arasında negatif bir korelasyon izlendiği, yaş ve cinsiyet açısından farklı sonuçlara ulaşıldığı gözlemlenmektedir (10-14).

Hastalığın ciddiyetini ve progresyonunu belirleyen çeşitli parametreler belirlenmiştir. Bunlar arasında en sık kullanılanlar; solunum fonksiyon testleri (SFT) ile solunumsal kapasitenin ölçümü, karbonmonoksit difüzyon kapasitesi (DLCO), 6 dakika yürüme testi (6-DYT) ile efor kapasitesinin değerlendirilmesi, arteriyel kanda parsiyel oksijen basıncı (PaO2) ile gaz değişiminin değerlendirilmesi ve pulse oksimetre ile efora bağlı desatürasyon varlığının araştırılması yer almaktadır (9, 10).

Solunum fonksiyonlarında yavaş azalma ve dispnede yavaş artış IPF'nin klasik prototipi olarak belirtilse de solunum fonksiyonlarının hızla bozulduğu ve semptomların hızla kötüleştiği bir diğer grup ve alevlenmelerle seyreden, ataklar arası kısmen stabil solunum fonksiyonları ve semptomları olan, ancak akut alevlenmelerin mortalitesinin yüksek olduğu fenotipik gruplar da tanımlanmıştır (1). Hastalığın tanı aşamasında, hastanın izleminin hangi gruba uygun ilerleyeceği, dolayısıyla prognozu öngörmek güçtür. Güncel çalışmalar eşliğinde hızlı veya yavaş progresyonlu hastalıktaki tedavi yaklaşımı farklı olmasa da hastaların izlem aralıkları, öngörülen yaşam süreleri, transplantasyon listesi için uygunluk araştırmaları ve transplantasyon zamanlaması açısından IPF hastasının semptomlarının, klinik bulgularının ve solunum fonksiyonlarının takibi önem taşımaktadır (10, 11).

IPF'li hastaların prognozunu değerlendirdiğimiz retrospektif çalışmamızın birincil sonlanım noktası, seri solunum fonksiyon testleri ile izlemde, zorlu vital kapasite (FVC) ve DLCO bazal değerlere göre saptanan değişikliklerin prognozu öngörmedeki değerinin gözlemlenmesidir. İkincil sonlanım noktası ise; yaş, cinsiyet gibi demografik özellikler yanı sıra sigara öyküsü ve pulmoner hipertansiyon gelişim göstergeleri ile prognoz arasındaki ilişkilerin solunum fonksiyon testlerine etkileri ile birlikte araştırılmasıdır.

GEREÇ ve YÖNTEM

Çalışmaya Mart 2003 - Mayıs 2013 tarihleri arasında Ege Üniversitesi Tıp Fakültesi, Göğüs Hastalıkları Anabilim Dalı, İnterstisyel Akciğer Hastalıkları Polikliniği'nde takip edilen IPF hastaları alınmıştır. Amerikan Toraks Derneği ve Avrupa Solunum Derneği'nin 2011 yılında yayınlamış olduğu IPF rehberindeki tanı kriterlerine uygun olarak IPF tanısı alan (biyopsili veya biyopsisiz) ve aralarında en az altı ay olmak üzere iki veya fazla SFT ile izlenen hastalar dahil edilmiştir. Hastaların hiçbiri, izlendikleri dönem itibariyle IPF'ye özel bir antifibrotik tedavi rutin kullanımda olmadığından, hastalığa spesifik tedavi almamıştır. Solunum fonksiyon testlerine koopere olamayan, düzenli SFT sonucu bulunmayan ve IPF tanısı kesinleşmemiş hastalar çalışma dışı bırakılmıştır.

Hastaların ilk başvurudaki ve izlemdeki kayıtları taranarak; ilk, 6.ay, 12.ay, 24.ay, 36.ay, 48.ay ve 60.aya karşılık gelen solunum fonksiyon testlerinden mevcut olanlar kaydedilmiştir. Ayrıca hastaların demografik verileri olarak yaş, cinsiyet, sigara öyküsü ve sağkalımları kaydedilmiştir. Ekokardiyografi (EKO) yapılan hastaların sistolik pulmoner arteriyel basınçları (SPAB) veri tabanına eklenmiştir. Birden fazla EKO yapılan olgularda, başlangıçta normal olan SPAB düzeyi izlemde yüksek saptandıysa 36 mmHg'nın üzerinde olan ilk değer ve tarih kaydedilmiştir.

Verilerin analizi IBM SPSS (Statistical Package for Sociel Sciences, SPSS Inc,, Chicago, Illinois, USA) 16,0 Windows istatistik paket programı kullanılarak yapılmıştır.

Kategorik değişkenler için ki-kare testi ve Fisher's exact test, normal dağılım özelliği gösteren değişkenler için Student's t test kullanılmış, korelasyon analizleri yapılmış, sağkalım analizleri için Cox regression analizleri, sağkalım eğrileri için Kaplan-Meier eğrileri kullanılmıştır. İstatistiksel anlamlılık için p değerinin <0,05 olması kabul edilmiştir.

Çalışma için Ege Üniversitesi Tıp Fakültesi Etik Kurulu'ndan onay alınmıştır (Onay no: 12-11,1/8)

BULGULAR

İdiyopatik pulmoner fibrozis hasta listesi epikriz sisteminden ICD kodu "J84,1" ve epikriz metninden "kelime ile tarama" şeklinde belirlenmiştir. İlk taramaya göre 249 hasta saptanmıştır. Kliniğimize IPF ön tanısıyla yönlendirilen, ancak ileri incelemelerle interstisyel akciğer hastalıkları konseyinde diğer nedenlere bağlı akciğer fibrozisi saptananlar, izlem süresi altı aydan kısa olanlar, SFT bulunmayan veya tek SFT olanlar ile tanıda radyolojik ve klinik korelasyonu olmayanlar çalışma dışı bırakılmıştır (Şekil-1).

Çalışmaya dahil edilen 94 olgunun yaş ortalaması 62,8±10,3 (17-83) yıl olup %71,3'ü (67 olgu) erkektir. Kadınların yaş ortalaması 65,3±5,8, erkeklerin ise 61,4±12 yıldır. Hastaların ortalama izlem süreleri 41,0±27 (6-139) aydır. Olguların %63,8'si (60 olgu) çalışmanın kapsadığı tarih aralığında ölmüş olup, %36,2 (34 olgu) ise çalışma sonunda hayattadır. Olgulardan 51'inin (%54,2) sigara öyküsü mevcuttur. Ortalama sigara tüketimi 33,7±17 paket-yılı olarak saptanmıştır. Otuz iki olgu (%34) akciğer biyopsisi ile kanıtlanmış IPF tanısı almış, 62 olguya ise (%66) klinik ve radyolojik bulgularla IPF tanısı konulmuştur. Biyopsi ile IPF tanısı kanıtlananlarla, klinik ve radyolojik bulgularla tanı alanlar arasında sağkalım farkı saptanmamıştır (p=0,38). Olguların demografik özellikleri Tablo-1'de verilmiştir.

Tablo-1. Olguların demografik özellikleri ve ortalama bazal solunum fonksiyon değerleri.

Demografik özellikler		**Sonuçlar**
Yaş (Yıl Ort±SD)		62,8±10,3
Cinsiyet n (%)	Erkek	67 (71,3)
	Kadın	27 (28,7)
İzlem süresi (Ay Ort±SD)		41,0±27,0
Biyopsi n (%)		32 (34,0)
Sigara n (%)	Bırakmış	51 (54,2)
	İçmemiş	43 (43,6)
FEV1 (L Ort±SD) (%)		2,10±0,7 (80,6)
FVC (L Ort±SD) (%)		2,59±0,9 (87,6)
FEV1/FVC (% Ort±SD)		83,1±8,1
DLCO (ml/mmHg/dk Ort±SD) (%)		13,9±5,3 (58,2)
DLCO/VA (% Ort±SD)		79,5 ± 28,3

FEV1: Zorlu ekspiryumun 1, saniyesinde çıkarılan hava hacmi, FVC: Zorlu vital kapasite, DLCO: Karbonmonoksit difüzyon kapasitesi, DLCO/VA: Karbonmonoksit difüzyon kapasitesinin alvoler volüme oranı

Tablo-2. Olguların cinsiyete göre FVC, DLCO ve DLCO/VA değişimleri (12 ay).

	Cinsiyet	N	Ortalama	Std, Sapma	P
FVC değişimi (ml)	erkek	67	-447,95	505,26	0,04
	kadın	27	-255,46	329,62	
FVC değişimi (%)	erkek	67	-14,61	16,89	0,8
	kadın	27	-15,43	22,90	
DLCOdeğişimi (ml/mmHg/dk)	erkek	32	-2,81	4,12	0,61
	kadın	12	-2,35	3,62	
DLCO değişimi (%)	erkek	32	-16,18	33,21	0,68
	kadın	12	-22,10	29,99	
DLCO/VA değişimi (%)	erkek	32	4,68	21,02	0,84
	kadın	12	4,12	24,51	

Tablo-3. Olguların cinsiyete göre yaş, sigara paket yılları ve izlem süreleri.

	Cinsiyet	N	Ortalama	Std, Sapma	p
Sigara paket-yılı	erkek	49	33,82	16,97	0,09
	kadın	2	13,50	6,12	
İzlem Süresi (ay)	erkek	67	33,85	26,39	0,61
	kadın	27	31,70	19,97	
Yaş	erkek	67	61,40	12,06	0,03
	kadın	27	65,33	5,80	
Sağkalım (ay)	erkek	39	30,22	25,86	0,9
	kadın	21	32,30	22,12	

Çalışmaya alınan hastaların demografik özellikleri ile klinik ve laboratuvar bulgularının prognoza ve SFT parametrelerine etkileri gözden geçirilmiş, aşağıdaki bulgulara ulaşılmıştır.

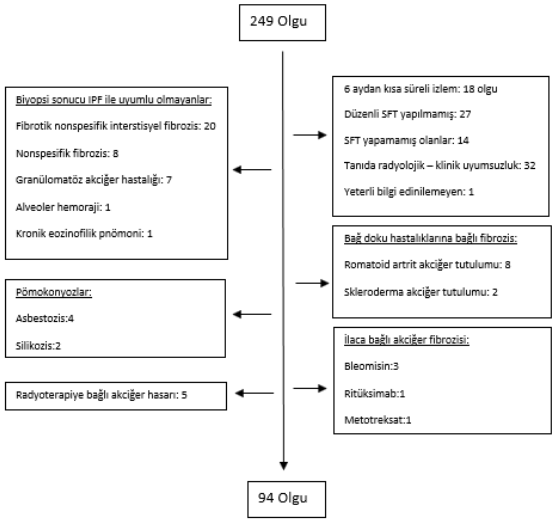
Yaş: Olguların yaşları incelendiğinde, yaşın genel sağkalımla veya izlemdeki SFT parametreleri kayıplarıyla ilişkisiz olduğu saptanmıştır. Ancak 50 yaş altı olgu grubu ayrı incelendiğinde (10 olgu), ortalama sağkalımın bu grupta 20,7±21,6 ay, 50 yaş üzerinde (84 olgu) ise 33,3±23,2 ay olduğu saptanmıştır (p= 0,036). Olguları 50 yaş altı ve üzeri olarak gruplandırılarak oluşturulan sağkalım eğrisi Şekil-2'e gösterilmiştir.

Cinsiyet: Kadın ve erkek hastalar arasında izlem süresi, sağkalım açısından fark saptanmamışken, kadın olguların tanı yaşının erkeklere göre anlamlı oranda daha ileri olduğu saptanmıştır (p=0,03) ve izlem süresince olan SFT kayıpları açısından erkeklerde FVC'deki kaybın fazla olması dışında anlamlı fark saptanmamıştır. Olguların cinsiyete bağlı FVC, DLCO ve DLCO/VA düzeyleri Tablo-2'de, yaş, sigara paket-yılları ve izlem süreleri ise karşılaştırmalı olarak Tablo-3'te gösterilmiştir.

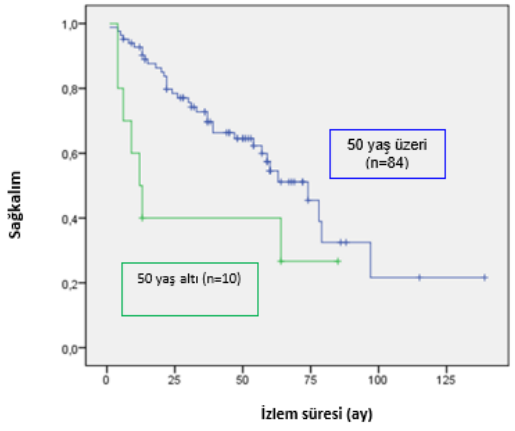
Sigara: Olguların %54,2'sinin sigara öyküsü olup ortalama paket-yılı 33,7±17,0'dır. Erkek olguların %73,71'inde, kadın olguların ise %7,4'ünde sigara öyküsü bulunmaktadır ve aralarında anlamlı istatistiksel farklılık saptanmıştır (p<0,001). Hastaların solunum fonksiyon parametrelerindeki kayıp yüzdeleri ve sağkalımları ile sigara öyküsü arasında ilişki saptanmamıştır ancak sigara içenlerin FEV1/FVC oranlarının anlamlı düşük olduğu saptanmıştır (p=0,005). Olguların sigara öyküleri olup olmamasına göre gruplandırılarak oluşturulmuş izlem süreleri, başlangıç SFT parametreleri, izlemdeki değişimleri ve sağkalımları Tablo-4'te verilmiştir.

Tablo-4. Olguların sigara öyküleri olup olmamasına göre gruplandırılarak oluşturulmuş izlem süreleri, başlangıç SFT parametreleri ve izlemdeki değişimleri ve sağkalımları

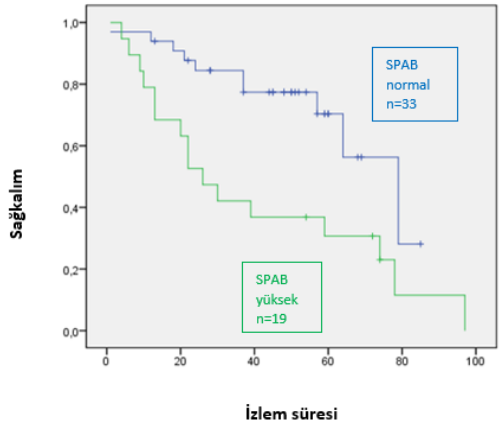
	Sigara Kullanımı	N	Ortalama	P
İzlem Süresi (ay)	var	51	41,28±25,40	0,77
	yok	43	40,70±22,98	
Başlangıç FEV1 (L)	var	51	2,46±0,64	<0,0001
	yok	43	1,71±0,53	
Başlangıç FEV1 (%)	var	51	82,56±17,21	0,71
	yok	43	81,00±24,13	
Başlangıç FVC (L)	var	51	3,01±0,79	<0,0001
	yok	43	2,01±0,66	
Başlangıç FVC (%)	var	51	96,53±11,52	0,31
	yok	43	76,07±22,43	
Başlangıç FEV1/FVC (%)	var	51	81,53±7,86	0,005
	yok	43	86,23±7,58	
Başlangıç DLCO ml/mmHg/dk	var	44	15,48±4,98	0,004
	yok	33	12,08±4,12	
Başlangıç DLCO (%)	var	44	60,79±20,77	0,39
	yok	33	56,72±19,21	
Başlangıç DLCO/VA (%)	var	44	77,65±26,62	0,55
	yok	33	81,23±25,53	
FVC değişim (ml)	var	51	-462,38±517,14	0,07
	yok	43	-294,33±367,60	
FVC değişim (%)	var	51	-14,62±16,51	0,93
	yok	43	-15,19±21,96	
DLCO değişim (ml/mmHg/dk)	var	24	-3,50±4,06	0,02
	yok	14	-1,69±3,70	
DLCO değişim (%)	var	24	-19,24±32,32	0,13
	yok	14	-14,43±31,95	
DLCO/VA değişim (%)	var	24	-0,50±18,95	0,16
	yok	14	9,86±26,15	
Sağkalım (ay)	var	36	30,54±24,84	0,95
	yok	24	31,00±25,47	



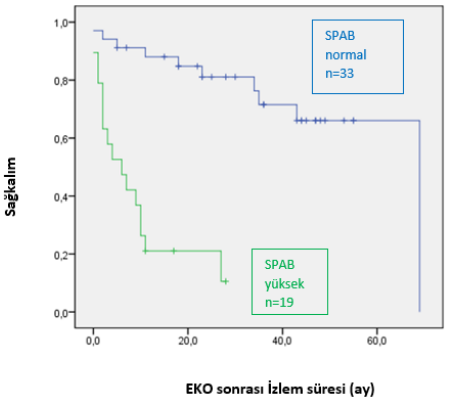
Şekil-1. Taranan hasta grubundan çalışmaya dahil edilmeyen hastalar ve nedenleri.



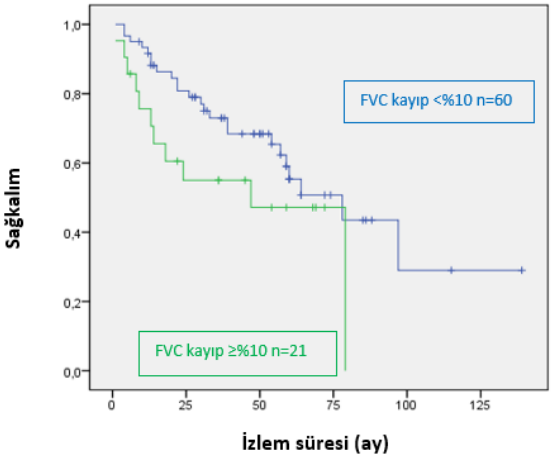
Şekil-2. Olguların 50 yaş altı ve üzeri olarak gruplandırılmasıyla oluşturulan sağkalım eğrisi



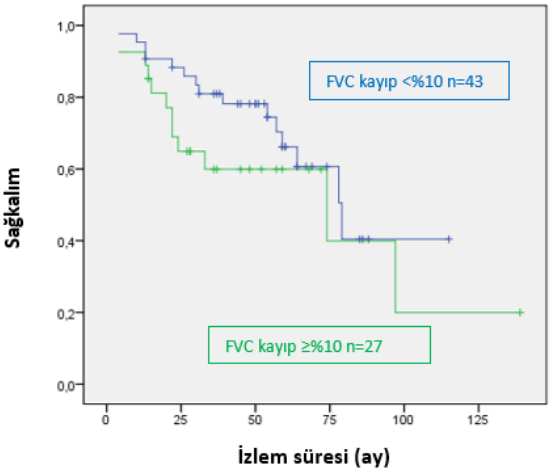
Şekil-3. Olguların SPAB düzeylerinin normal veya yüksek olmasına göre oluşturulan genel sağkalım eğrisi



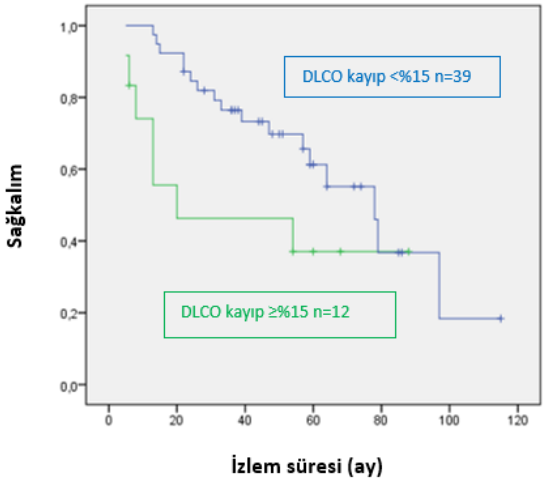
Şekil-4. Olguların SPAB düzeylerinin normal veya yüksek olmasına göre EKO sonrası sağkalım eğrisi



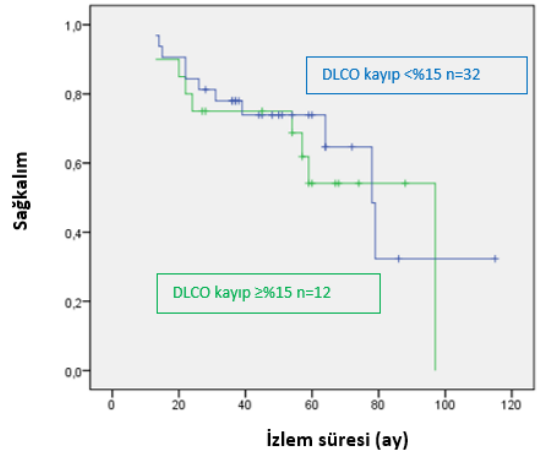
Şekil-5. Altı aylık FVC kaybının %10'un üzerinde olup olmamasına göre oluşturulan sağkalım eğrisi.



Şekil-6. On iki aylık FVC kaybının %10'un üzerinde olup olmamasına göre oluşturulan sağkalım eğrisi



Şekil-7. Altı aylık DLCO kaybının %15'in üzerinde olup olmamasına göre oluşturulan sağkalım eğrisi.



Şekil-8. On iki aylık DLCO kaybının %15'in üzerinde olup olmamasına göre oluşturulan sağkalım eğrisi.

Sistolik Pulmoner Arter Basıncı: Hastaların 52'sine (%55,3) IPF tanısı sonrasında en az bir kere EKO yapılmıştır ve SPAB'nin 36 mmHg'nın üzerinde olduğu değerler yüksek kabul edilmiştir. Bu değer Avrupa Kardiyoloji Derneği (ESC) Pulmoner Hipertansiyon Rehberi'ne göre belirlenmiştir, EKO yapılan olguların %64,2'sinin SPAB düzeyi normalken %34,8'inin ise yüksek saptanmıştır.

Sağkalım analizlerinde, EKO'da SPAB düzeyi normal saptanan olguların EKO sonrası ortalama sağkalım süresi 26,3±16,4 ayken, SPAB düzeyi 36 mmHg'nın üzerinde saptanan olguların EKO sonrası ortalama sağkalım süresi 5,6±6,1 ay saptanmıştır (p<0,001). Anlamlılık yalnızca EKO sonrasında değil genel sağkalımda da gözlenmiştir (p=0,006, Şekil-3 ve 4).

Solunum Fonksiyon Testi Parametrelerinde Zamana Bağlı Değişim: Olguların FVC kayıpları yüzde olarak ele alındığında, başlangıç FVC değerinin %10'unu ilk altı ay içerisinde kaybeden 21 olgu ile bu kaybı yaşamamış olan 60 olgunun sağkalımları arasında anlamlı fark gözlenmemiştir (p=0,10). Benzer şekilde başlangıç FVC değerinin %10'unu ilk yıl içerisinde kaybeden 27 olgunun sağkalımları bu kaybı yaşamamış olan 43 olgudan farklılık göstermemiştir (p=0,22). DLCO takibi bulunan 51 olgudan 12'sinin başlangıç DLCO değerinin izlemin ilk altı ayında %15'ini kaybettiği saptanmış ve bu kaybı yaşamamış olan olgularla aralarında anlamlı sağkalım farkı gözlenmemiştir (p=0,06). Bir yıllık DLCO takibi yapılan 44 olgunun ise 12'si başlangıç DLCO değerinin %15'ini ilk yıl içinde kaybetmiş olup bu kaybı yaşamamış olgularla aralarında anlamlı sağkalım farkı saptanmamıştır (p=0,62). Altı aylık ve bir yıllık FVC kaybının %10'un üzerinde olup olmamasına göre oluşturulan sağkalım eğrileri Şekil-5 ve 6'da, aynı sürelerde DLCO'da %15'in üzerinde kayıp olup olmamasına göre oluşturulmuş sağkalım eğrileri ise Şekil-7 ve 8'de yer almaktadır.

TARTIŞMA

Çalışmamızda IPF'de prognozu belirleyen parametrelerin 50 yaşın altında tanı almak ve SPAB'nin yüksek saptanması olduğu belirlenmiştir, 50 yaş altı grubun prognozu solunum fonksiyonlarındaki değişimle ilişkisiz, SPAB yüksekliği ise DLCO/VA ile ilişkili bulunmuştur.

İdiyopatik pulmoner fibrozisli hastaların prognozlarını öngörmek amacıyla yapılan çalışmalarda yaş sıkça irdelenmiş ve farklı sonuçlara ulaşılmıştır. IPF tanısı için ortalama yaşın 66 civarında olduğu meta analiz çalışmalarında gözlemlenmiştir (1, 3), Çalışma popülasyonumuzun ortalama yaşı 62,5±10,7 yıl olup en yaşlı olgu 83 yaşında, en genç olgu ise 17 yaşında bir ailesel IPF hastasıdır. İleri yaş hastalığı olarak kabul edilen IPF'de, hasta yaşı ne kadar ileri ise prognozun o kadar kötü olduğunu gösteren çalışmalar mevcut olmakla birlikte, 50 yaşından önce tanı alan grubun prognozunun daha kötü olduğunu saptayan araştırmalar da mevcuttur (3, 15, 16). Çalışmamızda ise yaşın genel sağkalımla veya

izlemdeki solunum fonksiyon testleri kayıplarıyla ilişkisiz olduğu saptanmıştır. Ancak 50 yaşın altında tanı alan olgu grubunun sağkalımının anlamlı olarak düşük olduğu gözlemlemiştir, Klinik olarak hastalığın hızlı seyrettiği bu genç olgu grubunun transplantasyona zamanında yönlendirilmesi önemlidir.

Erkek cinsiyette daha sık gözlenen bir hastalık olan IPF ile ilgili bazı çalışmalarda kadınların sağkalım avantajına sahip olduğu yönünde bulgular mevcuttur (1, 13, 15). Çalışmamızda erkek olgular çoğunluğu oluşturmakla beraber sağkalım açısından kadın olgulardan anlamlı bir farkları bulunmamıştır. Erkek ve kadın olguların solunum fonksiyonlarının yüzde cinsinden kayıpları ayrı ayrı değerlendirildiğinde, cinsiyetler arası fark olmadığı görülmüştür. Mutlak değerlerdeki erkekler lehine olan istatistiksel anlamlı farklılıkların, cinsiyetler arası vücut yüzey alanı farklılıklarına bağlı olduğu düşünülmüştür.

Sigara içen popülasyonda IPF'nin daha sık gözlendiği bilinmektedir (15-17). Çalışmamızda popülasyonun %54,2'ünde sigara öyküsü mevcuttur. Ortalama sigara tüketimi 33,7±17 paket-yılı gibi yüksek bir oranda saptanmış, sigara öyküsü olanların büyük oranda erkek olgulardan oluştuğu gözlenmiştir (%96). Çalışmamızda sigara öyküsünün sağkalım, izlemdeki FVC, DLCO ve DLCO/VA'da yüzde olarak kayıplar üzerinde anlamlı etkisi olmadığı görülmüştür. Ayrıca sigara ve cinsiyetin pulmoner hipertansiyonun dolaylı bir göstergesi kabul edilen SPAB düzeylerinin normal veya yüksek bulunmasıyla da ilişkili olmadığı saptanmıştır. Sigara kullanmış olan grubun başlangıç FEV1 ve FVC mutlak değerlerindeki anlamlı yüksekliğin yine cinsiyetler arası vücut yüzey alanı farklılıklarına bağlı olduğu, sigara içen grupta FEV1/FVC'nin içmeyen gruba göre anlamlı düşük olmasının ise sigara içimiyle ilişkili olduğu düşünülmüştür.

Olguların altı aylık ve bir yıllık SFT parametreleri incelendiğinde, bazal FVC ve DLCO değerlerinin sırasıyla %10 ve %15'ini ilk altı ve on iki ay içerisinde kaybeden olguların sağkalımlarının bu kaybı yaşamayanlarla farklı olmadığı görülmüştür.

Fibrotik akciğer hastalıklarında, akciğerlerdeki parankimal ve vasküler yeniden şekillenmenin, pulmoner hipertansiyonun bu grup hastada sıkça gözlenmesini açıklayan mekanizma olduğu düşünülmektedir (18). Pulmoner arteriyel hipertansiyon, interstisyel akciğer hastalıklarında fonksiyonel kapasitenin kötüleşmesine katkı sağlamaktadır. Pulmoner hipertansiyon tanısı ancak sağ kalp kateterizasyonu yapılarak ortalama pulmoner arter basıncı ölçümüyle konmaktadır. Fakat invaziv bir işlem olması nedeni ile tarama çalışmalarında öncelikle EKO yapılarak SPAB düzeyi ve pulmoner hipertansiyonun sağ kalp üzerine olan etkileri araştırılmaktadır. Avrupa Kardiyoloji Derneği (ESC) Pulmoner Hipertansiyon Rehberi'ne göre EKO'da SPAB değerinin 36 mmHg'nın üzerinde olduğu değerler yüksek kabul edilmiştir (19). Bu değerin üzerinde ve klinik bulgular varlığında sağ kalp kateterizasyonu gündeme gelmektedir. Sağ kalp kateterizasyonu, invaziv olması ve IPF'ye sekonder saptanan pulmoner hipertansiyonun etkin bir tedavisinin olmaması nedeni ile rutinde kullanılan pratik bir yöntem değildir.

Sağkalım analizlerinde, EKO'da SPAB düzeyi normal olan olguların EKO sonrası ortalama sağkalım sürelerinin, SPAB yüksek olan olgulara göre anlamlı olarak yüksek olduğu saptanmıştır. Olguların başlangıçta ya da izlemin herhangi bir zamanında yapılan EKO sonucunda yüksek SPAB düzeyi saptanması prognozun kötü olabileceği konusunda uyarıcı olmalıdır. Nathan ve arkadaşlarının 110 IPF tanılı hastaya EKO ve sağ kalp kateterizasyonu yaptıkları çalışmada; EKO ile ölçülen SPAB değerinin, IPF'de pulmoner hipertansiyon değerlendirmesi için tek başına yeterli bir test olmasa da SFT ve 6 DYT gibi parametrelerle birlikte değerlendirildiğinde klinisyenlere risk sınıflandırmasında yardımcı olabileceği sonucuna ulaşmışlardır (20).

Solunum fonksiyon testi parametreleri ile SPAB arasındaki ilişki incelendiğinde; SPAB yüksekliğinin FVC ve DLCO değişimi ile ilişkisiz, DLCO/VA ile istatistiksel olarak anlamlı şekilde ilişkili olduğu saptanmıştır. Restriktif akciğer hastalıklarında DLCO/VA normal olabilir ya da artabilir (21). Pulmoner hipertansiyonda ise DLCO/VA'nın düşmesi beklenir. Çalışmamızda SPAB düzeyi yüksek olan olguların normal saptananlara göre DLCO/VA düzeylerinin anlamlı olarak düşük olduğu görülmüştür. Peelen ve arkadaşlarının IPF'yi de içeren primer fibrotik akciğer hastaları ile FVC, DLCO ve DLCO/VA değişimleri üzerinden yaptıkları sağkalım analizi çalışmasında; altı aylık izlemde DLCO/VA'da %10'luk azalma ve on iki aylık izlemde FVC'de %10'luk azalma saptanan olguların prognozlarının belirgin düşük olduğunu saptamışlardır (22). DLCO'daki %15 ve üzerinde olan değişimde ise bu prognoz farklılığını

gözlememişlerdir. Olgulardan DLCO/VA değerlerinde düşme gözlenenlerin sağkalımlarının kötü olmasını vasküler inflamasyon, düz kas hücre proliferasyonu, vasküler yeniden yapılanma ve anjiyogenik disfonksiyonun sonucu artan pulmoner arteryel basınçla açıklamışlardır.

Çalışmamız geniş IPF'li hasta sayısına sahiptir. Hastaların tanısı interstisyel akciğer hastalıkları konseyinde tartışılmış, romatolojik hastalıklar dikkatli bir şekilde dışlanmış, yeterli izlem süreleri elde edilmiş, hastaların yaklaşık üçte birinin tanısı biyopsi ile doğrulanmıştır.

Çalışmamızın zayıf yönü retrospektif olmasıdır. Sağkalım araştırmalarının prospektif olması, olguların vizit aralıklarının düzenli olmasına ve klinik kötüleşmeye kadar olan zamanının daha net belirlenmesine olanak sağlayabilir.

SONUÇ

İdiyopatik pulmoner fibrozisli hastaların prognozları hakkında fikir sahibi olmak, hastaya uygulanan tedavilerin başarısı, transplantasyon için değerlendirilmesi ve listeye alınma zamanı ile ilgili hekime önemli bilgiler verebilir. IPF hastalarında, SFT'de FVC ve DLCO yanı sıra DLCO/VA değerine de dikkat etmek ve düşme gözlenen hastalara sistolik pulmoner arter basıncı değerini saptamak adına ekokardiyografi planlamak uygun bir yaklaşım olabilir. Ayrıca 50 yaşın altında tanı alan hastaların prognozlarının ileri yaşta tanı alanlara göre kötü olabileceğinin göz önünde bulundurulması izlemde klinisyen için faydalı olabilir.

Çıkar çatışması: Yazarların çalışmada herhangi bir çıkar çatışması yoktur.

Kaynakça

1. American Thoracic Society, Idiopathic Pulmonary Fibrosis, Diagnosis and treatment, International Concensus Statement. Am J Respir Crit Care Med 2000; 161: 646-64.
2. Raghu G, Weycker D, Edelsberg J et al, Incidence and Prevalence of Idiopathic Pulmonary Fibrosis. Am Journal of Respir Crit Care Med 2006; 174: 810-6.
3. Gribbin J, Hubbard RB, Le Jeune I, et al, Incidence and mortality of idiopathic pulmonary fibrosis and sarcoidosis in the UK, Thorax 2006; 61: 980–5.
4. Karakatsani A, Papakosta D, Rapti A, et al, Hellenic Interstitial Lung Diseases Group, Epidemiology of interstitial lung diseases in Greece, Respir Med 2009; 103: 1122-9.
5. King TE Jr, Tooze JA, Schwarz MI, et al, Predicting survival in idiopathic pulmonary fibrosis, Scoring system and survival model, Am J Respir Crit Care Med 2001; 164: 1171-81.
6. Fell CD, Martinez FJ, Liu LX, et al, Clinical predictors of a diagnosis of idiopathic pulmonary fibrosis, Am J Respir Crit Care Med 2010; 181: 832-7.
7. Iommi M, Faragalli A, Bonifazi M, et al, Prognosis and Survival in Idiopathic Pulmonary Fibrosis in the Era of Antifibrotic Therapy in Italy: Evidence from a Longitudinal Population Study Based on Healthcare Utilization Databases, Int J Environ Res Public Health, 2022; 19: 16689.
8. Mogulkoc N, Martin HB, Bishop PW et al, Pulmonary Function in Idiopathic Pulmonary Fibrosis and Referral for Lung Transplantation, Am J Respir Crit Care Med 2001; 164: 103-8.
9. An Official ATS/ERS/JRS/ALAT Statement: Idiopathic Pulmonary Fibrosis: Evidence-based Guidelines for Diagnosis and Management, Am J Respir Crit Care Med 2011; 183: 788-824.
10. Ley B, Collard H, King T Jr, Clinical Course and Prediction of Survival in İdiopathic Pulmonary Fibrosis, Am J Respir Crit Care Med 2011; 183: 431-40.
11. Erbes R, Schaberg T, Loddenkemper R, Lung function tests in patients with idiopathic pulmonary fibrosis, Are they helpful for predicting outcome? Chest 1997; 111: 51-57.
12. Nalysnyk L, Ruzafa J, Rotella P, et al, Incidence and prevalence of idiopathic pulmonary fibrosis: review of the literature, Eur Respir Rev 2012; 21: 355-61.
13. Han MK, Murray S, Fell CD, et al, Sex differences in physiological progression of idiopathic pulmonary fibrosis, Eur Respir J 2008; 31: 1183-8.
14. Mejia M, Carrillo G, Rojas-Serrano J, et al, Idiopathic pulmonary fibrosis and emphysema: decreased survival associated with severe pulmonary arterial hypertension, Chest 2009; 136: 10-15.
15. Kitamura H, Ichinose S, Hosoya T et al, Inhalation of inorganic particles as a risk factor for idiopathic pulmonary fibrosis: elemental microanalysis of pulmonary lymph nodes obtained at autopsy cases, Pathol Res Pract 2007; 203: 575-85.

16. Mejia M, Carrillo G, Rojas-Serrano J, et al, Idiopathic pulmonary fibrosis and emphysema: decreased survival associated with severe pulmonary arterial hypertension, Chest 2009; 136: 10-15.
17. Ryu JH, Colby TV, Hartman TE, et al, Smoking-related interstitial lung diseases: a concise review, Eur Respir J 2001; 17: 122-32.
18. Nadrous HF, Pellikka PA, Krowka MJ, et al, Pulmonary hypertension in patients with idiopathic pulmonary fibrosis, Chest 2005; 128: 2393-9.
19. Galie N, Hoeper M, Humbert M, et al, Guidelines on diagnosis and treatment of pulmonary hypertension, European Heart Journal 2009; 30: 2493-537.
20. Nathan SD, Shlobin OA, Barnett SD, et al, Right ventricular systolic pressure by echocardiography as a predictor of pulmonary hypertension in idiopathic pulmonary fibrosis, Respir Med, 2008; 102: 1305–10.
21. Latsi PI, du Bois RM, Nicholson AG, Fibrotic Idiopathic Interstitial Pneumonia: The Prognostic Value of Longitudinal Functional Trends Am J Respir Crit Care Med 2003; 168: 531-7.
22. Peelen L, Wells AU, Prijs M, at al, Fibrotic idiopathic intestitial pneumonias: Mortality is linked to a decline in gas transfer Respirology 2010; 15: 1233-43.

Araştırma Makalesi / *Research Article*

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Akut kolesistitin ciddi bir komplikasyonu olan safra kesesi perforasyonunun klinik yönetimi: yüksek volümlü tek merkez sonuçlarımız

Clinical management of gallbladder perforation, a serious complication of acute cholecystitis: our high-volume single-center results

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ÖZ

Amaç: Safra kesesi perforasyonu akut kolesistitin (AK) morbidite ve mortalite oranlarını artıran en ciddi komplikasyonudur. Akut perfore kolesistit (APK) yönetiminde net bir algoritma olmamakla birlikte erken dönemde operasyona uygun olmayan hastalar konservatif takip edilebilmektedir. Çalışmamızda akut perfore kolesistit nedeniyle takip edilen hastaların sonuçlarını değerlendirerek klinik deneyimimizi sunmayı amaçladık.

Gereç ve Yöntem: Çalışmaya Aralık 2018- Aralık 2023 yılları arasında akut kolesistit nedeniyle kliniğimizde takip edilen 532 hasta dahil edildi. Safra kesesi perforasyonu olmayan hastalar dışlanarak 118 hastanın verileri retrospektif tarandı.

Bulgular: Hastaların 53(%44,9)'ü kadın, 65(%55,1) erkek idi. Ortalama yaş 67,7(23-93) idi. Hastaların 17(%14,4)'sine medikal tedavi,73(%61,9)'üne perkutan kolesistostomi (PK),15'ine (%12,7) perkütan kolesistostomi uygulama sonrası operasyon ve 13(%11)'üne operasyon uygulandı. Operasyon uygulananların 13(%46,4)'ü interval dönemde, 15(%53,6)'i elektif opere edildi. Elektif operasyon planlanan hastalar ortalama akut perfore kolesistit tanısından 116 gün sonra opere edildi. Operasyon zamanlamasının yatış süresi, operasyon türü ve sağkalıma etkisi saptanmadı. Hastaların tedavi türleri ve laboratuvar değerleri karşılaştırıldığında C-Reaktif Protein/Albumin (CRP/ALB) değerinin anlamlı olarak perkütan kolesistostomi uygulananlarda daha yüksek olduğu görüldü(p=0,008). Hastaların 13(%11)'ü yatışında eksitus oldu. Eksitus olan hastaların yaş ortalaması 80,1 iken olmayanlarınki 66,1 olarak saptandı (p=0,0007). Bunların 11(%84,6)'ine perkütan kolesistostomi uygulanırken 2(%15,4)'si yalnızca medikal tedavi ile takip edildi.

Sonuç: Akut perfore kolesistit tanısı alan erken dönemde operasyona uygun olmayan hastalarda konservatif tedavi seçenekleri tercih edilebilmekte ancak perkütan kolesistostomi uygulaması hastane yatış sürelerini uzatmaktadır. Akut perfore kolesistit mevcut olan hastalarda hastaya spesifik tedavi tercihlerinin yapılmasının doğru olacağını düşünmekteyiz.

Anahtar Sözcükler: Akut Kolesistit, akut perfore kolesistit, perkutan kolesistostomi, kolesistektomi, safra kesesi.

ABSTRACT

Aim: *Gallbladder perforation is the most severe complication acute cholecystitis of acute cholecystitis (AC), increasing morbidity and mortality rates. Although there is no precise algorithm for managing acute perforated cholecystitis (APC), patients who are not suitable for surgery in the early period can be followed conservatively. Our study aimed to present our clinical experience by evaluating the results of patients who were followed up due to acute perforated cholecystitis*

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Materials and Method: *The study included 532 patients who were followed up in our clinic due to acute cholecystitis between December 2018 and December 2023. Patients without gallbladder perforation were excluded, and the data of 118 patients were retrospectively reviewed.*

Results: *53 (44.9%) of the patients were female, 65 (55.1%) were male. The mean age was 67.7 (23-93). 17 (14.4%) of the patients received medical treatment, 73 (61.9%) received percutaneous cholecystostomy (PC), 15 (12.7%) underwent surgery after percutaneous cholecystostomy, and 13 (11%) underwent surgery. 13 (46.4%) of those who underwent surgery were operated on in the interval period, and 15 (53.6%) were operated on electively. Patients who were planned for elective surgery were operated on an average of 116 days after the diagnosis of acute perforated cholecystitis. No effect of the timing of the surgery on the length of stay, type of surgery, and survival was detected. When the treatment types and laboratory values of the patients were compared, it was seen that the C-reactive protein/Albumin (CRP/ALB) value was significantly higher in those who underwent percutaneous cholecystostomy (p=0.008). Thirteen (11%) of the patients died during hospitalization. The mean age of the patients who died was 80.1, while the mean age of those who did not was 66.1 (p=0.0007). While 11 (84.6%) underwent percutaneous cholecystostomy, 2 (15.4%) were followed only by medical treatment.*

Conclusion: *Conservative treatment options can be preferred in patients diagnosed with acute perforated cholecystitis who are not suitable for surgery in the early period. Still, percutaneous cholecystostomy application prolongs the hospital stay. Making patient-specific treatment preferences in patients with acute perforated cholecystitis would be correct.*

Keywords*: Acute cholecystitis, acute perforated cholecystitis, percutaneous cholecystostomy, cholecystectomy, gallbladder.*

GİRİŞ

Safra kesesi perforasyonu, batın içine safra kaçağına bağlı peritonit ve sepsis gelişimi gibi yaşamı tehdit eden durumlara yol açması nedeniyle ciddi morbid ve mortal bir safra kesesi patolojisidir. Safra kesesi perforasyonu en sık akut kolesistitin bir komplikasyonu olarak görülmekle birlikte maligniteler, travma, immunsupresyon vs. perforasyona neden olabilmektedir (1-3).

Akut perfore kolesistit (APK) akut kolesistitin nadir görülen ancak klinik yönetimi zor ve yaşamı tehdit eden bir komplikasyonudur. Akut perfore kolesistit tüm akut kolesistitli hastaların %2-11'inde görülürken APK'e bağlı mortalite oranları %10-40 saptanmıştır (4-5). İleri yaş, kötü performans durumu, ciddi komorbiditeler, immunsupresif ilaç kullanımı gibi faktörler akut kolesistitli hastalarda APK gelişim riskini artırmakta ve APK mevcut olan hastalarda ise prognozun daha kötü seyretmesine neden olmaktadır (6).

Akut perfore kolesistit klinik olarak sıklıkla akut kolesistite benzer şekilde sağ üst kadran ağrısı, ateş gibi semptomlarla karşımıza çıkmaktadır. Lökositoz, sola kayma, akut faz reaktanlarında yükselme enflamasyonun sonucu olarak görülebilmekteyken akut kolesistitli hastalara nispeten laboratuvar değerleri daha yüksek görülebilmektedir. Peritonitin ilerlemesiyle birlikte hastalarda yaygın karın ağrısı, akut karın bulguları ve daha komplike hastalarda sepsis bulguları görülebilmektedir (7-8). Ultrasonografi ile safra kesesi perforasyonu tespit edilebilmekle birlikte bilgisayarlı tomografi (BT) ve manyetik rezonans kolanjiopankreatografi (MRKP) tanı ve perforasyon durumunu değerlendirmede önemli rol oynamaktadır (9).

Safra kesesi perforasyonlarının, tedavi planında direkt rolü olmamakla birlikte gelişim şekillerine göre evrelemeleri mevcuttur. İlk olarak Niemeier'in 1934 yılında önerdiği ve daha sonra modifiye edilen sınıflaması hala safra kesesi perforasyonlarının sınıflandırılmasında en yaygın kabul gören sistem olmaya devam etmektedir. Bu sınıflamaya göre Tip I (periton boşluğuna akut serbest perforasyon), Tip II (lokalize apse ile subakut perforasyon) ve Tip III (kolesistoenterik fistül ile kronik perforasyon) şeklinde üç tip mevcuttur. Anderson daha sonra kolesistobilier fistüller için bir Tip IV sınıflandırması ekleyerek modifiye etmiştir (10, 11).

Akut perfore kolesistit tedavisinde erken dönem ve uygun hasta grubunda en etkin tedavi seçeneği acil cerrahi olmakla birlikte acil cerrahiye uygun olmayan hastalarda perkütan safra drenajı ve antibiyoterapi ile akut dönemde

hastalığı kontrol ederek elektif cerrahi planlanabilir (12, 13).

Çalışmamızda APK nedeniyle takip edilen hastaların sonuçlarını değerlendirerek klinik deneyimimizi sunmayı amaçladık.

GEREÇ VE YÖNTEM

Çalışmaya Aralık 2018 ile Aralık 2023 tarihleri arasında akut kolesistit tanısıyla Ege Üniversitesi genel cerrahi kliniğinde yatırılan 532 hasta dahil edildi. Yapılan görüntülemelerde safra kesesi perforasyonu saptanmayan hastalar dışlandı. APK mevcut olan 118 hastanın verileri retrospektif tarandı.

Veri Toplama

Hastaların yaş, cinsiyet, komorbidite, American Society of Anesthesiologists (ASA) skoru, tedavi ve operasyon, yoğun bakım ve hastane yatış süresi, laboratuvar parametreleri (AST, ALT, ALP, GGT, Albumin, Bilirubin, CRP, Lökosit, Nötrofil sayısı, Lenfosit sayısı, Trombosit), morbidite ve mortalite verileri tarandı. Safra kesesi perforasyon sınıflandırılmasında Niemeier Sınıflama Sistemi kullanıldı. Buna göre hastalar;

-Tip I: Periton boşluğuna akut serbest perforasyon

-Tip II: Lokalize apse ile subakut perforasyon

-Tip III: Kolesistoenterik fistül ile kronik perforasyon

şeklinde sınıflandırıldı. Opere edilen hastalarda gelişen komplikasyonların tanımlanmasında Clavien-Dindo sınıflaması kullanıldı.

Tedavi

Hastalara uygulanan tedavi türleri yalnızca antibiyoterapi uygulananlar için medikal tedavi (MT), perkütan kolesistostomi (PK), operasyon ve perkütan kolesistostomi sonrası operasyon olarak sınıflandırıldı. Perkütan kolesistostomi uygulaması sonrası operasyon zamanı aynı yatışta veya daha ileri dönemde farklı yatışta yapılması göz önünde bulundurularak bu hastalar interval ve elektif dönem olarak sınıflandırıldı. Operasyonlar açık ve laparoskopik olarak uygulandı. Tedavi türleri Niemeier Sınıflama Sistemine ve hastaların sağkalım durumuna göre karşılaştırıldı.

İstatistiksel Analiz

Veriler Microsoft Excel (Sürüm 16.82) belgesinde toplanarak istatistiksel analize hazır hale getirildi. İstatistiksel analizde IBM SPSS Statistics v29 (IBM, Armonk, New York, USA) ve OpenAI GPT-4 Data Analyst kullanıldı. Analizde çalışma grupları arasında sürekli değişkenleri karşılaştırmak için bağımsız örneklem T-testi (normal dağılımlı veriler için) ve Mann-Whitney U testi (anormal dağılımlı veriler için) kullanıldı. Kategorik değişkenler için Ki-kare testi kullanıldı. $P<0.05$ istatistiksel olarak anlamlı kabul edildi.

Etik Onay

Çalışma Helsinki Deklarasyonu ilkelerine uygun olarak yürütülmüş ve Ege Üniversitesi Hastanesi Etik Kurulu tarafından 24-7T/89 belge numarası ile etik onay verilmiştir.

BULGULAR

Hastaların 53 (%44,9)'ü kadın, 65 (%55,1)'i erkek idi. Ortalama yaş 67.7 (23-93) idi. 48 (%40,7) hasta ASA1, 60 (%50,8) hasta ASA2, 10 (%8,5) hasta ise ASA3 idi. Hastaların 57 (%48,3)'sinde hipertansiyon, 31 (%26,3)'inde diabetes mellitus, 22'sında (%18,6) ise koroner arter hastalığı mevcuttu (Tablo-1).

Niemeier sınıflamasına göre 8 (%6,8) hasta Tip 1, 107 (%90,7) hasta Tip 2, 3 (%2,5) hasta Tip 3 olarak sınıflandırıldı. Hastaların 17 (%14,4)'sine MT, 73 (%61,9)'üne PK, 15 (%12,7)'ine PK uygulama sonrası operasyon ve 13 (%11)'üne operasyon uygulandı. (Tablo-2) Tedavi türleri Niemeier sınıflamasına göre karşılaştırıldığında medikal tedavi uygulanan hastaların 1 (%5,9)'i Tip 1, 16'sı (%94,1) Tip 2 idi. Perkütan kolesistostomi uygulanan hastaların 4 (%5,4)'ü Tip 1, 66'sı (%90,4) Tip 2, 3 (%4,2)'ü Tip 3 idi. Perkütan kolesistostomi sonrası opere edilen hastaların 1 (%6,7)'i Tip 1, 14 (%93,3)'ü Tip 2 idi. Opere edilen hastaların 2 (%15,4)'si Tip 1, 11 (%84,6)'i Tip 2 idi (Tablo-3).

Hastaların tedavi türleri ve laboratuvar değerleri karşılaştırıldığında CRP/ALB değerinin anlamlı olarak PK uygulanan hastalarda daha yüksek olduğu görüldü (p=0,008) (Şekil-1).

Tedavi türleri hastane yatış süresine göre değerlendirildiğinde medikal tedavi uygulanan hastaların ortalama hastane yatış süresi 9,6 gün, PK uygulanan hastaların 16,1 gün, operasyon uygulanan hastaların 5,4 gün, PK sonrası operasyon uygulanan hastaların ise 17,9 gün saptandı. Tedavi türleri arasında hastane yatış süresi açısından anlamlı fark izlenmedi (Şekil-2).

Operasyon uygulanan hastaların 13 (%46,4)'ü interval dönemde, 15 (%53,6)'i elektif dönemde

opere edildi. Elektif operasyon planlanan hastalar ortalama APK tanısından 116 gün sonra opere edildi. İnterval dönemde opere edilen hastaların 8 (%61,5)'i açık, 5 (%38,5)'i laparoskopik teknikle opere edildi. Elektif dönemde opere edilen hastaların 6 (%40)'sı açık, 9 (%60)'u laparoskopik teknikle opere edildi. İnterval dönemde opere edilen hastaların ortalama hastane yatış süresi 13,8 gün iken elektif dönemde opere edilenlerinki ise 10,5 gün idi. Elektif operasyon uygulanan hastalardan 1 (%6,7) Clavien Dindo 3 komplikasyon gelişti. Operasyon zamanlamasının yatış süresi, operasyon türü ve sağkalıma anlamlı etkisi saptanmadı (Tablo-4).

Hastaların 13 (%11)'ü ilk yatışında eksitus oldu. Eksitus olan hastaların yaş ortalaması 80,1 iken olmayanlarınki 66,1 olarak saptandı (p=0,0007). Eksitus olan hastaların 1 (%7,7)'i ASA1, 9 (%69,2)'u ASA2, 3 (%23,1)'ü ASA3 idi. Bu hastaların 11 (%84,6)'ine PK uygulanırken 2 (%15,4)'si medikal tedavi ile takip edildi. Sağ olan hastaların 15 (%14,3)'ine MT, 62 (%59,1)'sine PK,

13 (%12,3)'üne operasyon, 15 (%14,3)'ine PK sonrası operasyon uygulandı. Eksitus olan hastalarda ortalama hastane yatış süresi 23,8 gün iken olmayan hastalarda 13,1 gün idi (Tablo-5).

Tablo-1. Demografik veriler.

	Sayı(N)
Cinsiyet	
Kadın	65 (%55,1)
Erkek	53 (%44,9)
ASA skoru	
1	48 (%40,7)
2	60 (%50,8)
3	10 (%8,5)
Komorbidite	
HT	57 (%48,3)
DM	31 (%26,3)
KAH	22 (%18,6)
KOAH	8 (18,6)
KBH	4 (%3,4)
KKY	7 (%5,9)
MG	13 (%11,1)
SVH	13 (%11,1)
Malignite	8 (%6,8)
Aritmi	1 (%2,5)

ASA: AmericanSociety of Anesthesiologists, HT: Hipertansiyon; DM: Diyabetes Mellitus; KAH: Koroner Arter Hastalığı; KOAH: Kronik Obstruktif Akciğer Hastalığı; KBH: Kronik Böbrek Hastalığı; KKY: Konjestif Kalp Yetmezliği; SVH: Serebrovasküler Hastalık; MG: Myastenia Graves

Tablo-2. Sınıflama ve tedavi türleri.

Niemeier Sınıfı	**Sayı (N)**
Tip 1	8 (%6,8)
Tip 2	107 (%90,7)
Tip 3	3 (%2,5)
Tedavi türü	
Medikal tedavi	17 (14,4)
PK	73 (61,9)
Operasyon	13 (11,1)
PK sonrası operasyon	15 (12,6)
Eksitus	13 (%11)

PK: Perkutan Kolesistostomi

Tablo-3. Tedavi türlerinin sınıflamaya göre karşılaştırılması.

Tedavi türü	Tip 1	Tip 2	Tip 3	p
Medikal tedavi	1 (%5,9)	16 (%94,1)		0,05
Perkütan kolesistostomi	4 (%5,4)	66 (%90,4)	3 (%4,2)	0,05
Operasyon	2 (%15,4)	11 (%84,6)		0,05
PK sonrası operasyon	1 (%6,7)	14 (%93,3)		0,05
Operasyon türü				0,05
Açık	3 (%10,7)	11 (%39,3)		
Laparoskopik	0	14 (%50)		

PK: Perkutan Kolesistostomi

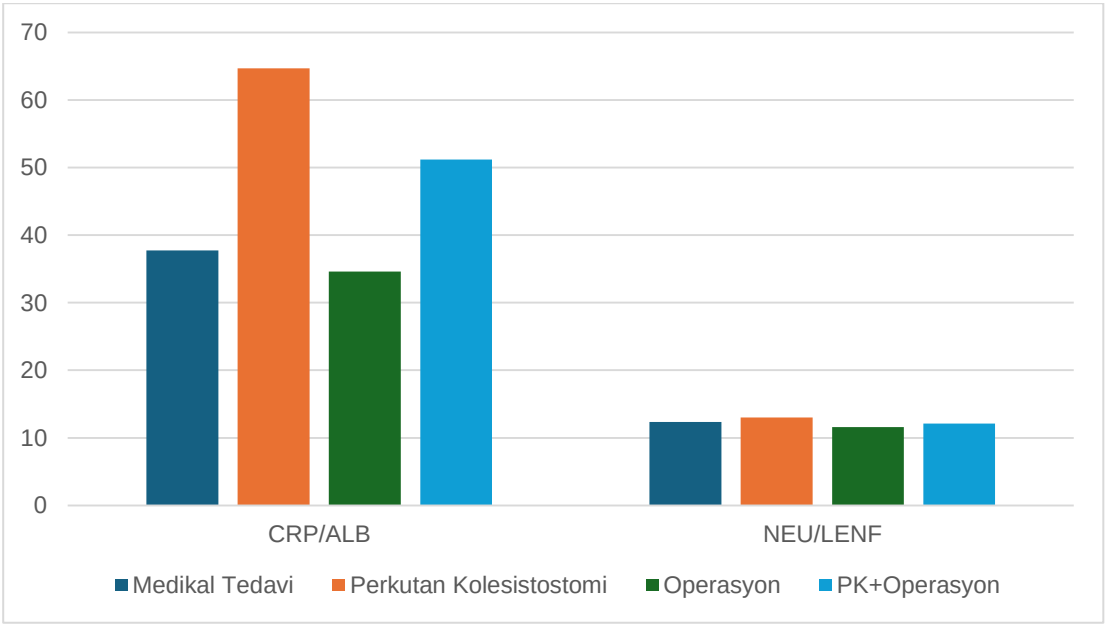
Tablo-4. Operasyon zamanı ve karşılaştırılması.

	İnternal (n=13)	Elektif (n=)15	p
Operasyon			
Açık	8 (%61,5)	6 (%40)	>0,05
Laparoskopik	5 (%38,5)	9 (%60)	
Tanı ile operasyon arası süre (gün)	7,7 (0-45)	116 (30-270)	>0,05
Hastane yatış süresi(gün)	13,8 (3-49)	10,5 (2-30)	>0,05
Komplikasyon, Clavien Dindo	0	1 (%6,7), 3	>0,05
Eksitus	0	0	

Tablo-5. Sağkalıma göre hastaların karşılaştırılması.

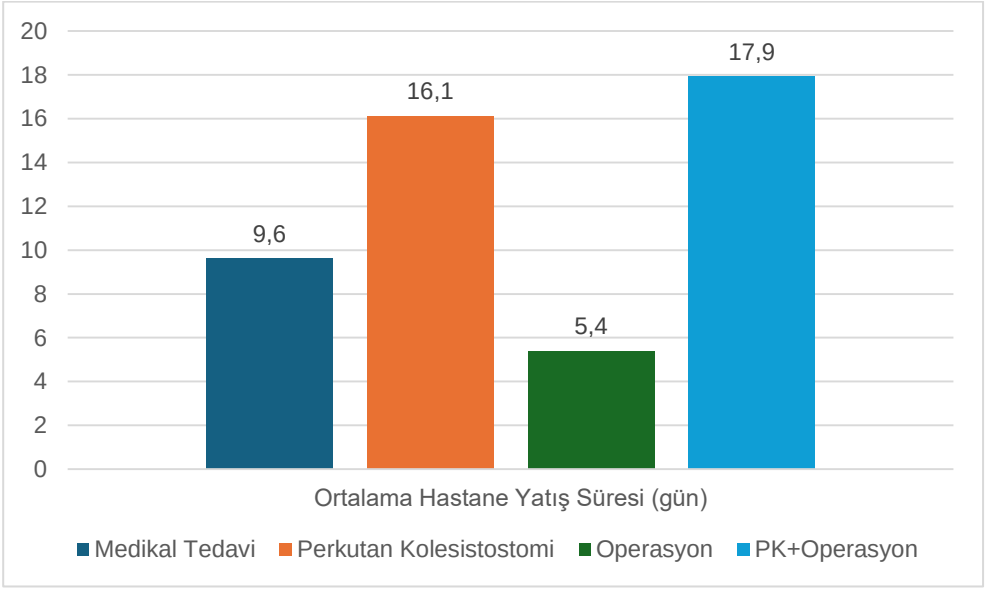
	Eksitus(n=13)	Sağ(n=105)	p
Cinsiyet			>0,05
Erkek	7 (%53,8)	58 (%55,2)	
Kadın	6 (%46,2)	47 (%44,8)	
Ortalama yaş	80.1(49-93)	66.1(23-88)	**=0,0007**
ASA skoru			>0,05
1	1 (%7,7)	47 (%44,8)	
2	9 (%69,2)	51 (%48,6)	
3	3 (%23,1)	7 (%6,6)	
Tedavi türü			>0,05
Medikal tedavi	2 (%15,4)	15 (%14,3)	
PK	11 (%84,6)	62 (%59,1)	
Operasyon	0	13 (%12,3)	
PK sonrası operasyon	0	15 (%14,3)	
Hastane yatış süresi(gün)	23.8(2-85)	13.1 (1-49)	>0,05

ASA: AmericanSociety of Anesthesiologists, PK: PerkutanKolesistostomi



Şekil-1. İnflamasyon belirteçleri ile tedavi arasındaki ilişki.

CRP/ALB: C-Reaktif protein/Albumin, NEU/LENF: Nötrofil/lenfosit oranı, PK: perkütan kolesistostomi



Şekil-2. Hastane yatış süresi ile tedavi şekli arasındaki ilişki.

PK: Perkütan kolesistostomi

TARTIŞMA

Akut perfore kolesistit, akut kolesistitin en ciddi komplikasyonudur ve mortalite oranlarını artırmaktadır. Literatürde akut kolesistitli hastaların %2-11'inde perforasyon geliştiği bildirilmiştir (14). Çalışmamızda 5 yıl içerisinde takip edilen akut kolesistit tanılı hastaların %22,2'sinde safra kesesi perforasyonu geliştiğini gördük. Kliniğimizin tersiyer ve yüksek volümlü hasta başvurusu olan bir merkez olması nedeniyle komplike hasta sayısı daha fazla izlenmektedir.

Erkek cinsiyetin semptomatik kolelitiaziste ve akut kolesistit gelişiminde risk faktörü olduğunu gösteren birçok çalışma yayınlanmıştır (15, 16). Buna bağlı olarak erkek hastalarda APK gelişim riski de artış göstermektedir. Derici ve arkadaşlarının yaptığı bir çalışmada daha önceki

yapılan çalışmalara benzer nitelikte erkeklerde daha fazla APK geliştiği gösterilmiştir. (14) 2021 yılında yapılan Meksika merkezli bir sistematik derleme çalışmasında erkek cinsiyette safra kesesi perforasyon oranlarının daha fazla olduğu saptanmıştır. (17) Yine bu çalışmalarla birlikte literatürde APK'in 60 yaş ve üzerinde daha fazla görüldüğü saptanmıştır. (2) Çalışmamızda perforasyonun literatüre uyumlu olarak erkek hastalarda ve 60 yaş üzeri hastalarda daha fazla görüldüğünü saptadık.

Derici ve arkadaşlarının yaptığı başka bir çalışmada kardiyovasküler komorbiditesi mevcut olan hastalarda safra kesesi perforasyonunun daha fazla izlendiği saptanmıştır. (18) Bununla birlikte diyabetes mellitus, immunsupresif hastalıklar, maligniteler de perforasyon riskini artırmaktadır. (19,20) Çalışmamızda hastaların yarısından fazlası ASA2 ve ASA3 skoruna sahipken %48,3'ünde hipertansiyon, %26,3'ünde diyabetes mellitus, %18,6'sında ise koroner arter hastalığı mevcuttu.

1934 yılında Niemeier ve arkadaşlarının yaptığı sınıflama ile safra kesesi perforasyonları 3 tipte sınıflandırılmış ve tedavi planlamasında yol gösterici olmuştur. İngiltere merkezli yapılan bir sistematik derlemede Tip 2 perforasyonlarla daha sık karşılaşıldığı gösterilmiştir. (5) Rajput ve arkadaşlarının yaptığı Hindistan merkezli çalışmada da hastaların yarısından fazlasında Tip 2 perforasyon olduğu görülmüştür (21). Çalışmamızda hastaların %90'dan fazlasında Tip 2 safra kesesi perforasyonu olduğunu ve literatüre göre çok daha fazla Tip 2 perforasyonlu hastayla karşılaştığımızı gördük. Bununla birlikte yapılan çalışmalarda sık rastlanmadığı görülen Tip 3 perforasyonlu 3 hasta çalışmamızda mevcut idi.

Tokyo 2018 kılavuzunda ileri evre akut kolesistitli hastalarda öncelikle konservatif tedavi önerilmektedir. Ancak hastanın yaş, komorbidite ve klinik durumunun tedavi planında göz önünde bulundurulması gerektiği ve buna bağlı olarak erken dönemde uygun hastalara cerrahi tedavi uygulanabileceği bildirilmektedir (22). Kılavuza uyumlu olarak APK'lı hastalar için yapılan çalışmaların birçoğu da hastaya spesifik tedavi tercihi yapılmasını önermektedir. Buna bağlı olarak Niemenier'in tanımladığı sınıflama tedavi planlamasında yol gösterici olabilir. Akut biliyer peritoniti olan Tip 1 APK'li hastalarda hastanın diğer risk faktörleri de göz önüne alınarak acil cerrahi planlanabilirken cerrahiye uygun olmayan hastalarda konservatif tedavi tercih edilmelidir. Kronik bilioenterik fistül gelişen Tip 3 hastalarda daha ileri tetkik-tedavi ve deneyimli ekip tarafından uygulanacak drenaj veya cerrahi prosedürler ön plandadır. Lokalize peritonit gözlenen Tip 2 hasta grubunda ise tedavi tartışmalıdır. Erken dönemde ilk adım olarak uygulanacak antibiyoterapi ve gereklilik halinde perkütan kolesistostomi uygulaması hastanın mevcut enflamasyon tablosunun gerilemesini sağlayacaktır ancak kesin bir tedavi seçeneği değildir. Elektif cerrahiye uygun olmayan hastalar için palyasyon amacıyla uygulanan drenaj işlemleri dışında bu gruptaki hastalara kolesistektomi planlanması gerekmektedir. (2, 5, 14, 17, 22-25) Çalışmamıza dahil ettiğimiz hastalara uygulanan tedavi türlerine bakıldığında Tip 1 APK'li hastaların 3'ü opere edilmiş olup bunlardan 1'i acil opere edilmiş, 1'i antibiyoterapi sonrası taburcu edilerek elektif opere edilmiş, 1'i ise perkütan kolesistostomi uygulaması sonrası elektif opere edilmiştir. Tip 2 APK'li hasta grubunda hastaların yarısından fazlasına perkütan kolesistostomi uygulanmıştır. Hastaların %13'ü perkütan drenaj sonrası opere olurken %10'una drenaj prosedürü uygulanmaksızın operasyon uygulanmıştır. Perkütan sonrası kolesistektomi uygulanan hastaların %25'ine aynı yatışta yani interval dönemde kolesistektomi uygulanmış, diğer hastalara elektif kolesistektomi uygulanmıştır. Drenaj uygulanmayan hastalarda ise durum tam tersi şekilde hastaların %75'i ilk yatışında kolesistektomi uygulandıktan sonra taburcu edilmiştir. Tip 3 APK'lı hastaların tamamına perkütan kolesistostomi uygulanmıştır. Çalışmamızın bulgularına bakıldığında literatüre benzer şekilde Tip 2 APK'lı hasta grubunun tedavisinde konservatif tedavi seçeneklerinin daha fazla tercih edildiğini ve bu hastalara ilerleyen dönemde kolesistektomi uygulandığını görmekteyiz.

Perkütan kolesistostomi uygulaması sonrası enflamasyonun gerileme süreci ve antibiyoterapi süresinin tamamlanması nedeniyle bu hastalarda hastane yatışlarının daha uzun olduğu görülmüştür.

Safra kesesi perforasyonuna bağlı mortalite oranları Glenn ve arkadaşları tarafından 1942 yılında %42'lerde gösterilirken güncel literatürde %10-40 arasında değiştiği bildirilmiştir (4, 5, 26). Bununla birlikte Almanya merkezli APK nedeniyle kolesistektomi uygulanan 5000 hasta ile yapılan bir çalışmada mortalite oranı %4 olarak bildirilmiştir (2). Çalışmamızda hastaların %11'i

eksitus olurken opere edilen hastaların hiçbirinde eksitus görülmemiştir. Tersiyer merkez deneyiminin bu oranların düşük olmasında etkisi olduğu düşünmekteyiz. Aynı zamanda daha önce bahsedildiği üzere yüksek yaşın hastalığın şiddetini ve buna bağlı mortalite ve morbidite oranlarını artırdığını gösterir şekilde çalışmamızda anlamlı olarak eksitus olan hastaların yaşı daha yüksek saptanmıştır.

SONUÇ

Akut perfore kolesistit özellikle yaşlı ve komorbid hasta grubunda morbid ve mortal seyredebilen ciddi bir safra kesesi patolojisidir. Erken dönemde tanı alan ve cerrahiye uygun hasta grubunda kolesistektomi önerilen tedavi seçeneğiyken cerrahiye uygun olmayan veya şiddetli kliniği mevcut olan hastalarda konservatif tedavi seçenekleri ön planda tercih edilmektedir. Ancak palyasyon amaçlı konservatif tedaviler dışında hastalığın yegâne tedavisi kolesistektomidir. Literatürü destekler nitelikte sonuç aldığımız çalışmamız gibi daha yüksek hasta sayılı ve deneyimli merkezler tarafından yapılacak çalışmalar ile akut perfore kolesistit tedavisinde etkin tedavi algoritmaları oluşturulabileceğini düşünmekteyiz.

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Kaynaklar

1. Ausania, F., Suárez, S., Garcia, H., Rio, P., & Núñez, E. (2015). Gallbladder perforation: morbidity, mortality and preoperative risk prediction. *Surgical Endoscopy*, 29, 955-960. https://doi.org/10.1007/s00464-014-3765-6.
2. Jansen, S., Doerner, J., Macher-Heidrich, S., Zirngibl, H., & Ambe, P. (2017). Outcome of acute perforated cholecystitis: a register study of over 5000 cases from a quality control database in Germany. *Surgical Endoscopy*, 31, 1896-1900. https://doi.org/10.1007/s00464-016-5190-5.
3. Menakuru, S.R., Kaman, L., Behera, A., Singh, R. and Katariya, R.N. (2004), Current management of gall bladder perforations. ANZ Journal of Surgery, 74: 843-846. https://doi.org/10.1111/j.1445-1433.2004.03186.x
4. Pak M, Lindseth G. (2016) Risk factors for cholelithiasis. Gastroenterol Nurs 39:297–309. https://doi.org/10.1097/SGA.0000000000000235.
5. Date, R., Thrumurthy, S., Whiteside, S., Umer, M., Pursnani, K., Ward, J., & Mughal, M. (2012). Gallbladder perforation: case series and systematic review.. *International journal of surgery*, 10 2, 63-8 . https://doi.org/10.1016/j.ijsu.2011.12.004.
6. Wani, A., Iqbal, J., & Parihar, S. (2023). A retrospective study of diagnosis and management of gallbladder perforation: 10-year experience from a tertiary health care centre.. *Turkish journal of surgery*, 39 2, 102-106 . https://doi.org/10.47717/turkjsurg.2023.5962.
7. Morris BS, Balpande PR, Morani AC, Chaudhary RK, Maheshwari M, Raut AA. (2007) The CT appearances of gallbladder perforation. Br J Radiol 80:898–901.https://doi.org/10.1259/bjr/28510614.
8. Aydin, C., Altaca, G., Berber, I. *et al.* Prognostic parameters for the prediction of acute gangrenous cholecystitis. *J Hepatobiliary Pancreat Surg* 13, 155–159 (2006). https://doi.org/10.1007/s00534-005-1042-8
9. Ong, C., Wong, T., & Rauff, A. (1991). Acute gall bladder perforation--a dilemma in early diagnosis.. *Gut*, 32, 956 - 958. https://doi.org/10.1136/GUT.32.8.956.
10. Niemeier, O. W. M.D., F.R.C.S. (edin.). ACUTE FREE PERFORATION OF THE GALL-BLADDER. Annals of Surgery 99(6):p 922-924, June 1934.https://doi.org/10.1097/00000658-193499060-00005
11. Anderson BB, Nazem A. Perforations of the gallbladder and cholecy- stobiliary fistulae: A review of management and a new classification. J Natl Med Assoc 1987; 79(4): 393-9.
12. Takada, T., Yasuda, H., Uchiyama, K., Hasegawa, H., Asagoe, T., & Shikata, J. (1989). Pericholecystic abscess: classification of US findings to determine the proper therapy.. *Radiology*, 172 3, 693-7 . https://doi.org/10.1148/RADIOLOGY.172.3.2672094.
13. G. Wakabayashi et al. "Tokyo Guidelines 2018: surgical management of acute cholecystitis: safe steps in laparoscopic cholecystectomy for acute cholecystitis (with videos)." *Journal of Hepato-Biliary-Pancreatic Sciences*, 25 (2018). https://doi.org/10.1002/jhbp.517.

14. Derıcı, H., Kara, C., Bozdağ, A., Nazlı, O., Tansuğ, T., & Akca, E. (2006). Diagnosis and treatment of gallbladder perforation.. *World journal of gastroenterology*, 12 48, 7832-6 . https://doi.org/10.3748/WJG.V12.I48.7832.
15. Lein, H.-H. and Huang, C.-S. (2002), Male gender: Risk factor for severe symptomatic cholelithiasis. World J. Surg., 26: 598-601. https://doi.org/10.1007/s00268-001-0275-1
16. Ambe, P., & Köhler, L. (2015). Is the male gender an independent risk factor for complication in patients undergoing laparoscopic cholecystectomy for acute cholecystitis?. *International surgery*, 100 5, 854-9 . https://doi.org/10.9738/INTSURG-D-14-00151.1.
17. Quiroga-Garza, A., Álvarez-Villalobos, N., Angeles-Mar, H., García-Campa, M., Muñoz-Leija, M., Salinas-Alvarez, Y., Elizondo-Omaña, R., & Guzmán-López, S. (2021). Localized gallbladder perforation: a systematic review of treatment and prognosis.. *HPB : the official journal of the International Hepato Pancreato Biliary Association*. https://doi.org/10.1016/j.hpb.2021.06.003.
18. Derıcı, H., Kamer, E., Kara, C., Ünalp, H., Tansuğ, T., Bozdağ, A., & Nazlı, O. (2011). Gallbladder perforation: clinical presentation, predisposing factors, and surgical outcomes of 46 patients.. *The Turkish journal of gastroenterology : the official journal of Turkish Society of Gastroenterology*, 22 5, 505-12 . https://doi.org/10.4318/TJG.2011.0246.
19. Strohl EL, Diffenbaugh WG, Baker JH, Chemma MH: Collective reviews: gangrene and perforation of the gallbladder. Int Abstr Surg. 1962, 114: 1-7.
20. Wang AJ, Wang TE, Lin CC, Lin SC, Shih SC. Clinical predictors of severe gallbladder complications in acute acalculous cholecystitis. World J Gastroenterol 2003; 9(12): 2821-823. https://doi.org/10.3748/wjg.v9.i12.2821
21. Rajput, D., Gupta, A., Kumar, S., Singla, T., Srikanth, K., & Chennatt, J. (2022). Clinical spectrum and management outcome in gallbladder perforation-a sinister entity: Retrospective study from Sub-Himalayan region of India.. *Turkish journal of surgery*, 38 1, 25-35 . https://doi.org/10.47717/turkjsurg.2022.5325.
22. K. Okamoto et al. "Tokyo Guidelines 2018: flowchart for the management of acute cholecystitis." *Journal of Hepato-Biliary-Pancreatic Sciences*, 25 (2018). https://doi.org/10.1002/jhbp.516.
23. Kochar K, Vallance K, Mathew G, Jadhav V. (2008) Intrahepatic perfo-

ration of the gall bladder presenting as liver abscess: case report, review of literature and Niemeier's classification. Eur J Gastroenterol Hepatol 20:240–244. https://doi.org/10.1097/MEG.0b013e3282eeb520.
24. HussainT,AdamsM,AhmedM,ArshadN,SolkarM.(2016)Intrahepatic perforation of the gallbladder causing liver abscesses: case studies and literature review of a rare complication. Ann R Coll Surg Engl 98: e88–e91. https://doi.org/10.1308/rcsann.2016.0115.
25. Jansen, S., Stodolski, M., Zirngibl, H., Gödde, D., & Ambe, P. (2018). Advanced gallbladder inflammation is a risk factor for gallbladder perforation in patients with acute cholecystitis. *World Journal of Emergency Surgery : WJES*, 13. https://doi.org/10.1186/s13017-018-0169-2.
26. Glenn F, Moore SW. Gangrene and perforation of the wall of the gallbladder. A sequele of acute cholecystitis. *Arch Surg*1942; 44: 677-686

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The application of BLUE (bedside lung ultrasound in emergency) protocol in the emergency department

Acil serviste BLUE (acil durumda yatakbaşı akciğer ultrasonu) protokolünün uygulanması

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ABSTRACT

Aim: This study aimed to evaluate the effectiveness of lung ultrasonography (US) in detecting the cause of acute respiratory distress in the emergency department.

Materials and Methods: This cross-sectional analytical study was carried out on 195 adult patients who were admitted to the Emergency Department of a University Hospital with acute respiratory failure in 6months period. The validity of the US diagnoses was assessed by comparing the decisions made by researchers according to the BLUE protocol classification with the final judgments made by the primary doctors using gold-standard diagnostic techniques suggested by the guidelines.

Results: The diagnostic accuracy of chest ultrasound was 89.7%. Specifically, ultrasound demonstrated 95.6% sensitivity and 99% specificity for diagnosing Congestive Heart Failure (CHF), 94.3% sensitivity and 97.2% specificity for Chronic Obstructive Pulmonary Disease (COPD), 94.2% sensitivity and 91.2% specificity for pneumonia, and 100% sensitivity and specificity for Pneumothorax (PTX). In contrast, the sensitivity for Pulmonary Embolism (PE) diagnosis was 66.7%. Ultrasound also identified pneumonia associated with CHF with 83.3% sensitivity and 96.0% specificity, and pneumonia associated with COPD with 54.6% sensitivity and 98.4% specificity. The diagnostic accuracy of routine physical examination and chest X-ray, which are standard for assessing respiratory distress at the bedside in the emergency department, was compared with ultrasound. The accuracy rates for CHF were 89.2%/81.9%/97.4%; for COPD were 90.8%/77.8%/96.4%; for pneumonia were 76.9%/93.8%/92.3%; for PE were 90.8%/90.7%/96.4%; and for PTX were 99.5%/100%/100%, respectively. Additionally, the average time difference between the requests and screenings for X-ray and chest CT was 1.36 hours and 2.26 hours, respectively.

Discussion: Our study demonstrated that chest ultrasound is an effective and feasible diagnostic tool for diagnosing CHF, COPD, pneumonia, PE, and PTX. Compared to gold standard tests, ultrasound reduced the diagnostic time and provided more reliable results than physical examination.

Keywords: Lung ultrasonography, blue protocol, emergency department, pulmonary edema, COPD

ÖZ

Giriş: *Bu çalışmada acil serviste akut solunum sıkıntısının nedeninin saptanmasında akciğer ultrasonografisinin (US) etkinliğinin değerlendirilmesi amaçlandı.*

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Gereç ve Yöntem: *Bu kesitsel analitik çalışma, bir Üniversite Hastanesi Acil Servisi'ne 6 aylık sürede akut solunum yetmezliği nedeniyle başvuran 195 yetişkin hasta üzerinde gerçekleştirildi. US teşhislerinin geçerliliği, araştırmacıların BLUE protokol sınıflandırmasına göre verdiği kararlar ile primer doktorların kılavuzların önerdiği altın standart teşhis tekniklerini kullanarak verdikleri nihai kararların karşılaştırılması yoluyla değerlendirildi.*

Bulgular: *Akciğer ultrasonunun tanısal doğruluğu %89,7 olarak bulunmuştur. Özellikle, ultrasonun Konjestif Kalp Yetmezliği (KKY) tanısındaki duyarlılığı %95,6 ve özgüllüğü %99; Kronik Obstrüktif Akciğer Hastalığı (KOAH) tanısındaki duyarlılığı %94,3 ve özgüllüğü %97,2; pnömoni tanısındaki duyarlılığı %94,2 ve özgüllüğü %91,2; ve Pnömotoraks (PTX) tanısındaki duyarlılığı ve özgüllüğü %100 olarak tespit edilmiştir. Buna karşın, Pulmoner Tromboembolizm (PTE) tanısındaki duyarlılık %66,7'dir. Ultrason ayrıca, KKY ile ilişkili pnömoniyi %83,3 duyarlılık ve %96,0 özgüllükle, KOAH ile ilişkili pnömoniyi ise %54,6 duyarlılık ve %98,4 özgüllükle teşhis etmiştir. Acil serviste yatak başında solunum sıkıntısını değerlendirmek için kullanılan rutin fizik muayene ve akciğer grafisinin tanısal doğruluğu ultrason ile karşılaştırılmıştır. KKY için tanısal doğruluk oranları sırasıyla %89,2/%81,9/%97,4; KOAH için %90,8/%77,8/%96,4; pnömoni için %76,9/%93,8/%92,3; PTE için %90,8/%90,7/%96,4; ve PTX için %99,5/%100/%100 olarak saptanmıştır. Ek olarak, çalışmamızda X-ray ve toraks BT istekleri ile tarama süreleri arasındaki ortalama fark sırasıyla 1,36 saat ve 2,26 saat olarak hesaplanmıştır.*

Tartışma: *Çalışmamız; akciğer US'un KKY, KOAH, pnömoni, PTE ve PTX tanısında etkili ve uygulanabilir bir tanısal araç olduğunu gösterdi. US, altın standart testlerle karşılaştırıldığında tanı süresini kısalttı ve fizik muayeneye göre daha güvenilir sonuçlar ortaya koydu.*

Anahtar Sözcükler: *Akciğer ultrasonografisi, BLUE protokolü, acil servis, akciğer ödemi, KOAH.*

INTRODUCTION

Respiratory distress is one of the most common reasons for visiting the Emergency Departments (ED) among all age groups (1). Also, acute dyspnea is a leading symptom of many diseases that may cause morbidity and mortality. A rapid distinction of the underlying pathologies causing dyspnea may sometimes be difficult in EDs (2). However, it is crucial to differentiate reasons of high morbidity and mortality in the EDs.

It is known that physical examination and bedside radiography during the evaluation of dyspnea in the ED may be insufficient for the diagnosis and treatment process, and the application of further tests may lead to serious time loss (3). Therefore, it has been suggested that the lung B-mode ultrasound (US) can be used for rapid diagnosis in patients with acute dyspnea (4). The low running cost, bedside availability, repeatability, and absence of radiation are emphasized as the advantages of US. In most studies, it has been highlighted that lung US is highly sensitive to the variations of pulmonary content and air-fluid balance (5, 6). Additionally, it has been reported that lung US may be a useful technique for the diagnosis of some pulmonary diseases based on the differences in air and fluid contrast (3, 7, 8).

BLUE protocol is the application of lung US based on the grouping of artifacts, pleural changes, alveolar consolidation, and pleural effusions to make an accurate diagnosis (3, 8).

This study aimed to investigate the efficacy of the BLUE protocol in identifying the underlying cause in patients referred to the EDs with acute respiratory failure.

MATERIALS and METHODS

Study design and Patient selection

This cross-sectional analytical study was carried out on 195 adult patients who were admitted to the ED of a University Hospital with acute respiratory failure in six months. The ethical approval was obtained Ege University, Ethical Committee (Approval number: 10-9.1/4). Patients under the age of 18, having structural lung disease, had undergone surgical intervention (pneumonectomy or lobectomy) or pleurodesis were excluded from the study.

Investigators

All patients were evaluated with lung US by investigators who did not participate in the primary follow-up and treatment of the individual patient. The investigators were emergency medicine resident doctors who had received "basic emergency ultrasonography" training and had experience of using the US in emergency patient care for at least two years. The diagnosis and treatment of the patients were carried out by other ED physicians who were blind to the US results. The investigators and treating physicians filled two different data collection forms, which were collected in two separate closed boxes.

Table-1. Gold standards according to guideline recommendations (9-13).

Pneumothorax	Chest radiography, CT (if necessary)
Cardiogenic edema	ECHO, functional tests, AHA recommendations
PTE	Wells criterion, D-dimer, Thorax angio CT
COPD attack	PFT (Respiratory function test)
Pneumonia	Infectious profile, radiological asymmetry, microorganism isolation, response to antibiotics

CT: Computed Tomography, ECHO: Echocardiography, AHA; American Heart Association, PTE: Pulmonary Thromboembolism, COPD: Chronic Obstructive Pulmonary Disease

Additionally, a cardiologist (for diagnosis of CHF), a radiologist (for diagnosis of PTX and PTE), and a chest physician (for diagnosis of COPD and Pneumonia) who are experts in their fields examined the filled forms and evaluated whether an accurate diagnosis was made according to the gold standards recommended by the current guidelines (Table-1).

Ultrasonographic Evaluation and Procedure

The US Device

The ultrasonographic evaluation was performed by a portable USG device (Sonosite Micromaxx, SonoSite Inc., USA) using a 5-MHz micro convex probe and a 7.5-MHz linear probe.

Procedure

US was performed without interruption during the admission of the patient to the ED (within the first 10 minutes).

Each hemithorax was divided into three regions by anterior and posterior axillary lines (Figure-1a-b). All three areas of both lungs were longitudinally scanned (Figure-2a-b). Findings such as artifacts (A-line, B-line), lung sliding (present/absent), pleural effusion (present/absent), alveolar consolidation (present/absent) were recorded according to the systematic analysis per the BLUE protocol (14).

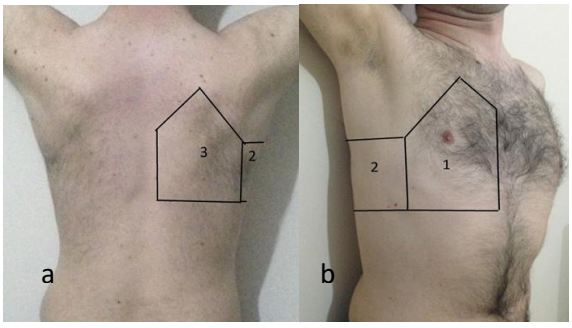

Figure-1a-b. Three lung regions based on anterior and posterior axillary lines

Venous study, a part of the BLUE protocol, was carried out after performing the US. Subclavian, jugular, femoral, and popliteal veins of the patients were detected by the linear probe, followed by the compression method and Doppler. Veins which could not be compressed or did not show blood flow were accepted as positive for DVT (1, 15).

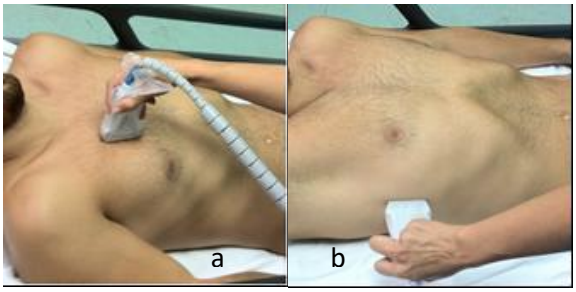

Figure-2a-b. Longitudinal scanning of the chest wall during lung US.

Interpretation of Lung Ultrasonographic Images:

In the ultrasonographic image, it was confirmed that when the pleural line (a hyperechoic white horizontal line located 0.5 cm below the rib line) was visible, the US probe had seen the parenchyma.

The surface appearance of the normal lung consists of the bat sign (normal intercostal appearance of the pleura and lung parenchyma), lung sliding (a movement in rhythm with respiration, indicating sliding of the visceral pleura against the parietal pleura), the A-line (hyperechoic horizontal artifacts arising from the pleural line), and comet-tail artifacts (irrelevant with lung sliding, and not erasing A-lines). The A-profile was defined as the presence of anterior lung sliding with A-lines (3, 16, 17).

Pleural effusion: The roughly quadrangular shapes and sinusoid signs with a regular lower border (the visceral pleura) was required for the diagnosis of pleural effusion (18–20).

Chronic Obstructive Pulmonary Disease (COPD): An A-profile without DVT or "posterior and/or lateral alveolar and/or pleural syndrome" (PLAPS) (the nude profile) was the typical profile indicating asthma or COPD.

Interstitial edema: The B-profile is a profile where three or more B-lines are observed between two ribs. The B-line always arises from the pleural line and moves in concert with lung sliding. Additionally, it isa well-defined, always long, laser-like, and hyperechoic comet-tail artifacts erasing A-lines (17, 21).

Pneumonia: Alveolar consolidations (hypoechoic tissue-like sign - C profile), bronchograms (internal hyperechoic punctiform appearance corresponding to air-filled bronchi), A profile plus PLAPS (the evaluation of pleural effusions and alveolar consolidations), and A/B profile (B-lines on one side, A-lines on the other) were the typical profiles indicating pneumonia (3, 22, 23).

Pneumothorax (PTX): Abolished lung sliding, the absence of B-lines, loss of "seashore sign," and detection of lung-point were the typical profiles indicating pneumothorax (3,24).

Pulmonary Thromboembolism (PTE): In the venous analysis, the detection of A-profile plus DVT positivity was connected to PTE (3, 16, 25).

Statistical Analysis

Statistical analysis was performed with the Statistical Package for the Social Sciences (SPSS, version 20, IBM, Armonk, New York 10504, NY, USA). Data were expressed as numbers, percentages, and mean±SDs (standard deviations). Sensitivity, specificity, positive (PPV) and negative (NPV) predictive values, accuracy, and likelihood ratios (LR) were calculated with 95% confidence intervals in relation to the final diagnosis, confirmed with recommended gold standards according to the guidelines. The McNemar Test was used to compare the sensitivity and the specificity of lung US and conventional ED evaluation. The level of significance, p, was set at 0.05.

RESULTS

The study included 215 patients with acute respiratory failure. Although 20 patients were included in the study during the initial evaluation, they were excluded according to the exclusion criteria (Table-2).

Table-2. Patient flow diagram.

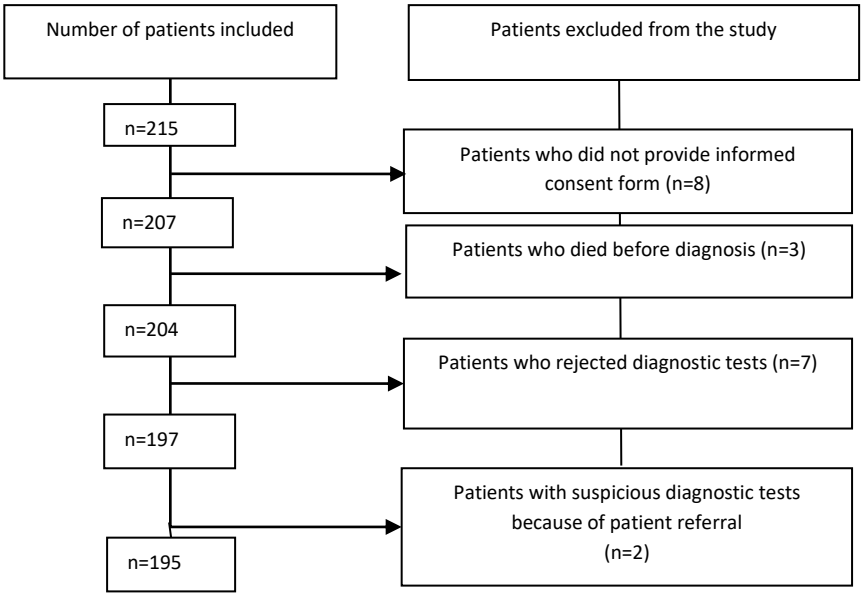

Table-3. Sensitivity, specificity, positive-negative predictive values, and diagnostic accuracy rates of US diagnoses.

Diagnoses	Ultrasound Signs	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	+LR	- LR	Accuracy
Pulmonary edema	Diffuse bilateral anterior B-1 lines associated with lung sliding (B profile)	95.6 (89.1-98.8)	99 (94.8-99.9)	98.9 (92.5-99.8)	96.3 (90.8-98.5)	99.4 (14.1-699.57)	0.04 (0.02-0.1)	97.4 (94.1-99.2)
COPD	Predominant anterior A lines without PLAPS and with lung sliding (normal profile), or with absent lung sliding without lung point	94.3 (84.38-98.8)	97.2 (92.9-99.3)	92.6 (82.6-97.05)	97.9 (93.9-99.3)	33.5 (12.7-88.2)	0.06 (0.02-0.18)	96.4 (92.7-98.5)
Pneumonia	Alveolar consolidation	94.2 (85.8-98.4)	91.2 (84.92-95.6)	85.6 (77.0-91.3)	96.6 (91.7-98.7)	10.79 (6.12-19.0)	0.06 (0.02-0.16)	92.3 (87.6-95.6)
PTE	Predominant anterior bilateral A lines plus venous thrombosis	66.7 (41.0-86.7)	99.4 (96.9-100)	92.3 (62.3-98.9)	96.7 (93.9-98.2)	118 (16.3-855.9)	0.34 (0.18-0.65)	96.41 (92.7-98.5)
PTX	Absent anterior lung sliding, absent anterior B lines, and present lung point	100 (47.8-100)	100 (98.1-100)	100	100	0	0	100 (98.1-100)
COPD +Pneumonia		83.3 (62.6-95.39	96.0 (91.7-98.3)	74.0 (57.5-85.8)	97.6 (94.4-99.0)	20.36 (9.64-42.68)	0.17 (0.07-0.42)	94.4 (90.1-97.1)
Pulmonary edema + Pneumonia		54.6 (23.4-83.3)	98.4 (95.3-99.7)	66.7 (36.4-87.4)	97.3 (94.99-98.6)	33.5 (9.63-116.2)	0.46 (0.24-0.88)	95.6 (92.1-98.2)

COPD: Chronic Obstructive Pulmonary Disease, PLAPS: Posterior and/or lateral alveolar and/or pleural syndrome, PTE: Pulmonary Thromboembolism, PTX: Pneumothorax, PPV: Positive Predictive Value, NPV: Negative Predictive Value, LR: Likelihood Ratio

Of the patients, 129 (66.2%) were male, and 66 (33.8%) were female. The mean age was 66.7±13.6 years (range 23 to 90 years).

When all diagnostic groups were taken into account, the diagnostic accuracy of lung US was 89.7%. Further, this diagnostic accuracy was not affected by patient-related variables such as age, gender, comorbidity, and vital status.

A statistically significant similarity/accuracy was detected in patients diagnosed with pulmonary edema, COPD, pneumonia, PTE, and PTX when the ultrasonographic diagnosis was compared to the gold standard diagnostic tests ($p<0.001$). Ultrasonographic accuracy rates are given in Table-3.

According to the gold standard tests, patients who were diagnosed with Congestive Heart Failure (CHF) (n = 91), COPD (n = 53), pneumonia (n = 69), and PTX (n = 5) could be diagnosed by US with high sensitivity and specificity. On the other hand, ultrasonographic specificity was quite low in patients diagnosed with PTE (n = 18).

Besides, US has revealed combined pathologies such as pneumonia associated with CHF, or pneumonia associated with COPD, with high sensitivity and specificity (Table-3).

In our study, we calculated the sensitivity, specificity and diagnostic accuracy rates of lung auscultation and radiography separately for each disease and compared them with lung US. These results are shown in Table-4.

Finally, we separately calculated the average of the difference between XR and CT request and scan times to give an idea of the time it takes to reach a diagnosis in traditional diagnostic processes. The mean difference between XR and thorax CT request and scanning times was calculated as 1.36 and 2.26 hours, respectively.

Table-4. Comparison of sensitivity, specificity, and diagnostic accuracy rates of lung auscultation, radiography, and US.

Diagnoses	Lung auscultation			Radiography			US		
	Sensitivity (95% CI)	Specificity (95% CI)	Accuracy	Sensitivity (95% CI)	Specificity (95% CI)	Accuracy	Sensitivity (95% CI)	Specificity (95% CI)	Accuracy
CHF	85.7 (76.8-92.2)	92.3 (85.4-96.6)	89.2 (84.0-93.2)	64.4 (53.6-74.3)	97.1 (91.8-99.4)	81.9 (75.8-87.1)	95.6 (89.1-98.8)	99 (94.8-99.9)	97.4 (94.1-99.2)
COPD	84.9 (72.4-93.3)	93.0 (87.4-96.6)	90.8 (85.8-94.4)	24.3 (13.7-38.3)	97.9 (93.9-99.6)	77.8 (71.3-83.5)	94.3 (84.38-98.8)	97.2 (92.9-99.3)	96.4 (92.7-98.5)
Pneumonia	39.7 (28.0-52.3)	96.8 (92.1-99.1)	76.9 (70.4-82.6)	92.6 (83.7-97.6)	94.4 (88.9-97.7)	93.8 (89.4-96.7)	94.2 (85.8-98.4)	91.2 (84.92-95.6)	92.3 (87.6-95.6)
PTE	0 (0-18.5)	100 (97.5-100)	90.8 (85.8-94.4)	11.1 (1.37-34.7)	98.8 (95.9-99.8)	90.7 (85.7-94.4)	66.7 (41.0-86.7)	99.4 (96.9-100)	96.4 (92.7-98.5)
PTX	80.0 (28.36-99.5)	100 (98.1-100)	99.5 (97.2-100)	100 (47.8-100)	100 (98.1-100)	100 (98.12-100)	100 (47.8-100)	100 (98.1-100)	100 (98.1-100)

DISCUSSION

Results of our study concerning sensitivity and specificity rates were similar to the study of Lichtenstein et al. and other scientific studies which utilized the BLUE protocol (3, 26). This result proved the reliability of lung US, which was applied in patients from different contexts.

In our study; COPD, CHF and pneumonia were identified with high accuracy, sensitivity and specificity rates. This result is similar to literature (3-5). The study also recognized cases where these diseases were combined with pneumonia with high accuracy and specificity. The ability of lung US to distinguish several accompanying pathologies with high accuracy, unlike traditional diagnostic methods, may be an important result for ED practice. There is no US study in the literature with which we can compare this result regarding combined diagnoses.

In this study, it was found that both the sensitivity and specificity rates of US in detecting pneumothorax were 100 %. Therefore, lung US can be defined as a rapid, accurate, and effective tool in the detection of pneumothorax (2,3,14,27). These results suggest the use of bedside lung US as a first-line diagnostic tool in patients with suspected PTX.

According to our results, lung US had low sensitivity and high specificity in detecting PTE. The proportions observed in our study were lower than those reported in the literature (3,28–30). This decrease might be due to the small number of cases, the application in a position that the patient can tolerate instead of the recommended position or the US operator. On the other hand, the negative predictive value of US was detected as 97%. This result suggested that lung US may be safe with D-Dimer to rule out PTE. However, larger studies are needed to reach a definitive conclusion.

In our study, sensitivity, specificity, and diagnostic accuracy of lung US were compared with lung auscultation and chest radiography. Lung US revealed superior results regarding diagnostic accuracy in patients diagnosed with pulmonary edema, COPD, PTE, and pneumonia. The sensitivity and specificity of lung US in the definition of pneumonia was higher compared to auscultation but similar to chest radiography. When these findings were evaluated together with the results of other similar studies, it was thought that the combined use of physical examination and lung US could reduce the need for additional imaging procedures or specific tests to recognize the underlying reason of acute respiratory distress (19, 31–35).

One of the study results is; the mean difference between the request and scanning times for XR and thorax CT were calculated as 1.36 and 2.26 hours, respectively. These results proved to us that routine use of lung US, which were can complete in first 10 minutes of admission, in patient with respiratory distress will make a great contribution in terms of correct time management.

CONCLUSION

Lung US has a high diagnostic accuracy rate in the EDs. It prevents loss of time due to incorrect differential diagnoses by providing a more reliable preliminary diagnosis than auscultation. It also minimizes radiation exposure by reducing the need for chest radiography and CT. Additionally; it reduces the requirements of advanced techniques such as V/Q scintigraphy. This bedside diagnostic method is fast, inexpensive, and repeatable. For emergency services, the BLUE protocol can be considered as a viable algorithm. Finally, lung US can be performed in a routine emergency service setting after a standard training.

Limitations

Since our study group consisted of acute respiratory distress patients, US imaging had to be performed in positions that the patient could tolerate, instead of the recommended positions. This has made it challenging to evaluate especially diseases, which were identified using focal US findings.

In emergency services, procedures for detecting the underlying pathology are frequently postponed to resuscitation procedures (such as providing airway, breathing and circulation safety). If US administration could adversely affect or prolong the diagnosis or treatment process of any patients, those patients were not included in the study, which limited the number of participants.

Conflict of interest: The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

References

1. Marx J, Adams J, Walls R, Rosen P, Hockberger R. Rosen's Emergency Medicine Concept and clinical practice. Elsevier; 2009. 129 p.
2. Zanobetti M, Scorpiniti M, Gigli C, Nazerian P, Vanni S, Innocenti F, et al. Point-of-Care Ultrasonography for Evaluation of Acute Dyspnea in the ED. Chest. 2017;151(6):1295–301.
3. Lichtenstein DA, Mezière GA. Relevance of lung ultrasound in the diagnosis of acute respiratory failure the BLUE protocol. Chest. 2008;134(1):117–25.
4. Cardinale L, Volpicelli G, Binello F, Garofalo G, Priola SM, Veltri A, et al. Clinical application of lung ultrasound in patients with acute dyspnoea: differential diagnosis between cardiogenic and pulmonary causes. Radiol Medica. 2009;114(7):1053–64.
5. Copetti R, Soldati G, Copetti P. Chest sonography: A useful tool to differentiate acute cardiogenic pulmonary edema from acute respiratory distress syndrome. Cardiovasc Ultrasound. 2008;29:6–16.
6. Volpicelli G. Lung sonography. J Ultrasound Med. 2013;32(1):165–71.
7. Noble VE, Lamhaut L, Capp R, Bosson N, Liteplo A, Marx JS, et al. Evaluation of a thoracic ultrasound training module for the detection of pneumothorax and pulmonary edema by prehospital physician care providers. BMC Med Educ. 2009;12(9):3–4.
8. Lichtenstein D. Lung ultrasound in acute respiratory failure an introduction to the BLUE-protocol. Minerva Anestesiol. 2009;75(5):313–7.
9. Helen E Davies, Robert J O Davies, Christopher W H Davies; BTS Pleural Disease Guideline Group, Management of pleural infection in adults: British Thoracic Society Pleural Disease Guideline, Thorax. 2010 Aug:65 Suppl 2:ii41-53. doi: 10.1136/thx.2010.137000.
10. Konstantinides SV, Meyer G, Becattini C, et al. 2019 ESC Guidelines for the diagnosis and management of acute pulmonary embolism developed in collaboration with the European Respiratory Society (ERS). European Heart Journal. August 2019. doi:10.1093/eurheartj/ehz405
11. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE Jr, et al. 2013 ACCF/AHA guideline for the management of heart failure: executive summary: a report of the American College of Cardiology Foundation/American Heart Association Task Force on practice guidelines. Circulation. 2013 Oct 15;128(16):1810-52. doi: 10.1161/CIR.0b013e31829e8807

12. Shireen Mirza, Ryan D Clay, Matthew A Koslow, Paul D Scanlon COPD Guidelines: A Review of the 2018 GOLD Report Mayo Clin Proc. 2018 Oct;93(10):1488-1502. doi: 10.1016/j.mayocp.2018.05.026.
13. Metlay JP, Waterer GW, Long AC, et al. Diagnosis and Treatment of Adults with Community-acquired Pneumonia. An Official Clinical Practice Guideline of the American Thoracic Society and Infectious Diseases Society of America. Am J Respir Crit Care Med. October 2019:e45-e67. doi:10.1164/rccm.201908-1581st
14. Lichtenstein DA, Mezière G, Lascols N, Biderman P, Courret JP, Gepner A, et al. Ultrasound diagnosis of occult pneumothorax. Crit Care Med. 2005;33(6):1231–8.
15. Lichtenstein D. General ultrasound in the critically ill. 2nd Editio. Heidelberg: Springer-Verlag; 2005. 70-95 p.
16. Lichtenstein D. Lung ultrasound in the critically ill. Curr Opin Crit Care. 2014;4:1–5.
17. Stefanidis K, Dimopoulos S, Nanas S. Basic principles and current applications of lung ultrasonography in the intensive care unit. Respirology. 2011;16(2):249–56.
18. Joyner CR, Herman RJ, Reid JM. Reflected Ultrasound in the Detection and Localization of Pleural Effusion. JAMA J Am Med Assoc. 1967;200:399–402.
19. Lichtenstein D, Goldstein I, Mourgeon E, Cluzel P, Grenier P, Rouby JJ. Comparative Diagnostic Performances of Auscultation, Chest Radiography, and Lung Ultrasonography in Acute Respiratory Distress Syndrome. Anesthesiology. 2004;100(1):9–15.
20. Radzina, M., & Biederer, J. (2019). Ultrasonography of the Lung. Rofo. 2019 Oct;191(10):909-923. doi: 10.1055/a-0881-3179. Epub 2019 Apr 4.
21. Lichtenstein D, Mézière G, Biderman P, Gepner A, Barré O. The comet-tail artifact: An ultrasound sign of alveolar-interstitial syndrome. Am J Respir Crit Care Med. 1997;156:1640–6.
22. Weinberg B, Diakoumakis EE, Kass EG, Seife B, Zvi ZB. The air bronchogram: Sonographic demonstration. Am J Roentgenol. 1986;147:593–5.
23. Lichtenstein DA, Lascols N, Mezière G, Gepner A. Ultrasound diagnosis of alveolar consolidation in the critically ill. Intensive Care Med. 2004;30:276–81.
24. Lichtenstein DA, Menu Y. A bedside ultrasound sign ruling out pneumothorax in the critically ill: Lung sliding. Chest. 1995;108:1345–8.
25. Lichtenstein DA. BLUE-Protocol and FALLS-Protocol. Chest. 2015;147(6):1659–70.
26. Patel CJ, Bhatt HB, Parikh SN, Jhaveri BN, Puranik JH. dside Lung Ultrasound in Emergency Protocol as a Diagnostic Tool in Patients of Acute Respiratory Distress Presenting to Emergency Department. J Emerg Trauma Shock. 2018;11(2):125–9.
27. Staub LJ, Biscaro RRM, Kaszubowski E, Maurici R, Chest ultrasonography for the emergency diagnosis of traumatic pneumothorax and haemothorax: A systematic review and meta-analysis, Injury. 2018 Mar;49(3):457-466. doi: 10.1016/j.injury.2018.01.033.
28. Mathis G, Metzler J, Fussenegger D, Sutterlütti G, Feurstein M, Fritzsche H. Sonographic observation of pulmonary infarction and early infarctions by pulmonary embolism. Eur Heart J. 1993;14(6):804–8.
29. Mathis G. Ultrasound in thromboembolism. Praxis (Bern 1994). 2015;104(19):1013–8.
30. Mathis G, Bitschnau R, Gehmacher O, Scheier M, Kopf A, Schwärzler B, et al. Chest ultrasound in diagnosis of pulmonary embolism in comparison to helical CT. Ultraschall der Medizin. 1999;20(2):54–9.
31. Volpicelli G, Mussa A, Garofalo G, Cardinale L, Casoli G, Perotto F, et al. Bedside lung ultrasound in the assessment of alveolar-interstitial syndrome. Am J Emerg Med. 2006;24(6):689–96.
32. Cortellaro F, Colombo S, Coen D, Duca PG. Lung ultrasound is an accurate diagnostic tool for the diagnosis of pneumonia in the emergency department. Emerg Med J. 2012;122:130–41.
33. Pagano A, Numis FG, Visone G et al. Lung ultrasound for diagnosis of pneumonia in emergency department Intern Emerg Med. 2015 Oct;10(7):851-4. doi: 10.1007/s11739-015-1297-2. Epub 2015 Sep 7.
34. Biagi C, Pierantoni L, Baldazzi M. et al. Lung ultrasound for the diagnosis of pneumonia in children with acute bronchiolitis. BMC Pulm Med. 2018 Dec 7;18(1):191. doi: 10.1186/s12890-018-0750-1.
35. Murali A, Prakash A, Dixit R. et al. Lung Ultrasound: A Complementary Imaging Tool for Chest X-Ray in the Evaluation of Dyspnea. Indian J Radiol Imaging. 2023 Jan 6;33(2):162-172. doi: 10.1055/s-0042-1759850.

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Our clinical experience with labia majoraplasty

Labia majoraplasti ile ilgili klinik deneyimimiz

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ABSTRACT

Aim: To share our experiences and surgical results of our labia majoraplasty technique and its complications

Material and Methods: Sixty-three patients who applied with complaints of sagging labium majus, decreased adipose tissue, excessive wrinkling, folding of the skin were included in the study. The appearance of the labia majora after the procedure was evaluated with photographs and patient satisfaction questionnaires including the female genital self-image scale (FGSIS).

Results: There were no life-threatening complications or serious sequelae after the procedure. So, post-op had better results for genital beauty since a higher FGSIS score indicated a more positive genital self-image and significantly correlates with a woman's sexual function, sexual behavior, and sexual and genital health-care behaviors. At 6 months of follow-up, the mean total FGSIS score was 20.4 ± 1.2 in the pre-op and 22.4 ± 1.4 in the post-op, indicating a statistically significant difference ($p = 0.026$)

Conclusion: Labia majoraplasty is a surgical procedure that contributes positively to functions, hygiene, and aesthetic appearance. There is a lack of major complications, that leave life-threatening permanent sequelae after the current surgical technique and minor complications can be controlled in a short time, all of which suggest that that the technique applied in the present study is easily applicable. At the same time, a positive significant improvement was observed in FGSIS results and was correlated with an increase in self-confidence in patients.

Keywords: Complication, FGSIS, labia majoraplasty, sexual health.

ÖZ

Giriş: *Bu çalışmanın arka planı, labia majoraplasti tekniğimiz ve komplikasyonları ile ilgili deneyimlerimizi ve cerrahi sonuçlarımızı paylaşmaktır.*

Gereç ve Yöntem: *Çalışmaya labium majus sarkması, yağ dokusunda azalma, ciltte aşırı kırışıklık ve katlanma şikayeti ile başvuran 63 hasta dahil edildi. İşlem sonrası labia majora'nın görünümü fotoğraflarla ve kadın genital benlik imajı ölçeğini (FGSIS) içeren hasta memnuniyet anketleriyle değerlendirildi.*

Bulgular: *İşlemden sonra hayatı tehdit eden herhangi bir komplikasyon veya ciddi sekeller görülmedi. Dolayısıyla, daha yüksek FGSIS puanı daha olumlu bir genital benlik imajına işaret ettiğinden ve kadının cinsel işlevi, cinsel davranışı ve cinsel davranışı ile önemli ölçüde ilişkili olduğundan, ameliyat sonrası genital güzellik açısından daha iyi sonuçlar elde edildi.*

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Cinsel ve genital sağlık bakım davranışları 6 aylık takipte ortalama toplam FGSIS skoru ameliyat öncesi 20,4 ± 1,2 ve ameliyat sonrası 22,4 ± 1,4 olup istatistiksel olarak anlamlı bir farka işaret etmektedir (p = 0,026).

Sonuç: *Labium minus estetiği, fonksiyonlara, hijyene ve estetik görünüme olumlu katkı sağlayan bir cerrahi işlemdir. Mevcut cerrahi teknikte yaşamı tehdit eden kalıcı sekel bırakan majör komplikasyonların görülmemesi ve minör komplikasyonların kısa sürede kontrol altına alınabilmesi bu çalışmada uygulanan tekniğin rahatlıkla uygulanabilir olduğunu göstermektedir. Aynı zamanda FGSIS sonuçlarında da olumlu yönde anlamlı bir iyileşme gözlendi ve hastalarda özgüven artışıyla ilişkilendirildi.*

Anahtar Sözcükler: *Cinsel sağlık, FGSIS, komplikasyon, labia majoraplasti.*

INTRODUCTION

The awareness and the demand of genital plastic surgery applications are increasing day by day. According to the International Society of Aesthetic Plastic Surgery, an increase of 73% from 2015 to 2020 of labiaplasty cases. (1). The prediction in the market of genital rejuvenation shows 34% growth until 2026 (2). The most important motivation that leads people to surgery is concerns about appearance. This is followed by physical discomfort, self-confidence problems and critical comments from sexual partners (3). The reason for the increase in interest in cosmetic gynecology is associated with the development of such procedures as waxing and other epilation methods, also the ease of access to indecent photographs and movies, the increased awareness of differences in the genital area, and the increased search for the ideal appearance (4). The available evidence suggests that as human beings, we are subconsciously obsessed with our genitals (5).

Due to aging, rapid weight gain and loss, decrease in the amount of collagen, slowing of hyaluronic acid production, acceleration of hyaluronic acid destruction, volume reduction in adipose tissue, sagging and wrinkling of the skin begin to be seen in the labium majus. With the increasing interest in genital aesthetic applications, the frequency of rejuvenation surgery for labium majus hypotrophy is increasing. The present study aims to share our experiences and surgical results of our labia majoraplasty technique.

MATERIALS and METHODS

This study was approved by the Institutional Review Board of Sancaktepe Şehit Prof. Dr. İlhan Varank Training and Research Hospital, Sancaktepe, Istanbul (Institutional Review Board Approval Number: E-46059653-050.99-243919737)

Sixty-three patients who applied to our clinic with complaints of sagging labium majus, decreased adipose tissue, excessive wrinkling and folding of the skin were included in the study. The ages of the patients ranged between 37 years and 56 years. Written informed consent was obtained from all participants. This study was conducted according to the standards of Good Clinical Practice (ICH-E6) and the principles of the Declaration of Helsinki. None of the patients had undergone labia majora surgery before. In addition, none of the patients had ever undergone autologous fat filling or hyaluronic acid filling for labia majora augmentation. And none of the participants had undergone any non-surgical cosmetic gynecology procedure such as high- intensity focused ultrasound or radiofrequency procedure for labia tissue tightening. Of the 23 patients, 5 smoked less than 10 cigarettes and 8 smoked more than 10 cigarettes per day. The remaining 10 patients were non-smokers. None of the patients had a history of chronic disease or chronic drug use that might affect wound healing. In addition, supplementary food consumption such as herbal tea and multivitamins was stopped at least 2 weeks before the procedure because it may affect wound healing. This diet was continued for one month after the procedure. The appearance of the labia majora after the procedure was evaluated with photographs and patient satisfaction questionnaires including the female genital self-image scale (FGSIS).

Surgical technique

Before the procedure, 15 cc infiltration anesthesia was applied to both labium majora. For infiltration anesthesia, 2 ml of local anesthetic (Jetocaine) containing 20 mg Lidocaine Hydrochloride and 0.0125 mg Epinephrine base

per ml and 3 ml bupivacaine were added into 25 cc ringer lactate. A total of 30 cc of infiltration anesthesia solution was obtained. After infiltration anesthesia was applied to both labium majora, the anesthesia was allowed to reach maximum efficacy for 7 minutes. Afterward, the excess skin to be removed from both labium majora was marked with a sterile skin pen in the form of a semi-balloon starting from the interlabial sulcus between the labium majus and labium minus to the lateral surface of the labium majus. Although the amount of tissue to be removed varies according to the surface area of the labium majus tissue and the level of sagging, it is planned to remove an average of 8-10 cm of skin and subcutaneous tissue vertically and 2.5-3 cm of skin and subcutaneous tissue horizontally. Then, a marked area incision was made with a No. 15 scalpel, and the skin, subcutaneous fascia, connective tissue, and adipose tissue were removed in the coagulation mode of the cautery at a power setting of 30 W for tissue excision up to a total depth of 0.5 cm. Bleeding control was performed after the procedure. The defects identified in the adipose sac were sutured separately with 3.0 Vicryl sutures to maintain the integrity of the adipose sac. After ensuring the integrity of the adipose sac, the adipose tissue that had herniated was removed with a fine cautery tip at 30 w even though it was minimal between the sutures. Plication of the fascia and connective tissue on the adipose tissue was again achieved with 3.0 vicryl sutures, leaving a 0.5 cm gap from top to bottom. After plication, 4.0 Vicryl was used to approximate the skin. A sub-cuticular suture was applied to the skin with 5.0 Rapid Vicryl (Figure-1).

After the patients were taken to their rooms after the procedure, ice application was performed as 30 min on and 30 min off for the first 4 hours. Patients were discharged from the hospital 12 hours after the procedure. Wound care training was given at the time of discharge. At discharge, the patients were prescribed metronidazole 500 gr twice a day for 1 week and paracetamol 500 mg twice a day for 3 days.

Postoperative Follow-Up

For post-procedure control, the patients were called on the 3rd, 5th, and 7th days in the early period, on the 15th and 30th days, and in the 3rd, 6 th months in the last period. The effect of surgery on the labia majora after the procedure was evaluated with photographs and patient satisfaction questionnaires including the female genital self-image scale (FGSIS). The FGSIS is a 7-item questionnaire that has respondents rate each question on a 4-point response scale (strongly disagree [1 point], disagree [2 points], agree [3 points], or strongly agree [4 points]). An individual's total score is obtained by adding the scores of individual questions and can range from 7 to 28. A higher score indicates a more positive genital self-image and significantly correlates with a women's sexual function, sexual behavior, and their sexual and genital health-care behaviors. Since patients' satisfaction was questioned in our study, there may be a possible response bias. FGSIS, an international. (6). valid and validated scoring system was used to reduce this.

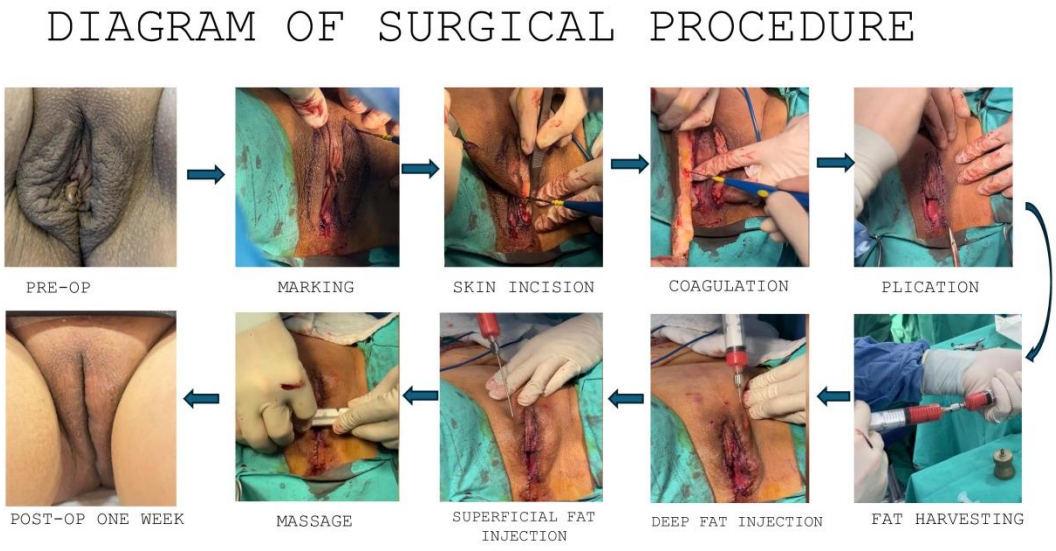

Figure-1. Diagram of the surgical procedure.

RESULTS

A total of 63 patients were included in the study. The mean age was 33.8 ± 5.2 years, and the mean body mass index (BMI) was 24.1 ± 3.6 kg/m2. There were no life-threatening complications or serious sequelae after the procedure. There were 5 complications in 4 patients. Three of the four patients were smokers, and one was a non-smoker. The first complication (patient A) was a subcuticular skin suture dehiscence that occurred in the first week. There was a 2-cm dehiscence in the left labium majus subcuticular suture. As soon as it was detected, a single suture was applied with 4.0 vicryl under local anesthesia. Another one (patient B) was a subcutaneous hematoma of about 3 cm in the midline of the right labium majus, which was detected on the 3rd day. The patient was referred to radiology and the superficial ultrasound report was consistent with a hematoma covering an area of 3.5 cm at a depth of 1 cm into the skin. The hematoma was followed up. The hematoma did not grow the next day and was absorbed and disappeared spontaneously during follow-up.

Another complication (Patient C) was a superficial skin infection of the patient, who came for the control on the 5th day, in which the incision line opened spontaneously up to 0.3 cm and purulent fluid came from the inside. The culture was taken and sent to the laboratory. In our interrogation, we realized that the patient did not use the antibiotic we prescribed. Antibiotic treatment was started quickly after the culture. Rifamycin (Rif 250) was started to be applied morning and evening to the 0.3 cm defective area from which purulent fluid was coming. The antibiotic administered according to the culture result was not changed and it was continued. The defective area was secondarily closed without the need for any surgical suturing. In another patient (Patient D), on the 5th day of control, a seroma covering an area of 1.8 cm in the left labium majus was confirmed by superficial ultrasound and a severe superficial hematoma-like ecchymosis covering the right labium majus was observed at the same time. While the ecchymosis disappeared on spontaneous follow-up with mucopolysaccharide polysulphate gel, the 1.8 cm seroma was aspirated with a superficial ultrasound and sent to the laboratory. No growth was observed. Repeat superficial ultrasound performed one week later showed no seroma. Information about the complications for patients is shown in Table-1.

The mean preoperative FGSIS scores were similar between the pre-op and post-op periods (p = 0.532). The mean total FGSIS score was 18.6 ± 1.2 in the pre-op and 18.5 ± 1.4 in the post-op . However, the mean FGSIS scores at 1, 3, and 6 months were significantly higher in the post-op than the pre-op (p = 0.03, p = 0.01, and p = 0.008, respectively). So, post-op had better results for genital appearance since a higher FGSIS score indicated a more positive genital self-image and significantly correlates with a women's sexual function, sexual behavior, and genital health-care behaviors. At 6 months of follow-up, the mean total FGSIS score was 20.4 ± 1.2 in the pre-op and 22.4 ± 1.4 in the post-op, indicating a statistically significant difference (p = 0.026) (Table-1). The mean FGSIS score results of the two groups were also shown as a line graphic

Table-1. Complications after labia majoraplasty

Patient A	Skin suture opening (Post-op 1st week) **(Smoker)**
Patient B	Hematoma (Post-op 3rd.day) **(Smoker)**
Patient C	Skin infection (Post-op 5th. day) **(Non-Smoker)**
Patient D	Seroma & ecchymosis (Post-op 5th. day) **(Smoker)**

DISCUSSION

When the literature is reviewed, there is no agreed labium majus measurement or ideal ratio that defines how the ideal labium majus should be. In the literature, the ideal vulva is described as a symmetrical and full labium majus appearance and invisible labium minora when the patient is standing. However, there is no measurement-based classification in the literature (7). As a contribution to the literature, the presentation of both the technique and the management of complications shows the importance of the present study.

The most commonly used method for labiaplasty is a surgical removal of excess skin and adipose tissue (8). Another method is autologous micro and nano fat transfer for Labia majora rejuvenation (9). However, since the sagging skin tissue is not excised in this method, the problem of sagging skin reappears when the adipose tissue transferred starts to melt. In addition, it may cause the formation of lipoma under the skin

after fat transfer (10). In the current study, the resection of excess skin tissue and correction of defects in the subcutaneous connective and adipose tissue were prioritized. In contrast to adipose tissue transfer, some of the adipose tissue with increased amount and volume was resected. Thus, no complications such as lipoma were encountered, and no extra procedure was added to the surgical process by eliminating the fat harvesting process that should be performed before fat transfer. Moreover, if we had done the fat harvesting process, we would have to use materials that would create additional costs specific to this process. However, in the current study, the surgical technique can be performed with the materials available in the operating room routinely. This can be considered as an advantage of the technique in terms of easier applicability. In addition, the absence of using extra equipment also reduces the cost of the procedure.

In a study on labia majoraplasty, suturing was performed directly on the midline of labia majora from top to bottom after excess skin was removed (11). It does not seem from an aesthetic point of view that the scar area that will remain after the procedure is present in a visible place. In the current study, the suture in the interlabial sulcus between the labium minus and labium majus is concealed so that the scar area is not visible from the outside. This can be considered as an advantage of the technique. In addition, in another study of labia majoraplasty, the scar line remained visibly in the inguinal canal in the postoperative period as the incision was made very close to the inguinal canal (12). This seems to be an advantage of the technique in the present study when compared to the other technique.

Similar to the technique in the present study, in their study, Alter et al., aimed to provide a more aesthetic wound healing and appearance by leaving the incision line in the interlabial sulcus (13). Our similarity to the study can be considered as an advantage of our study. Labia minora reduction and labia majoraplasty surgeries can be performed simultaneously (14). In the present study, it was aimed to perform isolated labia majoraplasty surgery to evaluate both the healing process and the complications that may occur only in terms of labia majoraplasty. Thus, it was possible to follow the results in terms of a single surgical technique without adding surgery.

Ostrzenski defined labiopexy as a new surgical intervention that reduces the size of the Colles' fascia and the size of the labium majus without excising the adipose tissue (15). This technique consists of the reconstruction of site-specific defects of the adipose sac and excision of the cutis just above the adipose sac tissue. The difference from this technique, which is very similar to the present study, is that routine tightening plication for the Colles' fascia was performed after labiopexy. In addition, the adipose tissues which were located between the correction suture during the correction of the adipose sac and minimally herniated were excised via cautery, so that the contour would have a flatter appearance.

In the study described by Ostrzenski, it was stated that the tension of the labium majus tissue was provided by three parameters. These are skin tightness, intact adipose sac tissue, and intact and tense Colles' fascia, respectively. In the present study, a standard plication was applied for the repair of all these adipose sacs and Colles defects and additionally, for tightening. Thus, it was intended to provide more tension in the labium majus tissue. In the traditional concept of labia majoraplasty, only excess skin and subcutaneous adipose tissue are excised followed by primary suturing (16). The present study may be seen advantageously than other techniques as it includes the correction of the integrity of the adipose sac, which was discovered in 2016, and plication of the Colles' fascia for standard tightening, apart from traditional applications. In another study, a de-epithelized fasciocutaneous flap for labia majora augmentation during thigh lift technique was applied in a surgical technique for correction and rejuvenation of labia majora appearance (17). This technique is not easy to apply and involves a different discipline, which makes it more difficult than our technique. In another study, a dermal fat graft was used in the surgical technique performed for the correction and rejuvenation of the labia majora appearance, but this technique seems to be more difficult compared to our technique because it is not easily applicable and practicable (18).

The technique in the present study is easy to apply and the tissue used as a graft does not lose volume over time since there is no graft application in our technique, all of which seem to be the advantages of the technique in terms of permanence and easy applicability.

CONCLUSION

Labia majoraplasty is a surgical procedure that contributes positively to functions, hygiene, and aesthetic appearance. There is a lack of major complications, that leave life-threatening permanent sequelae after the current surgical technique and minor complications can be controlled in a short time, all of which suggest that that the technique applied in the present study is easily applicable. With the increasing interest in cosmetic gynecology every passing day, it is foreseen that the number of labia majoraplasty surgeries and the number of techniques to be defined will increase. At the same time, a positive significant improvement was observed in FGSIS results and was correlated with an increase in self-confidence in patients Further studies with a larger number of patients should be conducted to confirm the data of the present study.

Conflict of interest: The authors declared no conflict of interest.

References

1. Isaps.org [homepage on the Internet]. International Society of Aesthetic Plastic Surgery. International Survey on Aesthetic/Cosmetic Procedures Performed in 2019. [updated 02 May 2024; cited 28 May 2024]. Available from www.isaps.org/discover/about-isaps/global-statistics.
2. Gminsights.com [homepage on the Internet]. Vaginal Rejuvenation Market Size By Treatment (Labioplasty, Vaginoplasty, Hymenoplasty, Perineoplasty, Hoodectomy, g-spot Amplification), By End-use (Hospitals, Plastic Surgery Centers), Industry Analysis Report, Regional Outlook, Application Potential, Price Trends, Competitive Market Share & Forecast, 2020 – 2026 [updated 04 May 2024; cited 28 May 2024]. Available from www.gminsights.com/industry-analysis/vaginal-rejuvenation-market.
3. Özer M, Mortimore I, Jansma EP, Mullender MG. Labiaplasty: motivation, techniques, and ethics. Nat Rev Urol 2018;15(3):175-89.
4. Alter GJ. Aesthetic labia minora and clitoral hood reduction using extended central wedge resection. Plast Reconstr Surg 2008;122(6):1780-9
5. Cassell WA. Body awareness and somatic delusions involving sexual organs. *Am J* Psychoanal 1980;40(2):125-35
6. Herbenick D, Reece M. Development and validation of the female genital self-image scale. J Sex Med 2010;7(5):1822-30
7. Clerico C, Lari A, Mojallal A, Boucher F. Anatomy and Aesthetics of the Labia Minora: The Ideal Vulva? [published correction appears in Aesthetic Plast Surg 2017;41(3):714-9.
8. Alter GJ. Pubic contouring after massive weight loss in men and women: correction of hidden penis, mons ptosis, and labia majora enlargement. Plast Reconstr Surg 2012;130(4):936-47
9. Menkes S, SidAhmed-Mezi M, Meningaud JP, Benadiba L, Magalon G, Hersant B. Microfat and Nanofat Grafting in Genital Rejuvenation. Aesthet Surg J 2021;41(9):1060-7.
10. Jabbour S, Kechichian E, Hersant B, Levan P, El Hachem L, Noel W, Nasr M. Labia Majora Augmentation: A Systematic Review of the Literature. Aesthet Surg J 2017;37(10):1157-64.
11. Felicio Yde A. Labial surgery. Aesthet Surg J 2007;27(3):322-8.
12. Mottura AA. Labia majora hypertrophy. Aesthetic Plast Surg 2009;33(6):859-63
13. Alter GJ. Management of the mons pubis and labia majora in the massive weight loss patient. Aesthet Surg J 2009;29(5):432-42.
14. Miklos JR, Moore RD. Simultaneous labia minora and majora reduction: a case report. J Minim Invasive Gynecol 2011;18(3):378-80.
15. Ostrzenski A. Labiopexy and labioplasty for labium majus rejuvenation in light of a newly discovered anatomic structure. Aesthetic Plast Surg 2014;38(3):554-60.
16. Di Saia JP. An unusual staged labial rejuvenation. J Sex Med 2008;5(5):1263-7.
17. El Danaf AAH. Deepithelized fasciocutaneous flap for labia majora augmentation during thigh lift. Eur J Plast Surg 2010;33(6):373-6.
18. Salgado CJ, Tang JC, Desrosiers AE. Use of dermal fat graft for augmentation of the labia majora. J Plast Reconstr Aesthet Surg 2012;65(2):267-70.

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Evaluation of lipid profile and statin therapy in patients with atrial fibrillation

Atriyal fibrilasyonlu hastalarda lipid profilinin ve statin tedavisinin değerlendirilmesi

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ABSTRACT

Aim: Dyslipidemia is a modifiable risk factor of atrial fibrillation (AF). However, the majority of patients either do not receive low-density lipoprotein cholesterol (LDL-C) lowering treatment or do not meet their LDL-C and non-high-density lipoprotein cholesterol (non-HDL-C) goal. We aimed to search whether patients with AF are being treated for dyslipidemia and/or are at target LDL-C and non-HDL-C levels if treated.

Materials and Methods: This cross-sectional analysis includes 675 AF patients and was performed between 20 May 2023 and 25 November 2023, in cardiology outpatient clinics of a tertiary hospital. The demographic and clinical features of the patients were recorded. Systemic coronary risk estimation-2 (SCORE2) and old person version algorithms were used for cardiovascular disease (CVD) risk estimation. Primary prevention (PP) group involved patients with low-to-moderate, high and very high CVD risk without established atherosclerotic cardiovascular disease (ASCVD) and secondary prevention (SP) group consisted of patients with established ASCVD.

Results: The mean age of the participants was 71.98± 9.01 and 54.5% (n=368) of patients were females. 207 (30.7%) of patients were paroxysmal AF, and 468 (69.3%) were permanent AF. Prevalence of dyslipidemia and hypertriglyceridemia were 364 (53.9%) and 248 (36.7%) respectively. 9 (1.3%) and 152 (22.5%) of patients were on fibrate and statin treatment respectively. Mean LDL-C and non-HDL-C were 107.81±35.97 and 135.42±41.19 and their target attainment rates were 62 (9.2%) and 107 (15.9%), respectively.

Conclusion: Control of dyslipidemia in patients with atrial fibrillation was severely poor and the most common cause was physician inertia.

Keywords: Atrial fibrillation, dyslipidemia, low-density lipoprotein cholesterol, statin therapy.

ÖZ

Amaç: *Dislipidemi, atriyal fibrilasyonun (AF) değiştirilebilir bir risk faktörüdür. Ancak hastaların büyük çoğunluğu ya düşük yoğunluklu lipoprotein kolesterol (LDL-C) düşürücü tedavi almıyor ya da LDL-C ve yüksek yoğunluklu olmayan lipoprotein kolesterol (non-HDL-C) hedeflerine ulaşamıyor. AF'li hastaların dislipidemi için tedavi edilip edilmediğini ve/veya tedavi edilirlerse hedef LDL-C ve non-HDL-C düzeylerinde olup olmadıklarını araştırmayı amaçladık.*

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Gereç ve Yöntem: *Bu kesitsel analiz 675 AF hastasını içermektedir ve 20 Mayıs 2023 ile 25 Kasım 2023 tarihleri arasında üçüncü basamak bir hastanenin kardiyoloji polikliniklerinde gerçekleştirildi. Hastaların demografik ve klinik özellikleri kaydedildi. Kardiyovasküler hastalık (CVD) risk tahmini için sistemik koroner risk tahmini-2 (SCORE2) ve yaşlı kişi versiyonu algoritmaları kullanıldı. Birincil koruma (PP) grubu, belirlenmiş aterosklerotik kardiyovasküler hastalığı (ASCVD) olmayan düşük-orta, yüksek ve çok yüksek CVD riski olan hastaları içermektedir ve ikincil önleme (SP) grubu, belirlenmiş ASCVD'si olan hastalardan oluşmaktadır.*

Bulgular: *Katılımcıların yaş ortalaması 71,98± 9,01 olup hastaların %54,5'i (n=368) kadındı. Hastaların 207'si (%30,7) paroksismal AF, 468'i (%69,3) kalıcı AF idi. Dislipidemi ve hipertrigliseridemi prevalansı sırasıyla 364 (%53,9) ve 248 (%36,7) idi. Hastaların 9'u (%1,3) fibrat, 152'si (%22,5) ise statin tedavisi görüyordu. Ortalama LDL-C ve non-HDL-C sırasıyla 107,81±35,97 ve 135,42±41,19 olup, hedeflenen oranlara ulaşma oranları sırasıyla 62 (%9,2) ve 107 (%15,9) idi.*

Sonuç: *Atriyal fibrilasyonu olan hastalarda dislipideminin kontrolü oldukça zayıftı ve en yaygın neden doktor ihmaliydi.*

Anahtar Sözcükler: *Atriyal fibrilasyon, dislipidemi, düşük yoğunluklu lipoprotein kolesterol, statin tedavisi.*

INTRODUCTION

Atrial fibrillation is the most common cardiac arrhythmia disease, affecting more than 33 million people worldwide and is a significant cause of morbidity and mortality as it increases the likelihood of stroke and heart failure (1).

AF is a complex disease that develops as a result of the interaction of genetic and environmental factors. Several risk factors and comorbidities have been identified that can predispose to the development and progression of AF. These risk factors can be classified into non-modifiable (age and genetics), partially modifiable (coronary artery disease, heart failure, valvular heart disease, and chronic obstructive pulmonary disease), and modifiable (hypertension, diabetes, obesity, obstructive sleep apnea, alcohol, dyslipidemia, physical activity, and smoking) (1).

Although the clinical significance and pathophysiological mechanism of lipid level is controversial in the context of AF development. Dyslipidemia contributes to the development and progression of AF directly through the left atrial remodeling, and indirectly through the development of ASCVD (1-3). In addition, dyslipidemia is a clinical risk factor for stroke in patients with AF (1, 3).

Lipid-lowering therapy, especially statins, has been shown to have beneficial effects on both AF and ASCVD (3,4). Statins can reduce the incidence and recurrence of AF by improving the lipid profile, stabilizing the atrial membrane potential, and exerting pleiotropic effects, such as anti-inflammatory, antioxidant, antithrombotic, and anti-fibrotic actions. Statins may also prevent and treat ASCVD by lowering LDL-C and non-HDL cholesterol levels, lipid-lowering therapy's primary and secondary targets (3-7).

However, despite the strong evidence and clear recommendations, the use and effectiveness of lipid-lowering therapy in AF patients are suboptimal. Many AF patients do not receive adequate lipid-lowering treatment or do not achieve their lipid goals. The reasons for this gap are multifactorial, including patient-related factors (such as low awareness, poor adherence, and intolerance), physician-related factors (such as low awareness and inertia), and health system-related factors (such as lack of guidelines, resources, and incentives) (8,9).

Our main aim in this assay is to try to raise awareness about the management of dyslipidemia, which is an important part of the multidisciplinary approach in AF patients. In this work, we evaluate the lipid profile and statin therapy in patients with AF, using real-life data from a tertiary hospital in Türkiye. We assessed the prevalence of dyslipidemia and hypertriglyceridemia, the rate of use and adherence of lipid-lowering therapy, the achievement of lipid goals, and the factors associated with these outcomes. Furthermore, we discuss the implications and limitations of our findings and suggest possible ways to improve the management of dyslipidemia in AF patients.

MATERIALS and METHODS

The study was approved by the local Research Ethics Committee (P202300024/19.05.2023) and conducted by the Declaration of Helsinki. Written consent was obtained from all subjects.

The present study is a cross-sectional analysis of 675 consecutive AF patients who were admitted to a cardiology outpatient clinic of a tertiary hospital between 20 May 2023 and 25 November 2023. Inclusion criteria were having a diagnosis of AF confirmed by electrocardiogram or Holter monitoring and having sufficient data to calculate a 10-year ASCVD risk score. Exclusion criteria included being under 40 years of age, and having

contraindications to statin therapy such as liver failure or cirrhosis.

AF type was classified as paroxysmal or permanent according to the relevant guideline (1). The comorbidities, such as hypertension, diabetes, coronary artery disease, and stroke, were defined according to the standard criteria. The smoking status was self-reported by the patients. Medications taken by patients including statin and oral anticoagulant therapy was also recorded.

The blood samples were taken from the patients after overnight fasting and analyzed for fasting blood glucose, HbA1c, total cholesterol, HDL-C, triglycerides, and creatinine levels using standardized biochemical methods. The LDL-C level was estimated using the Friedewald formula and the non-HDL-C level was calculated by subtracting the HDL-C level from the total cholesterol level. The Cockcroft-Gault equation was used to estimate the glomerular filtration rate.

Dyslipidemia was defined as having a fasting total cholesterol level> 240 mg/dL, or an LDL-C level> 160 mg/dL, or taking lipid-lowering drugs. Hypertriglyceridemia was defined as having a serum triglyceride level ≥ 150 mg/dL or taking lipid-lowering drugs. The lipid-lowering therapy, including statins and fibrates, was recorded. The intensity of statin therapy was classified as moderate or high according to the relevant guidelines (2, 3). No patient was taking ezetimibe or proprotein convertase subtilisin/kexin type 9 inhibitors.

We used the Systematic Coronary Risk Estimation (SCORE) charts to estimate the 10-year risk of ASCVD in patients aged 40-69 years and the SCORE2-OP charts to estimate the risk in patients aged ≥70 years, according to the relevant guidelines (3). We used the high-risk countries versions of the charts, as Türkiye is considered a high-risk country for ASCVD (10, 11).

We divided the study sample into two groups: primary prevention (PP) group and secondary prevention (SP) group. The PP group included patients with low-to-moderate, high, and very high CVD risk without established ASCVD, and the SP group included patients with established ASCVD. The CVD risk categories were defined according to relevant guidelines (3) as follows:

Very high-risk: Patients with established CAD, PAD, stroke, or severe chronic kidney disease (eGFR<30mL/min/1.73m2), or diabetic patients with eGFR<45mL/min/1.73m2, or apparently healthy participants <50 years, 50-69 years, and ≥70 years of age with an estimated ASCVD risk score of ≥7.5%, ≥10%, and ≥15%, respectively.

High-risk: Patients with long-standing (>10 years) DM, or moderate chronic kidney disease (eGFR 45-59 mL/min/1.73m2), or apparently healthy participants <50 years, 50-69 years, and ≥70 years of age with an estimated ASCVD risk score of 2.5 to <7.5%, 5 to <10% and 7.5 to <15%, respectively.

Low-to-moderate risk: Apparently healthy participants <50 years, 50-69 years, and ≥70 years of age with an estimated ASCVD risk score of <2.5%, <5% and <7.5%, respectively.

The target LDL-C levels were determined as <100 mg/dL, <70 mg/dL, and <55 mg/dL, and the corresponding non-HDL-C levels were determined as <131 mg/dL, <100 mg/dL, and <85 mg/dL for low-to-moderate, high, and very high CVD risk categories, respectively.

Patients were questioned whether their cholesterol levels were high, whether they knew their cholesterol levels, whether they took lipid-lowering medication, and whether they thought taking long-term cholesterol medication caused diabetes, dementia or cancer and why they not receiving cholesterol medication.

RESULTS

We included 675 AF patients (207 (30.7%) with paroxysmal AF and 468 (69.3%) with permanent AF, mean age 71.98 ± 9.01 years, 54.5% (n=368) females) in the study. Of these, 457 (67.7%) were in the PP group and 218 (32.3%) were in the SP group. According to the 2021 ESC-CVD prevention guideline, 46 (6.8%), 238 (35.3%), and 173 (25.6%) of the PP group had low-to-moderate, high, and very high CVD risk, respectively.

Table-1 shows the demographic and clinical characteristics and medication data of the study population. The number of women was significantly higher in each category of the PP group and lower in the SP group. The median age in the low-to-moderate CVD risk category was significantly lower than the other categories. There was no significant difference between the groups in terms of hypertension, hypothyroidism, COPD, smoking, and AF type (paroxysmal vs. permanent). As expected, ASCVD such as CAD, PAD, and stroke was only present in the SP group. Dyslipidemia was observed in 364 (53.9%) of the patients, and it was significantly more common in the SP group than the PP group. The only medications that showed a significant difference between the groups were antidiabetic drugs, statins, and ACE-I/ARBs. 152 (22.5%) patients were on statin treatment, of whom 119 (78.3%) were on moderate-intensity statin and 33 (21.7%) on high-intensity statin. Only 9 (1.3%) patients were on fibrate treatment. No patient was on ezetimibe or PCSK9 inhibitor or a combination of these molecules with statins. The post hoc analysis of the intergroup significant variables is given in detail in Supplementary Table-1.

Table-1. Data on demographic and clinical characteristics and medications of patients.

Variables	Total study population	Primary prevention			Secondary prevention	P
		Low-to-moderate CVD risk n:46 (6.8%)	High CVD risk n:238 (35.3%)	Very high CVD risk without established ASCVD n:173 (25.6)	Very high CVD risk with established ASCVD n:218 (32.3)	
Gender (F) n, (%)	368 (54.5)	33 (71.7)	146 (61.3)	89 (51.4)	100 (45.9)	0.001
Age (years), ±SD	71.98±9.01	61.59±6.32	73.74±8.91	72.06±7.87	72.20±9.05	<0.001
AF type						
• Paroxysmal AF	207 (30.7)	11 (23.9)	77 (32.4)	57 (35.8)	92 (26.1)	0.134
• Permanent AF	468 (69.3)	35 (76.1)	161 (67.6)	161 (64.2)	111 (73.9)	
CAD n, (%)	145 (21.5)	0 (0)	0 (0)	0 (0)	145 (66.5)	<0.001
PAD n, (%)	13 (1.9)	0 (0)	0 (0)	0 (0)	13 (6)	<0.001
Stroke n, (%)	95 (14.1)	0 (0)	0 (0)	0 (0)	95 (43.6)	<0.001
HT n, (%)	522 (77.3)	36 (5.3)	184 (27.3)	130 (19.3)	172 (25.5)	0.845
DM n, (%)	311 (46.1)	38 (82.6)	129 (54.2)	43 (24.9)	101 (46.3)	<0.001
Hypothyroidism n, (%)	62 (9.2)	8 (17.4)	22 (9.2)	12 (6.9)	20 (9.2)	0.190
COPD n, (%)	41 (6.1)	1 (2.2)	19 (8)	8 (4.6)	13 (6)	0.335
Dyslipidemia n, (%)	364 (53.9)	26 (56.5)	116 (48.7)	79 (45.7)	143 (65.6)	<0.001
Hypertriglyceridemia	248 (36.7)	24 (52.2)	84 (35.3)	57 (32.9)	83 (38.1)	0.104
Smoking n, (%)	236 (35)	16 (34.8)	80 (33.6)	68 (39.3)	72 (33)	0.575
Beta-blockers n, (%)	479 (71)	33 (71.7)	164 (68.9)	116 (67.1)	166 (76.1)	0.201
OADs n, (%)	226 (33.5)	32 (69.6)	104 (43.7)	0 (0)	90 (41.3)	<0.001
Insulin n, (%)	54 (8)	4 (8.7)	24 (10.1)	0 (0)	26 (11.9)	<0.001
ACEI/ARB n, (%)	437 (64.7)	27 (65.2)	154 (64.7)	97 (56.1)	156 (71.6)	0.017
CCB n, (%)	300 (44.4)	16 (34.8)	105 (44.1)	72 (41.6)	107 (49.1)	0.240
Digoxin n, (%)	126 (18.7)	14 (30.4)	46 (19.3)	30 (17.3)	36 (16.5)	0.163
Amiodarone n, (%)	38 (5.6)	6 (13)	14 (5.9)	7 (4)	11 (5)	0.125
Fibrate n, (%)	9 (1.3)	1 (2.2)	5 (2.1)	0 (0)	3 (1.4)	0.301
Statins n, (%)	152 (22.5)	4 (8.7)	42 (17.6)	20 (11.6)	86 (39.4)	<0.001
Statins intensity						
• High intensity statins n, (%)	33 (4.9)	2 (50)	5 (11.9)	7 (35)	19 (22.1)	0.096
• Moderate intensity statins n, (%)	119 (17.6)	2 (50)	37 (88.1)	13 (65)	67 (77.9)	
OACs						
• NOACs n, (%)	581 (86.1)	39 (84.8)	210 (88.2)	150 (86.7)	182 (83.5)	0.519
• Warfarin n, (%)	94 (13.9)	7 (15.2)	28 (11.8)	23 (13.3)	36 (16.5)	

ACEI: angiotensin-converting enzyme inhibitor, AF: atrial fibrillation, ARB: angiotensin receptor blocker, ASCVD: atherosclerotic cardiovascular disease, CAD: coronary artery disease, CCB: calcium channel blocker, COPD: chronic obstructive pulmonary disease, CVD: cardiovascular disease, DM: diabetes mellitus, F: female, HT: hypertension, NOAC: non-vitamin K anticoagulant, OAD: oral antidiabetic, PAD: peripheral artery disease, SD: standard deviation.

Table-2 shows the physical examination and laboratory data of the patients according to the ASCVD risk categories. There was no significant difference in BMI between the groups, but there was a significant difference in height and weight between the groups. SBP was not significantly different between the groups, but DBP and HR were significantly different between the groups. FG and HbA1c levels were also significantly different between the groups. Except for triglycerides, the lipid profile was significantly different between the groups. The lipid parameters were generally above the desired limits; the mean LDL-C and non-HDL-C levels were higher than the target levels recommended by the 2021 ESC-CVD prevention guidelines. Moreover, the rates of achieving both LDL-C and non-HDL-C targets were very low in all categories. Only 54 (8%) of the patients had the guideline-recommended target LDL-C level. In contrast, 621 (92%) patients were out of target LDL-C. The patients without established ASCVD

but with very high CVD risk had the highest LDL-C level and the highest percentage of LDL-C out of the target. The mean GFR was significantly different between the groups, as well. The mean CRP level in the SP group was not significantly different from the PP group with very high CVD risk, but it was significantly higher than the PP group with low-to-moderate and high CVD risk. The detailed intergroup significant differences are presented in Supplementary Table-1.

Table-3 shows the lipid profile in patients on statin treatment. The mean LDL-C and non-HDL-C levels of these patients were 92.94±39.93 mg/dL and 121.59 ± 46.33 mg/dL, respectively. In this group, only 25 (16.4%) and 42 (27.65%) patients achieved their target LDL-C and non-HDL-C levels, respectively, according to the guideline. As seen, patients in all categories were inadequately protected in terms of high LDL-C and non-HDL-C levels.

Figure-1 illustrates the reasons for patients not receiving statin treatment. 77.5% (n=523) of patients were not on statin treatment. The most common reason was physician inertia, which accounted for 56.79% of cases (n=297). The second most common reason was the failure to meet the conditions for the statins to be reimbursed by the social security system, which affected 38.62% of cases (n=202). The least common reason for discontinuing treatment was patient-related factors in 4.59% of cases (n=24), of these 24 cases, 9 discontinued statin therapy due to misinformation in the media, 5 due to side effects, 6 due to the advice of non-cardiologists, and 4 due to polypharmacy.

Table-2. Physical examination and laboratory data of patients with AF according to atherosclerotic cardiovascular disease risk categories.

Variables	Total study population	Primary prevention		Secondary prevention	P
		Low-to-moderate CVD risk n:46 (6.8%)	High CVD risk n:238 (35.3%)	Very high CVD risk without established ASCVD n:173 (25.6)	Very high CVD risk with established ASCVD n:218 (32.3)	
Hight (cm)	163 (12)	160 (10)	161.50 (12.25)	165 (12)	165 (12)	0.009
Weight (kg)	80 (18)	75 (19.75)	78 (19.25)	82 (15)	80 (18)	0.030
BMI (kg/m^2)	29.75 (6.71)	29.87 (8.31)	29.36 (6.48)	30.06 (7.58)	29.76 (6.18)	0.835
SBP (mmHg)	135 (25)	130 (22.50)	130 (20.50)	140 (29.50)	140 (20.50)	0.244
DBP (mmHg)	80 (20)	75 (21.25)	80 (15)	80 (20)	80 (15)	0.019
HR (beats/minute)	81±16	84.11±15.67	82.74±17.93	79.09±16.41	79.40±14.76	0.035
FG (mg/dL)	109 (37)	129 (56)	112 (39)	101 (19)	112.50 (52.50)	<0.001
HBA1c (%)	6 (1.07)	6.73 (1.79)	6.2 (1.26)	5.74 (0.40)	6.13 (1.52)	<0.001
TC (mg/dL), ±SD	183±42.55	187.07±37.42	185.21±40.12	193.17±42.73	173.04±44.02	<0.001
HDL-C (mg/dL)	46 (17)	45 (28.3)	46 (10.1)	49 (3.5)	45 (8.7)	0.019
LDL-C (mg/dL)	107.81±35.97	112.35±29.42	109.48±33.18	117.12±37.54	97.64±36.59	<0.001
LDL-C goal attainment	62 (9.2)	13 (1.3)	24 (3.6)	6 (0.9)	19 (2.8)	<0.001
Non-HDL-C (mg/dL)	135.42±41.19	142.59±32.68	137.09±38.37	143.09±43.24	126.02±42.53	<0.001
Non-HDLC goal attainment	107 (15.9)	16 (34.8)	45 (18.9)	12 (6.9)	34 (15.6)	<0.001
Both LDL-C and non-HDL-C goal attainment	52 (7.7)	10 (21.7)	23 (9.7)	4 (2.3)	15 (6.9)	<0.001
Triglyceride (mg/dL)	125 (87)	150.50 (95)	124.50 (89)	124 (81.50)	123.50 (85.50)	0.101
CRP (mg/dL)	7.54±6.51	5.39±3.09	6.66±6.15	8.13±6.40	8.47±7.28	0.002
Creatinine (mg/dL)	0.94 (0.39)	0.92 (1.22)	0.94 (0.39)	0.92 (0.53)	0.95 (0.32)	0.848
GFR (ml/minute)	84.06±33.74	99.79±36.09	80.07±31.69	84.42±36.82	84.81±32.01	0.004

ASCVD: atherosclerotic cardiovascular disease, BMI: body mass index, CRP: C-reactive protein, CVD: cardiovascular disease, DBP: diastolic blood pressure, FG: fasting glucose, GFR: glomerular filtration rate, HBA1c: hemoglobin-A1c, HDL-C: high-density lipoprotein cholesterol, HR: heart rate, LDL-C: low-density lipoprotein cholesterol, SBP: systolic blood pressure, TC: total cholesterol.

Table-3. Distribution of the lipid profile in patients using statin treatment.

Variables	Total	Primary prevention		Secondary prevention		P
		Low-to-moderate CVD risk n:4 (%)	High CVD risk n:42 (%)	Very high CVD risk without established ASCVD n:20 (%)	Very high CVD risk with established ASCVD n:86 (%)	
TC (mg/dL), ±SD	168.40±80.19	145.50±63.29	180.51±50.17	180.20±56.24	160.80±43.83	0.106
HDL-C (mg/dL)	45 (19)	48.50 (20.50)	43 (24.75)	45.50 (17.50)	45 (17.50)	0.891
LDL-C (mg/dL)	92.94±39.93	85.5±14.20	102.48±39.62	103.85±49.51	86.14±37.24	0.083
LDL-C goal attainment n, (%)	25 (16.4)	3 (75)	8 (19)	2 (10)	12 (14)	0.011
Non-HDL-C (mg/dL)	121.59±46.33	117.25±30.67	132.08±46.70	133.85±57.65	113.82±42.82	0.180
Non-HDLC goal attainment n, (%)	42 (27.6)	3 (75)	12 (28.6)	5 (25)	22 (25.6)	0.190
Both LDL-C and non-HDL-C goal attainment	21 (13.8)	2 (50)	8 (19)	2 (10)	9 (10.5)	0.093
Triglyceride (mg/dL)	123.5 (90.75)	166 (107.25)	130 (85.75)	126 (99)	115 (94.50)	0.437

ASCVD: atherosclerotic cardiovascular disease, CVD: cardiovascular disease, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, TC: total cholesterol.

Supplementary Table-1. Post hoc analysis of significantly different variables among groups.

Age	Low-to-moderate CVD risk - High CVD risk	<0.001
	Low-to-moderate CVD risk - Very high CVD risk without established ASCVD	<0.001
	Low-to-moderate CVD risk - Very high CVD risk with established ASCVD	<0.001
Heart rate	High CVD risk - Very high CVD risk with established ASCVD	0.030
	High CVD risk - Very high CVD risk without established ASCVD	0.026
Hight	High CVD risk - Very high CVD risk without established ASCVD	0.033
Weight	High CVD risk- Very high CVD risk without established ASCVD	0.023
Diastolic blood pressure	High CVD risk - Very high CVD risk without established ASCVD	0.043
Glomerular filtration rate	Low-to-moderate CVD risk - Very high CVD risk with established ASCVD	0.036
	Low-to-moderate CVD risk - Very high CVD risk without established ASCVD	0.035
	Low-to-moderate CVD risk – High CVD risk	0.002
C-reactive protein	Very high CVD risk with established ASCVD - Low-to-moderate CVD risk	0.020
	Very high CVD risk with established ASCVD - High CVD risk	0.018
High-density lipoprotein cholesterol	Very high CVD risk with established ASCVD - Very high CVD risk without established ASCVD	0.018
Fasting glucose	Very high CVD risk without established ASCVD - Very high CVD risk with established ASCVD	<0.001
	Very high CVD risk without established ASCVD – High CVD risk	<0.001
	Very high CVD risk without established ASCVD – Low-to-moderate CVD risk	<0.001
Hemoglobin-A1c	Very high CVD risk without established ASCVD - Very high CVD risk with established ASCVD	<0.001
	Very high CVD risk without established ASCVD – High CVD risk	<0.001
	Very high CVD risk without established ASCVD -Low-to-moderate CVD risk	<0.001
	Very high CVD risk with established ASCVD – Low-to-moderate CVD risk	0.042
	High CVD risk -low-to-moderate CVD risk	0.043
Total cholesterol	Very high CVD risk with established ASCVD - Very high CVD risk without established ASCVD	<0.001
	Very high CVD risk with established ASCVD - High CVD risk	0.012
Low-density lipoprotein cholesterol	Very high CVD risk with established ASCVD - Very high CVD risk without established ASCVD	<0.001
	Very high CVD risk with established ASCVD - High CVD risk	0.002
Non-high-density lipoprotein cholesterol	Very high CVD risk with established ASCVD - Very high CVD risk without established ASCVD	<0.001
	Very high CVD risk with established ASCVD - High CVD risk	0.023

ASCVD: atherosclerotic cardiovascular disease, CVD: cardiovascular disease.

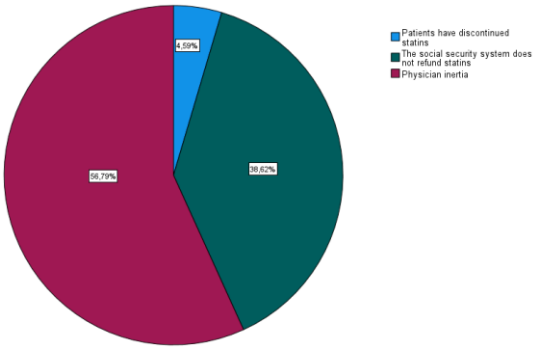

Figure-1. The reasons for patients not receiving statin treatment.

DISCUSSION

Our main aim in this study is to try to raise awareness about the management of dyslipidemia, which is an important part of the multidisciplinary approach in AF patients**.** We evaluate the lipid profile and statin therapy in patients with AF, using real-life data from a tertiary hospital. We found that dyslipidemia and hypertriglyceridemia were common in AF patients, but the use and effectiveness of lipid-lowering therapy were very low. Only 23.6% of the patients were on lipid-lowering therapy, mostly statins, and only 9.2% and 15.9% of the patients achieved their target LDL-C and non-HDL-C levels, respectively. The main reason for not receiving statins was physician inertia.

Dyslipidemias, primarily hypercholesterolemia and hypertriglyceridemia are independent and strong predictors of cardiovascular events. Additionally common in the general population and AF patients in Türkiye (12-14). The prevalence of hypercholesterolemia defined as a LDL cholesterol >130 and/or ≥130 mg/dL, is reported as 29.1% in the general population, 30.2% in females, and 27.8% in males. The prevalence of hypertriglyceridemia (>150 mg/dL) is reported as 36.5% in general, 32.0% in females and 41.3% in males (12). Our results are in consistence with previously published works that have reported a high prevalence of dyslipidemia and hypertriglyceridemia in AF patients, ranging from 30% to 50% (12, 13). Considering the results of the present analysis and a recent meta-analysis on the prevalence of dyslipidemia and lipid values in Türkiye (12), it appears that the frequency of dyslipidemia in AF patients is more common than in the general population.

Dyslipidemia is a modifiable risk factor for AF, as it can induce atrial remodeling and inflammation, and increase the risk of stroke and mortality (15). However, the relationship of lipid levels with the risk of AF development s controversial, some papers have suggested a paradoxical inverse relationship between cholesterol levels and AF incidence (13, 14, 16). This may be due to confounding factors, such as age, sex, ethnicity, and metabolic profile, and does not imply a causal relationship (15). The management of dyslipidemia is important for primary and secondary prevention of complications in AF patients.

Lipid-lowering therapy, especially statins, has been shown to have beneficial effects on both AF and ASCVD (1-3). Statins can reduce the occurrence and recurrence of AF by improving the lipid profile, stabilizing the atrial membrane potential, and exerting pleiotropic effects such as anti-inflammatory, antioxidant, antithrombotic, and anti-fibrotic actions (1-7,17). Statins are highly effective in preventing and treating ASCVD by significantly reducing LDL-C and non-HDL-C levels, which are lipid-lowering therapy's primary and secondary targets, respectively (2,3). However, despite the strong evidence and clear recommendations, in the present study the use and effectiveness of lipid-lowering therapy in AF patients are suboptimal, which is consistent with other studies that have reported low rates of statin prescription and target attainment in AF patients (18). LDL targets and risk stratification schemes in AF patients are similar to those in the general population (1, 2). The 2021 ESC Guidelines on cardiovascular disease prevention in clinical practice recommend statin therapy for AF patients with high or very high CVD risk and suggest target LDL-C levels of <70 mg/dL and <55 mg/dL, respectively (2, 3). However, in our study, only 22.5% of the patients were on statin therapy, and only 8% of the patients achieved the guideline-recommended target LDL-C level. Moreover, no patient was on ezetimibe or PCSK9 inhibitor, which are effective adjunctive therapies for lowering LDL-C levels (3).

The underutilization and inefficacy of lipid-lowering therapy in AF patients have various factors, such as patient, physician, and health system factors (8, 9). In our study, the most common reason for not receiving statins was physician inertia, which may reflect a lack of awareness, reluctance to prescribe statins for AF patients. This may be due to the focus on anticoagulant therapy in AF patients, while little attention is paid to the multidisciplinary approach and treatment of comorbidities. Therefore, more education and guidance are needed for physicians who manage AF patients, to emphasize the importance and benefits of lipid-

lowering therapy for AF prevention and treatment.

Our study is one of the few studies that have evaluated the lipid profile and statin therapy in AF patients in Türkiye. Our findings are consistent with the AFTER study, which was a multicenter study that included 2242 AF patients with a mean age of 66.8 ± 12.3 years, female predominance, and permanent AF (18). According to the AFTER study, the average levels of TC, TG, HDL-C, and LDL-C were 177 ± 43, 136 ± 80, 42 ± 13, and 111 ± 34 mg/dL, respectively. The study also found that only 14.2% of patients received statin therapy (18). The most common comorbidity was hypertension. In our study, the lipid profile and the comorbidity pattern of our sample were very similar to the AFTER study, except that the statin usage rate was slightly higher in our study (22.5% vs. 14.2%).

However, our study also showed that the lipid control was poor in AF patients, especially in terms of LDL-C and non-HDL-C levels. According to clinical practice guidelines for preventing cardiovascular disease, only 9.2% and 15.9% of the patients achieved their target LDL-C and non-HDL-C levels (3). Moreover, the rate of reaching both LDL-C and non-HDL-C targets in the same person was even lower. These findings are in contrast with other studies that have reported higher rates of statin prescription and target attainment in AF patients in different geographical regions (19). The difference in the statin use and effectiveness may depend on various factors, such as income level, health care system, guideline adherence, and sample size.

Physician inertia is defined as the physicians' failure to initiate the treatment or intensify the dose or change the medication despite a higher level of a clinical parameter than levels established by guidelines. The main reason for physicians' inertia may be, inability to obtain adequate anamnesis and spare enough time to implement guideline recommendations for each patient, due to time constraints and large number of patients in daily outpatient clinics. Additionally, concerns about the negative side effects of statins and the thought that patients may be using statins may also cause physicians' inertia.

Reasons for patients to quit statins may include factors such as drug-related side effects, fear of adverse effect, psychological diseases, misinformation learned from the media, forgetting to take their medication, polypharmacy, problems in obtaining the drug, reaching the LDL-C target and resting the liver. It is important for physicians to provide adequate information to patients about the complications caused by high cholesterol, aiming for regular use of statins. Patients should know that the benefits of statins outweigh their potential side effects.

Turkey's social security institution reimburses statins in the following cases: in cases where the LDL level is above 190 mg/dl, or the LDL level is above 160 mg/dl with two additional risk factor from: hypertension, a family history of premature cardiovascular disease, and being 65 years of age or older, or in cases where the LDL level is above 130 mg/dl with there are three additional factors which are mentioned earlier, or in cases where the LDL level is above 70 mg/dl; Those with diabetes mellitus, acute coronary syndrome, previous stroke, coronary artery disease, peripheral artery disease, abdominal aortic aneurysm and carotid artery disease. The mentioned conditions do not fully meet the statin recommendations according to the SCORE category proposed by the ESC. As a possibility of improvement in statin provision, the social insurance institution in Turkey may implement the ESC recommendations. In addition, statin treatment could be initiated by primary care physicians.

Relatively large sample size, the use of real-life data from a tertiary hospital, and the use of the SCORE charts to estimate the CVD risk and categorize the patients according to the latest guidelines were the strengths of the present work. However, our study has several limitations; 1- The cross-sectional design that avoids causal inference. 2- The lack of the duration and adherence to lipid-lowering therapy. 3- The use of a single hospital records and self-reports, which can introduce measurement errors and bias.

CONCLUSION

Our study revealed that dyslipidemia and hypertriglyceridemia were common in AF patients, but the use and effectiveness of lipid-lowering therapy were very low. The main reason for not receiving statins was physician inertia. These findings suggest that there is a need for more education and guidance for physicians who manage AF patients, to improve the management of dyslipidemia and prevent AF and its complications.

Conflict of interest: The authors declared no conflict of interest.

References

1. 2020 ESC Guidelines for the diagnosis and management of atrial fibrillation, European Heart Journal (2020) 00, 1_126 doi:10.1093/eurheartj/ehaa612.
2. 2019 ESC-EAS Guidelines for the management of dyslipidemia. European Heart Journal (2019)00-,1-78, doi: :10.1093/eurheartj/ehz455
3. 2021 ESC Guidelines on cardiovascular disease prevention in clinical practice. European Heart Journal (2021) 00, 1_111, doi:10.1093/eurheartj/ehab484
4. Flint AC, Conell C, Ren X et al. Statin adherence is associated with reduced recurrent stroke risk in patients with or without atrial fibrillation. Stroke. 2017;48:1788-94.
5. Li ZZ, Du X, Guo XY et al. Association Between Blood Lipid Profiles and Atrial Fibrillation: A Case-Control Study. Med Sci Monit. 20[18] Jun 9;24:3903-3908. doi: 10.12659/MSM.907580. PMID: 29885277; PMCID: PMC6024732
6. Fauchier L, Pierre B, de Labriolle A, Grimard C, Zannad N, Babuty D. Antiarrhythmic effect of statin therapy and atrial fibrillation. A meta-analysis of randomized controlled trials.J Am Coll Cardiol. 2008;51:828-35.10
7. Liu T, Li L, Korantzopoulos P, Liu E, Li G. Statin use and developmentof atrial fibrillation: a systematic review and meta-analysis ofrandomized clinical trials and observational studies. Int J Cardiol. 2008;126:160-70.
8. Başaran Ö, Doğan V, Mert KU et al. How did the updated 2019 European Society of Cardiology/European Atherosclerosis Society risk categorization for patients with diabetes affect the risk perception and lipid goals? A simulated analysis of real-life data from EPHESUS study. Anatol J Cardiol. 2023;27(2):78-87. DOI:10.14744/AnatolJCardiol.2022.2012.
9. Mert GÖ, Başaran Ö, Mert KU et al. The reasons of poor lipid target attainment for secondary prevention in real life practice: results from EPHESUS. Int J Clin Pract. 2019;73(9):1-9.
10. SCORE2 working group and ESC Cardiovascular risk collaboration. SCORE2 risk prediction algorithms: new models to estimate 10-year risk of cardiovascular disease in Europe. Eur Heart J 2021;42:24392454.
11. SCORE2-OP working group and ESC Cardiovascular risk collaboration. SCORE2-OP risk prediction algorithms: estimating incident cardiovascular event risk in older persons in four geographical risk regions. Eur Heart J 2021;42:24552467
12. Kayıkçıoğlu M, Tokgözoğlu L, Kılıçkap M, et al. Türkiye'de dislipidemi sıklığı ve lipit verileri: Kardiyovasküler risk faktörlerine yönelik epidemiyolojik çalışmaların sistematik derleme ve meta-analizi [Data on prevalence of dyslipidemia and lipid values in Turkey: Systematic review and meta-analysis of epidemiological studies on cardiovascular risk factors]. Turk Kardiyol Dern Ars. 2018 Oct;46(7):556-74. Turkish. doi: 10.5543/tkda.2018.23450. PMID: 30391985.
13. Hyun-Jung Lee, So-Ryoung Lee, Eue-Keun Choi, Kyung-Do Han, Seil Oh. Low cholesterol levels and high cholesterol variability were associated with a higher risk of AF development. (J Am Heart Assoc. 2019;8:e012771. DOI: 10.1161/JAHA.119.012771.)
14. Qi Jiang, Ling Yang, Ming-Long Chen, Fei Hua, Jian-Jun Li. Lipid Profile and Atrial Fibrillation: Is There Any Link?. Rev. Cardiovasc. Med. 2022, 23(8), 272. https://doi.org/10.31083/j.rcm2308272
15. Li F, Du X, He L et al. Relationship between serum lipid levels and ischemic stroke in patients with atrial fibrillation: a nested case–control study based on the China Atrial Fibrillation Registry. BMC Cardiovasc Disord. 21, 424 (2021). https://doi.org/10.1186/s12872-021-02237-6
16. Harrison SL, Lane DA, Banach M et al. Lipid levels, atrial fibrillation and the impact of age: Results from the LIPIDOGRAM2015 study. Atherosclerosis. 2020; 312: 16–22.
17. Fauchier L, Pierre B, de Labriolle A, Grimard C, Zannad N, Babuty D. Antiarrhythmic effect of statin therapy and atrial fibrillation. A metaanalysis of randomized controlled trials. J Am Coll Cardiol. 2008;51: 828-35.
18. Faruk Ertaş, Hasan Kaya, Zekeriya Kaya, Serkan Bulur, Nuri Köse, Mehmet Gül. Epidemiology of atrial fibrillation in Turkey: preliminary results of the multicenter AFTER∗ study. Türk Kardiyol Dern Arş- Arch Turk Soc Cardiol. 2013;41(2):99-104 doi: 10.5543/tkda.2013.18488
19. Hanna IR, Heeke B, Bush H et al. Lipid-lowering drug use is associated with reduced prevalence of atrial fibrillation in patients with left ventricular systolic dysfunction. Heart Rhythm. 2006 Aug;3(8):881-6. doi: 10.1016/j.hrthm.2006.05.010. Epub 2006 May 9. PMID: 16876733; PMCID: PMC3164215.

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Impact of KLF4, SHH, and Hif1a knockdown on miRNA expression in malign melanoma cancer stem cells

KLF4, SHH ve Hif1a susturulmasının malign melanom kanser kök hücrelerinde miRNA ekspresyonuna etkisi

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ABSTRACT

Aim: microRNAs (miRNAs) play a pivotal role in gene regulation, influencing various cellular processes such as differentiation, proliferation, and apoptosis. This study investigated the expression of three specific miRNAs (Hsa-miR-21-5p, Hsa-miR-9-5p, and Hsa-miR-200a-5p) in malignant melanoma stem cells (CSCs) and non-stem cells (NSCs).

Materials and Methods: CSCs and NCSCs were sorted from CHL-1 cells based on CD133 marker, a malignant melanoma cell line. CD133+ cells were treated with Hif1a, KLF4, and SHH siRNA and the expression levels of three different miRNAs were compared between groups.

Results: Our findings indicated that Hsa-miR-200a-5p expression was similar in both cell groups. Conversely, Hsa-miR-21-5p and Hsa-miR-9-5p were significantly upregulated in NCSCs. Further analysis showed that the knockdown of KLF4 did not significantly affect the expression levels of these miRNAs. However, silencing SHH resulted in a substantial downregulation of Hsa-miR-21-5p and a significant upregulation of Hsa-miR-9-5p. Additionally, Hif1a knockdown led to the downregulation of both Hsa-miR-21-5p and Hsa-miR-9-5p.

Conclusion: These findings highlight the complex regulatory mechanisms of miRNA expression in different cellular contexts and suggest potential roles for these miRNAs in response to specific gene silencing.

Keywords: Malignant melanoma, cancer stem cell, miRNA, Hif1a, KLF4, SHH.

ÖZ

Amaç: *mikroRNA'lar (miRNA'lar) gen regülasyonunda önemli bir rol oynar ve farklılaşma, proliferasyon ve apoptoz gibi çeşitli hücresel süreçleri etkiler. Bu çalışmada, malign melanom kök hücrelerinde (CSC'ler) ve kök hücre olmayan hücrelerde (NSC'ler) üç spesifik miRNA'nın (Hsa-miR-21-5p, Hsa-miR-9-5p ve Hsa-miR-200a-5p) ekspresyonu araştırılmıştır.*

Gereç ve Yöntem: *Malign melanoma hücre hattı olan CHL-1 hücrelerinden CSC ve NCSC hücreleri CD133 belirteci baz alınarak elde edilmiştir. CD133+ hücreler HIF1a, KLF4, ve SHH siRNA ile muamele edilerek üç farklı miRNA ekspresyon seviyesi gruplar arası karşılaştırılmıştır.*

Bulgular: *Bulgularımız Hsa-miR-200a-5p ekspresyonunun her iki hücre grubunda da benzer olduğunu ortaya koymuştur. Buna karşılık, Hsa-miR-21-5p ve Hsa-miR-9-5p, NCSC hücrelerinde önemli ölçüde yüksek ifade edilmiştir. Daha ileri analizler KLF4'ün susturulmasının bu miRNA'ların ifade düzeylerini önemli ölçüde etkilemediğini göstermiştir.*

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Bununla birlikte, SHH'nin susturulması Hsa-miR-21-5p'nin önemli ölçüde düşük regülasyonu ve Hsa-miR-9-5p'nin önemli ölçüde yüksek ifadelenmesi ile sonuçlanmıştır. Ek olarak, Hif1a'nın susturulması hem Hsa-miR-21-5p hem de Hsa-miR-9-5p'nin aşağı regülasyonuna yol açmıştır.

Sonuç: *Elde edilen bulgular, farklı hücresel bağlamlarda miRNA ifadesinin karmaşık düzenleyici mekanizmalarını vurgulamakta ve spesifik gen susturmaya yanıt olarak bu miRNA'lar için potansiyel roller önermektedir.*

Anahtar Sözcükler: *Malign melanom, kanser kök hücreleri, miRNA, Hif1a, KLF4, SHH.*

INTRODUCTION

Cancer includes a wide range of diseases that is marked by the uncontrolled proliferation of abnormal cells, that can spread to other organs (metastasis), a leading cause of cancer mortality. Malignant melanoma, an aggressive skin cancer arising from melanocytes, exhibits a complex genetic landscape shaped by genetic and environmental influences. Malignant melanoma ranks as the fifth or sixth most prevalent malignancy and has the highest death rate among skin malignancies. It represents 5% and 4% of cancer cases in men and women, respectively (1) and is responsible for 75% of skin cancer-related deaths (2–4).

A particular subset of cells found in tumors with the potential to differentiate into different cell types and self-renewal is known as cancer stem cells (CSCs). These cells are believed to drive tumor initiation, growth, spread, and recurrence due to their stem cell-like characteristics (5). CSCs are marked by specific surface markers. Besides these cells regulate their signaling molecules, that they can be distinguished from the bulk tumor cells (6–8). Their resistance to conventional therapies poses a significant challenge in cancer treatment, as they can survive and regenerate the tumor even after the bulk of the tumor cells have been eradicated (9). They share similar signaling pathways with stem cells and regulate internal signaling through the differential expression of various molecules. The signaling pathways of KLF4, SHH, and Hif1a, which are more active in stem cells, are active in CSCs. However, the literature on the determination of changes related to the silencing of these gene expressions in malignant melanoma stem cells is limited. Another category of molecules that are differentially expressed by CSCs is microRNAs (miRNAs). These short RNAs regulate the expression patterns of targeted genes. miRNA expression is an emerging and increasingly significant area in cancer biology (10–15). Studies on malignant melanoma have shown that certain miRNA expressions are dramatically increased and decreased in malignant melanoma cells compared to melanocytes (16).

miR-21 is an oncogenic miRNA and is frequently upregulated by different subset of cancers, including malignant melanoma. miR-21 is involved in stimulating proliferation, invasion, and avoidance of apoptosis (17). Additionaly, miR-21 has been assocciated with the metastatic behaviour of the melanoma (18). miR-9 has shown in the regulation of cancer progression in addition to neural development. miR-9 modulates gene regulations related with cell adhesion and migration. In different cancer types, miR-9 affects the progression of the cancer differently. In breast cancer, miR-9 is correlated with metastasis, whereas in ovarian, gastric adenocarcinoma and medullablastoma it shows the opposite effect (12). A study on malignant melanoma tissue and cell culture reported miR-9 downregulation in cancerous cells compared to normal cells and miR-9 directly targets NRP1 (12, 19). Another miRNA family is miR-200, known to suppress epithelial-mesenchymal transition (EMT), an important process in cancer metastasis. miR-200 targets ZEB1 and ZEB2 transcription factors that promote EMT (20). The miR-200 family members are dysregulated in cancer tissue (21). Compared to healthy cells, miR-200 is differentially expressed in various cancer types; specifically, in melanoma, cancer cells exhibit lower expression of miR-200 than healthy cells (21, 22).

The tumor microenvironment is another factor affecting tumor characteristics; extracellular matrix molecules are responsible for tumor progression. Therefore, it is extremely important to establish the tumor microenvironment to create specific target therapies. To increase the metastatic capacity, melanoma cells manipulate the extracellular matrix and secrete extracellular factors for this purpose (23). Each change or modification in the extracellular matrix affects

cancer cell behavior and has to be examined (24).

Here, we examined the differential roles of specific miRNAs in regulating gene expression in malignant melanoma CSCs and NCSCs, particularly in response to the silencing of key genes such as Hif1a, KLF4, and SHH, thereby contributing to the development of targeted therapies that could potentially disrupt the CSC-driven tumor progression.

MATERIALS AND METHODS

Cell Culture

Non-pigmented human melanoma cell lines, specifically CHL-1 (ATCC® CRL-9446TM), were maintained in EMEM (Eagle's Minimum Essential Medium) (Biowest L0416) with 10% fetal bovine serum (Biowest, S1810). The cells were cultured in a humidified incubator set to 37°C with 5% CO_2. For flow cytometry sorting, cells between passages 6 and 8 were used to preserve experimental reliability and integrity. Regular validation of cell line identity and mycoplasma contamination testing were performed.

Flow Cytometry

Cells were detached from the flask surface using trypsinization, a common enzymatic technique to release adherent cells. Post-detachment, the harvested cells were then resuspended to a concentration of 10^6 cells/ml in 10 ml of cold 1X PBS. Subsequently, the cells were incubated with 10 µL of CD133 phycoerythrin (PE)-conjugated antibody (Miltenyi Biotec Ltd. 130-113-186) and 10 µL of DAPI for 15 minutes at 4°C. After the incubation, the cells were washed with 1X PBS containing 1% dialyzed fetal bovine serum (FBS). Control samples were stained with DAPI alone, without any antibodies. Cell sorting was then carried out using BD FACS Diva 8.0. The sorted cells labeled as CD133+ were identified as CSCs, while CD133- cells were classified as NCSCs. Within the malignant melanoma cell population, the CD133+ subset ranged from 0.1% to 0.4%. CD133+ cells, ranging from passage 2 to passage 4, were used in subsequent experiments. Post-sorting and experimental procedures, cell counting was performed using the Muse® Cell Analyzer, an automated system for cell counting and analysis.

siRNA treatment

To achieve the silencing of Hif1a, KLF4, and SHH genes, CD133+ malignant melanoma CSCs were transfected with varying concentrations (0-200 nM) of siRNA (On-Targetplus Human siRNA, Smartpool, L-005089-00-0005, L-004018-00-0005, L-006036-00-0005 Horizon). The transfection process was carried out to determine the optimal siRNA dosage, which was subsequently validated by RT-PCR for validation. A fold-change cut-off of 2 was established, with changes below this threshold considered negligible. In the experiment, siRNA concentrations ranging from 0-200 nM were utilized. Specifically, the selected doses were 5 nM for Hif1a and KLF4 siRNAs, and 25 nM for SHH siRNA and negative control siRNA (25,26).

RT-PCR

miRNA isolation was performed on cells treated with siRNA and control cells using the miRNeasy Kit 96 kit (Qiagen, 217061). The cells were seeded for 24 hours post-siRNA on Matrigel Basement Membrane Matrix (Corning) at a concentration of 10^6 cells/ml. They were washed with 1X PBS, detached using StemPro™ Accutase™ Cell Dissociation Reagent (Thermo Fisher), and then resuspended in RNA buffer. After that following steps were performed according to the isolation kit (Qiagen, 217061). The quantity and purity of the isolated miRNAs were measured by Nanodrop spectrophotometer (MaestroGen). Ideal RNA purity was indicated by A230/A260 and A260/A280 absorbance ratios of 1.9-2.1. The eluted miRNAs were stored at -80°C. cDNA synthesis was operated using the microScript microRNA cDNA synthesis kit (Norgen, 54410) with miRNAs of suitable quantity and purity (27). The analysis of Hsa-miR-21-5p, Hsa-miR-9-5p, and Hsa-miR-200a-5p with oncogenic or tumor-suppressor roles in malign melanoma stem cells was performed using RT-PCR by LightCycler® 480 SYBR Green I Master (Roche, 4707516001).

Statistical Analysis

The statistical analysis of expressed miRNAs was conducted in three replicates. miRNA expressions were computed as $\text{relative gene expression} = 2-\Delta\Delta CT$, where $\Delta\Delta CT = [(\text{CT gene} - \text{CT cel} - \text{miR} - \text{u6})\text{treated} - (\text{CT gene} - \text{CT cel} - \text{miR} - \text{u6})]\text{control}$. The relative gene expression values were listed in Table 1. The housekeeping miRNA U6 served as the reference gene in the data analysis. The quantity of the expression was calculated by fold change and fold regulation. Here, we compared CD133+ cell group with other experimental

groups so fold change was calculated as ($Fold\ change = \frac{Expression\ level\ of\ experimental\ group}{Expression\ level\ of\ control\ group}$) where CD133+ cell group is control. Fold regulation was calculated with $d\ Regulation = -\frac{1}{Fold\ change}$. The fold change and fold regulation values of the groups were listed in Table-2.

RESULTS

CD133+ cells were utilized as a CSC group after CHL-1 cells were sorted using the CD133 marker. The CD133+ cells were subjected to Hif1a, KLF4 and SHH separately examining the Hsa-mir-21-5p, Hsa-mir-9-5p and Hsa-mir-200-5p expression. In order to find out relative gene expression $2^{(-Avg.(Delta(Ct))}$ values were calculated (Table-1).

Table-1. Relative expression of the miRNAs calculated by $2^{(-Avg.(Delta(Ct))}$ method.

Symbol	$2^{(-Avg.(Delta(Ct))}$					
	CD133+	CD133-	CD133+/Hif1a-	CD133+/KLF4-	CD133+/SHH-	CD133+/negc
u6	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
Hsa-mir-21-5p	0.000907	0.002312	0.000350	0.001420	0.000168	0.000833
Hsa-mir-9-5p	0.006287	0.023793	0.001460	0.003016	0.047476	0.003023
Hsa--mir-200a-5p	0.000577	0.000618	0.000547	0.001026	0.000231	0.000410

Table-2. Fold regulation and fold change of the experimental groups' values. As a reference, the fold change and fold regulation values were calculated according to CD133+ group. CD133+ cell group was the control group of siRNA treated and CD133- groups and CD133+/negc group was the negative siRNA treated group.

	CD133+		CD133-		CD133+/Hif1a-		CD133+/KLF4-		CD133+/SHH-		CD133+/negc	
Symbol	Fold Change	Fold Regulation	Fold Change	Fold Regulation	Fold Change	Fold Regulation	Fold Change	Fold Regulation	Fold Change	Fold Regulation	Fold Change	Fold Regulation
u6	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Hsa-mir-21-5p	1.00	1.00	**2.55**	**2.55**	**0.39**	**-2.59**	1.57	1.57	**0.18**	**-5.41**	0.92	-1.09
Hsa-mir-9-5p	1.00	1.00	**3.78**	**3.78**	**0.23**	**-4.31**	**0.48**	**-2.08**	**7.55**	**7.55**	**0.48**	**-2.08**
Hsa--mir-200a-5p	1.00	1.00	1.07	1.07	0.95	-1.05	1.78	1.78	**0.40**	**-2.50**	0.71	-1.41

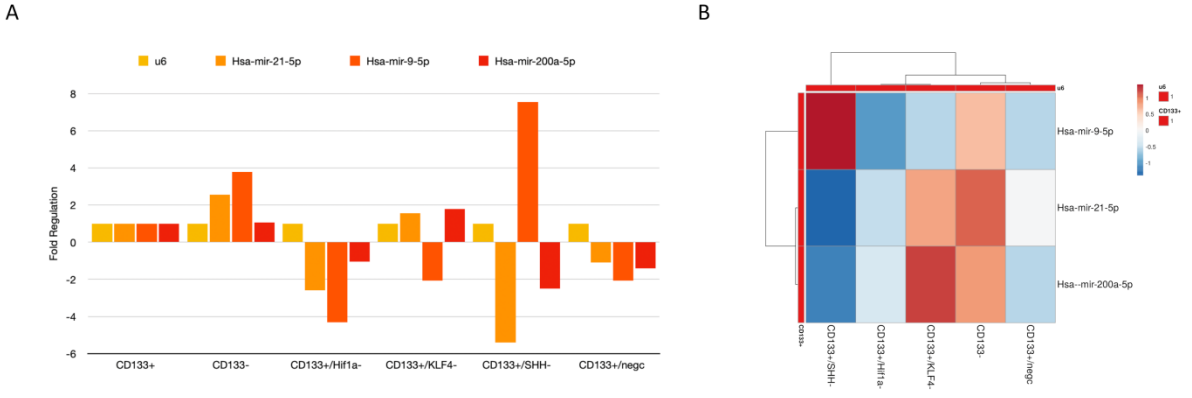

Figure-1. Fold regulation values of the experimental groups were shown in A) bar graph and B) heatmap generated by Clustvis.

The expression of four miRNAs, one of which was control miRNA (u6), was examined for expression analysis. These miRNAs were Hsa-miR-21-5p, Hsa-miR-9-5p, and Hsa-miR-200a-5p. The expression of Hsa-miR-200a-5p was similar in CD133+ and CD133- cell groups (1.07x), whereas Hsa-miR-21-5p and Hsa-miR-9-5p expression were upregulated in CD133- cells (2.55x and 3.78x, respectively) (Table-2). The expression of all three miRNAs did not significantly change in the KLF4 siRNA treated group. While Hsa-miR-21-5p is negatively regulated (-5.41x) in SHH siRNA application, Hsa-miR-9-5p is positively regulated (7.55x). Hsa-miR-21-5p and Hsa-miR-9-5p were both negatively regulated (-2.59x and -4.31x, respectively) in the Hif1a siRNA group. Fold regulation graph and heatmap were illustrated in Figure-1. The heatmap was generated by Clustvis (28).

DISCUSSION

Malignant melanoma is an aggressive and lethal type of skin cancer that originated from melanocytes. Melanoma accounts for fewer than 5% of all skin cancer cases, but due to its high metastatic potential and resistance to conventional treatments, it is the primary cause of skin cancer-related mortality. A promising area of research for addressing malignant melanoma from an epigenetic perspective focuses on miRNAs. These small non-coding RNA molecules control gene expression post-transcriptionally, influencing epigenetic modifications that can affect cancer progression or repression (29–31). Altered expression of miRNAs can contribute to various hallmarks of cancer, including uncontrolled cell proliferation, evasion of growth suppressors, resistance to apoptosis, and enhanced metastatic capability (32). In a review article, it was reported that the characteristics of initiating primary tumors are found only in melanoma cell subgroups characterized by CD133 expression, while CD133- melanoma cells do not possess tumorigenic properties (33). Another study showed that both CD133+ and CD133- cell groups have potential to form colonies, but CD133+ group more potent besies both could initiates tumor formation in mice (34). Additionally, a meta-analysis study demonstrated that both CD133+ and CD133- groups can form tumors; however, CD133- cells derived from patient samples did not metastasize (34). Even, the CD133 marker is utilized for the precise isolation and characterization of malignant melanoma stem cells, distinguishing them from non-stem cancer cell populations, there should be additional markers for better characterization and tracing the cell lineage.

Here, we targeted three molecules for silencing. The selection of KLF4, Hif1a, and SHH for this study is based on their significant roles in cancer biology and their potential impact on malignant melanoma stem cells. KLF4 is a critical factor in development, cellular reprogramming, and cancer. This molecule can play role as an oncogene or a tumor suppressor (35). It is involved in various signalling pathways. KLF4 expression was shown to be reduced in gastric cancer, hepatocellular carcinoma, and lung cancer, where it serves as a positive prognostic marker. On the other hand, elevated KLF4 levels are linked to poor outcomes in breast cancer, prostate cancer, colorectal cancer, and skin squamous cell carcinoma, indicating a potential oncogenic role (36). Hif1a upregulation has been implicated in the aggressive phenotypes and worse survival rates observed in various cancers. While a higher level of Hif1a is linked with elevated tumor suppressive signs such as apoptosis and anti-tumor inflammation, it shows a stronger connection with immune-response signs, highlighting its dual role in cancer development (37). The SHH signaling pathway is crucial in the progression of various cancers, including malignant melanoma. SHH signaling has been implicated in promoting cell proliferation, survival, and metastasis by modulating the tumor microenvironment and enhancing EMT. The hedgehog signaling pathway is modulated by miRNAs. Specific miRNAs, such as miR-200 and miR-21, have been shown to influence SHH signaling in melanoma cells. For instance, miR-21 is known to enhance the expression of SHH pathway components, thereby promoting melanoma cell survival and metastasis (38).

In our study, we selected three miRNAs (mir9, mir21, and mir200) from among oncogenic and protooncogenic miRNAs. We then assessed the variations in their expression across the experimental groups. u6 was used as a control for miRNA expression assessment. While no alterations were observed in the expression of the chosen miRNAs upon KLF4 silencing, a

noteworthy outcome emerged, revealing a significant increase in mir9 expression upon the silencing of SHH. miR9 features a conserved sequence spanning from insects to humans. Investigations into neurogenesis have unveiled mir9 expression during various stages of the developmental process (39), as SHH signalling active in neurogenesis. While mir9 expression was high in gastric and neural cancers, it was found to be low in ovarian cancer (39). In addition, mir21 expression decreased with SHH silencing by 5.41x fold. High expression of SHH was associated with GBM and it was determined that the increase of its ligand, PTCH protein, created a resistance to temozolomide in GBM treatment. The GBM study additionally revealed an association between mir9 and PTCH, impacting the SHH pathway. Notably, this association was observed to be independent of the SHH expression level (40). In a study conducted in GBM CSCs, it was stated that mir9 is expressed at high levels in CSCs (41). mir200 has been indicated as a tumor suppressor in various types of cancer (21). The mir 200 family can be classified as mir200a, mir200b, mir200c. Studies comparing healthy tissue and melanoma have shown that the expression of these three mir200s is lower than in healthy tissue (21). In here only with SHH silencing CSCs lowered expression mir-200a-5p, while there was no change in Hif1a and KLF4 silencing.

CONCLUSION

This study demonstrates the differential expression of miRNAs Hsa-mir-21-5p, Hsa-mir-9-5p, and Hsa-mir-200a-5p in CD133+ and CD133- cell populations. Hsa-mir-21-5p and Hsa-mir-9-5p were significantly upregulated in CD133- cells, suggesting their potential roles in the differentiation state of these cells. The knockdown of KLF4 did not affect miRNA expression significantly, indicating that KLF4 might not be a major regulator of these miRNAs under the studied conditions. SHH siRNA treatment showed contrasting effects on Hsa-mir-21-5p and Hsa-mir-9-5p, with the former being downregulated and the latter upregulated, indicating a complex regulatory mechanism between miRNA expression. The modulation of SHH signaling affects these miRNAs in opposite directions of regulation. Hif1a silencing led to the downregulation of both Hsa-mir-21-5p and Hsa-mir-9-5p, highlighting its role in the regulation of these miRNAs. These findings provide perspective on the regulatory networks of miRNAs in various cell types especially cancer stem cells and their possible implications for cellular differentiation and gene silencing.

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References

1. Moran B, Silva R, Perry AS, Gallagher WM. Epigenetics of malignant melanoma. Semin Cancer Biol [Internet]. 2018;51:80–8. Available at: https://www.sciencedirect.com/science/article/pii/S1044579X1730130X
2. Apalla Z, Lallas A, Sotiriou E, Lazaridou E, Ioannides D. Epidemiological trends in skin cancer. Dermatol Pract Concept [Internet]. 30 Nisan 2017;7(2):1–6. Available at: https://pubmed.ncbi.nlm.nih.gov/28515985
3. Hessler M, Jalilian E, Xu Q, Reddy S, Horton L, Elkin K, vd. Melanoma biomarkers and their potential application for in vivo diagnostic imaging modalities. Int J Mol Sci. 2020;21(24):9583.
4. Liu Z, Wang H, Sun C, He Y, Xia T, Wang J, vd. ZWZ-3, a Fluorescent Probe Targeting Mitochondria for Melanoma Imaging and Therapy [Internet]. C. 13, Frontiers in Pharmacology . 2022. Available at: https://www.frontiersin.org/article/10.3389/fphar.2022.829684
5. Kuşoğlu A, Biray Avcı Ç. Cancer stem cells: A brief review of the current status. Gene [Internet]. 2019;681:80–5. Available at: https://www.sciencedirect.com/science/article/pii/S0378111918310163
6. Jain P, Pillai M, Duddu AS, Somarelli JA, Goyal Y, Jolly MK. Dynamical hallmarks of cancer: Phenotypic switching in melanoma and epithelial-mesenchymal plasticity. Semin Cancer Biol [Internet]. 2023;96:48–63. Available at: https://www.sciencedirect.com/science/article/pii/S1044579X23001293

7. Boudreault J, Wang N, Ghozlan M, Lebrun J-J. Transforming Growth Factor-β/Smad Signaling Inhibits Melanoma Cancer Stem Cell Self-Renewal, Tumor Formation and Metastasis. C. 16, Cancers. 2024.
8. Wu J, Li W, Su J, Zheng J, Liang Y, Lin J, vd. Integration of single-cell sequencing and bulk RNA-seq to identify and develop a prognostic signature related to colorectal cancer stem cells. Sci Rep [Internet]. 2024;14(1):12270. Available at: https://doi.org/10.1038/s41598-024-62913-3
9. Yang L, Shi P, Zhao G, Xu J, Peng W, Zhang J, vd. Targeting cancer stem cell pathways for cancer therapy. Signal Transduct Target Ther [Internet]. 2020;5(1):8. Available at: https://doi.org/10.1038/s41392-020-0110-5
10. Bu P, Luo C, He Q, Yang P, Li X, Xu D. MicroRNA-9 inhibits the proliferation and migration of malignant melanoma cells via targeting sirituin 1. Exp Ther Med [Internet]. 2017/06/13. Ağustos 2017;14(2):931–8. Available at: https://pubmed.ncbi.nlm.nih.gov/28810544
11. Bustos MA, Ono S, Marzese DM, Oyama T, Iida Y, Cheung G, vd. MiR-200a Regulates CDK4/6 Inhibitor Effect by Targeting CDK6 in Metastatic Melanoma. J Invest Dermatol [Internet]. 2017;137(9):1955–64. Available at: http://www.sciencedirect.com/science/article/pii/S0022202X17315282
12. Liu S, Kumar SM, Lu H, Liu A, Yang R, Pushparajan A, vd. MicroRNA-9 up-regulates E-cadherin through inhibition of NF-κB1-Snail1 pathway in melanoma. J Pathol. Ocak 2012;226(1):61–72.
13. Melnik BC. MiR-21: an environmental driver of malignant melanoma? J Transl Med [Internet]. 27 Haziran 2015;13:202. Available at: https://pubmed.ncbi.nlm.nih.gov/26116372
14. Satzger I, Mattern A, Kuettler U, Weinspach D, Niebuhr M, Kapp A, vd. microRNA-21 is upregulated in malignant melanoma and influences apoptosis of melanocytic cells. Exp Dermatol. Temmuz 2012;21(7):509–14.
15. van Kempen LC, van den Hurk K, Lazar V, Michiels S, Winnepenninckx V, Stas M, vd. Loss of microRNA-200a and c, and microRNA-203 expression at the invasive front of primary cutaneous melanoma is associated with increased thickness and disease progression. Virchows Arch. Ekim 2012;461(4):441–8.
16. Gajos-Michniewicz A, Czyz M. Role of miRNAs in Melanoma Metastasis. Cancers (Basel). Mart 2019;11(3).
17. Rhim J, Baek W, Seo Y, Kim JH. From Molecular Mechanisms to Therapeutics: Understanding MicroRNA-21 in Cancer. Cells. Eylül 2022;11(18).
18. Yang CH, Yue J, Pfeffer SR, Handorf CR, Pfeffer LM. MicroRNA miR-21 regulates the metastatic behavior of B16 melanoma cells. J Biol Chem. Kasım 2011;286(45):39172–8.
19. Xu D, Chen X, He Q, Luo C. MicroRNA-9 suppresses the growth, migration, and invasion of malignant melanoma cells via targeting NRP1. Onco Targets Ther. 2016;9:7047–57.
20. Park S-M, Gaur AB, Lengyel E, Peter ME. The miR-200 family determines the epithelial phenotype of cancer cells by targeting the E-cadherin repressors ZEB1 and ZEB2. Genes Dev. Nisan 2008;22(7):894–907.
21. Klicka K, Grzywa TM, Mielniczuk A, Klinke A, Włodarski PK. The role of miR-200 family in the regulation of hallmarks of cancer. Front Oncol [Internet]. 2022;12. Available at: https://www.frontiersin.org/articles/10.3389/fonc.2022.965231
22. Chen W-Y, Xu Y-Y, Zhang X-Y. Targeting GOLM1 by microRNA-200a in melanoma suppresses cell proliferation, invasion and migration via regulating PI3K/Akt signaling pathway and epithelial-mesenchymal transition. Eur Rev Med Pharmacol Sci. Ağustos 2019;23(16):6997–7007.
23. Botti G, Cerrone M, Scognamiglio G, Anniciello A, Ascierto PA, Cantile M. Microenvironment and tumor progression of melanoma: new therapeutic prospectives. J Immunotoxicol. 2013;10(3):235–52.
24. Yue B. Biology of the extracellular matrix: an overview. J Glaucoma. 2014;23(8 Suppl 1):S20-3.
25. Ozdil B, Biray Avci C, Calik-Kocaturk D, Gorgulu V, Uysal A, Guler G, vd. Modulating cancer stem cell characteristics in CD133+ melanoma cells through HIF1α, KLF4, and SHH silencing Berrin. unpublished. 2024.
26. Ozdil Bay B. MALİGN MELANOMA KANSER KÖK HÜCRE MODELİNDE HİF1A GENİNİN SUSTURULMASI İLE İNCELENMESİ Doktora Tezi HİF1A GENİNİN SUSTURULMASI İLE. 2023.
27. Gunel NS, Birden N, Kurt CC, Bagca BG, Shademan B, Sogutlu F, vd. Effect of valproic acid on miRNAs affecting histone deacetylase in a model of anaplastic thyroid cancer. Mol Biol Rep [Internet]. 2021;48(8):6085–91. Available at: https://doi.org/10.1007/s11033-021-06616-2

28. Metsalu T, Vilo J. ClustVis: a web tool for visualizing clustering of multivariate data using Principal Component Analysis and heatmap. Nucleic Acids Res [Internet]. 01 Temmuz 2015;43(W1):W566–70. Available at: https://doi.org/10.1093/nar/gkv468
29. Varrone F, Caputo E. The miRNAs Role in Melanoma and in Its Resistance to Therapy. C. 21, International Journal of Molecular Sciences. 2020.
30. Poniewierska-Baran A, Zadroga Ł, Danilyan E, Małkowska P, Niedźwiedzka-Rystwej P, Pawlik A. MicroRNA as a Diagnostic Tool, Therapeutic Target and Potential Biomarker in Cutaneous Malignant Melanoma Detection-Narrative Review. Int J Mol Sci. Mart 2023;24(6).
31. Esquela-Kerscher A, Slack FJ. Oncomirs—microRNAs with a role in cancer. Nat Rev cancer. 2006;6(4):259–69.
32. Ghafouri-Fard S, Gholipour M, Taheri M. MicroRNA Signature in Melanoma: Biomarkers and Therapeutic Targets. Front Oncol [Internet]. 2021;11. Available at: https://www.frontiersin.org/journals/oncology/articles/10.3389/fonc.2021.608987
33. Schatton T, Frank MH. Cancer stem cells and human malignant melanoma. Pigment Cell Melanoma Res. Şubat 2008;21(1):39–55.
34. Madjd Z, Erfani E, Gheytanchi E, Moradi-Lakeh M, Shariftabrizi A, Asadi-Lari M. Expression of CD133 Cancer Stem Cell Marker in Melanoma: A Systematic Review and Meta-Analysis. Int J Biol Markers [Internet]. 01 Nisan 2016;31(2):118–25. Available at: https://doi.org/10.5301/jbm.5000209
35. He Z, He J, Xie K. KLF4 transcription factor in tumorigenesis. Cell Death Discov [Internet]. 2023;9(1):118. Available at: https://doi.org/10.1038/s41420-023-01416-y
36. Luo X, Zhang Y, Meng Y, Ji M, Wang Y. Prognostic significance of KLF4 in solid tumours: an updated meta-analysis. BMC Cancer [Internet]. 2022;22(1):181. Available at: https://doi.org/10.1186/s12885-022-09198-9
37. Chen B, Li L, Li M, Wang X. HIF1A expression correlates with increased tumor immune and stromal signatures and aggressive phenotypes in human cancers. Cell Oncol (Dordrecht, Netherlands). Ekim 2020;43(5):877–88.
38. Xu L, Li K, Li J, Liu L, Xu F, Xu Y, vd. MiR-21/Sonic Hedgehog (SHH)/PI3K/AKT Pathway is Associated with NSCLC of Primary EGFR-TKI Resistance. Oncol (Tech Sci Press. 2022;24(3).
39. Yuva-Aydemir Y, Simkin A, Gascon E, Gao F-B. MicroRNA-9: functional evolution of a conserved small regulatory RNA. RNA Biol [Internet]. 2011/07/01. 2011;8(4):557–64. Available at: https://pubmed.ncbi.nlm.nih.gov/21697652
40. Munoz JL, Rodriguez-Cruz V, Ramkissoon SH, Ligon KL, Greco SJ, Rameshwar P. Temozolomide resistance in glioblastoma occurs by miRNA-9-targeted PTCH1, independent of sonic hedgehog level. Oncotarget. Ocak 2015;6(2):1190–201.
41. Munoz JL, Rodriguez-Cruz V, Rameshwar P. High expression of miR-9 in CD133+ glioblastoma cells in chemoresistance to temozolomide. J cancer stem cell Res [Internet]. 2015;3:e1003. Available at: https://europepmc.org/articles/PMC4917210

Research Article / *Araştırma Makalesi*

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Size-dependent toxicological effects comparison of Aluminum oxide nanoparticles (Al_2O_3 NPs)

Alüminyum oksit nanopartiküllerinin (Al_2O_3 NP'leri) boyuta bağlı toksikolojik etkilerinin karşılaştırılması

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ABSTRACT

Aim: Modification of nanomaterials with different synthesis methods can affect their biological response, as well as their use as nanotherapeutics. It is necessary to address and understand the safety issue of these particles through toxicological evaluation with an underlying mechanism of interaction. With the fast entry of aluminum-based nanoparticles into the industry, their potential exposure has also increased significantly. Aluminum oxide nanoparticles (Al_2O_3 NPs) are among the priority materials by international organizations. Studies have not yet elucidated the toxic response of Al_2O_3 NPs depending on their size range.

Materials and Methods: Therefore, this study aimed to investigate toxicological effects of Al_2O_3 NPs depending on size range on MCF-10 and MCF-7 cells by WST-1 test, hemolytic activity on red blood cells and irritation effects by HET-CAM test.

Results: As a result of tests, all size ranges of Al_2O_3 NPs didn't show any cytotoxic effects on MCF-10 and MCF-7 cells, also none of sizes of Al_2O_3 NPs were caused hemolysis (<2%). It was observed that there was no irritating effect in all size ranges on HET-CAM test.

Conclusion: In conclusion, risk assessments in terms of characteristic features as the size of Al_2O_3 NPs showed that they have the potential to provide safe use in drug delivery systems and immobilization studies.

Keywords: Aluminum oxide nanoparticles, Size-depended toxicity, Biocompatibility, HET-CAM.

ÖZ

Amaç: *Farklı sentez yöntemleri kullanılarak nanomateryallerin modifiye edilmesi, biyolojik yanıtlarını ve nanoterapötik olarak kullanımlarını etkileyebilir. Bu parçacıkların güvenliği konusunu, altta yatan etkileşim mekanizması ile toksikolojik değerlendirme yoluyla ele almak ve anlamak gerekmektedir. Alüminyum bazlı nano parçacıkların endüstriye hızlı bir şekilde girmesiyle, potansiyel maruziyetleri de önemli ölçüde arttı. Alüminyum oksit nano parçacıkları (Al2O3 NPs) uluslararası kuruluşlar tarafından öncelikli malzemeler arasında yer almaktadır. Araştırmalarda, Al2O3 NP'lerin boyut aralığına bağlı olarak toksik yanıtı henüz ortaya konulmamıştır.*

Gereç ve Yöntem: *Bu nedenle bu çalışmada, Al2O3 NP'lerinin boyut aralığına bağlı olarak MCF-10 ve MCF-7 hücreleri üzerindeki toksikolojik etkilerinin WST-1 testi ile, kırmızı kan hücreleri üzerindeki hemolitik aktivitesinin ve HET-CAM testi ile tahriş etkilerinin araştırılması amaçlanmıştır.*

Bulgular: *Test sonuçlarında, Al2O3 NP'lerin tüm boyut aralıklarında MCF-10 ve MCF-7 hücreleri üzerinde herhangi bir sitotoksik etki göstermediği, ayrıca Al2O3 NP'ler hiçbir boyutunda hemolize neden olmamıştır (<2%).*

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HET-CAM testinde tüm boyut aralıklarında tahriş edici bir etki olmadığı gözlemlendi.

Sonuç: *Sonuç olarak, Al_2O_3 NP'lerinin boyut olarak karakteristik özellikleri açısından yapılan risk değerlendirmesinde, ilaç taşıma sistemlerinde ve immobilizasyon çalışmalarında güvenli kullanım sağlama potansiyeline sahip olduklarını göstermiştir.*

Anahtar Sözcükler: *Alüminyum oksit nanopartiküller, boyuta bağlı toksisite, biyouyumluluk, HET-CAM.*

INTRODUCTION

The physico-chemical features of nanoparticles as size, shape, surface area, surface charge as well as route and frequency of exposure are among the factors that affect their behavior in biological systems and their toxicity. There is a need to investigate these nanomaterials depending on their application areas and their potential effects on the environment and human health. Particularly metallic nanoparticles attract attention with their wide usage areas. Metal oxide nanoparticles have recently been produced at an industrial level and have widespread applications in water purification, medicine, cosmetics, and engineering (1,2). One of these materials is aluminum oxide nanoparticles that have been the subject of research due to their unique properties. The Organization for Economic Cooperation and Development (OECD) has presented 14 nanomaterials including aluminum oxide nanoparticles (Al_2O_3 NPs) as priority materials for the investigations (3). Al_2O_3 NPs are used in many fields such as enzyme immobilization, drug delivery, biosensors, wastewater management with produced approximately 20% of the 2005 world market of nanoparticles (4). This shows that their use in application areas will grow over the years with increased synthesis types of these materials. However, despite the widespread use of such a priority material, there is limited information available on size-depend potential hazards. It is necessary to determine the potential effects of the physico-chemical properties as a result of increased exposure and also bring the deficiencies into the literature.

In this study, the cellular response after exposure to different sizes of aluminum oxide nanoparticles (Al_2O_3 NPs) was discussed in order to determine the effect of size, which is an important characteristic parameter in the toxicity and effectiveness of nanomaterials.

MATERIAL AND METHODS

Materials

Aluminum oxide nanoparticles with primary sizes (Al_2O_3-48nm; Al_2O_3-78nm; Al_2O_3-100nm) were obtained from *Nanografi* Company with characteristic features data as spherical morphology and 99.95% purity. All other chemicals were obtained from Sigma-Aldrich (USA).

Characterization analyses

In order to determine the size distribution of Al_2O_3 NPs in different pH conditions (pH 4, pH 7.5 and pH 10) were determined with ZETASizer (Malvern ZETA ZS, England).

In vitro studies

Cell Culture and Cytotoxicity Assay

MCF-10 (Human mammary epithelial cell line) and MCF-7 (Human breast cancer cell line) were kindly provided by Dr. Balcan (Manisa Celal Bayar University) and Dr. Karataş (Erzurum Technical University). The cell lines were cultured in DMEM culture medium supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin/ streptomycin in T75-cm^2 culture flasks. The cells were incubated at 37°C in 5% CO_2. The culture was changed every 2 days until cells were confluent.

The WST-1 assay, which gauges mitochondrial reductase activity, was used to conduct the cytotoxicity test (5). 2×10^4 cells/mL were seeded in 96-well plates and then, different treatment concentrations were applied to the cultivated cells (6.25 µg -500 µg /ml) of Al_2O_3 NPs for 24h at 37 ºC. Using an inverted light microscope, the cells in each treatment group were inspected for morphological changes, and THERMO microplate reader was used to assess the optical density of each plate at 450 nm. The percentage representation of the relative viability was calculated using untreated cells as the negative control. The formula below was used to determine the viability (%):

(%) Viable cells= ([(the absorbance of the treated cells)-(the absorbance of the blank)])/([(the absorbance of the control)-(the absorbance of the blank)) x100

Hemolysis assay

The blood sample obtained from New Zealand Albino rabbit with the approval of Ege University Animal Experiments Local Ethics Committee (EÜHADYEK- July 27, 2022/no. 2022-055) to be used in the study. Test was performed according to Bakan (2020) protocols (5) and ASTM standards (6). First, 6.0 mL of phosphate buffered saline (PBS) was added to the 3 mL of blood and centrifuged at 3000 g for 10 minutes. Then, the pellet containing red blood cells (RBC) was separated and washed 3 times with an equal volume of PBS. The remaining volume after washing was diluted 1:1 with PBS. The test material was applied in different dose ranges (20-160 µg/ml) depending on the therapeutic dose. Subsequently, 0.8 mL of test samples at the applicable concentrations were combined with 0.2 mL of RBC suspension, and the tubes were incubated for 3 hours at 37 °C in a water bath, with 30 minutes mixing intervals. PBS was utilized as the negative control and Triton X-100 (1%) as the positive control. After incubation, the tubes were centrifuged at 3000 g for 4 minutes and the absorbance values of all sample supernatants were taken at 540 nm. According to the ASTM standard, <2% is considered as not hemolytic; 2%–5% slightly hemolytic; and>5% was accepted as hemolytic.

The percentage of hemolysis was calculated with the following formula;

Hemolysis %=(A sample -A0)/(A100-A0) X 100

A100 represents the absorbance of fully lysed red blood cells, A0 represents the absorbance of non-blood samples, and A sample is the absorbance value of the sample.

Hen's egg test on chorio-allantoic membrane (HET-CAM) test

The test is an alternative test developed by EURL-ECVAM (European Union Reference Laboratory for Alternatives to Animal Testing) to determine the degree of irritation. It allows to see possible effects of substances by observing alterations in the egg's chorio-allantoic membrane following exposure to the test sample. Test was carried out in accordance with ICCVAM (7) on fertilized chicken eggs (50-60g) with three independent replicates and egg were incubated at 37±0.5°C'de, 70% humidity for 7 days. On the 7th day, an area (2x2cm) was opened at the equator of the eggs and 300 µL of each test material was dropped directly onto the CAM surface and left in contact for 0.5, 2 and 5 minutes. 0.9% NaCl solution was used as a negative control and 0.1N NaOH was used as a positive control. After exposure of each sample, the membrane was examined for vascular damage and the elapsed time was recorded. The possible irritation degree score (IS) was calculated as follows;

IS = [(301 − tH) × 5]/ 300 + (301 − tL) × 7]/ 300 + (301 − tC) × 9]/ 300

where tH, tL, and tC are the corresponding (in seconds) timespans for hemolysis, lysis, and coagulation. Formulations can be categorized as non-irritating (IS < 1), slightly irritating (1 ≤ IS < 5), moderately irritating (5 ≤ IS < 10), or extremely irritating (IS > 10), based on their IS values.

Statistical analysis

Statistical analyses were performed with GraphPad Prism 8 (GraphPad Software, LLC, Boston, MA, USA) and results compared with the control group using ONE-WAY analysis of variance (ANOVA). All values are expressed as means ± standard deviation (SD). Statistical significance was set to $p<0.05$.

RESULTS

Characterization analyses results

The sizes of Al_2O_3 NPs in different pH conditions were performed with ZETAsizer to observe their behaviour in diverse environments. As seen in all other types of nanoparticles, Al_2O_3 NPs tend to agglomerate at all dimension in different pH environments as presented in Figure-1.

Cytotoxicity test results

In the test results, there is no significant cytotoxic effects of all size range of Al_2O_3 NPs at applied doses on both of cell lines after 24h exposure as presented in Figure-2 and Figure-3.

Hemolysis test results

According to hemolysis test results, none of Al_2O_3 NPs showed hemolytic activity at applied doses (20, 40, 80 and 160 µg/ml) on erythrocytes as presented in Figure-4.

HET-CAM test results

The test, which was carried out to reveal the irritation effect of different sizes Al_2O_3 NPs were evaluated in terms of hemorrhage, lysis and coagulation parameters, and no irritant effects were observed at all size ranges of Al_2O_3 NPs as presented in Figure-5.

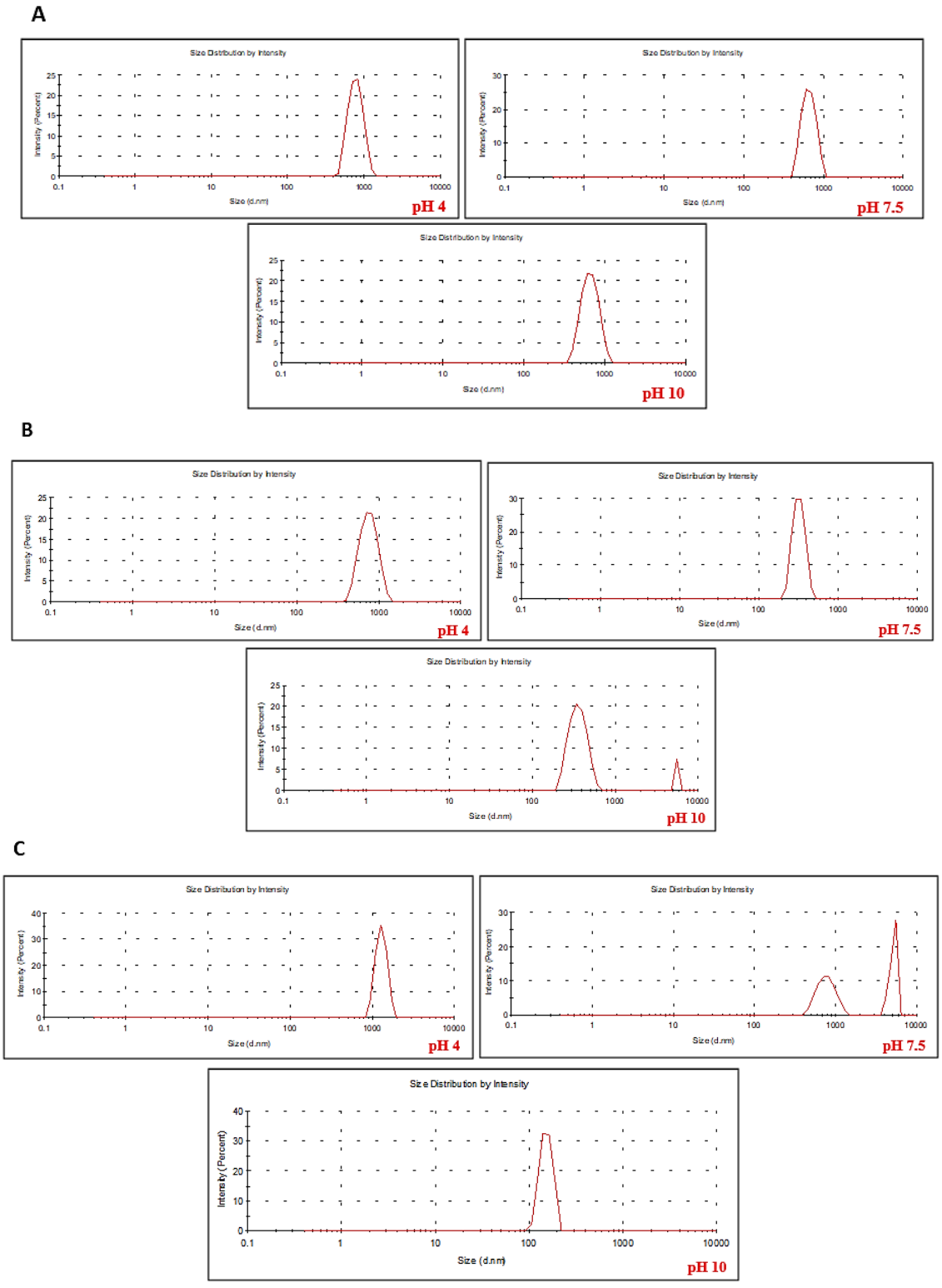

Figure-1. Size distribution of Al_2O_3 NPs (A. Al_2O_3-48nm; B. Al_2O_3-78nm; C. Al_2O_3-100nm) at different pH conditions (pH4, pH7.5 and pH10) by ZETASizer analyses.

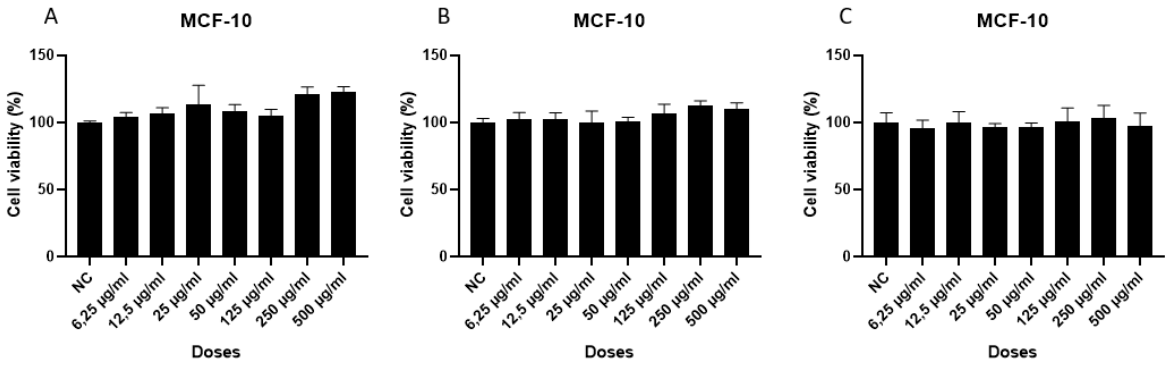

Figure-2. Cytotoxicity test results of Al_2O_3 NPs on MCF-10 cell line after 24h exposure time by WST-1 assay; **A.** Al_2O_3-48nm; **B.** Al_2O_3-78nm; **C.** Al_2O_3-100nm. All values are expressed as means ± SD with three independents repeated.

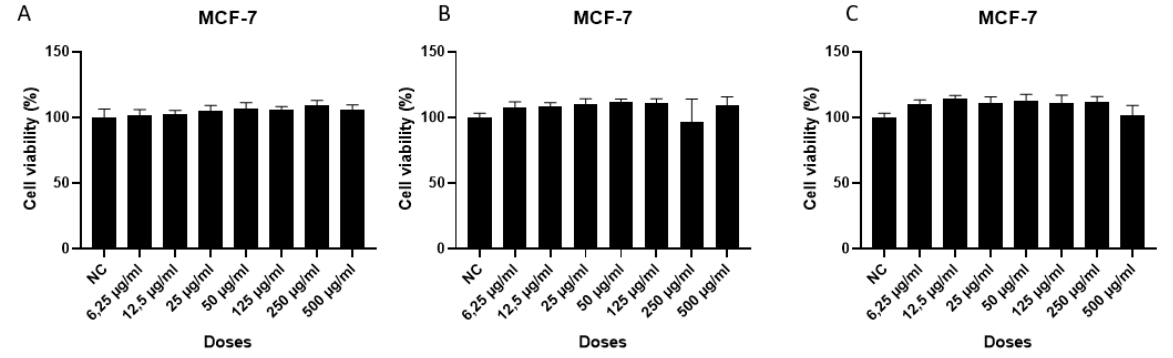

Figure-3. Cytotoxicity test results of Al_2O_3 NPs on MCF-7 cell line after 24h by WST-1 assay; **A.** Al_2O_3-48nm; **B.** Al_2O_3-78nm; **C.** Al_2O_3-100nm. All values are expressed as means ± SD with three independents repeated.

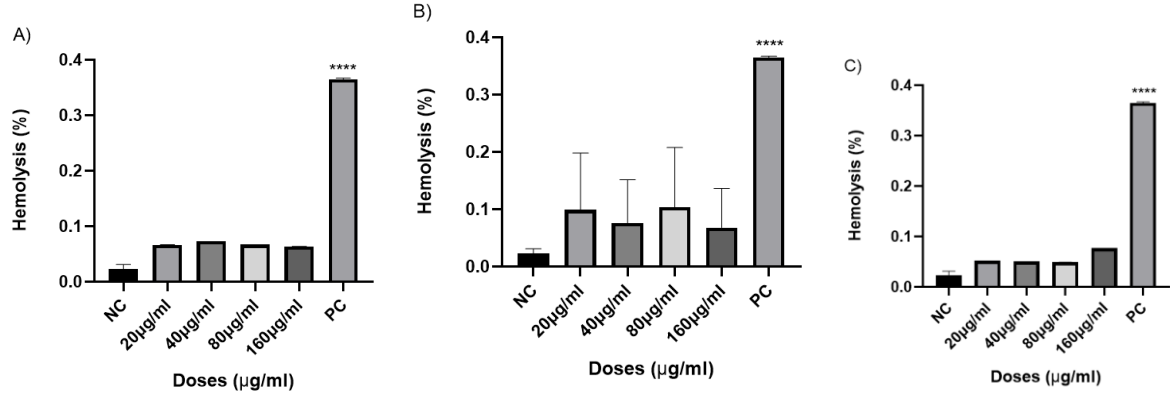

Figure-4. Hemolysis rates of Al_2O_3 NPs with different size range as **A.** Al_2O_3-48nm, **B.** Al_2O_3-78nm, **C.** Al_2O_3-100nm. Data are presented as mean ± SD from three repeats. PC, positive control; NC, negative control.

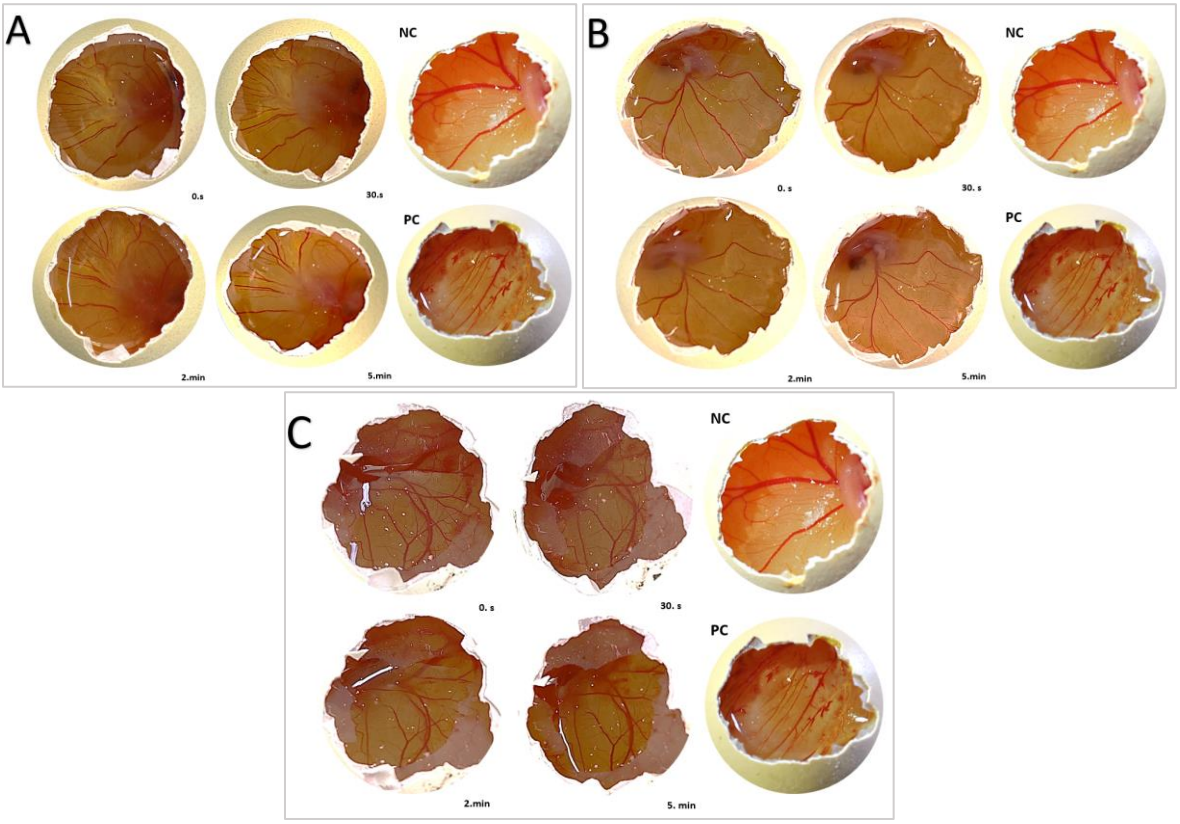

Figure-5. HET-CAM test results at different time points after Al_2O_3-48nm exposure (A); Al_2O_3-78nm (B); Al_2O_3-100nm (C). 0.9% NaCl was used as a negative control and 0.1 N NaOH was used as a positive control.

DISCUSSION

Nowadays, exposure to NPs have been increasing in many areas due to their widely use. These nanoparticles' potential toxicity is dictated by their physicochemical characteristics, including size, surface charge, and surface area, as well as the dosage, frequency of exposure, and mode of administration. In the presence of these parameters, post-exposure toxicity of nanoparticles can affect any tissue type in the body. In previous studies have shown that to exposed nanoparticles in different ways, may behave differently depending on their physicochemical properties. Size is one of these parameters which has a major impact on the toxicity of nanoparticles. As the size of NPs decreases, the surface area increases, leading to complex biophysiochemical interactions under the various environment conditions (8). Changes in the physicochemical and structural characteristics of NPs might impact their biological response, resulting in altered cytotoxicity, ROS generation, and genotoxicity (9). While the oxidative stress induced by NPs is generally caused by non-cellular factors such as particle size, surface, structure or the presence of metals in their structure, on the other hand, it is also responsible for ROS-mediated damage through biological reactions such immune cell activation and NP-cell interaction (9).

The industrial sector's adoption of nanoparticles based on aluminum has also greatly expanded their potential exposure, which is why the OECD listed Al_2O_3 NPs as high priority groups in its program on the safety of produced nanomaterials in 2007 (10). These such kind of engineered oxide nanoparticles (NPs) have the potential to exposed in many ways. In a conclusion that, these materials concern about their potential toxic effects in humans (11). Leading cause of cancer death due to breast cancer in women was increased in recently. Therefore, it was planned to study these particles in breast cells, due to their frequently potential exposure route. In addition, considering the studies conducted, there is limited in vitro studies focusing on the effects of Al_2O_3 NPs on breast cell lines, and in vivo activity as well. When NPs can enter the bloodstream and interact with blood components, they associate with blood components such as

red blood cells (RBCs) and hemoglobin (Hb). It is extremely important to investigate size-dependent toxic effects for their safety use especially in drug delivery systems. Physicochemical properties and behavior of nanoparticles in different environment conditions can be responsible to immune response, biodistribution, accumulation and clearance as well at the biological systems (12). The size distribution analysis used in the study to examine the impact of various pH levels on particle size and agglomeration revealed that the particles tended to group together. This alteration in physical properties may increase translocate through the endothelial liner and enter the circulatory system (13). Studies have shown that serum proteins have major effect on particle toxicity, probably due to agglomeration or changes in the surface chemistry (14). At the same time, the morphology feature is also may be effective on the tendency to agglomerate of the nanoparticles. In the study of Al-Gebory and Mengüç, (2018) (15), they investigate different pH conditions effects on TiO_2 NP_S and they showed that pH may have significant effect on the particle agglomeration behavior. Choi et al. (2011) (16) emphasized in their study that size and surface area of silver nanoparticles have effect on hemolytic activity. Al_2O_3 NPs have been reported by certain researchers to be less hazardous than other metal-based nanoparticles (17). On the contrary, some researches were asserted that pure aluminum has long been known to be a potential neurotoxin (18). In their study of Kim et al. (2018) showed that after 28-day repeated dose exposure of Al_2O_3 NPs as an inhaler, aluminum contents were determined at the highest level in lung tissues and there was a dose-dependent relationship in the exposure groups (19). In our study, it was revealed that Al_2O_3 NPs with different sizes (48nm, 78nm and 100nm) did not show any hemolytic activity in the applied dose range.

HET-CAM test is an ideal alternative test that can be used as an intermediate step between *in vitro* and *in vivo* preclinical evaluation. It can be used to screen the anti-inflammatory potential, irritation properties and ocular toxicity of compounds, especially on nanoparticles for fast and reliable response. HET-CAM assay provides data not only on the effectiveness of nanoparticles but also on membrane irritation-vascular bleeding, lysis and coagulation with toxicity (5). Especially, in the literature, studies on the irritation effect in particle toxicity evaluations are very limited and there isn't any study that has been found in particular where the size-dependent irritation effect of Al_2O_3 NPs. In our study, for the first time, irritation effects of Al_2O_3 NPs were evaluated with an alternative test-HET-CAM assay. The study's findings showed that there was no irritant impact in any of the three size ranges. This shows that the particle does not have any toxic effect in all three size ranges on different type of cells. However, further tests as genotoxicity and in vivo evaluation are needed to confirm that the particle is biocompatible across the entire size range.

CONCLUSION

Aluminum nanoparticles (Al_2O_3 NPs) have different characteristics under varied physiological conditions. It needs to be tested on erythrocytes, taking into account morphology and aggregation tendency, and the mechanisms that may cause hemolysis and even cytotoxicity need to be fully elucidated. Based on the tests performed within the scope of the study, it was observed that Al_2O_3 NPs in all sizes were exhibit biocompatibility. However, further studies are needed to reveal how different physico-chemical properties as other than size of Al_2O_3 NPs affect their biological properties. Also, supporting *in vitro* data with *in vivo* experiments will contribute to the risk assessment profile and safety use of these materials.

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Conflict of interest: The author(s) declare that no conflict of interest.

References

1. U.S. Environmental Protection Agency Nanotechnology White Paper (2005) http://www.epa.gov/osa/pdfs/EPA_nanotechnology_white_paper_external_review_draft_12-02-2005.pdf
2. Sadiq IM, Pakrashi S, Chandrasekaran N. et al. Studies on toxicity of aluminum oxide (Al_2O_3) nanoparticles to microalgae species: Scenedesmus sp. and Chlorella sp. J Nanopart Res, 2011; 13: 3287–299.
3. Park EJ, Lee GH, Shim JH, Cho MH, Lee BS, Kim YB, Kim JH, Kim Y, Kim DW. Comparison of the toxicity of aluminum oxide nanorods with different aspect ratio. Arch Toxicol. 2015; 89(10):1771-82.
4. Oesterling E, Chopra N, Gavalas V, Arzuaga X, Lim EJ, Sultana R, Butterfield DA, Bachas L, Hennig B, Alumina nanoparticles induce expression of endothelial cell adhesion molecules. Toxicol Lett. 2008; 30;178(3):160-6.
5. Bakan B, Gülcemal S, Akgöl S, Hoet PH, & Yavaşoğlu NÜK. Synthesis, characterization and toxicity assessment of a new polymeric nanoparticle, l-glutamic acid-gp (HEMA). Chemico-Biological Interactions, 2020; 315: 108870.
6. ASTM standard practice F 756-00: Assessment of hemolytic properties of materials,2013.
7. ICCVAM, 2006, ICCVAM Test Method Evaluation Report: In Vitro Ocular Toxicity Methods for Identifying Severe Irritants and Corrosives. NIH Publication No: 07-4517. Research Triangle Park, NC: National Institute of Environmental Health Sciences.
8. Raj S, Kumar D. Biochemical Toxicology: Heavy Metals and Nanomaterials, 2020; 90928
9. Manke A, Wang L, Rojanasakul Y, Mechanisms of nanoparticle-induced oxidative stress and toxicity. Biomed Res Int. 2023; 942916.
10. Park EJ, Lee SJ, Lee GH, Kim DW, Yoon C, Lee BS, Kim Y, Chang J, Lee K, Comparison of subchronic immunotoxicity of four different types of aluminum-based nanoparticles. J Appl Toxicol. 2018; 38(4):575-84.
11. Alarifi S, Ali D, Alkahtani S. et al. Iron Oxide Nanoparticles Induce Oxidative Stress, DNA Damage, and Caspase Activation in the Human Breast Cancer Cell Line. Biol Trace Elem Res., 2014; 159:416–24.
12. Ganesan K, Wang Y, Gao F, Liu Q, Zhang C, Li P, Zhang J, Chen J, Targeting Engineered Nanoparticles for Breast Cancer Therapy. Pharmaceutics. 2021; 3(4), 276-83.
14. Murdock RC, Braydich-Stolle L, Schrand AM, Schlager JJ, Hussain SM. Characterization of nanomaterial dispersion in solution prior to in vitro exposure using dynamic light scattering technique. Toxicol Sci. 2008; 101(2):239-53.
15. Al-Gebory L, Mengüç MP. The effect of pH on particle agglomeration and optical properties of nanoparticle suspensions. Journal of Quantitative Spectroscopy and Radiative Transfer, 2018; 219, 46-60.
16. Choi J, Reipa V, Hitchins VM, Goering PL, Malinauskas RA. Physicochemical characterization and in vitro hemolysis evaluation of silver nanoparticles. Toxicol Sci. 2021; 123(1):133-43.
17. Demir E, Burgucu D, Turna F, Aksakal S, Kaya B. Determination of TiO_2, ZrO_2, and Al_2O_3 nanoparticles on genotoxic responses in humanperipheral blood lymphocytes and cultured embryonic kidney cells.J. Toxicol. Environ. Health 2013; 76: 990–1002.
18. Kumar V, Gill KD, Aluminium neurotoxicity: neuro-behavioural and oxidative aspects.Arch. Toxicol. 2009; 83: 965–78.
19. Kim YS, Chung YH, Seo DS, Choi HS, Lim CH, Twenty-Eight-Day Repeated Inhalation Toxicity Study of Aluminum Oxide Nanoparticles in Male Sprague-Dawley Rats. Toxicol Res. 2018; 34(4):343-54.

Research Article / *Araştırma Makalesi*

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Retrospective results of our non-invasive prenatal test (NIPT) experience

Non-invaziv prenatal test (NIPT) deneyimimize ait retrospektif sonuçlar

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ABSTRACT

Aim: Non-invasive prenatal test (NIPT) has become widespread over the years with higher probabilities of detection and fewer false positives with regard to traditionally used screening techniques. We aimed to document the experience of introducing this kind of equipment into clinical practice, evaluate its impact on the detection of fetal-aneuploidies, analyze the demographic characteristics of females undergoing 1.trimester fetal-aneuploidy screening testing with those choosing the NIPT, and assess elements influencing cfDNAfetal fraction.

Materials and Methods: Our research was designed as anobservational, retrospective research of 406 pregnant females who underwent fetal-aneuploidy screening in the course of pregnancy, from January 2019 to April 2023. Some patients had the 1.trimester fetal-aneuploidy screening test between 11-13.weeks, while another group of patients chose to undergo the NIPT at their own request. Any abnormalities in trisomy 13,18,21 were reported in the NIPT results. Maternal age, parity, history of miscarriage, presence of hypertension, fetal anomaly detected on ultrasound were questioned.

Results: The average age of females who chose the 1.trimester fetal-aneuploidy screening test was 31.17±4.00, and that of those who chose NIPT was 32.84±5.09, and it was seen to be significantly higher in the NIPTgroup ($p<0.01$). The history of miscarriage in patients undergoing NIPT was significantlyhigher with regard to the other group ($p=0.027$). The presence of pregestational diabetes mellitus and hypertension in patients who underwent NIPT was found to be significantly higher than the other group ($p=0.016$, $p=0.024$, respectively). Age and body mass index (BMI) have a statistically significant negative connection versus cfDNA fetal fraction ($p<0.01$, $r=-0.506$) ($p<0.01$, $r=-0.509$).

Conclusion: Our study showed that the area of prenatal aneuploidy screening was greatly impacted by the introduction of NIPT, which replaced the 1.trimester screening test and decreased the number of intrusive testing. Our findings may be used as a reference for prenatal treatment and can offer clinics useful information when integrating NIPT into the prenatal screening flow.

Keywords: Non-invasive prenatal testing, fetal aneuploidi, fetal screening testing, fetal trisomy.

ÖZ

Amaç: *Non-invaziv prenatal test (NIPT), geleneksel olarak kullanılan tarama yöntemlerinden daha üstün saptama oranları ve düşük yanlış pozitiflik oranlarıyla yıllar içinde yaygınlaşmıştır.*

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Çalışmamızda, bu teknolojinin fetal anöploidilerin saptanmasına yönelik yaklaşımlarımıza etkisini raporlamak, 1. Trimester fetal anöploidi taraması yapılanların özelliklerini, NIPT testini seçenlerle karşılaştırmak ve serbest DNA fetal fraksiyonunu etkileyen faktörleri değerlendirmek amaçlanmıştır.

Gereç ve Yöntem: *Çalışmamız, Ocak 2019-Nisan 2023 arasında prenatal dönemde fetal anöploidi taraması yapılan 406 gebeye ilişkin gözlemsel, retrospektif bir çalışma olarak tasarlandı. Hastaların bir bölümü 11-13.hafta arasında 1. trimester fetal anöploidi tarama testi yaptırmış olup, bir grup hasta ise kendi isteğiyle NIPT yaptırmıştır. NIPT sonuçlarında trizomi 13,18 ve 21 kromozomlarındaki olası bir anomali rapor edildi. Anne yaşı, gebelik sayısı, abortus öyküsü, hipertansiyon varlığı, ultrasonda saptanmış fetal anomali varlığı gibi demografik veriler çalışmada sorgulandı.*

Bulgular: *1. trimester fetal anöploidi taraması testini seçen kadınların ortalama yaşı 31,17±4,00, NIPT'i seçenlerin ise 32,84±5,09 olarak saptanmış olup, NIPT grubunda anlamlı yüksek saptanmıştır (p<0.01). NIPT yapılan hastalarda abortus öyküsü, diğer gruba göre anlamlı şeklide yüksek olduğu tespit edilmiştir (p=0.027). NIPT yapılan hastalarda pregestasyonal diabetes mellitus ve hipertansiyon varlığı, diğer gruba göre anlamlı yüksek saptanmıştır (p=0.016, p=0.024 sırasıyla). Yaş ve vücut kitle indeksi ile hücre dışı serbest DNA fetal fraksiyonunu arasında sırasıyla negatif yönlü bir ilişki saptanmıştır (p<0.01, r=-0,506) (p<0.01, r=-0,509).*

Sonuç: *NIPT uygulamasının, 1. trimester fetal anöploidi taraması testlerinin yerini alarak ve invaziv testleri azaltarak doğum öncesi anöploidi taraması alanını önemli ölçüde etkilediğini göstermiştir. Çalışmamız, NIPT'nin prenatal tarama akışına entegrasyonu sürecinde kliniklere pratik bilgiler sunabilir ve doğum öncesi bakımda referans bilgiler verebilir.*

Anahtar Sözcükler: *Non-invaziv prenatal tanı testi, fetal anöploidi, fetal tarama testi, fetal trizomi.*

INTRODUCTION

Prenatal diagnosis enables the molecular and biochemical detection of hereditary diseases, allowing for their identification through advanced methods (1,2). Simultaneously, it provides the opportunity for prenatal treatment, if possible, and the implementation of necessary postnatal measures. It also facilitates the option of terminating the pregnancy within the legal timeframe when deemed necessary (1, 2). The increasing prevalence of delayed marriages and childbirth in societies worldwide, parallel to cultural development, has led to a rise in pregnancies at advanced maternal ages, indicating a growing tendency (3, 4). The significant increase in pregnancies at advanced ages has raised concerns regarding the heightened risk of fetal aneuploidy such as trisomy 13,18 and 21 (5, 6). The methods used for screening fetal chromosomal abnormalities can be divided into two categories. Invasive methods encompass direct intervention techniques performed on the fetus and its appendages. These techniques include fetal biopsies, amniocentesis, chorionic villus sampling, and cordocentesis (7, 8). Non-invasive tests, on the other hand, include fetal ultrasonography (Nuchal Translucency measurement during the 11-13th week screening) and biochemical tests analyzed from maternal blood (first trimester screening-test (FTST), second-trimester screening test (STST) (9, 10). These tests are still widely used as standard procedures (9,10). However, since the commercial release of the non-invasive prenatal test (NIPT) on the basis of cell-free-DNA (cfDNA) sequencing and its rapid global proliferation, private clinics have started providing this high-performing device to expectant mothers. The NIPT test has shown a detection rate of over 99% for fetal aneuploidies with an approximately 0.1% false positive rate and a 0.2% false negative rate (11-14). In comparison, the FTST has a 5% false positive probability and a detection probability of 95% (15, 16). However, recent discussions on the effectiveness of the test have arisen, suggesting that the false negative rates of the FTST and NIPT are comparable. This is because approximately 4% of patients undergoing NIPT have a low fetal fraction, which increases the false negative result risk (17). In our study, we retrospectively evaluated our experiences with patients who underwent NIPT at our hospital and aimed to report its impact on the detection of fetal aneuploidies in accordance with our current approaches. We also aimed to make an evaluation regarding clinically significant factors that influence the cfDNA-fetal fraction in NIPT.

MATERIALS and METHODS

The present study was designed as a retrospective observational study. The Helsinki-Declaration's Principles were followed in the composition of this research. Informed consent documents were received from all patients. The ethics committee approval numbered 2023/200 was obtained from the ethics committee. This is a retrospective observational study covering a total of 406 pregnant females who underwent fetal aneuploidy screening during January 2019 to April 2023 in a tertiary hospital. All patients in our study received information about the availability and limitations of FTST and NIPT, as well as their utilization in the medical field, during the visit before the 11th week of pregnancy. Some opted for FTST, while others voluntarily requested NIPT. Pregnant females were advised that if increased nuchal translucency (more than the 99th percentile) was observed in the 11th and 13th-week ultrasound, invasive tests could be considered instead of NIPT. Risk assessments for trisomy 21, 18 and 13 were included in the NIPT result reports. In accordance with legal procedures in our country, reporting fetal gender is permissible only in cases where abnormalities are detected. Since no abnormalities were identified in the gender chromosome, results regarding fetal gender were not disclosed. In case of positive results, as previously explained, for karyotyping, amniocentesis or chorionic-villus sampling was recommended. Retrospective queries were used to get data regarding maternal age, first trimester body mass index (BMI) value, parity, history of preterm birth, history of miscarriage, presence of pregestational diabetes mellitus, presence of pregestational hypertension, detection of fetal anomalies on ultrasound, and additional pertinent data from patient records and the hospital database.

Statistical analysis

The analyses were conducted with the SPSSx26.0 (IBM-Inc. Chicago, IL, USA). Normality analysis was conducted using the Kolmogorov-Smirnov-test. The quantitative data of the patients were reported as mean ± Standard-Deviation (SD). The Chi-Square test was employed to assess the categorical data and results were presented as counts and percentages (%). Pearson correlation test was used to determine correlations between variables. There was a 95% Confidence Interval (CI) used to analyze the results. The p-value, which was less than 0.05, was accepted as statistically significant.

RESULTS

During the examined four-year period, 406 females in total had either FTST or NIPT. Among these 406 females, while 269 females (66.3%) have chosen FTST as the primary serum screening technique, 137 females (33.7%) opted for NIPT. Among the females subjected to FTST, negative results were obtained in 92.9% (250/269), with 19 females identified as having a high risk of fetal aneuploidies.

Females at high risk established with FTST, 47.3% chose NIPT as the second screening method, while 36.8% underwent amniocentesis. 15.7% of females with high risk either refused further testing or discontinued follow-up. Of the patients who underwent amniocentesis, it was noted that 85.7% had a normal karyotype, while trisomy 21 was detected in 14.3% of cases. For the 9 females who had NIPT as the 2nd screening technique, all NIPT tests came back negative. Among the 137 females who chose NIPT as the main screening technique, in 130 patients (94.9%) negative results were obtained, positive results in 5 patients (3.7%), and in 2 patients (1.4%), results were deferred due to insufficient cfDNA fetal fractions despite repeated testing. Of the 5 patients with positive NIPT results, 4 (80%) were determined to have high-risk for trisomy 21, and 1 patient was determined to have high-risk due to sex chromosome abnormalities. Amniocentesis was recommended for the 4 patients with a high-risk of trisomy 21 based on NIPT results. Three patients accepted amniocentesis, and trisomy 21 was confirmed in all cases. The one patient who declined amniocentesis was found to have trisomy 21 in the newborn following giving birth. In a case where NIPT yielded a positive result for sex chromosome abnormalities, amniocentesis revealed a normal karyotype. For pregnancies in which NIPT was performed during the prenatal period and negative results were obtained, chromosomal analysis conducted due to suspected physical findings in two infants after birth revealed trisomy 21 in both cases, resulting in a false-negative rate of 1.4%. In the 137 females who underwent NIPT, the observed average cfDNA fetal fraction was 10.60 ± 3.85 (range 2% – 20%). During the four-year period, out of 137 NIPT tests conducted, results were determined as deferred in 8 cases because of cfDNA fetal fraction being under 5%. After repeat sampling in these 8 cases, negative results were obtained in 6 patients, while in 2 cases, results were deferred again because of insufficient cfDNA fetal fraction even following further samples. No abnormalities were detected in

pregnancies with insufficient cfDNA fetal fraction (Figure-1).

The mean age of females choosing first trimester screening test was 31.17±4.00, while those choosing NIPT was 32.84±5.09, and age was significantly higher in the NIPT group (p<0.01). The primigravida rate of females who chose the 1st trimester-screening-test was 62.5%, and the primigravida rate of those who opted NIPT was 51.9%, and the rate was seen to be significantly lower in the NIPT group (p=0.032). The miscarriage rate of females who chose the 1st trimester-screening test was 23.8%, and the miscarriage rate of those who chose NIPT was 35%, and the rate was seen to be significantly higher in the NIPT group (p=0.027). The presence of pregestational diabetes mellitus and pregestational hypertension in patients who underwent NIPT was seen to be significantly higher than the other group (p=0.016, p=0.024, respectively) (Table-1).

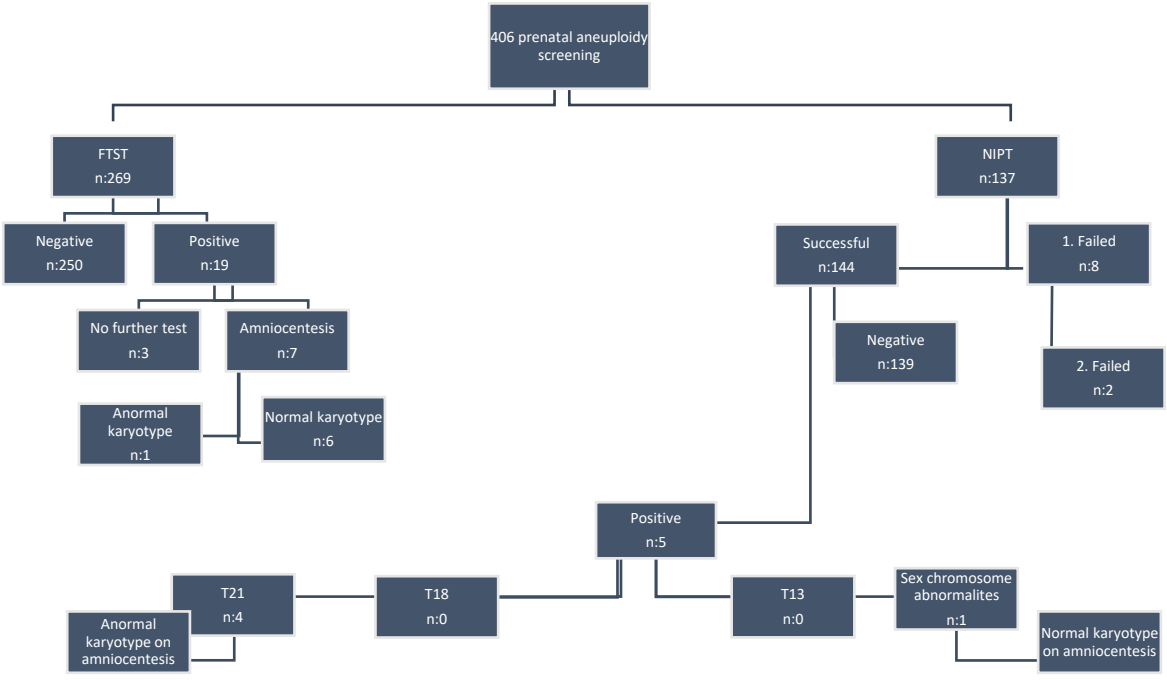

Figure-1. Outcomes from the diagnostic and Maternal serum screening tests

* NIPT: Non-invasive-prenatal test, FTST: First trimester screening test

Table-1. Descriptive statistics of demographics and pregnancy related variables between two groups.

		FTST n-% 269 (66.3%)	**NIPT n-% 137 (33.7%)**	***p*-value**
Maternal Age (year)	<30	66 (24.5%)	23 (16,8%)	<0.01*
	30-35	157 (58.4%)	66 (48,2%)	
	36-40	41 (15.2%)	31 (22.6%)	
	>40	5 (1.9%)	17 (12.4%)	
	Mean±SD	31.17±4.00	32.84±5.09	<0.01**
Primigravid		168 (62.5%)	71 (51.9%)	0.032*
Multi-fetal Pregnancy		15 (5.6%)	4 (2.9%)	0.322*
History of preterm birth		25 (25.8%)	14 (13.2%)	0.859*
History of miscarriage		64 (23.8%)	48 (35%)	0.027*
Pregestational Diabetes mellitus		3 (1.1%)	10 (7.3%)	0.016*
Pregestational Hypertension		2 (0.7%)	5 (3.6%)	0.024*
Fetal structural abnormality		4 (1.5%)	5 (3.6%)	0.172*

** :Two-sample t-test, * :Chi-squared test

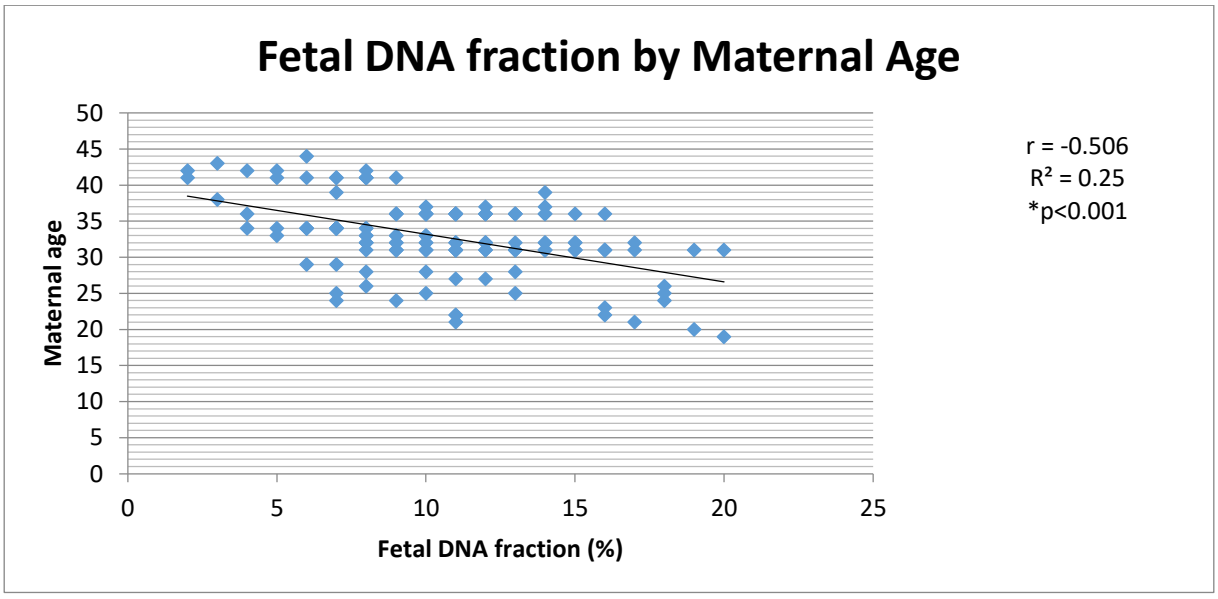

Figure-2. Fetal DNA fraction by maternal age.

*: Pearson correlation test

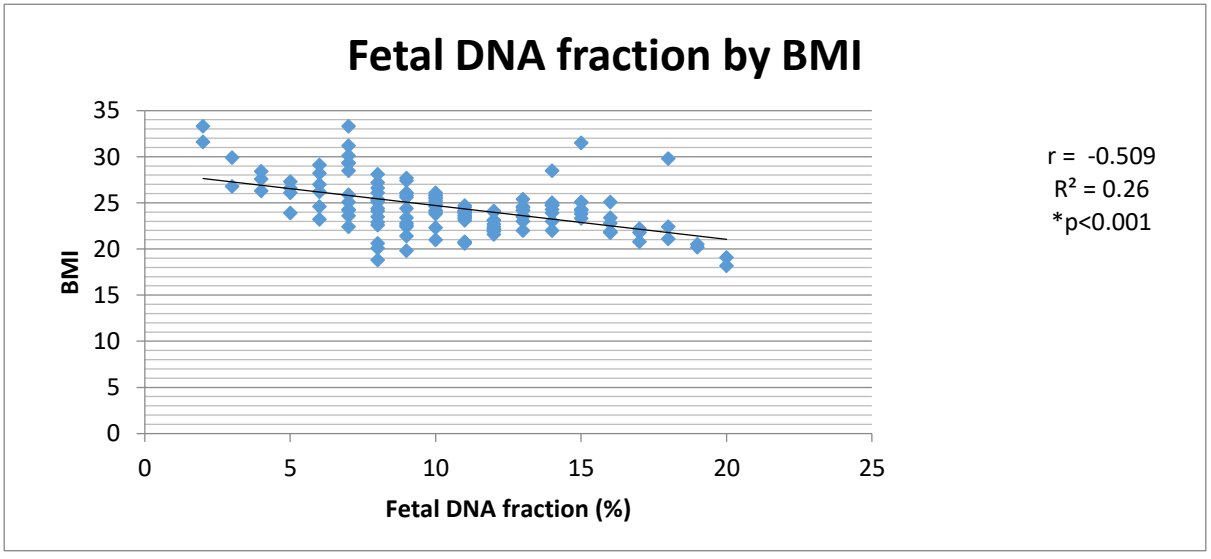

Figure-3. Fetal DNA fraction by BMI.

*: Pearson correlation test

A negative and statistically significant correlation was seen across age and cfDNA fetal-fraction ($p<0.01$, $r=-0.506$). According to regression analysis, the R2 value is 0.25. Therefore, 25% of the variance in fetal DNA fraction percentage is explained by age ($p<0.01$) (Figure 2).

The cfDNA fetal-fraction and BMI have a statistically significant negative connection ($p<0.01$, $r=-0.509$). According to regression analysis, the R2 value is 0.26. Therefore, 26% of the variance in fetal DNA fraction percentage is explained by BMI ($p<0.01$) (Figure 3).

DISCUSSION

Comparative analyses revealed that the Patients in the NIPT group were older and more had multiple fetal pregnancies. Additionally, the initial screening negative rate for fetal aneuploidy was 92.9% in the FTST group and 94.9% in the NIPT group, indicating similar rates. In the FTST group, the screening positive rate was 7.1%, while in the NIPT group, it was 3.7%, demonstrating a higher positivity rate in the group undergoing FTST. In NIPT, insufficient cfDNA fetal-fraction rates were found to be 5.6% in the first screening and 1.4%

in the second screening. It was observed that females who had previously had abortions preferred to select NIPT as the primary serum screening method instead of the combined test. Perhaps the most significant advantage of NIPT, compared to traditional serum screening methods, is its capacity to cut down on the quantity of invasive diagnostic procedures. The study's comparative analysis of demographic characteristics between the NIPT group and the combined test group revealed differences in terms of maternal age, history of pregnancy and abortion, presence of diabetes mellitus, and presence of hypertension. However, parameters related to fetal structural anomalies and multi-fetal pregnancies were similar between the groups. It is noteworthy that patients with a history of previous miscarriage opt for NIPT over the combined test. Further research is needed to investigate the precise reasons for choosing NIPT as the primary serum screening method. However, the great efficacy of NIPT, which is distinguished by a decreased false-positive rate in comparison to conventional screening tests, is thought to confer upon them the opportunity to alleviate concerns associated with false positives. The potential of negative results from NIPT because of inadequate cfDNA fetal fraction is one of the primary fears. In our study, the failure rate due to insufficient cfDNA fetal fraction was found to be high at 5.6%, compared to previously published series. This rate was higher than what is reported in the literature. In routine practice, both the ACOG and the ACMG suggest evaluating invasive diagnostic tests for individuals with low cfDNA fetal fraction in NIPT tests (18,19). It is known that in approximately half of the patients with test failures, the issue can be fixed by obtaining a 2nd sample afterwards. In this current study, interpretable results were obtained in 75% of females with insufficient cfDNA fetal fraction in the initial sampling after repeat sampling. There is limited knowledge about clinical and biological elements affecting this parameter, aside from gestational age and maternal weight (20). In this present research, a negative relation is detected between cfDNA fetal-fraction and weight and Maternal age. Cases with low cfDNA fetal fraction have been mostly omitted from several prior research looking at NIPT, and unable to obtain results. However, studies have reported an association between low cfDNA fetal fraction and increased aneuploidy risk (21,22). Therefore, When NIPT performance is analyzed, excluding a low cfDNA fetal percentage may lead to an overestimation of the fetal aneuploidy detection rate. To resolve the issue of the relation between low cfDNA fetal-fraction and fetal aneuploidy risk, further advanced studies with larger sample sizes are needed. In our study, 33.7% of all patients chose NIPT as the primary screening method. This rate is higher compared to the NIPT application rates reported in other studies in the literature (23). The high preference for NIPT over the combined test observed in this study may be attributed to the characteristics of tertiary care clinics where there is a higher prevalence of high-risk pregnancies. Additionally, this may be influenced by the patients' comparatively high socioeconomic class at our facility, which is situated in one of the most urbanized areas. The undeniable great performance and efficacy of NIPT notwithstanding, the procedure of integrating this test into real-world clinical settings requires further investigation and should be determined with cautious evaluation. At the moment, a number of recommendations, such as the ACOG's December 2012 Guidelines, state that low-risk ladies should not be provided NIPT (24). It is important to carefully choose the target group for this new screening technique while considering strong evidence from recently created guidelines. Generalizing the results of this study to the overall population is challenging. Firstly, the obtained results were derived from a tertiary medical center where the prevalence of high-risk-pregnancies is high. Secondly, a retrospective research design was employed, limiting our capability to identify the specific elements that influenced individual decisions regarding a particular test. As various elements, such as clinical conditions, economic status, and previous awareness of NIPT can influence the choices made by patients, and previous awareness of NIPT may affect the choices made by patients, future research should evaluate these aspects.

CONCLUSION

The present study outcomes indicate that the implementation of NIPT significantly impacts the field of prenatal aneuploidy screening by potentially swapping out combined tests and reducing invasive tests. Our research may provide practical insights to clinics and hospitals in the procedure of integrating NIPT into prenatal screening workflows and contribute valuable reference information to prenatal care.

Conflict of interest: The author(s) declare that there is no conflict of interest.

References

1. Yüreğir ÖÖ, Büyükkurt S, Koç F, Pazarbaşı A. Prenatal (Doğum Öncesi) Tanı. Aktd. 2012; 21(1).
2. Nussbaum RL MR, Willard HF. Principles of Clinical Cytogenetics Thompson and Thompson Genetics, in Medicine Sixth Edition. Philadelphia: W.B. Saunders Company.2001;307-8.
3. (KOSIS) KSIS. Basic demographic information of the nation. In: WaFA Moh, editor. Seoul, South Korea. 2016.
4. Bianchi DW, Chiu RWK. Sequencing of Circulating Cell-free DNA during Pregnancy. N Engl J Med. 2018 Aug 2;379(5):464-473. doi: 10.1056/NEJMra1705345. PMID: 30067923; PMCID: PMC10123508.
5. (KOSIS) KSIS. Prevalence of congenital anomalies in newborns. In: WaFA Moh, editor. Seoul, South Korea. 2006.
6. Oepkes D, Page-Christiaens GC, Bax CJ, Bekker MN, Bilardo CM, Boon EM, et al. Trial by Dutch laboratories for evaluation of non-invasive prenatal testing. Part I-clinical impact. Prenat Diagn. 2016; 36(12): 1083-90.
7. Balkan M, Erdemoğlu M, Alp MN, Budak T. Patau Sendromlu Bir Prenatal Tanı Olgu Sunumu. Diclemedj. Haziran 2008;35(2):145-148.
8. Yenilmez ED, Tuli A. İnvaziv Olmayan Bir Prenatal Tanı Yöntemi; Maternal Plazmadaki Serbest Fetal DNA. Arşiv Kaynak Tarama Dergisi. 2013; 22(3):317-34.
9. Fuchs F, Riis P. Antenatal sex determination. Nature.1956; 18(177).
10. Antonarakis SE, Skotko BG, Rafii MS., et al. Down syndrome. Nat Rev Dis Primers. 2020; 6(9).
11. Norton ME, Brar H, Weiss J, Karimi A, Laurent LC, Caughey AB, et al. Non-Invasive Chromosomal Evaluation (NICE) Study: results of a multicenter prospective cohort study for detection of fetal trisomy 21 and trisomy 18. Am J Obstet Gynecol. 2012; 207(2).
12. Nicolaides KH, Syngelaki A, Ashoor G, Birdir C, Touzet G. Noninvasive prenatal testing for fetal trisomies in a routinely screened first-trimester population. Am J Obstet Gynecol. 2012; 207(5).
13. Ashoor G, Syngelaki A, Wagner M, Birdir C, Nicolaides KH. Chromosome-selective sequencing of maternal plasma cell-free DNA for first-trimester detection of trisomy 21 and trisomy 18. Am J Obstet Gynecol. 2012; 206(4).
14. Van-Opstal D, Srebniak MI, Polak J, de-Vries F, Govaerts LC, Joosten M, et al. False Negative NIPT Results: Risk Figures for Chromosomes 13, 18 and 21 Based on Chorionic Villi Results in 5967 Cases and Literature Review. PLoS One. 2016; 11(1).
15. Malone FD, Canick JA, Ball RH, Nyberg DA, Comstock CH, Bukowski R, et al. First-trimester or second-trimester screening, or both, for Down's syndrome. N Engl J Med. 2005; 353(19): 2001-11.
16. Nicolaides KH. Screening for fetal aneuploidies at 11 to 13 weeks. Prenat Diagn. 2011; 31(1): 7-15.
17. Norton ME, Baer RJ, Wapner RJ, Kuppermann M, Jelliffe-Pawlowski LL, Currier RJ. Cell-free DNA vs sequential screening for the detection of fetal chromosomal abnormalities. Am J Obstet Gynecol. 2016; 214(6): 727.
18. Committee Opinion No. 640: Cell-Free DNA Screening for Fetal Aneuploidy. Obstet Gynecol. 2015; 126(3).
19. Gregg AR, Skotko BG, Benkendorf JL, Monaghan KG, Bajaj K, Best RG, et al. Noninvasive prenatal screening for fetal aneuploidy, 2016 update: a position statement of the American College of Medical Genetics and Genomics. Genet Med. 2016; 18(10): 1056-65.
20. Wang E, Batey A, Struble C, Musci T, Song K, Oliphant A. Gestational age and maternal weight effects on fetal cell-free DNA in maternal plasma. Prenat Diagn. 2013; 33(7): 662-6.
21. Norton ME, Jacobsson B, Swamy GK, Laurent LC, Ranzini AC, Brar H, et al. Cell-free DNA analysis for noninvasive examination of trisomy. N Engl J Med. 2015; 372(17): 1589-97.
22. Palomaki GE, Kloza EM, Lambert-Messerlian GM, van den Boom D, Ehrich M, Deciu C, et al. Circulating cell free DNA testing: are some test failures informative? Prenat Diagn. 2015; 35(3): 289-93.
23. Platt LD, Janicki MB, Prosen T, Goldberg JD, Adashek J, Figueroa R, et al. Impact of noninvasive prenatal testing in regionally dispersed medical centers in the United States. Am J Obstet Gynecol. 2014; 211(4).
24. Committee Opinion No. 545: Noninvasive prenatal testing for fetal aneuploidy. Obstet Gynecol. 2012; 120(6): 1532-4.

Research Article / *Araştırma Makalesi*

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Social and clinical reasons in patients who undergo labiaplasty surgery

Labiaplasti ameliyatı yapılan hastalarda sosyal ve klinik nedenler

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ABSTRACT

Aim: Although an increasing number of women in developed societies prefer genital plastic surgeries, the most common procedure is considered to be labiaplasty. The aim of this study is to reveal the social and clinical factors that cause the decision to operate in women undergoing labiaplasty.

Materials and Methods: In our study, 189 patients who had labia minora reduction surgery in our hospital between April 2018 - 2023 were evaluated retrospectively. The social and clinical factors that caused all patients included in the study to request surgery from the hospital database from their patient files were evaluated retrospectively.

Results: Patients participating in the study; Patients who underwent surgery for aesthetic reasons, aesthetic + functional reasons and psychological reasons were evaluated in 3 separate groups, and no significant difference was found in the average age between the groups (p=0.914). In our study, among the patients without a history of coitus, 16 (88.9%) patients were operated for individual reasons and 2 (11.1%) patients were operated for environmental reasons. The rate of operations performed for individual reasons in patients without a history of coitus was found to be significantly higher than the rate of operations performed for environmental reasons (p<0.001).

Conclusion: Women who undergo surgical intervention do not always do so for individual reasons. Therefore, it is necessary to make a comprehensive evaluation before considering labiaplasty surgery. A clear understanding of labiaplasty patients' motivations and expectations will facilitate better patient decision-making and increase the patient's likelihood of satisfaction with the result.

Keywords: Genital aesthetics, labiaplasty, body dysmorphic disorder.

ÖZ

Amaç: *Gelişmiş toplumlarda giderek artan sayıda kadın, genital estetik ameliyatları tercih etmekle birlikte en yaygın olan prosedür labiaplasti olarak kabul edilmektedir. Bu çalışmanın amacı labiaplasti operasyonu geçiren kadınlarda operasyon kararına neden olan sosyal ve klinik faktörleri ortaya koymaktır.*

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Gereç ve yöntem: *Çalışmamızda Nisan 2018 - 2023 döneminde hastanemizde labia minör küçültme ameliyatı olan 189 hasta retrospektif olarak değerlendirildi. Araştırmaya dahil edilen tüm hastaların hasta dosyalarından hastane veritabanından ameliyatı talep etmelerine neden olan sosyal ve klinik faktörler retrospektif olarak değerlendirildi.*

Bulgular: *Çalışmaya katılan hastalar; estetik nedenler, estetik + işlevsel nedenler ve psikolojik nedenlerle ameliyat olan hastalar olarak 3 ayrı grupta değerlendirilmiş olup, gruplar arasında yaş ortalaması olarak anlamlı fark saptanmamıştır (p=0.914). Çalışmamızda koitus öyküsü olmayan hastalardan 16 (%88.9) hasta bireysel nedenler, 2 (%11.1) hasta çevresel nedenlerden dolayı opere olmuştur. Koitus öyküsü olmayan hastalarda bireysel nedenlerle yapılan operasyon oranı, çevresel nedenlerle yapılan operasyon oranına göre anlamlı yüksek saptanmıştır (p<0.001)*

Sonuç: *Cerrahi müdahale geçiren kadınlar bunu her zaman kişisel nedenlerden dolayı yapmamaktadır. Bu nedenle labiaplasti operasyonunu düşünmeden önce kapsamlı bir değerlendirme yapmak gerekir. Labiaplasti hastalarının motivasyonlarının ve beklentilerinin net olarak anlaşılması, hastanın daha iyi karar vermesini kolaylaştıracak ve hastanın sonuçtan memnuniyet olasılığını arttıracaktır.*

Anahtar Sözcükler: *Genital estetik, labiaplasti, vücut dismorfik bozukluk.*

INTRODUCTION

Although an increasing number of women prefer genital aesthetic surgeries in developed societies, the most common procedure is labiaplasty (1, 2). Hypertrophy and/or asymmetry of the inner lips may cause functional, aesthetic, and/or psychosocial concerns in some adolescents and adult women (3). Labiaplasty is conducted particularly with aesthetic motives and is frequently introduced as a technique used to improve female's physical appearance (4-6). However, patients who have unilateral or bilateral labia minora enlargement may express complaints of discomfort, inflammation, dyspareunia, improper personal hygiene throughout the menstruation, and difficulties using a self-urinating catheter (7, 8). Many females who have asymmetry or hypertrophy of the labia minora also report higher self-awareness, anxiety or embarrassment, or increased sensations of abnormality when wearing tight clothing or in sexual settings (5, 9). This has led to increased demands for plastic surgery of the inner lips (4-11). Labiaplasty can improve the appearance of the labia in many women and additionally offer functional and psychological advantages (4–11). Labiaplasty is a cosmetic procedure that is becoming more and more common in our country. The study conducted by Herbenick et al. revealed that women who want to have labiaplasty are affected not only by functional concerns but also by external factors (12). A study that was conducted among university-graduate women reported that 4.2% of women experienced psychological problems including negative body image, anxiety, and loss of self-confidence (12). This is the reason why a thorough assessment is important for women who request labiaplasty before proceeding with the surgery. The purpose of this current investigation was to uncover the social and clinical factors that cause the decision to operate in women undergoing labiaplasty.

MATERIALS and METHODS

The present study was designed in a retrospective observational design following the principles of Helsinki declaration. Informed consent documents were received from all patients and the study was started after receiving ethics committee approval (2024/259) from our hospital's Ethics Committee. A total of 189 individuals who had labia minora reduction surgery in our hospital from April 2018 to 2023 were evaluated retrospectively in the present study. The social and clinical factors that caused the individuals accepted in the research to request surgery were evaluated retrospectively from the clinic database and patient files. The age, education level, and lifetime sexual partners of all patients were questioned. Their requests for surgery were categorized into 3 groups as aesthetic reasons, psychological reasons, and aesthetic + functional reasons. Aesthetic reasons were defined as lips being larger than normal and/or asymmetrical. Functional causes were defined as patients who described pain during intercourse and frequent vulvovaginitis symptoms. Psychological reasons were defined as a lack of self-confidence during sexual intercourse. The patients were asked whether the reason for the surgery was because of their own

needs or whether they were affected by other people including sexual partners, relatives, close companions, or the press. The relationship between the demographic data and the factors causing the surgery was evaluated.

Statistical Analysis

SPSS 26.0 (IBM Inc., Chicago, IL, USA) was employed for the statistical analysis. The normality of the distribution was evaluated with the Kolmogorov-Smirnov Test. The Mann-Whitney U-Test was utilized to analyze the not normally distributed parameters, and the Chi-Square and Fisher's Exact Tests were used in the analysis of the categorical data. The One-Way ANOVA Test was employed to test whether there were statistically significant differences between the averages of the independent groups. The quantitative data of the patients were given as Mean±Standard Deviation (SD). The qualitative data were presented as percentages (%) and numbers. The scores were considered at a 95% Confidence Interval (CI). The p-value was regarded statistically significant when it was <0.05.

RESULTS

The average age of the participants in the research was 30.7±7.4 years. Those who participated in the study were evaluated in 3 separate groups as those who underwent surgery for aesthetic reasons, aesthetic + functional reasons, and psychological reasons. No significant difference was found in the average age across the groups (p=0.914). The patients who participated in the study were evaluated in two separate groups as those who underwent surgery for individual reasons and environmental reasons. No significant difference was detected in the average age across the groups (p=0.085) (Table-1).

Table-1. Evaluation of the relationship between the reason for the operation and age.

Reasons for Surgery	Age Mean±SD	p
Aesthetic reasons	30.9±7	
Aesthetic + Functional reasons	30.7±7.3	0.914
Psychological reasons	30.2±7.2	
Individual reasons	29.8±6.9	0.085
Environmental reasons	31.6±7.3	

Table-2. Assessment of the relationship between the reason for the surgery and the presence of coitus.

Reasons for Surgery	Coitus (+) n - (%)	Coitus (-) n - (%)	Total n- (%)	p
Aesthetic reasons	49-(28.7%)	5-(27.8%)	54-(28.6%)	
Aesthetic + Functional reasons	99-(57.9%)	8-(44.4%)	107-(56.6%)	0.249
Psychological reasons	23-(13.5%)	5-(27.8%)	28-(14.8%)	
Individual reasons	83-(48.5%)	16-(88.9%)	99-(52.4%)	**<0.001**
Environmental reasons	88-(51.5%)	2-(11.1%)	90-(47.6%)	

Table-3. The assessment of the relationship between the reasons for the surgery and the level of education.

Reasons for Surgery	University n - (%)	High school n - (%)	Total n - (%)	p
Aesthetic Reasons	26-(34.7%)	28-(24.6%)	54-(28.6%)	
Aesthetic + Functional Reasons	33-(44%)	74-(64.9%)	107-(56.6%)	
Psychological Reasons	16-(21.3%)	12-(10.5%)	28-(14.8%)	0.013
Individual	34-(45.3%)	65-(57%)	99-(52.4%)	
Environmental	41-(54.7%)	49-(43%)	90-(47.6%)	0.077

Among the patients who had a history of coitus, 49 (28.7%) were operated on for aesthetic reasons, 99 (57.9%) were operated on for aesthetic + functional reasons, and 23 (13.5%) were operated on for psychological reasons in the present study. Among those who did not have a history of coitus, 5 (27.8%) were operated on for aesthetic reasons, 8 (44.4%) were operated on for aesthetic + functional reasons, and 5 (27.8%) were operated on for psychological reasons. No significant differences were detected in terms of the reasons for surgery between the groups (p=0.249). Among those who had a history of coitus, 83 (48.5%) patients were operated on for individual reasons, and 88 (51.5%) were operated on for environmental reasons. Among those who did not have a history of coitus, 16 (88.9%) patients were operated on for individual reasons and 2 (11.1%) were operated on for environmental reasons. In patients who did not have a history of coitus, the ratio of surgeries conducted for individual reasons was significantly greater than the rate of surgeries performed for environmental reasons (p<0.001) (Table-2).

Among the university-graduate patients, 26 (34.7%) patients were operated on for aesthetic reasons, 33 (44%) were operated on for aesthetic + functional reasons, and 16 (21.3%) were operated on for psychological reasons. Among those who were high school graduates, 28 (24.6%) patients were operated on for aesthetic reasons, 74 (64.9%) were operated on for aesthetic + functional reasons, and 12 (10.5%) were operated on for psychological reasons. The rate of patients who underwent surgery for aesthetic reasons was found to be significantly higher in the university graduate group (p=0.013). Among the university graduate patients, 34 (45.3%) patients were operated on for individual reasons and 41 (54.7%) were operated on for environmental reasons. Among the high school graduates, 65 (57%) patients were operated on for individual reasons and 49 (43%) were operated on because of environmental reasons. There was no significant difference in the reasons for surgery between the groups in terms of individual and environmental factors. (p=0.077) (Table-3).

DISCUSSION

As far as we found, this current study is the first research investigating the motives for undergoing labiaplasty in Turkey. A variety of elements can lead to Labia hypertrophy (e.g., congenital conditions such as labia asymmetry, repetitive pulling, or infection). The results of the present study demonstrate that the main reason why our participants resorted to labiaplasty surgery was both functional and aesthetic concerns, followed by functional reasons and psychological concerns, respectively. It has been revealed that women who describe their labiaplasty experiences request surgery because of media influence, negative comments and experiences, and physical and functional dysfunctions. Rouzier et al. reported in their study that most patients applied for surgery because of aesthetic complaints, followed by discomfort during clothing and exercise and dyspareunia (5). In a prospective research with 33 patients with psychological or physical problems, Crouch et al. discovered that the majority of the complaints had to do with functional pain or appearance (2). 4.2% of participants, according to Herbenick et al., experienced psychological issues including anxiety, low self-esteem, and unfavorable body image. The majority of individuals in this current investigation were highly educated and 81.4% were university graduate women (12). However, Herbenick et al. thought that there was no correlation between female genital self-image and educational background. (12). In the present study, 39.6% of the patients were university graduate women. Sharp et al. reported that the most commonly cited reason for surgery was the look of the labia, and relationship status had a significant influence on women's decisions to have surgery (13). Similarly, it was shown that 90.4% of the women in the present study had sexual partners, and 12.6% of the women who had sexual partners were affected by their partners. Furthermore, Sharp et al. reported that these ladies shied away from love connections and they were anxious because of their sex companions' reactions to their labia's appearance. In Veale et al.'s research, most of women stated that they decided to undergo surgery, usually because of a negative experience with a former sexual partner (10). Although some comments may be considered objective, it is clear that some of them were misinterpreted (10). These negative comments regarding lip appearance seem to cause emotional distress resulting in behaviors including not using tight clothes and/or swimming suits and staying away from studies on medicine

such as smear tests (4). Bramwell et al. performed a retrospective-qualitative investigation to analyze the expectations and experiences of 6 women who underwent labiaplasty surgery and reported that Every woman firmly followed societal norms and believed that their genital look was "weird" (14). Ackard et al. published the findings of a survey on sexual activity, body image, and self-image that was answered by 3627 women. They discovered that greater levels of sexual pleasure were linked to positive self- and body images (15). Nevertheless, not much study has been done on their relations, for this reason, it is possible to speculate that women are less likely to experience sexual pleasure if they perceive dissatisfaction with their bodies (15). For this reason, possessing a lovely or "standard" vulva could help to experience better results (15). In the present study, 47.6% of the patients who underwent labiaplasty were operated because of environmental factors. As the reason for surgery, the rate of psychological problems accounted for only 21.3% of the patients; however, all these psychological problems were affected by external elements, which shows that sexual partners and the media affect women's motivations for surgery. The effects of media on aesthetic understanding and culture are undeniable. However, it must be emphasized that the vulva does not have a standard appearance. The media's exposure to photographs of female genitalia makes people more conscious of their looks. The internet and advertising may conjure up images of a "normal" genital look, misguiding women into thinking that surgery is necessary to get the desired appearance. The majority of the patients in this research were young women, with an average age of 30.7±7.4. Also, 21.1% of the patients reported that they were influenced by the media. Many studies are reporting that the reason for the rising demand for labiaplasty is the media (13, 15). However, there are also several studies reporting the influence of online photos on females' decisions to have labiaplasties (16, 17). In the present study, consistent with the literature data, patients reported that their primary source of knowledge on labiaplasty was the internet. In their study, Markey et al. examined the motivations of young females' desire for plastic surgery and came to the conclusion that body dissatisfaction played a role in the aesthetic desire and that individuals had a higher propensity to internalize media messages on issues with physical appearance (18). According to Sharp et al., the assessment of labiaplasty was impacted by media exposure and contextual factors that affect genital appearance dissatisfaction. But because this study was carried out in a social setting, the results may have been influenced by the sociocultural context (17). Prior research indicates that reasons for having a labiaplasty might stem from both environmental factors and personal desires. In their study, Lowenstein et al. suggested that before considering labiaplasty, their patients should be sent for a consultation with a psychologist or psychiatrist for a thorough examination (19). Additionally, they noted that females who thought that having a labiaplasty surgery would boost their self-esteem may be more likely to feel self-conscious about their vulval look if they were told inaccurate information regarding certain morphological traits (19).

Also, the Cosmetic Surgery Committee of the International Society for the Study of Vulvovaginal Disease (ISSVD) recommended that body dysmorphic disorder must be considered and that surgeons should seek a multidisciplinary expert opinion in cases where they were unsure (19). Another theme that emerged was about women's reasons for having labiaplasty. Debates over the importance of labiaplasty get a lot of interest from the scientific and medical communities as well as the general public (20). Females who participated in many previous studies seemed to be aware of this topic and showed a lot of effort in voicing their reasons for undergoing labiaplasty. As reported in some previous studies, almost all women who were interviewed reported that they had physical and aesthetic concerns about the inner lips (2, 13). In Gimlin et al.'s interview study conducted with patients who underwent labiaplasty, women expressed their aesthetic concerns to a lesser extent because they thought that their decision to have labiaplasty might be considered superficial or shallow by others (21). In the present study, unlike the results of this study, patients freely expressed their desire for surgery because of aesthetic concerns, and the rate of surgery performed for aesthetic concerns was significantly higher when compared to the literature data. In the research conducted by Bramwell et al., some women emphasized their physical symptoms more than their appearance concerns when they described their discussions

with their doctors (14). This is particularly valid for females who access labiaplasty through a public healthcare system (14, 22). This distinction could not be made in our study because the present study was a multicenter study based on both public and private hospitals. The women contacted for this study usually showed high levels of satisfaction with the look and functionality of their inner lips following surgery, as has been observed in earlier studies on labiaplasty (23, 24). Additionally, this change in how they felt about their genitalia demonstrated a rise in general self-assurance and self-worth, which is in line with qualitative research on other forms of plastic surgery (25, 26). Though they were pleased with the enhancement in their genital look, over half of the ladies who participated in the interviews stated that it was not as "perfect" as they had hoped. It was evident from the descriptions provided that these ladies thought their inner lips should look perfect, much like the "after" pictures of labiaplasty they read on doctors' websites. While our interviews with these individuals did not expressly address body dysmorphic disorder, research indicates that some women who are considering labiaplasty may be suffering from this condition. When women seek labiaplasty, clinicians must check for signs of body dysmorphic disorder (24). After receiving cosmetic procedures, the majority of people with body dysmorphic disorder reported no improvement or deterioration of their symptoms (27, 28).

CONCLUSION

In Turkey, females are having more and more labiaplasties. Many patients are motivated by their sexual partners and the media, and many want surgery for both functional and cosmetic reasons. Not every woman who undergoes surgery does this for merely individual reasons. For this reason, a complete evaluation is necessary before considering labiaplasty surgery. It could be crucial for doctors and patients to talk about how the patients' genitalia might look following surgery to reduce the likelihood of patient dissatisfaction overall. Most females are happy with their labiaplasty outcomes, which often means improvement in psychological and sexual health. However, the expectations of women are not always met, especially when they take into account how it will affect their sexual interactions. Clinicians may be able to interact with women seeking labiaplasty more successfully as a consequence of the study's findings. Better patient decision-making will result from a thorough grasp of the goals and expectations of individuals undergoing labiaplasty and increase the likelihood of satisfaction of the patient with the outcomes.

Conflict of interest: The authors declared no conflict of interest.

References

1. Cosmetic Surgery National Data Bank Statistics. Aesthet Surg J. 2015;35(Suppl 2):1-24.
2. Crouch NS, Deans R, Michala L, Liao LM, Creighton SM. Clinical characteristics of well women seeking labial reduc tion surgery: A prospective study. BJOG. 2011;118:1507-1510.
3. Kelishadi SS, Elston JB, Rao AJ, Tutela JP, Mizuguchi NN. Posterior wedge resection: A more aesthetic labia plasty. Aesthet Surg J. 2013;33:847-853
4. Veale D, Eshkevari E, Ellison N, et al. Psychological characteristics and motivations of women seeking labiaplasty. Psychol Med. 2014;44:555-566.
5. Rouzier R, Louis-Sylvestre C, Paniel BJ, Haddad B. Hypertrophy of labia minora: Experience with 163 reduc tions. Am J Obstet Gynecol. 2000;182:35-40.
6. Rogers RG. Most women who undergo labiaplasty have normal anatomy; We should not perform labiaplasty. Am J Obstet Gynecol. 2014;211:218-218 e211.
7. Solanki NS, Tejero-Trujeque R, Stevens-King A, Malata CM. Aesthetic and functional reduction of the labia minora using the Maas and Hage technique. J Plast Reconstr Aesthet Surg. 2010;63:1181-1185.
8. Mayer HF, de Elizalde ML, Duh N, Loustau HD. Bidimensional labia minora reduction. Eur J Plast Surg. 2011;34:345-350.
9. Iglesia CB, Yurteri-Kaplan L, Alinsod R. Female genital cosmetic surgery: A review of techniques and outcomes. Int Urogynecol J. 2013;24:1997-2009.

10. Veale D,Eshkevari E, Ellison N, et al. A comparison of risk factors for women seeking labiaplasty compared to those not seeking labiaplasty. Body Image. 2014;11:57-62.
11. Veale D, Naismith I, Eshkevari E, et al. Psychosexual outcome after labiaplasty: a prospective case-comparison study. Int Urogynecol J. 2014;25:831-839.
12. Herbenick, D., et al., 2011. The Female Genital Self-Image Scale (FGSIS): results from a nationally representative probability sample of women in the United States. The Journal of Sexual Medicine, 8 (1), 158–166.
13. Sharp, G., Tiggemann, M. and Mattiske, J., 2016. Factors that influence the decision to undergo labiaplasty: media, relationships, and psychological well-being. Aesthetic Surgery Journal, 36 (4), 469–478.
14. Bramwell, R., Morland, C. and Garden, A. S., 2007. Expectations and experience of labial reduction: a qualitative study. BJOG, 114 (12), 1493–1499.
15. Ackard, D. M., Kearney-Cooke, A. and Peterson, C. B., 2000. Effect of body image and self-image on women's sexual behaviors. International Journal of Eating Disorders, 28 (4), 422–429.
16. Koning M, Zeijlmans IA, Bouman TK, van der Lei B. Female attitudes regarding labia minora appearance and reduction with consideration of media influence. Aesthet Surg J. 2009;29:65-71
17. Sharp G, Tiggemann M, Mattiske J. Predictors of consideration of labiaplasty: An extension of the Tripartite Influence Model of Beauty Ideals. Psychol Women Quart. 2015;39:182-193.
18. Markey, C. N. and Markey, P. M., 2009. Correlates of young Women's interest in obtaining cosmetic surgery. Sex Roles, 61 (3-4), 158–166.
19. Lowenstein, L., et al., 2014. Physicians' attitude toward female genital plastic surgery: a multinational survey. The Journal of Sexual Medicine, 11 (1), 33–39.
20. Hamori CA. Aesthetic surgery of the female genitalia: Labiaplasty and beyond. Plast Reconstr Surg. 2014; 134:661-673.
21. Gimlin D. Cosmetic surgery: Beauty as commodity. Qual Sociol. 2000;23:77-98.
22. Harding T, Hayes J, Simonis M, Temple-Smith M. Female genital cosmetic surgery: Investigating the role of thegeneral practitioner. Aust Fam Physician. 2015;44:822-825
23. Goodman MP, Placik OJ, Benson RH III., et al. A large multicenter outcome study of female genital plastic surgery. J Sex Med. 2010;7:1565-1577.
24. Oranges CM, Sisti A, Sisti G. Labia minora reduction techniques: A comprehensive literature review. Aesthet Surg J. 2015;35:419-431.
25. Klassen AF, Pusic AL, Scott A, Klok J, Cano SJ. Satisfaction and quality of life in women who undergo breast surgery: A qualitative study. BMC Womens Health. 2009;9:11.
26. Klassen AF, Cano SJ, Scott A, Johnson J, Pusic AL. Satisfaction and quality-of-life issues in body contouring surgery patients: A qualitative study. Obes Surg. 2012; 22:1527-1534.
27. Crerand CE, Phillips KA, Menard W, Fay C.Nonpsychiatric medical treatment of body dysmorphic disorder. Psychosomatics. 2005;46:549-555.
28. Crerand CE, Infield AL, Sarwer DB. Psychological considerations in cosmetic breast augmentation. Plast Surg Nurs. 2007;27:146-154.

Araştırma Makalesi / *Research Article*

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Tibialis anterior tendon transferi tespitinde çapa dikiş, askı düğme sistemi ve tünel yöntemlerinin karşılaştırmalı biyomekanik ve anatomik analizi

Comparative biomechanical and anatomical analysis of anchor, endobutton and tunnel methods in tibialis anterior tendon transfer fixation

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ÖZ

Amaç: Tendon transferleri, ortopedik cerrahide özellikle pediatrik deformiteler ve sinir hasarı sonrası fonksiyonel kapasiteyi arttırmak amacıyla sık kullanılan tekniklerdir. Tendon transferleri birkaç temel prensip etrafında şekillenmiştir. Bu prensipler transfer sonrası hareket beklenen eklemin esnek olması, transfer yapılacak yumuşak dokunun iyileşmeye elverişli olması, donör tendonun yeterli ekskürsiyona ve kuvvete sahip olması, doğrusal bir çekiş eksenine sahip olması ve aynı zamanda feda edilebilir olmasıdır. Bu prensiplerin çoğu iyi bir preoperatif planlama ile uyulabilecek sınırları ifade ederken intraoperatif değiştirilebilir temel değişken olarak transfer edilecek bölgedeki dokunun mahiyeti ve uygulanacak transfer tekniğinin bu doku ile etkileşimi olarak öne çıkmaktadır.

Gereç ve Yöntem: Çalışmamızda osseotendinöz bir iyileşme beklentisi ile tarsal kemiklere transfer edilerek tespit edilen tibialis anterior tendon transferi uygulamalarında üç farklı tespit yöntemini kıyaslamayı amaçladık. Bu teknikler: 1) Askı düğme sistemi ile tespit 2) Çapa dikiş ile tespit 3) Tünel tekniği ile tespit. Bunun için toplam dokuz kadavrada, üç farklı cerrahi teknik, üçer farklı kadavrada uygulanmıştır. Sonuç parametresi olarak tespit sonrası transfer edilen tendonun traksiyon kuvveti ile direnebildiği maksimum kuvvet, maksimum kuvvet etki ettiği andaki deplasman değerlendirilmiştir. Biyomekanik testin tamamlanmasının ardından tibialis anterior transfer edilen ayak bileği mediali disseke edilerek medial plantar sinirin hasarlanıp hasarlanmadığı araştırılmıştır.

Bulgular: Deneylerde elde edilen sonuçlara göre gruplar arasında kopma öncesi maksimum kuvvet değerinde ve maksimum kuvvet uygulandığı andaki deplasman miktarında anlamı bir fark olmadığı ortaya konulmuştur. Dokuz kadavranın hiçbirinde medial plantar sinir hasar görmemiştir.

Sonuç: Önerilen tekniğin karşılaştırılan teknikler ile benzer biyomekanik dayanım sunması, implant maliyeti olmaması, kalıcı tespit materyali bırakılmasını gerektirmemesi ve nörovasküler hasar yaratma olasılığı açısından risk oluşturmaması sebebiyle etkin ve güvenli bir yöntemdir.

Anahtar Sözcükler: Tendon transferi, tibialis anterior, çapa dikiş, askı düğme sistemi.

ABSTRACT

Aim: *Tendon transfers are frequently used techniques in orthopedic surgery to increase functional capacity, especially after pediatric deformities and nerve damage. Tendon transfers are shaped around a few basic principles.*

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These principles are that the joint in which movement is expected after the transfer is flexible, the soft tissue to be transferred is suitable for healing, the donor tendon must have sufficient excursion and strength, it must have a linear traction axis, and it must also be sacrificial. While most of these principles express the limits that can be followed with good preoperative planning, the main variables that can be changed intraoperatively are the nature of the tissue in the area to be transferred and the interaction of the transfer technique with this tissue.

***Materials and Methods**: In this study, we aimed to compare 3 different fixation methods in tibialis anterior transfer applications, which are transferred and fixed to the tarsal bones with the expectation of an osseotendinous recovery. These techniques are: 1) Fixation with endobutton technique 2) Fixation with suture anchor 3) Fixation with tunnel technique. For this purpose, 3 different surgical techniques were applied to 3 different cadavers' lower extremities. Nine extremities were used in total. As the result parameters, the maximum force that the transferred tendon could resist with the traction force after fixation and the displacement at the moment the maximum force acted were evaluated. After the biomechanical test was completed, the medial part of the tibialis anterior*

transferred ankle was dissected and it was investigated whether the median plantar nerve was damaged.

***Result:** According to the results obtained in the experiments, it was revealed that there was no significant difference between the groups in the maximum force before rupture and the amount of displacement when the maximum force was applied. The median plantar nerve was not damaged in any of the nine cadavers.*

***Conclusion**: The proposed technique is an effective and safe method because it offers similar biomechanical strength to the compared techniques, has no implant cost, does not require leaving permanent fixation material, and does not pose a risk of neurovascular damage.*

***Keywords:** Tendon transfer, tibialis anterior, suture anchor, endobutton technique.*

GİRİŞ

Ayak, ayak bileği çevresi tendon transferleri, ayak deformiteleri, basış bozuklukları ve travma sekelleri sonrası geniş olarak kullanılan cerrahi prosedürlerdir. Özelikle çocukluk çağındaki basış anormallikleri ve "club foot" deformiteleri için sıklıkla tibialis anterior tendon transferi (TAT) uygulanmaktadır. Tibialis anterior tendon transferi için literatürde aynı amaca yönelik değişik cerrahi teknikler tariflenmiştir. Askı düğme sistemi yardımlı teknik, düğme tekniği, çapa dikiş tekniği bu yöntemlerden bazılarıdır. Bu tekniklerin tamamında yardımcı materyal kullanımı ihtiyacı vardır. Geleneksel olarak en yaygın uygulanan teknik, üç cilt insizyonu yapılarak, ayak plantar dokularının kesilmesini gerektiren, bu sebeple nörovasküler yapılara zarar verilmesi riskini ve yara yeri problemi yaratma olasılığını barındıran, geleneksel düğme ile tespit tekniğidir. Düşük maliyetli ve kolay erişilebilir basit bir düğmeyle yapılabilen bu teknik son yıllarda implant teknolojisinin gelişmesinin de etkisiyle yerini daha farklı tespit yöntemlerine bırakmıştır (1, 2). Bu doğrultuda bu tekniğin yerini alacak altın standart bir transfer tekniği konusunda bir fikir birliği bulunmamaktadır. Çalışmamızda yenilikçi bir teknik olan tünel tekniğinde herhangi bir yardımcı materyale ihtiyaç duyulmayacak şekilde tariflediğimiz tekniğin, diğer tekniklerle biyomekanik yönden karşılaştırılması ve nörovasküler yapılara hasar vermesi açısından güvenliliğinin araştırılması amaçlanmıştır.

Sadece TAT özelinde değil, pek çok farklı endikasyonla yapılan farklı tendonların transferleri modellerinde farklı implantların birbirine olan üstünlükleri araştırılmıştır ve araştırılmaya devam etmektedir. Kullanılan tendon ve uygulanan tespit tekniğine bağlı olarak değişmekle birlikte, temel felsefe elde edilmek istenilen osseo-tendinöz/osseo-osseöz iyileşmeyi elde edene kadar transfer edilen tendonun yeni anatomik konumunda kalmasını ve fonksiyon görmesini mümkün kılacak sağlamlıkta bir tespit elde edebilmektir (4).

Tendon transferleri farklı anatomik lokasyonlarda farklı kaslarla klinik uygulamalara konu olsa da tespit yöntemleri çoğu anatomik bölgede benzerlik göstermektedir. Ancak tespit yapılan anatomik bölgedeki yapısal farklılıklar, transfer edilen tendonun ekskürsiyonu, çapı, anatomik yapısı gibi değişkenler farklı anatomik bölgelerdeki farklı tespit tekniklerinin farklı davranışlar göstermesine yol açabilmektedir. Çapa dikiş, interferans vidası, askı düğme sistemi

güncel olarak tanımlanmış aktif klinik kullanımda kabul görmüş tekniklerden bazılarıdır. Kabaca özetlemek gerekirse, çapa dikiş ile tespit yönteminde kemik içerisine yerleştirilen bir adet çapa dikiş ve bu çapaya bağlı güçlü dikişler ile tespit sağlanır.

Diğer bir teknik olan askı düğme sistemi yönteminde ise, Tendon transfer edileceği bölgeye kemik içerisinde hazırlanan bir tünel yardımıyla taşınmakta, bu konumdaki tespiti, tünel içerisine asılarak kalmasını sağlayacak implant ile sağlanmaktadır. Bu uygulamadaki temel felsefe transfer edilen tendonun hazırlanan kanal içerisinde asılması ve osseo-tendinöz iyileşme sağlanana kadar askı düğme sistemi mekanizmasının tespit görevi görmesidir (4). Pediatrik pes ekinovarus cerrahisi, tendon transferlerinin sık bir endikasyonu olup, düğme ile tespit yöntemi geleneksel olarak en çok tercih edilegelen yöntem konumundayken, ayak tabanında yüksek oranda yarattığı bası ve cilt sorunları sebebiyle yerini genel olarak askı düğme sistemine bırakmıştır. Ancak askı düğme sistemi de bu risklerden tamamen arınmış değildir. Düğme altında kalan biyolojik yapılar, pediatrik vakalarda implantı kalıcı olmasının getirebileceği olası sorunlar, implant maliyeti ve erişimi bu yöntemin optimal yöntem olmaktan uzaklaştıran özellikleridir.

Araştırmamızda, yaygın bir prosedür olarak uygulanan tibialis anterior tendon transferinde literatürde tanımlanmış ve kabul gören iki adet yöntemle, daha önce karşılaştırması yapılmamış olan yenilikçi tünel yöntemini karşılaştırmak, yöntemler arası biyomekanik ve anatomik olarak karşılaştırmalı bir analiz yapmak hedeflenmiştir.

GEREÇ ve YÖNTEM

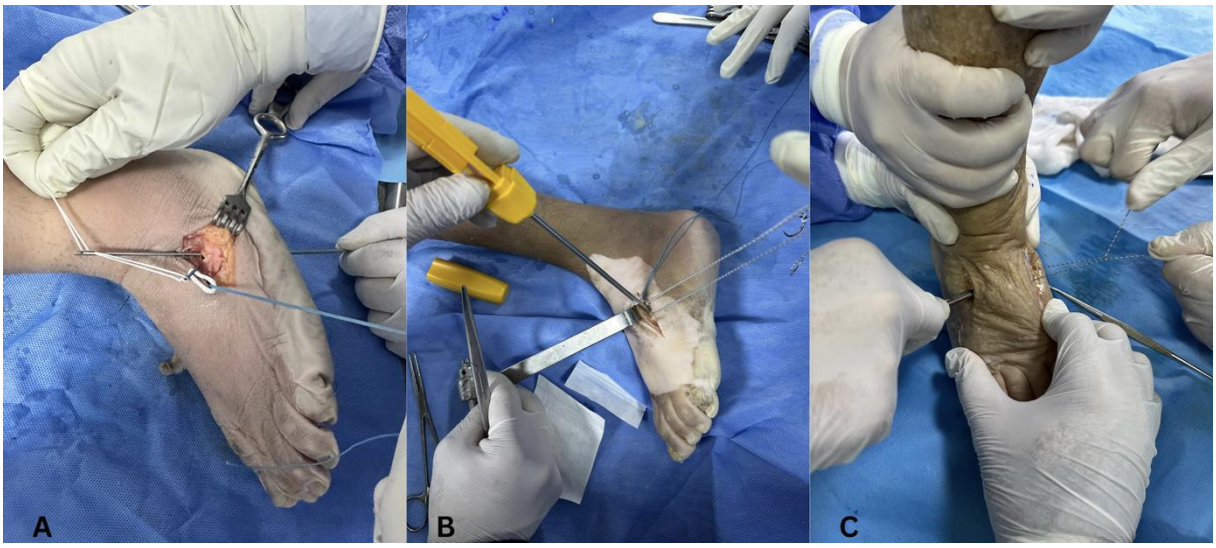
Proje için etik kurul onayı Ege Üniversitesi Tıbbi Araştırmalar Etik Kurulundan alınmıştır (Karar Nu: 23-10T/42 Tarih: 05.10.2023). Çalışma Ege Üniversitesi Bilimsel Araştırma Projeleri Koordinasyon Biriminin desteği ile 30777 Karar Numarası ile desteklenmiştir.

Araştırmanın tasarımı gereği kontrol grubu tasarlanmamıştır, karşılaştırma üç çalışma grubu arasında yapılmıştır. Üç grubun karşılaştırılması şeklinde tasarlanmıştır. Çalışma tasarımımız erişilebilir durumda olan, tümör nedeniyle diz altı seviyesinden ampute edilmiş, 12 adet alt ekstremite üzerinden kurgulanmıştır. Ancak üç ekstremitenin çalışmaya uygun olmadığının görülmesi sonrası dokuz ekstremite üzerinden çalışmaya devam edilmiştir. Kadavra çalışmalarındaki örneklem büyüklüğünün ne olması gerektiği konusunda literatürde bir uzlaşı bulunmasa da pek çok çalışma benzer örneklem sayısıyla tasarlanmış ve uygulanmıştır. Elimizdeki kullanıma uygun ekstremitelerin tamamı çalışmaya dahil edilmiştir.

Dokuz ekstremitenin dört tanesi kadın, beş tanesi erkek cinsiyete aitti. Amputasyon seviyeleri diz üstü olan olguların tümörlü dokuları patolojik tetkikler için uzaklaştırıldıktan sonra geriye kalan diz altı ampütasyon materyalleri Modifiye Larssen solüsyonlarında –20°C derecede muhafaza edilmekteydi (3, 4). Cerrahi prosedürler uygulanmadan önce kadavralar oda sıcaklığına alınarak 4 saat oda sıcaklığında çözünmeye bırakıldı. Ardından cerrahi uygulama aşamasına geçildi (Tablo-1).

Tablo-1. Kadavraların Özellikleri.

Kadavra No	Cinsiyet	Ampütasyon Seviyesi	Uygulanan TAT Tekniği
1	Kadın	Dizüstü ampütasyon	Çapa dikiş
2	Erkek	Dizüstü ampütasyon	Askı Düğme Sistemi
3	Kadın	Dizüstü ampütasyon	Çapa dikiş
4	Erkek	Dizüstü ampütasyon	Tünel
5	Kadın	Dizüstü ampütasyon	Tünel
6	Erkek	Dizüstü ampütasyon	Askı Düğme Sistemi
7	Kadın	Dizüstü ampütasyon	Çapa dikiş
8	Erkek	Dizüstü ampütasyon	Tünel
9	Erkek	Dizüstü ampütasyon	Askı Düğme Sistemi



Şekil-1. Cerrahi tekniklerin karılaştırılması A: Askı düğme sistemi ile tespit. B: Çapa dikiş ile tespit C: Tünel tekniği ile tespit.

İlk insizyon, tüm gruplarda aynı olacak şekilde, tibialis anterior tendonunun çıkarılmasına izin verecek şekilde tendonun yapışma yerinde tendonun izdüşümüne oblik olarak yapıldı. Bu aşamada alınacak tendonun extensor hallucis longus'a ait olmadığı iki farklı araştırmacı tarafından onaylanarak deneye devam edildi.

Sonrasında tendonun distaldeki yapışma yeri belirlenerek, yapışma yerinden kesilerek ayrıldı. Distal tutunma yerinden ayrılan tendon taşıyıcı amaçla kullanılacak, eriyebilen bir dikişle dikilerek tübülize edildi. Sonrasında tendon proksimale doğru subkutan disseke edilerek serbestleştirildi. Ardından ikinci bir kesi, yaklaşık beş santimetre olacak şekilde, ayağın lateral kısmında yapıldı.

Daha sonra ayak laterelindeki kesiden alınan tendonun transfer edileceği yer belirlendi. Bu yer genel TAT endikasyonu olan ekin deformitesi cerrahisi prensiplerine uygun olarak cuboid kemik üzerinde işaretlendi. Devamında medialdeki kesiden serbestleştirilen tibialis anterior tendonu cilt altından ayak lateraline transfer edildi.

Tespit aşamasına gelindiğinde üç farklı grupta üç farklı yöntemle tespit yapıldı (Şekil-1).

Kadavraların tibialis anterior tendonlarının yarı statik mekanik tepkilerini belirlemek için testler, oda sıcaklığında, 5 mm/dk hızında bir test makinesi (Autograph, Shimadzu Co, Japonya) kullanılarak uygulandı (5). Kuvvet ve yer değiştirme sinyalleri, 20 Hz örnekleme hızına sahip bir Shimadzu 5 kN yük hücresi kullanılarak elde edildi.

Açılan tünellerin medial nörovasküler yapılar ile olan uzaklığını objektif olarak ortaya koymak amacıyla dijital kumpas yardımıyla ölçümler yapıldı. Nörovasküler yapılara bir cm'den daha fazla bir uzaklık olması durumunda hasar oluşmadığı not edildi. Deney düzeneği Şekil-2'de gösterilmiştir.

Şekil-2. Çekme testi için kurulan deney düzeneği.

Yük hücresi ile elde edilen kuvvet-deplasman verileri her bir kadavra için analiz edildi. Maksimum kuvvet ve maksimum kuvvet anındaki yer değiştirme elde edilen eğriler üzerinden karakterize edildi. Sertlik, kuvvet-yer değiştirme eğrisinin eğimi olarak hesaplandı. Toplam enerji de bu eğrinin altında kalan alan olarak hesaplandı. Değerlendirme toplamda 4 değişken için yapıldı ve maksimum kuvvet Newton (N) cinsinden, yer değiştirme milimetre (mm) cinsinden sertlik (N/mm) cinsinden Enerji ise Joule cinsinden olmak üzere analiz edildi.

BULGULAR

Mekanik testlerde örnek sayısının azlığı nedeni ile istatistiksel farklılık olmamasına rağmen, mekanik olarak en güçlü tekniğin askı düğme sistemi tekniği olduğu (Ortalama: 272,07 Newton), tünel tekniğinin ise dayanıklılık gücünün askı düğme sistemi tekniğine çok yakın olduğu görülmüştür (Ortalama 204,62 Newton). Çekme testine karşı en dayanıksız tekniğin ise çapa dikiş tekniği olduğu gözlenmiştir. (Ortalama 162.55 Newton) Tablo-2. Hiçbir kadavrada nörovasküler yapıların hasarlanmadığı görülmüştür. Askı düğme sistemi tekniğinin daha güçlü bulunmasına rağmen, askı düğme sistemi materyalini plantar yüzeye yerleştirmek için, oldukça geniş bir insizyon uygulanması gereksinimi ve plantar bölgedeki yumuşak dokulara daha fazla hasar verme riski olduğu gözlenmiştir.

Tablo-2. Kadavralarda uygulanan çekme testinin sonuçları.

Kadavra No	Maksimum Kuvvet (N)	Maksimum Kuvvet anındaki Deplasman (mm)	Sertlik (N/mm)	Enerji (Joule)*
1	137.34	36.07	3.8075	3.3824
2	300.0 +	67.61	-	-
3	148.75	43.19	3.4440	3.7153
4	180.93	65.53	2.7610	6.0966
5	195.62	74.13	2.6388	10.089
6	219.53	42.05	5.220	6.6608
7	201.56	87.44	3.74	12.27
8	237.32	96.44	6.52	13.44
9	296.69	63.47	6.93	9.19

TARTIŞMA

Ortopedik cerrahide tendon transferleri sinir hasarı, motor ünite disfonksiyonu, doğumsal anomaliler deformiteler gibi pek çok farklı endikasyonda kullanılan cerrahi tekniklerin üst başlığıdır. Tendon transferi prosedürlerinin en sık uygulama alanlarından biri ayak-ayak bileğinde kullanılan tendon transferi prosedürü uygulamalarıdır. Tendon transferi sonrasında transfer edilen tendonun implante edileceği lokasyona, hastaya bağlı değişkenlere, postoperatif rehabilitasyon sürecine göre değişkenlik gösteren tendon tespit teknikleri tanımlanmıştır. Bu değişkenler üzerinden aynı endikasyon için kullanılan pek çok tendon transferi yöntemi kullanılabilmektedir. Sinerjistik farklı kasların tercih edilebilmesi, transfer edilecek bölgede farklı amaçlar gözetilerek çeşitlilik olabilmesi, transfer edilen tendonun farklı yöntemlerle tespit edilebilmesi gibi bağımsız değişkenler, farklı tendon transferi seçenekleri ve bu farklı seçeneklerin birbirine olan avantajları ve dezavantajlarını ortaya çıkarmıştır. İdeal tendon transferi tespit metodu; biyolojik, maliyet etkin, erişilebilir ve kolay uygulanabilir olmalıdır (6).

Çalışmamızın diğer çalışma gruplarını oluşturan askı düğme sistemi ile tespit, çapa dikiş ile tespit yöntemleri MPFL (medial patellofemoral ligaman) rekonstrüksiyonu, Ön çapraz bağ rekonstrüksiyonu, Biseps tendon rüptürü onarımı, aşil tendon rüptürü, patellar tendon rüptürü gibi pek çok farklı endikasyonla ortopedik cerrahinin farklı alanlarında aktif olarak kullanılmaktadır. Bu endikasyonlar ile opere edilen hasta gruplarında pes ekinovarus sebebiyle tendon transferi yapılan hastalardan farklı olarak erişkin hastalar ağırlıktadır ve uzun süre immobilizasyon mümkün olmamakta, fizyolojik yüklenmelerde implanta

binen yük tibialis tendon transferindeki yükten çok daha fazla olmaktadır (7).

Çalışmamızı tasarlarken yola çıkılan klinik problem, çoğunlukla pediatrik yaş grubunda yapılan, çoğu zaman birden fazla cerrahi gerektiren bu hasta grubunda düğme askı/ çapa dikiş gibi kalıcı materyallerin kullanımından kaçınabilmek için önerdiğimiz tekniğimizin biyomekanik olarak alternatiflerinden geride olmadığının ortaya konulabilmesi olmuştur (8). Bu sebeple, tibialis anterior tendon transferlerinde kullanılmakta olan iki farklı tespit tekniğinin (çapa dikiş ve askı düğme sistemi) önerdiğimiz tünel içine yerleştirme sonrası iki farklı tünel üzerinden cerrahi düğüm ile bağlanması tekniği ile biyomekanik olarak karşılaştırılmış, implant kullanılan diğer yöntemlerle kıyaslanabilir sağlamlıkta olduğu ortaya konmuştur.

Pediatrik ayak deformiteleri, tarihsel gelişim sürecinde farklı tedavi denemelerine konu olmuştur. Tarihsel olarak sadece cerrahi tedavi edilebileceği düşünülen pes ekinovarus olguları, Ponseti tarafından tanımlanan seri alçılama tekniğinin kullanıma girmesiyle çok ciddi oranlarda cerrahisiz tedavi edilebilir duruma gelmiştir. Ancak serebral palsi, glikojen depo hastalıkları gibi özellikli alt gruplarda cerrahi dışı yöntemlerin başarısızlık oranları sağlıklı popülasyona oranla daha fazladır. Bu anlamda bu özellikli alt gruplarda daha sık olmak üzere pes ekinovarus tedavisinde cerrahinin yeri önemini hala korumaktadır (9). Bu paralelde bu hasta grubunda tedaviyi optimize edecek, maliyeti azaltacak, komplikasyonları yönetilebilir olacak yöntemler ortaya koyabilmek son derece önemlidir. Tendon transferi hastalarında optimal klinik başarının anahtarının uygulanacak tendon transfer yönteminin detaylarından ziyade preop uygun değerlendirme, doğru hasta seçimi ve eşlik eden diğer çözülebilir ayak-ayak bileği sorunlarının çözülmesi olduğu bilinmektedir. Bu bazal gereksinimler ve prensipler yerine getirildikten sonra uygulanacak teknikler arasında teknik detaylar sonucu mükemmelleştirecek dokunuşlar olacaktır (10). Tanımladığımız tünel yöntemi çekme kuvvetine gösterdiği direncin yeterli olması dolayısıyla, alternatifleri arasında tercih edilebilecek bir yöntem olarak yerini alabilecektir.

Tibialis anterior tendon transferi prosedürleri için karşılaştırma konusu olabilecek önemli ancak bu araştırmanın konusu olmayan değişkenlerden biri transfer edilecek tendonun split (kısmi) veya tamamen alınarak transfer edilmesi hususudur. Yapılan pek çok araştırma her iki seçeneğin de iyi klinik sonuçlar ile uygulanabilir olduğunu bildirmiştir (11). Pes ekinovarusta cerrahi tedaviler de çeşitlilik göstermektedir. İzole posterior tibial tendon transferi bu seçeneklerden biridir ve nispeten basit ve kolay uygulanabilir bir tekniktir. Tibialis anterior ve ekstensör hallusis in sağlam olduğu ve fonksiyon gördüğü durumlarda tercih edilmektedir. Tibialis posterior tendon transferi izole olarak yapılabileceği gibi, fleksör hallusis ve fleksör digitorum longus ile de kombine olarak yapılabilmektedir. Ancak özellikle son 4 dekatta aşil gevşetme ile kombine uygulanan tibialis tendon transferi ekinovarus cerrahi tedavisinde başat konumdadır (11). Çalışmamızda elde ettiğimiz öncül olarak kabul edilebilecek veriler, bahsedilen alternatif klinik uygulamalarda test edilebilir, bu anlamda geniş çeşitlilikteki bir dizi klinik uygulama için çalışma konusu olabilir.

Tendon transferleri sonrası oluşabilecek komplikasyonlardan kaçınmak, serebral palsili hastaların çoğunlukta olduğu bu grupta fazlaca önem taşımaktadır. Pediatrik hasta gruplarında yapılan cerrahi prosedürler, erişkin hastalardan pek çok yönüyle ayrılmaktadır. Ayak seviyesinde tendon transferleri özelinde özelikle dikkat edilmesi gereken hususlardan biri kullanılan implanta bağlı yaşanan sorunlardır. Çocuklarda kalıcı tespit materyali kullanımı kararı verilirken, büyümeye devam eden uzuvda kalacak olan materyalin yaratması olası problemler de hesaba katılmalıdır (12, 13). Bu pencereden bakıldığında, tünel yöntemi bu anlamda güvenli bir seçenek olarak değerlendirilebilir.

SONUÇ

Sonuç olarak tünel yöntemin mekanik olarak güçlü olduğu, anatomik yapılara az hasar verdiği, en önemlisi materyal kullanımına ihtiyaç duyulmadığından ucuz ve etkili bir yöntem olduğu görülmüştür.

Tünel yönteminin karşılaştırılan diğer teknikler ile benzer biyomekanik dayanım sunması, implant maliyetinin olmaması, kalıcı tespit materyali gerektirmemesi ve nörovasküler hasar yaratma riski oluşturmaması gibi nedenlerden ötürü etkin ve güvenli bir yöntemdir.

Çalışmamızın verileri doğrudan bir ürün geliştirilmesi ve ticari bir ürün ortaya çıkmasını desteklememekle birlikte, implant kullanılan

tekniklerde olasılıkla görülebilecek olan implant ilişkili komplikasyonlardan kaçınabilmeyi mümkün kılması, implant maliyetlerinden tasarruf edilebilmesi ve tekrarlayan ameliyatların getireceği sağlık sistemi harcamalarının önüne geçilebilmesi açısından değerlendirilebilir.

Çıkar çatışması: Yazarlar bu makalenin yazarlığı ve/veya yayımlanmasıyla ilgili olarak herhangi bir çıkar çatışması beyan etmemiştir.

Kaynaklar

1. Ayub AAA, Firth GB, Green GL, Bijlsma P, Ramachandran M. Tibialis anterior tendon transfer using bone anchor for dynamic supination in congenital talipes equinovarus. J Pediatr Orthop B [Internet]. 2023 Jan 1 [cited 2024 Apr 14];32(1):15–20. Available from: https://pubmed.ncbi.nlm.nih.gov/35834789/
2. Liu GT, Balldin BC, Zide JR, Chen CT. A Biomechanical Analysis of Interference Screw Versus Bone Tunnel Fixation of Flexor Hallucis Longus Tendon Transfers to the Calcaneus. J Foot Ankle Surg [Internet]. 2017 Jul 1 [cited 2024 Apr 14];56(4):813–6. Available from: https://pubmed.ncbi.nlm.nih.gov/28633783/
3. Bilge O, Celik S. Cadaver embalming fluid for surgical training courses: modified Larssen solution. Surg Radiol Anat [Internet]. 2017 Nov 1 [cited 2024 Apr 14];39(11):1263–72. Available from: https://pubmed.ncbi.nlm.nih.gov/28497162/
4. Pekedis M, Yoruk MD, Binboga E, Yildiz H, Bilge O, Celik S. Characterization of the mechanical properties of human parietal bones preserved in modified larssen solution, formalin and as fresh frozen. Surg Radiol Anat [Internet]. 2021 Dec 1 [cited 2024 Apr 14];43(12):1933–43. Available from: https://pubmed.ncbi.nlm.nih.gov/33954823/
5. Ayzenberg M, Arango D, Gershkovich GE, Samuel PS, Saing M. Pullout strength of a novel hybrid fixation technique (Tape Locking ScrewTM) in soft-tissue ACL reconstruction: A biomechanical study in human and porcine bone. Orthop Traumatol Surg Res [Internet]. 2017 Jun 1 [cited 2024 Apr 14];103(4):591–5. Available from: https://pubmed.ncbi.nlm.nih.gov/28238964/
6. Walton L, Villani MF. Principles and Biomechanical Considerations of Tendon Transfers. Clin Podiatr Med Surg [Internet]. 2016 Jan 1 [cited 2024 Apr 14];33(1):1–13. Available from: https://pubmed.ncbi.nlm.nih.gov/26590719/
7. Núñez-Pereira S, Pacha-Vicente D, Llusá-Pérez M, Nardi-Vilardaga J. Tendon transfer fixation in the foot and ankle: a biomechanical study. Foot Ankle Int [Internet]. 2009 Dec [cited 2024 Apr 14];30(12):1207–11. Available from: https://pubmed.ncbi.nlm.nih.gov/20003881/
8. Sabonghy EP, Wood RM, Ambrose CG, McGarvey WC, Clanton TO. Tendon transfer fixation: comparing a tendon to tendon technique vs. bioabsorbable interference-fit screw fixation. Foot Ankle Int [Internet]. 2003 Mar 1 [cited 2024 Apr 14];24(3):260–2. Available from: https://pubmed.ncbi.nlm.nih.gov/12793491/
9. Masrouha K, Chu A, Lehman W. Narrative review of the management of a relapsed clubfoot. Ann Transl Med [Internet]. 2021 Jul [cited 2024 Apr 14];9(13):1102–1102. Available from: https://pubmed.ncbi.nlm.nih.gov/34423014/
10. Mulhern JL, Protzman NM, Brigido SA. Tibialis Anterior Tendon Transfer. Clin Podiatr Med Surg [Internet]. 2016 Jan 1 [cited 2024 Apr 14];33(1):41–53. Available from: https://pubmed.ncbi.nlm.nih.gov/26590723/
11. Bibbo C, Jaglan SS. Tendon transfers for equinovarus deformity in adults and children. Foot Ankle Clin [Internet]. 2011 Sep [cited 2024 Apr 14];16(3):401–18. Available from: https://pubmed.ncbi.nlm.nih.gov/21925358/
12. Hochstetter-Owen J, Stott S, Williams SA. The efficacy of split tibial tendon transfers on functional gait outcomes for children and youth with cerebral palsy and spastic equinovarus foot deformities. Bone Jt Open [Internet]. 2023 [cited 2024 Apr 14];4(5):283–98. Available from: https://pubmed.ncbi.nlm.nih.gov/37121581/
13. Vova JA, Davidson LT. Nerve and Tendon Transfers After Spinal Cord Injuries in the Pediatric Population: Clinical Decision Making and Rehabilitation Strategies to Optimize Function. Phys Med Rehabil Clin N Am [Internet]. 2020 Aug 1 [cited 2024 Apr 14];31(3):455–69. Available from: https://pubmed.ncbi.nlm.nih.gov/32624105/

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Management of a corneal perforation due to resistant peripheral ulcerative keratitis by repeated tectonic patch grafting combined with conjunctival resection

Dirençli periferik ülseratif keratite bağlı kornea perforasyonunun konjonktival rezeksiyon ile kombine tekrarlanan tektonik yama grefti ile yönetimi

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ABSTRACT

Tectonic graft patching and conjunctival resection is one of the effective treatment modalities in especially resistant peripheral ulcerative keratitis patients with corneal perforation additional to topical and systemic immunosuppressive treatment. A 44-year-old female patient with a 10-year history of rheumatoid arthritis, was referred to our clinic with peripheral corneal perforation. Her visual acuity was at the level of hand movement perception in the left eye. Slit-lamp examination revealed full-thickness circular area of 3x3 mm diameter peripheral ulcerative keratitis with corneal perforation. Tectonic patch grafting was performed. Two weeks later, because of small melting area at the inferior part of the graft with iris incarceration, an additional cornea-scleral graft transplantation was performed. Due to the immune nature of the peripheral ulcerative keratitis, limbal conjunctiva at the perforation site was also resected. Fourteen months after the re-grafting, there was no recurrence. Best corrected visual acuity (BCVA) was 5/10 log MAR in the left eye.

Keywords: Conjunctival resection; peripheral ulcerative keratitis, tectonic patch grafting.

ÖZ

Dirençli kornea perforasyonu olan periferik ülseratif keratit hastalarında, tektonik greft yama ve konjonktival rezeksiyon, topikal ve sistemik immünsüpresif tedaviye ek olarak, etkili tedavi yöntemlerinden biridir. On yıldır romatoid artrit öyküsü olan 44 yaşında kadın hasta periferik kornea perforasyonu ile kliniğimize sevk edildi. Görme keskinliği sol gözde el hareketlerini algılayacak düzeydeydi. Yarık lamba muayenesinde medial periferik ülseratif keratit ve korneal perforasyon saptandı. Tektonik yama grefti uygulanan hastanın iki hafta sonraki kontrolünde greftin alt kısmında erime alanı olması ve iris inkarseresyonu gelişmesi nedeniyle ilave kornea-skleral greft nakli yapıldı. Periferik ülseratif keratitin immun yapısı nedeniyle, perforasyon bölgesindeki limbal konjonktiva da rezeke edildi. 2 yıllık takipte nüks izlenmedi. En iyi düzeltilmiş görme keskinliği sol gözde 5/10 LogMAR idi.

Anahtar Sözcükler: *Konjonktival rezeksiyon; periferik ülseratif keratit, tektonik yama grefti.*

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INTRODUCTION

Peripheral ulcerative keratitis (PUK) is defined as the progressive thinning of the peripheral corneal stroma caused by limbal vasculitis (1). It is characterized by crescent-shaped inflammation of mostly inferior limbus, accompanied by an epithelial defect, may remain indolent or cause advanced melting and corneal perforation. Several autoimmune conditions have been linked to PUK, including rheumatoid arthritis (RA), Wegener's granulomatosis, large vessel vasculitis, lichen planus, pyoderma gangrenosum, autoimmune hepatitis. Systemic collagen vascular diseases such as RA are responsible for approximately half of all noninfectious PUK cases (2).

We present the management of resistant peripheral ulcerative keratitis (PUK) with corneal perforation with repeated tectonic patch grafting combined with conjunctival resection.

Case report

A 44-year-old female patient with a history of rheumatoid arthritis for 10 years, was referred to our clinic with corneal perforation due to peripheral ulcerative keratitis. According to her medical history, RA status was under controlled with systemic immunosuppression therapy (subcutaneous methotrexate 15 mg weekly).

Her visual acuity was at the level of hand movement perception in the left eye and 10/10 in the right eye. Slit-lamp examination of the left eye revealed medial PUK with corneal perforation. (Figure-1A) Anterior chamber was shallow. Inferonasal descemetocele, approximately 3x3 mm in size, with no signs of infectious infiltration and accompanied by full-thickness stromal perforation with iris plug was observed. Anterior segment examination of the right eye and fundus examination of both eyes were normal. Bandage contact lens, oral tetracycline 100 mg twice, oral lansoprazole, 0.5% moxifloxacin drops and autologous serum eight times a day, dorzolamide, timolol and 0.2% brimonidine drops twice a day were administered before surgical intervention.

Corneal tectonic patch grafting was performed. (Figure-1B) The host bed is first prepared by clearing the adjacent and overlying necrotic tissue and renewing the descemetocele margins. Depending on the size and shape of the defect, a lamellar, customized crescentic patch donor graft was transplanted. The recipient bed was irrigated with balanced salt solution and the graft sutured to its margins with interrupted 10-0 nylon sutures. Systemic immunosuppression was maintained postoperatively along with topical tobramycin and dexamethasone drops 8 times a day, and 0.05% cyclosporine drops 4 times a day.

At the follow-up postoperative 2 weeks, BCVA in the left eye was 2/10 log MAR with Snellen chart. In the anterior segment examination, the graft was in place, the sutures were stable. However, a small melting area with iris incarceration was observed at the inferior part of the graft, close to the limbus. (Figure-1C-D) The intraocular pressure was found to be hypotonic. An additional surgical intervention was planned. Due to the immune nature of the PUK, limbal conjunctiva at the perforation site was also resected and supplementary patch graft transplantation was performed. The scleral part of the second patch graft was positioned to the sclera at the conjunctival excision area to obtain a limbal barrier, an extending part was sutured to the cornea, over the previous graft. No extra pathology was observed in the postoperative first day and monthly controls. (Figure-1E-F)

Fourteen months after the re-grafting, the sutures and graft were stable, the anterior chamber depth was fair, and there was no recurrence. (Figure-1G) Intraocular pressure was 11 mm/hg and BCVA was 5/10 log MAR in the left eye.

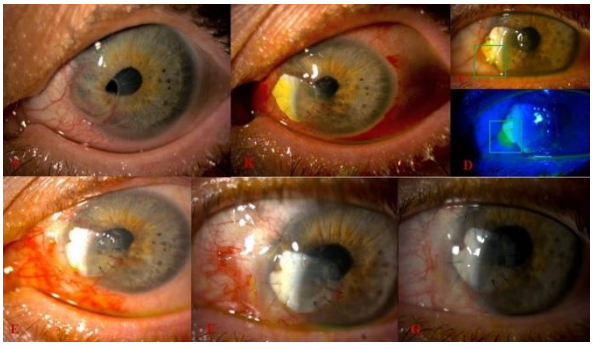

Figure-1. Preoperative slit lamp examination demonstrates medial PUK with corneal perforation (A). Graft and sutures appear to be stable on the first day after tectonic patch grafting (B). Postoperative second week, slit lamp examination reveals melting area at inferior part of the graft (C-D). Slit lamp examination shows graft in place on first day of second tectonic grafting with conjunctival resection (E). No recurrence is observed at the first (F) and 9 months (G) of the operation.

DISCUSSION

The triggers of corneal perforation can be categorized as either traumatic-nontraumatic or infectious-noninfectious causes. PUK located in nontraumatic, non-infectious category with an inflammatory etiology (3). The peripheral cornea has unique properties that facilitate the effects of systemic reactions, such as close conjunctival lymphoid tissue, anterior ciliary artery (unlike the avascular central cornea) supply, tighter collagen bundles (storing immune complexes), and greater thickness presence (4, 5). Therefore, management of inflammation after mechanical repair is important when PUK is combined with corneal perforation. In the present case, while the systemic and topical immunosuppressive therapy was insufficient, conjunctival resection was added to the surgical intervention to reduce inflammation.

Interruption of the rich vascular supply results with necrosis and ulceration which may cause corneal perforation. Perforations can be effectively treated with a variety of methods, depending on the size and location. The main therapeutic purpose is to provide tectonic support with various options such as tissue adhesives, bandage contact lenses, amniotic membrane transplantation, penetrating keratoplasty, or patch grafts. In cases of large paracentral or peripheral perforation and thinning, considering complications such as graft rejection and development of secondary glaucoma, small patch grafts can be used for the globe stabilization (6, 7). The use of a tectonic graft also eliminates the necrotic stroma, which secretes collagenase-like enzymes causing stromal degradation (8). In the present case, a patch graft suitable for the size of the corneal defect was transplanted for tectonic purposes.

Although perilimbal conjunctival resection, which is an old treatment method, is an effective method to control inflammation in PUK, it is neglected and not used frequently today (9). The value of this surgical method is thought to reduce the effects of immune complex sources, inflammatory cells and collagenolytic activity in the limbal conjunctiva. Thus, it induces healing of deep, non-infiltrative ulcers associated with RA (10). In the present case, conjunctival resection surgery which was combined with patch graft transplantation was performed because the inflammation was resistant and caused recurrent perforation.

CONCLUSION

In conclusion, corneal surgeons should keep in mind that conjunctival resection is one of the effective treatment modalities in especially resistant PUK patients with corneal perforation additional to immunosuppressive treatment and perforation management surgery.

Conflict of interest: The authors declared no conflict of interest.

References

1. Sabhapandit S, Murthy SI, Sharma N, et al. Surgical Management of Peripheral Ulcerative Keratitis: Update on Surgical Techniques and Their Outcome. *Clinical Ophthalmology*. 2022;16. doi:10.2147/OPTH.S385782.
2. Sharma N, Sinha G, Shekhar H, et al. Demographic profile, clinical features and outcome of peripheral ulcerative keratitis: a prospective study. *British Journal of Ophthalmology*. 2015;99:1503 LP - 1508. doi:10.1136/bjophthalmol-2014-306008.
3. Deshmukh R, Stevenson LJ, Vajpayee R. Management of corneal perforations: An update. *Indian J Ophthalmol*. 2020;68:7-14. doi:10.4103/ijo.IJO_1151_19.
4. Knop E, Knop N. The role of eye-associated lymphoid tissue in corneal immune protection. *J Anat*. 2005;206. doi:10.1111/j.1469-7580.2005.00394.x.
5. Gomes BF, Santhiago MR. Biology of peripheral ulcerative keratitis. *Exp Eye Res*. 2021;204:108458. doi:10.1016/j.exer.2021.108458.
6. Soong, H. Kaz M.D.; Katz, Douglas G. M.D.; Farjo, Ayad A. M.D.; Sugar, Alan M.D.; Meyer RFMD. Central Lamellar Keratoplasty for Optical Indications. *Cornea*. 18:249.
7. Soong HK, Farjo AA, Katz D, et al. Lamellar Corneal Patch Grafts in the Management of Corneal Melting. *Cornea*. 2000;19:126-134.
8. Vajpayee RB. Tectonic Grafts for Corneal Thinning and Perforations. *Cornea*. 2002;21:792-797.
9. Galor A, Thorne JE. Scleritis and Peripheral Ulcerative Keratitis. *Rheumatic Disease Clinics of North America*. 2007;33. doi:10.1016/j.rdc.2007.08.002.
10. Hassanpour K, ElSheikh RH, Arabi A, et al. Peripheral Ulcerative Keratitis: A Review. *J Ophthalmic Vis Res*. 2022. doi:10.18502/jovr.v17i2.10797.

Derleme / *Review*

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Yara iyileşmesi ve cilt rejenerasyonuna güncel bir yaklaşım: Kök hücre eksozom tedavisi

A current approach to wound healing and skin regeneration: Stem cell exosome therapy

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ÖZ

Yara iyileşme süreci, klinik uygulamada birtakım zorluklarla seyreden uzun bir süreç olup güncel tedavilerin etkileri halen sınırlıdır. Yara iyileşme süreci, hücrelerin göçü ve proliferasyonu, ekstraselüler matriksin yeniden şekillendirilmesi ve anjiyogenez ile ilişkilidir. Çeşitli risk faktörleri, kronik iltihaplanma ve bazı hastalıklar, yetersiz yara kapanmasına yol açarak fibrozisle sonuçlanabilecek bir yara izi oluşmasına neden olabilir. Son yıllarda, mezenkimal kök hücrelerin (MKH) yara iyileşmesi ve cilt yenilenmesi üzerinde güçlü terapötik potansiyele sahip olduğuna dair kanıtlar ortaya çıkmıştır. Ancak, MKH'lerin doğrudan uygulanmasında hala birçok sorunla karşılaşılmaktadır. Bununla birlikte son yıllarda, köken aldığı hücrelerden belirli bileşenler içeren lipid çift tabakalı membran yapısına sahip ve "granüler veziküller" olarak tanımlanan eksozomlar, MKH'ler için mükemmel bir alternatif olarak ortaya çıkmıştır. Çeşitli çalışmalarda özellikle MKH'lerden türetilen eksozomların (MKHE) yaraların iyileşmesi ve cilt rejenerasyonu için faydalı olduğu gösterilmiştir. Eksozomların cilt yaralarını iyileştirme sürecinde etkili olduğu mekanizmalar arasında inflamasyonu hafifletmek, damar oluşumunu uyarmak, epitel hücreleri ve fibroblastların proliferasyon ve göçünü uyarmak yer almaktadır. Bu nedenle, MKHE uygulanması, cilt yaralarının tedavisinde hücre tedavisine umut verici bir alternatif olabilir ve aynı anda birden fazla mekanizma aracılığıyla yara iyileşmesini teşvik edebilir. Bu derlemede, MKH'lerden türetilen eksozomların yara iyileşmesinde ve cilt rejenerasyonundaki rolü ve mekanizmaları hakkında güncel bilgiler sunulacak ve MKHE'lerin klinik uygulamalardaki potansiyelleri ayrıntılı olarak ele alınacaktır.

Anahtar Sözcükler: Yara iyileşmesi, cilt rejenerasyonu, mezenkimal kök hücre, eksozom**,** yaşlanma, eksozom tedavisi

ABSTRACT

The wound healing process is a long and challenging one in clinical practice, with current treatments showing limited effects. The wound healing process is associated with cell migration and proliferation, remodeling of extracellular matrix and angiogenesis. Various risk factors, chronic inflammation, and certain diseases can lead to insufficient wound closure, resulting in scar formation that may lead to fibrosis. In recent years, there has been evidence suggesting that mesenchymal stem cells (MSCs) have significant therapeutic potential for wound healing and skin regeneration. However, the direct application of MSCs still confronts many difficulties. Interestingly, exosomes, identified as "granular vesicles" with a lipid bilayer membrane structure involving specific components from their source cells, may reveal as an perfect alternative to MSCs. Various studies in recent years have shown that exosomes derived from MSCs are useful for wound healing and skin regeneration.

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The mechanisms by which exosomes are effective in the wound healing process include relieving inflammation, promoting vascularization, and the proliferation of epithelial cells and fibroblasts. Therefore, the application of MSC-exosomes may be a promising alternative to cell therapy in the treatment of skin wounds. This review will present current literature on the role and mechanisms of MSC-exosomes in wound healing and skin regeneration and will elaborate on the clinical potential of MSC-exosomes.

Keywords: *Wound healing, skin regeneration, mesenchymal stem cell, exosome, aging, exosome therapy.*

GİRİŞ

Vücudumuzun dış ortama karşı koruyucu bariyeri olan deri, güneşin ultraviyole ışınları ve çeşitli patojenler gibi çevresel tehditlere karşı savunmada önemli bir rol oynar. Ancak deri, travma veya yanıklara karşı çok hassas olup çeşitli patolojik durumlarda kronik yaralar veya ülserler geliştirmeye yatkındır. Günümüzde yara iyileşmesini uyarmak için standart tedavi stratejileri, büyüme faktörleri ve sitokinler gibi bazı biyolojik ajanlar kullanılmaktadır. Bununla birlikte, yara iyileşmesi, çeşitli hücre türlerini ve hücreler ile ekstraselüler matriks (ESM) arasındaki moleküler etkileşimi içeren karmaşık bir süreç olduğu için biyolojik ajanların tedavi edici etkileri sınırlıdır (1). Bu nedenle halen akut ve kronik cilt yaraları için yeni tedavi paradigmalarının keşfedilmesine ihtiyaç duyulmaktadır.

Son yıllarda yapılan deneysel ve klinik çalışmalar hasar görmüş dokuların tedavisinde kök hücre temelli yaklaşımların, büyüme faktörleri veya sitokin uygulamalarına dayalı tedavilere göre birçok avantaja sahip olduğunu desteklemektedir. Özellikle kendi kendini yenileme ve farklılaşma yetenekleri olan pluripotent mezenkimal kök hücreleri (MKH), çeşitli doku yaralanmaları üzerinde güçlü tedavi edici etkilere sahiptir (2, 3). Mezenkimal kök hücreleri kemik iliği, yağ dokusu, diş pulpası ve umblikal kordon gibi birçok dokudan elde edilebilmekte olup hücre göçü ve proliferasyonu, anjiyogenez, inflamasyonun baskılanması ve ESM'nin düzenlenmesi gibi birçok fizyopatolojik süreçte rol oynamaktadır (4). Bununla birlikte MKH'lerin elde edilmesi çoğu zaman invaziv ve zaman alıcı prosedürlerle gerçekleştirilmekte ve aynı zamanda sistemik ve lokal uygulamalarda hedef bölgelere ulaşma ve tutunma sorunları ortaya çıkmaktadır.

MKH'lerin cilt yaralarını tedavi edici özelliklerine yönelik yapılan yeni çalışmalarda kök hücrelerin çoğalma ve farklılaşma yeteneklerinden çok parakrin etkilerle bunu gerçekleştirdiği görülmüştür (5, 6). Bu sonuçlardan yola çıkarak hücre uygulamaları yerine MKH kaynaklı ekstraselüler veziküllerin (EV'ler), özellikle eksozomların uygulanması, cilt yaralarının iyileşmesinde ve cilt rejenerasyonunda umut verici yeni bir tedavi paradigması olacağı düşünülebilir.

Bu derlemede, MKH kaynaklı eksozomların (MKHE) yara iyileşmesindeki tedavi edici etkinlikleri ve cilt rejenerasyonundaki rolü, altta yatan hücresel ve moleküler mekanizmalar güncel literatür eşliğinde ele alınacaktır.

Yara iyileşmesinin fizyolojik temeli

Cilt yaraları, cildin yapısını veya bütünlüğünü bozan çeşitli içsel patolojik ve dış mekanik faktörler tarafından oluşabilir. Ciltte oluşan bir yaranın onarımı, hemostaz, inflamasyon, proliferasyon, anjiyogenez, yara kontraksiyonu, kolajen birikimi ve yeniden yapılanma (remodelasyon) gibi birçok faaliyetin gerçekleştiği karmaşık bir süreçtir (Şekil 1) (1,6-9). Bu süreçte, belirli aşamalarda işlev gören birçok farklı cilt hücreleri ve bağışıklık hücreleri arasında dinamik etkileşimler ortaya çıkar. Yara oluşumunu takiben dakikalar içinde hemostatik süreç gerçekleşir ve pıhtılaşma ile oluşan fibrin pıhtısı inflamatuar hücreler için bir iskele (skafold) görevi görür. Sonraki 24 saat içinde nötrofiller ve ardından makrofajlar yara bölgesine göç ederler ve yara yerindeki patojenleri, hücre kalıntılarını ve apoptotik hücreleri ortadan kaldırarak iyileşme için ortam hazırlarlar (1,6-8). Yara bölgesinde gelişen inflamasyon, M1 makrofajlarının M2 makrofajlarına dönüşümünü artırır. M2 makrofajları, keratin sentezleyen hücrelerin, fibroblastların ve endotel hücrelerinin çoğalmasını ve göçünü yöneterek doku onarımını ve ESM üretimini uyarır. Bu süreci takiben yara matriksi, kapillerler, fibroblastlar ve kolajen fibrillerini içeren ve hücrelerin göçü ve büyümesi için bir iskelet görevi görecek granülasyon dokusu ile yavaş yavaş değiştirilir (6-8). Daha

sonra, epitelizasyon aşamasına gelindiğinde, keratinositler hasarlı dermise göç ederler ve epitelyal bariyer işlevini yeniden oluştururlar. Hücreler hızla çoğalır, yeni damarlar ve epitel ortaya çıkar. Sonrasında, fibroblastlar miyofibroblastlara farklılaşır ve yara bölgesini daraltırlar. Kolajen birikimi aşamasında, fibroblastlar tarafından ilk olarak matriks içine yüksek konsantrasyonlarda olgunlaşmamış Tip III kolajen salgılanırken yeniden yapılanma (remodelasyon) aşamasında, Tip III kolajeni Tip I kolajene dönüştürülür, bu da yaranın kapanmasına izin verir. Süreç boyunca, fibroblastlar, adipositler, endotel hücreleri, keratinositler, makrofajlar ve diğer bağışıklık hücreleri gibi birçok cilt hücresi, yara iyileşmesini teşvik etmek için etkileşime girer. Remodelasyon fazı 3.haftada başlar ve uzun aylar hatta yıllar boyunca devam edebilir (1, 6-9).

Klinikte kronik yaralar, altı hafta içinde iyileşmeyen, derin, tam veya kısmi kalınlıkta yaralar olarak tanımlanır. Bu yaralar yavaş iyileşirler ve bazı kişilerde hiperplastik skar ve keloidlerle sonuçlanabilen ciddi fibröz doku oluşumu görülür. Kötü görsel görünümünün yanı sıra, skar çevresindeki doku, glandula sebacea, folliculus pili ve duyusal sinir reseptörleri gibi bazı temel dermal bileşenden yoksundur (9, 10). Skar oluşumuna neden olan birkaç risk faktörü vardır; bunlar arasında aşırı kolajen birikimi, azalmış fibroblast apoptozu, gecikmiş keratinosit fonksiyonu, artmış transforme edici büyüme faktörü β1 (TGF-β1) ekspresyonu, aşırı anjiyogenez, uzun süreli inflamasyon ve yaşlanma sayılabilir. İnflamatuar tepkinin erken yönetimi, yenilenme için önemlidir çünkü çözülmemiş uzun süreli inflamasyon, yenilenme yerine skar oluşumuna yol açabilir (11-13).

Cilt yaşlanmasının fizyolojik temeli

Yaşlanmakta olan cilt, çeşitli iç ve dış faktörler nedeniyle kaçınılmaz olarak yapısal ve fonksiyonel özelliklerini kaybeder. Araştırmalar, genel olarak dış faktörlerin cilt yaşlanmasının ana nedeni olduğunu ve yalnızca %3 oranında içsel faktörlerin yaşlanmaya katkıda bulunduğunu öngörmektedir. Dış faktörler arasında hava kirleticileri, yaşam tarzı seçimleri ve özellikle UV maruziyeti cilt yaşlanmasının başlıca nedenleridir. Yaşlanma, cilt elastikiyetini azaltır ve cilt kalınlığı ve kolajen dokusunu değiştirir, kırışıklıklara yol açar. UV maruziyetine bağlı yaşlanma, çoğunlukla ESM yapısındaki değişikliklere bağlı olarak ortaya çıkan düzensiz pigmentasyon, pürüzlülük, kuruluk ve kırışıklıklar ile kendini gösterir (14, 15).

Cilt yaşlanma mekanizmaları oldukça karmaşık olup genetik mutasyonlar, DNA hasarı, hücre yaşlanması, inflamasyon ve oksidatif stres süreçlerini içerir. DNA onarım mekanizmalarındaki yaşa bağlı eksiklikler, epidermal kök hücrelerin kendini yenileme kapasitesini zayıflatarak kromozomal yeniden düzenlemelere veya mutasyonlara yol açabilir ve böylece cilt yaşlanmasını hızlandırabilir ve/veya kanser gelişimini artırabilir. Oksidatif stresin cilt yaşlanması üzerinde etkileri uzun yıllardır bilinmekte olup melatonin, C vitamini, A vitamini ve glutatyon gibi çeşitli antioksidanlar cilt yenilenmesine yardımcı olma potansiyeline sahiptir (9, 15).

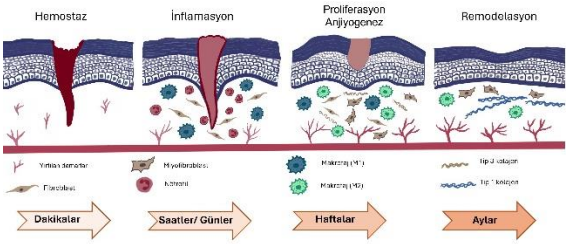
Yaşlanma sırasında tip I ve III kolajen üretimi etkilenir ve ayrıca kolajen parçalanması ve düzensizliği de meydana gelir. Yaşlanmış ciltte kolajende görülen değişiklikler, yeni kolajen üretiminin azalması ve fibroblast proliferasyonunun azalmasının yanı sıra matriks metalloproteinazların (MMP), özellikle MMP-1'in artan üretiminin bir sonucudur (16). Kolajen ve elastin yıkımı, cildin gücünü ve esnekliğini kaybetmesine neden olur, bu da klinik olarak kırışıklık ve sarkma olarak kendini gösterir. Aşırı üretilen reaktif oksijen türleri (ROT), hücre fonksiyonuna ve yapısına doğrudan zarar verebilir, inflamatuar tepkileri düzenleyebilir ve cilt yaşlanma sürecini hızlandırabilir. ROT tarafından uyarılan MMP sentezi, p38, ekstraselüler sinyal düzenleyici kinaz ve c-Jun-terminal kinaz içeren mitojenle aktive edilen protein kinaz (MAPK) sinyal yolu ile düzenlenir. Foto yaşlanma ve UV radyasyonuna yanıtı düzenleyen başka bir transkripsiyon faktörü ise inflamasyon ve MMP üretimine aracılık ederek yöneten nükleer faktör-κB (NF-κB) olarak bilinmektedir (17, 18).

Epidermisteki melanositler ve keratin oluşturan hücreler arasındaki etkileşim, cilt pigmentasyonundan sorumludur. Cilt UV radyasyonuna maruz kaldığında, keratin oluşturan hücreler, melanositleri melanin üretmeye teşvik eden endotelin-1 ve α-melanosit uyarıcı hormon (α-MSH) gibi parakrin hormonlar salgılar. Uygun miktarda melanin doğal bir güneş koruyucusu işlevi görürken, aşırı melanin üretimi UV kaynaklı pigmentasyon bozuklukları olarak görülen güneş lekesi hastalığı ve melazma gibi hiperpigmentasyona yol açar (9, 19).

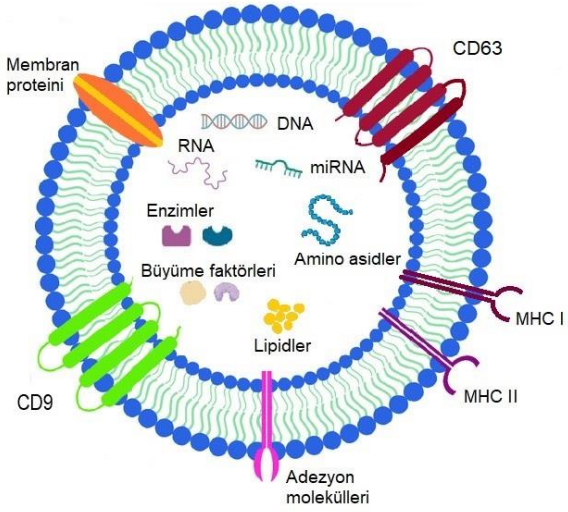
Mezenkimal kök hücre kaynaklı eksozomların yara iyileşmesi ve cilt rejenerasyonundaki etkileri

Mezenkimal kök hücreler (MKH), kendi kendini yenileyebilen, çok yönlü farklılaşma potansiyeline ve parakrin düzenleme yeteneğine sahip kök hücrelerdir. Kolay izole edilebilmeleri, çoğaltılabilmeleri ve çok yönlü farklılaşma potansiyelleri nedeniyle, MKH'ler doku onarımı da dahil olmak üzere rejeneratif tıp alanında önemli bir kök hücre kaynağı olarak kabul edilmektedir. MKH'ler kemik iliği dışında yağ dokusu, kas, göbek kordonu ve diş gibi diğer dokularda da bulunur. MKH'ler yüzey belirteci olarak CD105, CD90 ve CD73'ü eksprese ederlerken CD34, CD45, CD19, CD11b, CD79α veya CD14'ü eksprese etmezler. MKH'leri hücre terapisi için umut verici bir hedef haline getiren başlıca özellikleri bağışıklık modülasyonu, anti-inflamasyon ve rejenerasyon potansiyeli göstermeleridir (2, 3). Kök hücreler, cilt rejenerasyonu ve gençleştirilmesini, doku damarlanmasını, yumuşak doku rejenerasyonunu, kemik ve kıkırdak onarımını ve saç folikülü rejenerasyonunu uyarmak gibi güçlü terapötik etkilere sahiptir. Ancak, kök hücrelerin yara iyileşmesinde terapötik kullanımı, depolama zorlukları, mutasyonla ilişkili tümör oluşumu, immün rejeksiyon ve etik düzenlemeler nedeniyle sınırlıdır (4).

Tüm ökaryotik hücreler, normal fizyolojilerinin bir parçası olarak hücre dışı veziküller (EV'ler) salgılar. EV'ler, farklı boyutlardaki lipid çift katmanlı veziküler yapılar olarak tanımlanabilir. EV'ler geniş anlamda ektozomlar ve eksozomlar olmak üzere iki kategoriye ayrılabilir. Ektozomlar, plazma zarının yüzeyinden dışa doğru tomurcuklanma yoluyla kopan veziküllerdir ve mikroveziküller, mikropartiküller ve çapları ~50 nm ile 1 µm arasında değişen büyük vezikülleri içerir. Eksozomlar ise endozomal kökenli olup çapları ~40 ila 160 nm (ortalama ~100 nm) arasında olan EV'lerdir. Plazma zarının ardışık invajinasyonu sonucunda multiveziküler cisimlerin oluşumuna yol açar ve bu cisimler diğer hücre içi veziküller ve organellerle kesişebilir, eksozomların bileşenlerinde çeşitliliğe katkıda bulunur. Kaynak hücreye bağlı olarak, eksozomlar da dahil olmak üzere EV'ler, DNA, RNA, miRNA, lipidler, metabolitler ve sitozolik ve hücre yüzeyi proteinleri gibi birçok hücre bileşenini içerebilir (Şekil-2) (8, 20, 21).



Şekil-1. Yara iyileşme sürecinde gelişen hemostaz, inflamasyon, proliferasyon, anjiyogenez ve remodelasyon evreleri.



Şekil-2. Eksozomlar, tüm hücreler tarafından üretilen hücre dışı veziküller olup DNA, RNA, proteinler, lipitler, enzimler, büyüme faktörleri gibi çok çeşitli hücre bileşenini taşırlar.

Son yıllarda, özel bir EV kategorisi olarak eksozomlar, daha geniş ve derinlemesine incelenmektedir (22). Eksozomlar, serum, tükürük, süt, beyin omurilik sıvısı, idrar ve semen gibi vücut sıvılarında dağılmış olarak bulunabilirler. Eksozomlar, terapötik etkilerini genişletmek, değiştirmek veya iyileştirmek için biyokimyasal olarak modifiye edilebilirler. Eksozomların modifikasyonu, iç stratejiler (örneğin, ilaç yükleme) ve dış stratejiler (örneğin, yüzey modifikasyonu) olarak sınıflandırılır. Eksozomlar stabilite, bağışıklık yanıtı oluşturmama ve hedef hücrelere ulaşma avantajları nedeniyle ilaç, nükleik asitler ve aşıları taşımak için ideal bir terapötik taşıyıcı olabilirler. Özellikle, mezenkimal kök hücre eksozomları (MKHE), kök hücrelerin ürünleri oldukları için kök hücrelere benzer çok önemli biyolojik işlevlere sahiptir. MKHE, kök hücrelerden gelen parakrin

faktörlerin biyolojik etkilerinin önemli aracıları olup hücresiz terapötik yara iyileşmesi için ideal bir yaklaşım sunar. MKHE ayrıca vasküler endotelyal büyüme faktörü (VEGF), transforme edici büyüme faktörü-β1 (TGF-β1), interlökin-6 (IL-6) ve interlökin-10 (IL-10) gibi anjiyogenez ve immünmodülasyonu kolaylaştıran çok çeşitli sitokinleri içerir. Metabolitler, proteinler, DNA ve kodlamayan RNA'lar (ncRNA'lar) gibi MKHE'lerin bileşenleri fibroblastlar, keratinositler, bağışıklık hücreleri ve endotelyal hücreler gibi hücreler tarafından alınarak yara dolaşımının iyileştirilmesi, anjiyogenezin uyarılması, inflamasyonun düzenlenmesi ve kök hücrelerin uyarılması yoluyla yara onarımını hızlandırabilirler (6, 7, 9, 21, 23). Burada dikkat çekici olan, eksozomların yüksek derecede heterojenite göstermesidir; bu durum, hücrelerde çeşitli yolakların aktifleşmesine ve farklı biyolojik fonksiyonların uyarılmasına yol açar. Eksozomların heterojenliği, boyut, içerik ve köken hücresi gibi faktörlerden etkilenmektedir (24).

Cilt yaralarının iyileşmesi, keratinositler, fibroblastlar, endotelyal hücreler, adipositler, makrofajlar ve diğer bağışıklık hücreleri de dahil olmak üzere çeşitli cilt hücreleri arasında eksozomlar aracılığıyla gerçekleşen hücreler arası etkileşimlerle ortaya çıkar. Birçok yara iyileşme modelinde, çeşitli hücre tiplerinden elde edilen eksozomların yara iyileşme sürecinin inflamasyon, proliferasyon ve yeniden yapılanma aşamalarında faydalı etkiler gösterdiği kanıtlanmıştır. İnflamasyon aşamasında, nötrofiller önce mikrobiyal patojenleri temizlemek için yaralanma bölgesine sızar ve ardından apoptoza uğrar, ardından hücresel kalıntıları, apoptotik nötrofilleri ve diğer apoptotik hücreleri yutan makrofajların sızması izlenir (6, 7, 9, 21, 23). Örneğin, Li ve ark. makrofaj kaynaklı eksozomların, pro-inflamatuar sitokinler ve enzimlerin salgılanmasını azaltarak, yoğun anjiyogenez ve proliferasyon etkileriyle diyabetik yara iyileşmesini teşvik edebildiğini keşfetmişlerdir (25). Cilt rejenerasyon sürecinde belirgin ve önemli bir rol oynayan makrofajların iki farklı fonksiyonel fenotip gösterdiğini öne sürülmektedir. Bunlar pro-inflamatuar M1 fenotipi ve anti-inflamatuar M2 fenotipi. Yaralanmayı takiben, M1 makrofajları, hasarlı doku ve hücrelerin ortadan kaldırılması için gerekli olan pro-inflamatuar aktiviteleri uyarırken, M2 makrofajları, doku tamirini ve rejenerasyonu kolaylaştıran anti-inflamatuar aktiviteler gösterir. Bununla birlikte, aşırı pro-inflamasyon aktiviteleri ve yetersiz anti-inflamatuar aktiviteler, kronik yaraların veya fibrozis gelişme riskine yol açabilir. Bu konuyla ilgili yapılan çalışmalar eksozomların mikroRNA'ları (miRNA'lar) aktararak M2 polarizasyonunu tetikleyebileceğini göstermektedir. Örneğin, insan göbek kordonu MKHE'lerinin, yanık yarası modeli yapılan sıçanlarda miR-181c yoluyla makrofajlarda tümör nekroz faktörü α (TNF-α) ve interlökin-1β (IL-1β) seviyelerinin artışını ve IL-10 seviyelerinin azalmasını sağlayarak makrofajların M2 polarizasyonunu uyarabileceği gösterilmiştir (26). Bir başka çalışmada He ve arkadaşları, kemik iliği MKHE'lerinin makrofaj polarizasyonunu M2 fenotipine doğru indüklediğini bildirmiştir (27).

Yara iyileşmesi sürecinde proliferasyon fazında başlıca fibroblast proliferasyonu, ESM bileşenlerinin üretimi, yeniden epitelizasyon ve anjiyogenez olmak üzere başlıca 4 önemli olay meydana gelir. İnflamasyon sonrasında gelişen bu olaylar yeni dokuları oluşturmak ve cildin morfolojisini ve işlevini yeniden sağlamak için çok önemlidir. Çok sayıda kanıt, eksozomların bu dört süreç üzerinde olumlu terapötik etkileri olduğunu göstermiştir. Shabbir ve arkadaşlarının *in vitro* çalışmasında, MKHE'lerin fibroblastların proliferasyonunu ve göçünü artırabileceği göstermiştir. Etkilerin, yara iyileşmede önemli olduğu bilinen AKT, ERK ve STAT3'ü içeren hücre içi sinyal yollarının aktivasyonlarıyla tetiklendiği kanıtlanmıştır (28). Benzer şekilde Zhang ve arkadaşlarının çalışmasında, yağ dokusu kök hücre türevli eksozomların fibroblastlar üzerinde olumlu etkileri olduğu, kolajen birikimini ve fibroblast büyüme faktörü (bFGF) ve transforme edici büyüme faktörü-β1 (TGF-β1) gibi büyüme faktörlerinin ekspresyonunu teşvik ettiği hem *in vitro* hem de *in vivo* olarak gösterilmiştir (29). Bu sonuçlar, MKHE'lerin proliferasyon fazında yeniden epitelizasyon sürecini hızlandırabileceğini desteklemektedir. Ren ve arkadaşlarının daha kapsamlı bir çalışmasında, yağ dokusu kaynaklı MKH'lerden elde edilen mikroveziküllerin fibroblastlar, keratinositler ve endotel hücreleri üzerindeki etkileri hem *in vitro* hem de *in vivo* olarak incelenmiştir. Bu çalışmanın sonuçları, mikroveziküllerin bu hücrelerin proliferasyonunu ve göçünü AKT ve ERK sinyal yolları aracılığıyla teşvik ettiğini, vasküler endotel büyüme faktörü (VEGF), platelet kaynaklı büyüme faktörü (PDGF), epidermal büyüme faktörü ve FGF2'nin ekspresyonunu arttırdığını göstermiştir (30).

İnsan göbek kordonu MKH'lerinden türetilen ekzosomların, bir yara kesisine (çap 12 mm) uygulanmasından sonra epitel, sinirler ve kan damarlarının yenilenmesinin hızlandığı bildirilmiştir. Eksozomlar yara iyileşmesi sırasında kolajen liflerinin dağılımının düzenlenmesine katkıda bulunmuşlar, ayrıca hem *in vitro* hem de hayvan modellerinde deri hücrelerinin proliferasyonunu ve göçünü arttırmışlardır (31). Pomatto ve arkadaşlarının yaptıkları bir çalışmada kemik iliği ve yağ dokusu kaynaklı MKH'lerden türetilen eksozomlar diyabetik yara iyileştirme potansiyelleri açısından karşılaştırılmıştır. Çalışmanın sonuçları yağ dokusu MKHE'lerinin kemik iliği MKHE'lerine göre daha etkili olduğunu göstermiştir. Ayrıca yağ dokusu MKHE'lerinin anjiyogenezi uyarırken, kemik iliği MKHE'lerinin ise daha çok hücre proliferasyonunu uyardığı gözlenmiştir (5). Bir diğer çalışmada ise Zhou ve arkadaşları, yağ dokusu kaynaklı kök hücreleri ve bunlardan elde edilen eksozomları birlikte ve ayrı ayrı kullanarak yara iyileşmesi üzerindeki etkilerini incelemişlerdir. Çalışmanın sonuçları 2 ürünün birlikte uygulanması halinde epitelizasyonun ve anjiyogenezin daha iyi geliştiğini ve aynı zamanda skar oluşumunun da azaldığını göstermiştir (32).

Eksozomlar yara iyileşmesinin yanı sıra cilt yenilenmesi, pigmentasyonun düzenlenmesi ve kıl büyümesi gibi birçok fizyopatolojik süreçlerde de denenmiş olup çeşitli *in vitro* ve hayvan çalışmalarında olumlu sonuçlar bildirilmiştir. Örneğin, Hu ve arkadaşları, insan dermal fibrositlerinin (HDF) üç boyutlu kültüründen elde edilen eksozomların foto-yaşlanma modeli uygulanan farelerde TNF-α'yı baskılama ve TGF-β'yı uyarma yönünde düzenleyerek, tip I prokollajen artışına, MMP-1 ekspresyonunda azalmaya ve yaşlanma karşıtı etkilere neden olduğunu göstermişlerdir (33).

Klinik bakış açısı

Literatürde osteoartrit, kas ve tendon yaralanmaları, iskemik hasar, sinir yaralanmaları, Alzheimer hastalığı, Parkinson hastalığı gibi çeşitli deneysel hastalık modellerinde eksozom tedavisinin uygulandığı ve başarılı sonuçlar elde edildiği çok sayıda çalışma olduğu görülmektedir. Ancak insan çalışmaları bağlamında değerlendirildiğinde fazla sayıda klinik çalışma olmadığı ve eksozom tedavisinin henüz deneme aşamasında olduğu anlaşılmaktadır. Günümüzde eksozomların klinik olarak denendiği alanlar başlıca biyobelirteç, ilaç taşıyıcısı, kanser aşısı ve tedavi amaçlı kullanım olarak sıralanabilir. Eksozom tedavisinin uygulandığı klinik çalışmalar ClinicalTrials.gov (https://clinicaltrials.gov/) adresinde tarandığında yara iyileşmesi ve ciltle ilişkili patolojilerin tedavisine yönelik şu an için 27 adet çalışma yürütülmektedir. Konuyla ilgili yayınlanmış çalışmalar incelendiğinde, 12 haftalık randomize bir çift kör kontrollü klinik bir araştırmada yüzdeki atrofik akne skarlarına fraksiyonel CO_2 lazer uygulaması sonrası adjuvan tedavi olarak cilde jel formunda uygulanan yağ dokusu MKHE'lerinin klinik etkinliğini araştırılmıştır. Çalışmada yağ dokusu MKHE'leri ile tedavi edilen taraflarda, edilmeyen taraflara göre anlamlı derecede bir iyileşme olduğu görülmüştür. Aynı zamanda tedavi ile ilişkili eritem daha hafif ve tedavi sonrası iyileşme süresi daha kısa olmuştur. Bu çalışma MKHE'lerinin cilt yenilenmesinde kullanılan yüzey yenileme cihazları ile birlikte uygulanmasının hem etkinlik hem de güvenlik açısından atrofik akne izlerinin tedavisinde sinerjik etkiler sağlayabileceğini ortaya koymuştur (34).

Yakın zamanda yayınlanan bir olgu çalışmasında ise, kafa derisindeki anjiyosarkom nedeniyle neoadjuvan kemoterapi, ardından eşzamanlı kemoradyoterapi, geniş lokal eksizyon ve serbest flep ve kısmi kalınlıkta deri grefti uygulanan 60'lı yaşlarda bir erkek hastada 1 yıl boyunca iyileşmeyen 2 kronik yara geliştiği bildirilmiştir. Bu hastaya 7 ay boyunca debritman işlemi sonrası anti-enflamatuvar ve anjiyojenik büyüme faktörleri ile zenginleştirilmiş trombosit kaynaklı Saflaştırılmış Eksozom Ürünü (PEP) uygulanmıştır. Araştırmacılar bu olguya uygulanan PEP tedavisinin yaralarda %96 ve %100 oranında iyileşme sağladığını, herhangi bir yan etki ve komplikasyon görülmediğini bildirmişlerdir (35).

Bir başka olgu çalışmasında, akne, hafif akne izleri ve melazması olan 31 yaşındaki bir kadın hastanın yüz, göğüs ve sırt bölgelerine fraksiyonel non-ablatif lazer tedavisi yapılmıştır. Hasta son uygulamadan sonra rahatsızlık açısından değerlendirilmiş ve ısı rahatsızlığı, yanma ve batma için 10 üzerinden 8 ağrı seviyesi bildirilmiştir. Değerlendirmeden sonra, tedavi alanlarına toplam 3 mL insan plasentası kaynaklı MKH'lerden elde edilen eksozom serumu uygulanmıştır. Uygulamanın hemen ardından, hasta rahatsızlık şiddetinin 10 üzerinden 4'e düştüğünü bildirmiş olup hastanın ilgili

bölgelerindeki eritem ve şişliklerinin iyileşme süresi de azalmıştır. Aynı araştırmacıların bir başka olgusu ise alt dudağında köpek ısırığı olan 49 yaşında kadın hasta olup acil serviste yapılan yara dikişinden 20 saat sonra tedavi için dermatoloji kliniğine başvurmuş ve hastanın yara bölgesine 2.5 mL eksozom, her seferinde birkaç damla olacak şekilde, 10 dakika boyunca uygulanmıştır. Eksozom uygulamasından 18 saat sonra yara iyileşmesi gözlemlenmiş ve 10.günde ise yara tamamen kapanmış, fibrotik doku belirtisi olmadan, minimal iz ve duyusal ve motor fonksiyonları korunarak iyileşmiştir (36).

Cilt rejenerasyonu ve kozmetik dermatoloji açısından eksozom uygulamasının olumlu etkileri birkaç klinik araştırma ile desteklenmiştir. Bu çalışmalardan birinde, Proffer ve arkadaşları, insan trombositlerinden türetilmiş eksozomlar içeren topikal bir serum kullanarak 56 kişinin yer aldığı 6 haftalık bir klinik denemede cilt sağlığında önemli iyileşmeler ve kızarıklık, kırışıklık ve melanin üretiminde azalma gözlemlemişlerdir (37). Diğer bir çalışmada, Park ve arkadaşları, 28 kişi üzerinde, 12 haftalık tedavi sonrasında mikro iğneleme ile uygulanan insan yağ MKHE'lerinden türetilmiş eksozomların, yalnızca mikro iğneleme ile karşılaştırıldığında cilt estetiğini önemli ölçüde iyileştirdiğini bildirmişlerdir. Eksozom uygulanan hastalarda kolajen içeriği artışı, kırışıklıkların azalması, elastikiyet, nemlendirme ve pigmentasyon bozukluklarında anlamlı düzelme elde edilmiştir (38).

TARTIŞMA

Vücuttaki tüm hücrelerden kaynaklanabilen eksozomlar, biyolojik olarak aktif bileşenlerin taşınması, hücre içi bileşenlerin uzaklaştırılması ve ilaç taşıyıcıları olarak işlev görmektedirler. MKH kaynaklı eksozomlar, yara iyileşmesini ve cilt rejenerasyonunu teşvik etmede MKH'lere göre belirli uygulama avantajlarına sahip olabilirler. Eksozom kaynağı olarak insan yağ dokusu, kemik iliği ve göbek kordonu kaynaklı MKH'ler yara iyileşmesi için en çok çalışılmış ve olumlu sonuçlar alınmış hücreler olup gelecekte umut verici yeni terapötik strateji olarak kabul edilmektedir. MKH-eksozomlarının klinik uygulaması titiz bir kalite yönetimini gerektirir ve bu nedenle hücrelerin, kültür serumunun ve eksozomların izolasyonunu içeren yüksek derecede bir standart protokoller kullanılmalıdır. Eksozom izolasyonu için teknikler çeşitlidir ve ultra santrifüj, boyut filtrasyonu, boyut ayırma kromatografisi, polimer çöktürme ve birkaç yeni birleşik teknikleri içerir (8, 20, 21). Ancak, şu an için MKH-eksozomlarının izolasyonu, taşınması ve korunması veya tanımlanması için tam olarak standardize edilmiş bir protokol yoktur.

Günümüzde, piyasada cilt ve saç tedavisi için eksozom içeren çeşitli ürünler bulunmaktadır. Bu ürünlerin birçoğu lazer tedavileri ve mikro iğneleme gibi minimal invaziv prosedürlerle birlikte kullanılmak üzere tasarlanmıştır. Popülerliklerinin artmasına rağmen, klinik kanıtların yetersizliği, yara iyileşmesi ve kozmetik dermatolojide yaygın olarak kullanılmalarını henüz haklı çıkarmamaktadır.

SONUÇ

Sonuç olarak; eksozom tedavisinin güvenliği ve etkinliğini, tedavi dozunu, hücre kaynağını ve uygulama sıklığını optimize etmek için geniş kapsamlı klinik araştırmalara gereksinim vardır.

Çıkar çatışması: Bu makaleyle bağlantılı olarak herhangi bir çıkar çatışması yoktur.

Yazarların tümü, derlemenin tasarımına, yürütülmesine ve analizine katkıda bulunduklarını ve son halini onayladıklarını beyan etmektedir.

Kaynaklar

1. Sylakowski K, Bradshaw A, Wells A. Mesenchymal stem cell/multipotent stromal cell augmentation of wound healing: lessons from the physiology of matrix and hypoxia support. Am J Pathol 2020;190(7):1370-1381. doi:10.1016/j.ajpath.2020.03.017.
2. Taylor DA, Chacon-Alberty L, Sampaio LC, et al. Recommendations for nomenclature and definition of cell products intended for human cardiovascular use. Cardiovasc Res 2022;118(11):2428-2436. doi:10.1093/cvr/cvab270.
3. Saadh MJ, Ramírez-Coronel AA, Saini RS, et al. Advances in mesenchymal stem/stromal cell-based therapy and their extracellular vesicles for skin wound healing. Hum Cell 2023;36(4):1253-1264. doi:10.1007/s13577-023-00904-8.

4. Zakrzewski W, Dobrzynski M, Szymonowicz M, Rybak Z. Stem cells: past, present, and future. Stem Cell Res Ther 2019;10:68. doi: 10.1186/s13287-019-1165-5.
5. Pomatto M, Gai C, Negro F et al (2021) Differential therapeutic effect of extracellular vesicles derived by bone marrow and adipose mesenchymal stem cells on wound healing of diabetic ulcers and correlation to their cargoes. Int J Mol Sci 2021;22:1–26. doi:10. 3390/ijms2 20838 51.
6. Hu JC, Zheng CX, Sui BD, Liu WJ, Jin Y. Mesenchymal stem cell-derived exosomes: A novel and potential remedy for cutaneous wound healing and regeneration. World J Stem Cells 2022;14(5):318-329. doi:10.4252/wjsc.v14.i5.318.
7. Ding JY, Chen MJ, Wu LF. et al. Mesenchymal stem cell-derived extracellular vesicles in skin wound healing: roles, opportunities and challenges. Military Med Res 2023;10:36. doi:10.1186/s40779-023-00472-w.
8. Zhou C, Zhang B, Yang Y, et al. Stem cell-derived exosomes: emerging therapeutic opportunities for wound healing. Stem Cell Res Ther 2023;14(1):107. doi:10.1186/s13287-023-03345-0.
9. Li J, Liu Y, Zhang R, et al. Insights into the role of mesenchymal stem cells in cutaneous medical aesthetics: from basics to clinics. Stem Cell Res Ther 2024;15(1):169. doi:10.1186/s13287-024-03774-5.
10. Rani Raju N, Silina E, Stupin V, Manturova N, Chidambaram SB, Achar RR. Multifunctional and smart wound dressings-A review on recent research advancements in skin regenerative medicine. Pharmaceutics 2022;14(8). doi:10.3390/pharmaceutics14081574.
11. Nathan C. Points of control in inflammation. Nature. 2002;420(6917):846–852.
12. Serhan CN, Savill J. Resolution of inflammation: the beginning programs the end. Nat Immunol 2005;6(12):1191–1197. doi:10.1038/ni1276.
13. Landén NX, Li D, Ståhle M. Transition from inflammation to proliferation: a critical step during wound healing. Cell Mol Life Sci 2016;73(20):3861–3885. doi: 10.1007/s00018-016-2268-0.
14. Chaudhary M, Khan A, Gupta M. Skin ageing: pathophysiology and current market treatment approaches. Curr Aging Sci 2020;13(1):22-30. doi:10.2174/1567205016666190809161115.
15. Qian H, Shan Y, Gong R, et al. Mechanism of action and therapeutic effects of oxidative stress and stem cell-based materials in skin aging: Current evidence and future perspectives. Front Bioeng Biotechnol 2023;10:1082403. doi:10.3389/fbioe.2022.1082403.
16. Quan T, Fisher GJ. Role of age-associated alterations of the dermal extracellular matrix microenvironment in human skin aging: a mini-review. Gerontology 2015;61(5):427-434. doi:10.1159/000371708.
17. Gu Y, Han J, Jiang C, Zhang Y. Biomarkers, oxidative stress and autophagy in skin aging. Ageing Res Rev 2020;59:101036. doi:10.1016/j.arr.2020.101036.
18. Wang Y, Wang L, Wen X, et al. NF-κB signaling in skin aging. Mech Ageing Dev 2019;184:111160. doi:10.1016/j.mad.2019.111160.
19. Kim M, Shibata T, Kwon S, Park TJ, Kang HY. Ultraviolet-irradiated endothelial cells secrete stem cell factor and induce epidermal pigmentation. Sci Rep 2018;8(1):4235. doi:10.1038/s41598-018-22608-y.
20. Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. Science 2020;367:eaau6977.
21. Arbade G, Jose JV, Gulbake A. et al. From stem cells to extracellular vesicles: a new horizon in tissue engineering and regenerative medicine. Cytotechnology 2024;76,363–401. doi:10.1007/s10616-024-00631-4
22. Heo JS, Kim S, Yang CE, Choi Y, Song SY, Kim HO. Human adipose mesenchymal stem cell-derived exosomes: a key player in wound healing. Tissue Eng Regen Med 2021;18:537–48.
23. Xiong M, Zhang Q, Hu W, et al. The novel mechanisms and applications of exosomes in dermatology and cutaneous medical aesthetics. Pharmacol Res 2021;166:105490. doi:10.1016/j.phrs.2021.105490.
24. Ye H, Wang F, Xu G, Shu F, Fan K, Wang D. Advancements in engineered exosomes for wound repair: current research and future perspectives. Front Bioeng Biotechnol 2023;11:1301362. doi:10.3389/fbioe.2023.1301362.
25. Li M, Wang T, Tian H, Wei G, Zhao L, Shi Y. Macrophage-derived exosomes accelerate wound healing through their anti-inflammation effects in a diabetic rat model. Artif Cells Nanomed Biotechnol 2019;47(1):3793-3803. doi:10.1080/21691401.2019.1669617.
26. Li X, Liu L, Yang J, et al. Exosome derived from human umbilical cord mesenchymal stem cell mediates mir-181c attenuating burn-induced excessive inflammation. EBioMedicine 2016;8:72-82. doi:10.1016/j.ebiom.2016.04.030.
27. He X, Dong Z, Cao Y, et al. MSC-derived exosome promotes M2 polarization and enhances cutaneous wound healing. Stem Cells Int 2019;2019:7132708. doi:10.1155/2019/7132708.

28. Shabbir A, Cox A, Rodriguez-Menocal L, Salgado M, Van Badiavas E. Mesenchymal stem cell exosomes ınduce proliferation and migration of normal and chronic wound fibroblasts, and enhance angiogenesis in vitro. Stem Cells Dev 2015;24:1635-1647. doi:10.1089/scd.2014.0316.
29. Zhang W, Bai X, Zhao B, Li Y, Zhang Y, Li Z, Wang X, Luo L, Han F, Zhang J, Han S, Cai W, Su L, Tao K, Shi J, Hu D. Cell-free therapy based on adipose tissue stem cell-derived exosomes promotes wound healing via the PI3K/Akt signaling pathway. Exp Cell Res 2018;370:333-342. doi:10.1016/j.yexcr.2018.06.035.
30. Ren S, Chen J, Duscher D, Liu Y, Guo G, Kang Y, Xiong H, Zhan P, Wang Y, Wang C, Machens HG, Chen Z. Microvesicles from human adipose stem cells promote wound healing by optimizing cellular functions via AKT and ERK signaling pathways. Stem Cell Res Ther 2019;10:47. doi:10.1186/s13287-019-1152-x.
31. Zhang Y, Pan Y, Liu Y, Li X, Tang L, Duan M, Li J, Zhang G. Exosomes derived from human umbilical cord blood mesenchymal stem cells stimulate regenerative wound healing via transforming growth factor-β receptor inhibition. Stem Cell Res Ther 2021;12:434. doi:10.1186/s13287-021-02517-0.
32. Zhou Y, Zhao B, Zhang X-L, Lu Y, Lu S-T, Cheng J, et al. Combined topical and systemic administration with human adipose-derived mesenchymal stem cells (hADSC) and hADSC-derived exosomes markedly promoted cutaneous wound healing and regeneration. Stem Cell Res Ther 2021;12:257.
33. Hu S, Li Z, Cores J, Huang K, Su T, Dinh PU, Cheng K. Needle-free injection of exosomes derived from human dermal fibroblast spheroids ameliorates skin photoaging. ACS Nano 2019;13(10): 11273–11282. doi:10.1021/acsnano.9b04384.
34. Kwon HH, Yang SH, Lee J, Park BC, Park KY, Jung JY, Bae Y, Park GH. Combination treatment with human adipose tissue stem cell-derived exosomes and fractional CO2 laser for acne scars: a 12-week prospective, double-blind, randomized, split-face study. Acta Derm Venereol 2020;100(18):adv00310. doi:10.2340/00015555-3666.
35. Pumford AD, Staricha KL, Kunkel ET, Armstrong MF, Behfar A, Van Abel KM. Exosome therapy for a nonhealing scalp wound following chemoradiation and surgical therapy. Mayo Clin Proc 2024;99(6):1006-1012. doi: 10.1016/j.mayocp.2024.04.011.
36. Peredo M, Shivananjappa S. Topical human mesenchymal stem cell-derived exosomes for acceleration of wound healing following tissue trauma and aesthetic procedures: a case series. J Drugs Dermatol 2024;23(4):281-284. doi:10.36849/JDD.C7395.
37. Proffer SL, Paradise CR, DeGrazia E, et al. Efficacy and tolerability of topical platelet exosomes for skin rejuvenation: six-week results. Aesthet Surg J 2022;42(10):1185-1193. doi:10.1093/asj/sjac149.
38. Park GH, Kwon HH, Seok J, et al. Efficacy of combined treatment with human adipose tissue stem cell-derived exosome-containing solution and microneedling for facial skin aging: A 12-week prospective, randomized, split-face study. J Cosmet Dermatol 2023;22(12):3418-3426. doi:10.1111/jocd.15872.

Review / *Derleme*

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Nonclinical safety assessment of vaccines: up to date applications

Aşıların klinik dışı güvenlik değerlendirmesi: güncel uygulamalar

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ABSTRACT

Vaccines have a great impact on global health. These pharmaceutical products are prophylactic agents administered to healthy individuals, involving infants and children. Therefore, it is important to demonstrate the safety of them with nonclinical studies before the start of clinical trials. Nonclinical assessment includes product characterization, both in vitro and in vivo testing of vaccines, adjuvanted vaccines or vaccine adjuvants. In vivo safety studies include pharmacology studies, pharmacokinetic studies, general toxicity studies, developmental and reproductive toxicity, genotoxicity and carcinogenicity studies, and immunogenicity assessment. These tests should be conducted in compliance with GLPs. Nonclinical studies are conducted to determine safety and appropriate dose to induce an immune response in animal models. A benefit-to-risk profile is considered for each vaccine because of many factors that affect nonclinical and clinical toxicities. Herewith, the non-clinical safety evaluation of vaccines, including toxicity testing, has been focused. Nonclinical testing requirements are an essential tool to determination of the safety and efficacy of vaccines.

Keywords: Nonclinical safety assessment, in vitro studies, in vivo studies, toxicity.

ÖZ

Aşıların küresel sağlık üzerinde büyük etkisi vardır. Bu farmasötik ürünler, bebekleri ve çocukları da kapsayan sağlıklı bireylere uygulanan profilaktik ajanlardır. Bu nedenle klinik araştırmalara başlanmadan önce bunların güvenliğinin klinik öncesi çalışmalarla ortaya konması önemlidir. Klinik dışı değerlendirme, aşıların, adjuvanlanmış aşıların veya aşı adjuvanlarının hem in vitro hem de in vivo testlerini içeren ürün karakterizasyonunu içerir. İn vivo güvenlik çalışmaları farmakoloji çalışmalarını, farmakokinetik çalışmaları, genel toksisite çalışmalarını, gelişimsel ve üreme toksisitesini, genotoksisite ve karsinojenisite çalışmalarını ve immünojenisite değerlendirmesini kapsar. Bu testler İLU'ya uygun olarak yapılmalıdır. Hayvan modellerinde immün tepkiyi tetiklemek için güvenliği ve uygun dozu belirlemek amacıyla klinik dışı çalışmalar yürütülmektedir. Klinik dışı ve klinik toksisiteleri etkileyen birçok faktör nedeniyle her aşı için bir fayda-risk profili dikkate alınır. Bu derlemede aşıların toksisite testleri de dahil olmak üzere klinik dışı güvenlik değerlendirmesine odaklanılmıştır. Klinik dışı test gereklilikleri, aşıların güvenliğinin ve etkinliğinin belirlenmesinde önemli bir araçtır.

Anahtar Sözcükler: *Klinik dışı güvenlik değerlendirmesi, in vitro çalışmalar, in vivo çalışmalar, toksisite.*

INTRODUCTION

Vaccination of healthy people against childhood or infectious diseases from the first year of their lives is a very important issue for public health. Over the years, many diseases are largely controlled by effective vaccination programs. For example, while the number of paralytic cases of polio around the world before vaccine was over 350,000 per year, the disease was eliminated with vaccination in the 1960s and 70s.

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According to 2016 data, the disease was still prevalent in 125 countries in the world and the annual number of cases with paralysis was reduced by more than 99% with 42 cases (1). Nowadays, over two billion people suffer from infectious diseases prevented by vaccinations. It is a large fact that prophylactic vaccines prevent disabilities and diseases on public for years. In addition to this, new generation therapeutic vaccines are recently used for noninfectious and chronic diseases such as cancer. However, the concerns about potential risks of overall vaccines often prevent the perception of their benefits (2). Therefore, it is very important to demonstrate the safety as well as the efficacy of vaccines. From this point of view, a process was started to identify a nonclinical assessment approach in vaccine development in the 1990s (3).

Vaccines are special pharmaceutical products that may include inactivated bacteria or virus (inactivated whole-cell), inactivated toxoid, or live-attenuated vaccine strains. Live- attenuated vaccines with long-term immune response are among the most effective vaccines against human infectious disease according to European Medicines Agency (EMEA). Live recombinant vectored vaccines are produced using bacteria or viruses and live recombinant vectors express heterologous antigens by the antigen-encoding genes (4,5). Attenuation and recombination events in live- attenuated or recombinant vaccine strains may carry risks related to the reversion of vaccine strains to virulence (6). Therefore, the attenuation mechanisms of vaccine strains should be well defined. New generation vaccines produced by recombinant DNA technology have provided better protection than some conventional vaccines and they are safer. Among these vaccines, subunit vaccines consist of purified, recombinant or engineered proteins, or peptides (7). Polysaccharide and conjugated vaccines can also be considered in this group. These vaccines differ from inactivated vaccines contain only the antigenic parts of the pathogen and they are safer than the live-attenuated vaccines. Also, nucleic acid vaccines and therapeutic vaccines currently used for immune response (8,9). In DNA vaccines, genetically engineered DNA (DNA plasmid having antigens) is used to stimulate both humoral and cellular immunity (10).

Vaccines also contain other components such as adjuvants, stabilizers, preservatives, and trace substances produced during the manufacturing process alongside highly purified antigens (11). Adjuvants are pharmacological or immunological agents included in vaccine formulations to enhance the immunogenicity of vaccine antigens. Although not all vaccines need adjuvants, many vaccines -especially live-attenuated vaccines- often include adjuvants/adjuvant systems. These components consist of heterogeneous materials such as salts (e.g., aluminum), oil emulsions (e.g., squalene), lipid A derived from lipopolysaccharide (LPS), saponin-based mixtures and oligonucleotides (9, 12) and they are not considered active ingredients (11). Adjuvants used in vaccines must be determined in keeping with the type of immune response and should be used in accordance with pharmacopoeia to avoid toxicity. The effects of adjuvants should be revealed in nonclinical immunogenicity studies (13-15). The safety of vaccine adjuvant is evaluated according to the specific vaccine in which it is used (13). Therefore, each vaccine should be evaluated individually and the safety assessment of them should be thorough and continuous.

During the production and as end-product, vaccines are tested in a number of nonclinical and clinical evaluation studies (16). Nonclinical assessments are considered as the initial step of a vaccine guiding from the laboratory tests to the clinical assessment (17, 18). For the nonclinical assessments of vaccines, several guidelines have been produced since 1997 by the major regulatory and public health agencies such as World Health Organization (WHO), the European Medicines Agency (EMEA), the International Conference on Harmonization (ICH), the USA Food and Drug Administration-Center for Biologics Evaluation and Research (FDA-CBER), and other regulatory agencies. These guidelines are shown in Table-1. In all guidelines, the general principles of nonclinical evaluations of vaccines and the regulatory authorities' expectations for new vaccines are discussed. These guidelines have a similar scope, and their nonclinical programs are with significant alignment across agencies (19). According to WHO Guideline on nonclinical evaluation of vaccines (2005), nonclinical evaluations of vaccines contain "all in vivo and in vitro testing performed before and during clinical development of vaccines" (15). The definition of preclinical evaluation in this guideline is described as "all in vivo and in vitro testing carried out prior to the first testing of vaccines in humans". When both definitions are considered, it is understood that nonclinical evaluation includes preclinical studies as well as nonclinical tests performed during the clinical trial phase.

Table-1. Guidelines for the nonclinical assessment of vaccines for human use (Modified from Sun et al., 2012 (50)

Vaccine type	Guidelines
All vaccines	EMEA, 1997. Note for guidance on Preclinical pharmacological and toxicological testing of vaccines, EMA/CPMP/SWP/465/95.
	WHO Guidelines on nonclinical evaluation of vaccines, 2005 (WHO Technical Report Series No 927, Annex 1). WHO/BS/03.1969.
	FDA-CBER, 2006. Guidance for Industry: Consideration for developmental toxicity studies for preventive and therapeutic vaccines for infectious disease indications.
DNA and vector-based vaccines	EMEA, 2001. Note for Guidance on the Quality, Preclinical and Clinical Aspects of Gene Transfer Medicinal products, CPMP/BWP/3088/99
	EMEA, 2008a. Guideline on the non-clinical studies required before first clinical use of gene therapy medicinal products.
	EMEA, 2010. Guideline on quality, non-clinical and clinical aspects of live recombinant viral vectored vaccines, EMA/CHMP/VWP/141697/2009
	WHO Guidelines for assuring the quality and nonclinical safety evaluation of DNA vaccines, 2007 (WHO Technical Report Series, No 941)
	FDA-CBER, 2007. Guidance for Industry: Considerations for Plasmid DNA Vaccines for Infectious Disease Indication.
Recombinant vaccines	ICH Harmonized Tripartite Guideline, ICH S6 (R1), 1997: Preclinical safety evaluation of biotechnology-derived pharmaceuticals (Addendum 12 June 2011)
Viral vaccines	EMEA, 2002. Note for Guidance on the development of vaccinia virus-based vaccines against smallpox.
	EMEA, 2007. Guideline on influenza vaccines prepared from viruses with the potential to cause a pandemic and intended for use outside of the core dossier context.
	EMEA, 2008b. Guideline on dossier structure and content for pandemic influenza vaccine marketing authorization application.
	FDA-CBER, 2010. Guidance for Industry: Characterization and qualification of cell substrates and other biological materials used in the production of viral vaccines for infectious disease indications.
Adjuvants in vaccines	EMEA, 2005. Guideline on adjuvants in vaccines for human use, CHMP/VEG/134716/2004.
	WHO Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines, 2013a (WHO Technical Report Series, TRS 987, Annex 2, 2014)

EMEA: European Medicines Agency; WHO: World Health Organization; ICH: International Conference on Harmonization; FDA-CBER: United States Food and Drug Administration

A clearly defined vaccine-specific developmental strategy is crucial to ensure the efficient and successful development before initiation of nonclinical and clinical evaluations (18-20). Similar with chemical drugs, vaccine development process typically comprises many phases. These phases are shown in Figure-1.

The nonclinical assessment of a vaccine development process is carried out in multiple stages and is a complex multidisciplinary activity (21). The vaccine components and the final vaccine product are tested for purity, sterility, potency, consistency, activity, and stability. Also, vaccines are assessed for efficacy, toxicity, immunogenicity and safety. These tests are conducted both in vitro (in the laboratory) and in vivo (in animal models), and both studies contribute to vaccine characterization and safety evaluation (12). Nonclinical assessment studies in relevant animal models are more valuable for identifying potential risks of the vaccines.

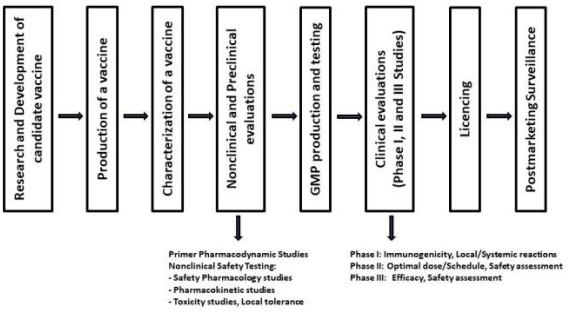

Figure-1. A vaccine development, production, evaluation and marketing process. This process carries out depending on Good Manufacturing Practices (GMP), Good Laboratory Practices (GLP), Good Clinical Practices (GCP), Guidelines of Major Regulatory Agencies and National Regulatory Authorities.

However, animal tests should be performed according to the national and international animal welfare acts, appropriate biosafety necessities and compliance with Good Laboratory Practice (GLP) (OECD Principles on GLP, 1998 (22); WHO Manual of Laboratory Methods, 1997 (23); WHO Laboratory Biosafety Manual, 2020 (24); Code of Federal Regulations 21 CFR 58, 2024 (25)). Nevertheless, there are some limitations in animal testing. The immune responses in animal models may not project human studies due to species specific susceptibility to infection by viruses, bacteria, and other microorganisms. Despite this, animal models in toxicology and pathophysiology can be used to predict human outcomes (26). As a result, there has been an increased focus on nonclinical evaluation of vaccines in recent years (2). The candidate vaccine must be tested in comprehensive nonclinical studies and appropriately designed clinical trials (21). Nonclinical assessment requirements of a candidate vaccine include:

1. Characterization of candidate vaccine (quality control testing program)
2. Pharmacodynamics studies (Primary pharmacodynamics studies: proof of concept testing and protective efficacy studies in animal models, secondary pharmacodynamics and safety pharmacology studies)
3. Pharmacokinetic studies
4. Preclinical safety testing (toxicity studies in animal models)

All these assessments play a crucial role in providing safety of vaccines and, they eliminate candidate vaccines that have inadmissible risks for clinical assessment testing on human (5).

NONCLINICAL ASSESSMENT

According to WHO, the potential toxicity of a vaccine should be assessed not only prior to initiation of human trials, but also throughout preclinical studies. Preclinical assessment is essential in moving a vaccine from the laboratory to the clinical studies and this assessment includes all test procedures such as characterization of vaccines, primary pharmacodynamics and safety testing on animals carried out prior to human clinical trials (18, 19). However, nonclinical assessment may only be needed when changes in the manufacturing process or product formulations are made or to further study potential safety concerns that may have arisen from phase I and II clinical studies or that have been reported in the literature for similar products (15).

Characterization of Candidate Vaccine

Vaccines are a unique category of pharmaceuticals, and they have to be both effective and extremely safe. The biological nature and the manufacturing process of the candidate vaccine are important factors to be considered in the plan of nonclinical assessment of vaccines. The quality, potency and safety of vaccines may vary depending on the manufacturing conditions. In case of any modification in the manufacturing process of a vaccine, the quality, efficacy and safety should be re-evaluated (15). Therefore, the manufacturing process of vaccines must be carried out in accordance with Good Manufacturing Practices (GMP).

Vaccines are derived from well-characterized materials and include disease-specific antigens such as live/attenuated viruses or bacteria, viral vector-based products, virus-like particles, virosomes, purified protein antigens (natural or produced by recombinant DNA technology), peptides, glycoproteins, protein conjugates, novel nucleic acid systems, polysaccharides etc. All these elements pose challenges for characterization (27). Occasionally, purified antigens can produce weak immune responses, so it is very important to choose an appropriate vaccine delivery system that enhances and encourages a protective immune response (21).

Some of vaccines are produced using prokaryotic or eukaryotic microorganisms (15). These organisms can be highly immunogenic and stimulate an immune response like a natural infection (21). To identify antigens against

infectious disease, information about structure of pathogen, route of entry into the body; interaction with host cells and cellular receptors, and mechanisms of pathogenicity should be identified. Any possible alteration in these organisms may affect the vaccine product and for this reason the establishment of main seed strains and seed-stocks is required for vaccine production. Using appropriate characterization methods for candidate vaccine depending on its component is very important for their clinical use. Therefore, effective physico-chemical and biological characterization methods are needed for the vaccine candidate. Also, vaccines should be tested for content uniformity (28).

For vaccine safety, it is crucial to characterize the physicochemical and functional properties of vaccine antigens and vaccine adjuvants as well as formulation and antigen–adjuvant interactions in the final vaccine formulation (27). The quality and stability of the antigen, adjuvant or adjuvanted vaccine formulation must be comprehensively evaluated before their use in a nonclinical toxicity study (29). The characterization of antigens and adjuvants used in the primary pharmacology, nonclinical safety pharmacology, nonclinical toxicology and human clinical studies should be consistent and well-documented. It is recommended that the same lots of antigen and adjuvant used in the final formulation for clinical trials should also be used in non-clinical toxicology studies (28).

Characterization of a Vaccine Antigen

It is important to monitoring specific parameters with in-process control during the process and to quantify the characteristics of the final vaccine antigen once all process stages are completed (27). Although the systems and processes used for production of vaccine antigens may vary, a number of physicochemical parameters such as size, homogeneity, purity, quantity, identity and stability should be measured for vaccine antigens. The vaccine characterization methods are based on the study of physical-chemical properties using analytical methods. For antigen characterization, physico-chemical and immunochemical techniques are used (27).

For production of protein/glycoprotein-based vaccine antigens, different expression systems are used. Multi-step purification process is required for this step. Protein analysis and characterization process for these vaccine antigens include protein structure analysis, activity, physico-chemical and immunochemical properties, protein quantity, potency and biological activity, purity/impurities and contaminants determination (30). Purity which is one of the main physicochemical parameters is used to determine the percentage of active vaccine antigens in the final bulk (27). Electrophoretic and chromatographic methods (for peptide length, isoelectric pH, size, charge, polarity etc. determination), sedimentation and light scattering analyses (for mass/size, mass/charge measurement) are used to assess the purity of recombinant-protein/glycoprotein-based vaccine antigens (27, 31).

Viral material has the propensity for particle formation and aggregation. For the investigation of these circumstances during manufacture and especially storage, analytical techniques comprising chromatographic methods such as liquid chromatography (LC), liquid chromatography - mass spectrometry (LC-MS) are used. (32). These techniques allow the studies of the entire virus or profile of the viral proteome (32). Viral proteome fingerprinting can be done by chromatography (such as HPLC), Matrix-Assisted Laser Desorption/Ionization (MALDI) mass spectrometry and gel electrophoresis (such as SDS-PAGE) techniques. For vaccines containing oligonucleotide, accurate revealing of physicochemical characteristics such as identity, purity, quality, strength, structure characterization etc. are required (32). Molecular weight and molecular sequencing are used for assurance of the identity of an oligonucleotide (33). The purity and impurities analysis of the oligonucleotide are performed with chromatographic methods (34).

Throughout the entire vaccine development process -from initial characterization to final manufacturing and testing- these technologies are invaluable. The methods used in the characterization and control of currently licensed conventionally produced vaccines are probably not applicable to new vaccine products developed using advanced technology to protect against the same infection (15). Also, specific guidelines have been improved for the production, characterization and quality control and evaluation of vaccines. These guidelines and standards are described for each vaccine by “The Expert Committee on Biological Standardization” in the WHO.

Stability Tests of a Vaccine

The stability of a vaccine refers to its ability to maintain its physico-chemical and biological

properties within defined limits throughout its shelf life. Vaccines are complex mixture and unique. Therefore, stability of each vaccine should be evaluated specifically. The stability of a vaccine has a great impact on immunization. For this reason, the potency of a vaccine is evaluated during stability studies. Also, the use of physico-chemical characteristics of a vaccine in stability evaluation allows monitoring of any changes in vaccine antigen over time (35,36).

Stability evaluation of a vaccine is a continuous process at all stages from the development of the vaccine to post-license monitoring (See Fig 1) (37,38). In the past, stability tests had been focused on efficacy of vaccines at different temperatures. This is because vaccines are very sensitive to inactivation by environmental factors such as temperature, time, handling and storage conditions (15,39). As some vaccines are oversensitive to light factors such as light also should be considered in the development of new vaccines (35). Stress testing studies that are not regularly performed as part of a stability evaluation, are used to detect the intrinsic stability of a vaccine (35). Stress testing is performed under extreme conditions such as extreme temperatures or light.

Sufficient data to elicit the stability of a vaccine entering human clinical studies should be collected during nonclinical assessment. Vaccine stability data are usually collected in two stages: Real storage condition studies in suggested storage temperature and accelerated stability studies in higher temperatures (35). In these tests, vaccine characteristics including biological activity especially potency, are determined. For licensing purposes, long-term stability data should be obtained under real storage conditions and these results should be supported by accelerated stability studies (35,38).

Potency Tests of a Vaccine

Potency of a vaccine is defined as the measure of specific ability or biological activity using a proper quantitative biological test such as laboratory tests or experimental animals (15). The immunogenicity of a vaccine is determined by potency and immunogenicity (primary pharmacodynamics) tests (See Section 2.2.1). Potency tests are based on the measure of the biological activity to demonstrate the protective immunity of a vaccine however do not guarantee that the vaccine will provide a protection in all cases. Even the well characterized, highly purified or synthetic antigens may lack the ability to activate the innate immune system. Due to the complex structure and immune response of the pathogen, the efficacy of the vaccine in potency tests may not always accurately predict vaccine efficacy. In some cases, vaccines that have passed control potency tests may not always provide sufficient efficacy (40). Therefore, potency evaluation is used to confirm the consistency of the manufacturing process, and this action is performed on vaccine lots (15). Potency tests of a vaccine is the measurement of the biological activity of the vaccine according to the well-defined reference materials with known bioactivity.

In routine potency evaluation, classical challenge tests are conducted on animals. The animals are first immunized with the candidate vaccine and then infected with the pathogen organism. The control group is only exposed to the pathogen. As a result of the infection, the percentage of animals that show specific symptoms or die in the test groups is recorded. This method has been shown to be very effective in demonstrating the potency of the vaccine. However, it needs to find alternatives to the use of laboratory animals. In addition to this, where no proper animal model exists for challenge tests, potency is based on measurement of immunogenicity with generally serological tests (15, 41). Potency tests for live attenuated vaccines generally differs from the others. In the measure of potency for live attenuated viral vaccines, the infectious titer in cell culture or chicken embryos is considered. In live attenuated bacterial vaccines, the number of colony forming unit (CFU) is measured for potency. These methods may not be adequate for vectored vaccines that express heterologous vaccine antigens and, in this case, other methods such as the quantitation of the expression of the insert should be used (15).

Standard and reference materials should be used in all processes (immunogenicity, potency etc.) within the scope of quality control test program of vaccines. Numerous guidelines and recommendations that outline the fundamental principles for the formulation and production of vaccines, characterizations of vaccine antigens and adjuvants, quality control of vaccine formulations, and antigen–adjuvant interactions are available (27). The European Pharmacopoeia for pharmacopeial requirements of vaccines is also established (15).

Pharmacodynamics Studies

Pharmacology studies as part of the nonclinical assessment of vaccine have been conducted for

many years (42). In the development of pharmaceutical products, pharmacodynamics tests are performed to detect pharmacological responses. Pharmacodynamics studies are carried out in three main categories: Primary pharmacodynamics, secondary pharmacodynamics, and safety pharmacology studies (42,43). Primary pharmacodynamic studies are generally carried out during the discovery stage of a pharmaceutical product development and not generally carried out in accordance with GLP requirements, while the other pharmacology studies are expected to be conducted to GLP standards, when their results are used for human safety testing (44). Data of the primary and secondary pharmacodynamics studies of the vaccine also contributes to the safety evaluation of the vaccine. In these studies, vaccine immunogenicity (protective efficacy) for the desirable immune response and vaccine immunotoxicity for the undesired/unexpected immune response are evaluated (45).

Primary Pharmacodynamics Studies

During a vaccine development, vaccine immunogenicity should be evaluated by primary pharmacodynamics studies (3). In vitro/in vitro primary pharmacodynamics studies are proof-of-concept testing in animals and are performed to investigate the mode of action and primary action in target system of the vaccine, while secondary pharmacodynamics studies are performed to reveal the resultant action in these systems (29, 44).

Immunogenicity data obtained from small animal species (e.g. mice, rat and ferret) are expected before clinical studies, because these studies are crucial because the ability of the vaccine to elicit an immune response cannot be fully assessed in humans without initial animal testing. Therefore, to provide evidence regarding the potential protective efficacy of a vaccine, challenge (or protection) studies with the infectious agent should be carried out in a proper animal model (46). These studies should be conducted using the strain intended for the candidate vaccine and should be involved an assessment of immune responses according to dose and dosing interval of vaccine. Immunization studies for protective effect of a vaccine conducted in animal models should be planned to evaluate related immune responses (antibody production level, class and subclass of antibody produced, duration of immune response and cell-mediated immunity) (15,47). Functional immunogenicity leading to protection such as the formation of neutralizing antibodies, immune complex formation, and interactive relation with immune cells should also be investigated in vaccinated animals (47). In determining the immunological characteristics of the vaccine, immunogenicity data generated from the animal models are useful. This data help about the dose selection, dosing (vaccination) schedules and administration routes of the vaccine to support for both nonclinical and clinical study plan (15,44). Determining the dosing schedules for vaccines, in vaccinated animals, seroconversion rate, seroprotectivity, mean antibody titers or cell-mediated immunity of the biologically active component in the vaccine are assessed apart from the primary pharmacodynamics studies (48). Immune response studies in animal models are also beneficial to document consistency of production, especially during the verification stage of a vaccine manufacturing process.

To confirm whether the animal model is suitable for immunogenicity studies, challenge studies could be used (15). It should be taken in consideration that some animal models often fail to foresee immune response and efficacy in humans, because humans and animals have different immune systems, their mechanisms of antibody induction vary depending on the origin and the immunological characteristics of the vaccine. For this reason, appropriate reference materials should be used in all processes for comparative immunogenicity assessment (49). Pharmacodynamics studies may also be planned to determine interference between vaccine antigens and live organisms (15,45,50). When the candidate vaccine consists of many defined antigen, the response to each antigen should be assessed separately (15,51). If a vaccine interacts with other vaccines, reciprocal antagonism may occur, so co-administration of two or more vaccines should also be assessed (47).

The pharmacology of an adjuvant; if used; should be evaluated by pharmacodynamics studies according to the “Guideline on adjuvants in vaccines for human use” (14) or “Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines” (29). Proof-of-concept studies are also recommended to support the use of an adjuvant in vaccine formulations. Vaccine adjuvants can induce or modify an immune response and the immunogenicity to the antigen

could be enhanced by the adjuvant (45). Therefore, relevant animal models should demonstrate the increased immune response to the adjuvant/antigen combination and ensure protection against a challenge of infectious agent (14, 52). Besides, mechanism of action of the adjuvant should also be assessed in the absence of the vaccine antigen (29). In vitro assays may provide valuable insights in understanding the mechanism of action of a particular adjuvant and may also provide precious supplementary data to animal studies. These assays are important especially when there are limitations such as species-specific differences in animal models (29). For this purpose, antigen-expressing cells, other immune system cells or complex tissue culture systems mimicking lymphoid tissue are used to evaluate the effects of adjuvants by quantifying activation parameters (45, 53).

Safety Pharmacology Studies

Safety pharmacology studies are carried out to investigate the secondary pharmacological effects, potential undesirable (adverse) pharmacodynamic and pathophysiological effects and to show any functional effects of vaccines on the major physiological systems (9,54). This assessment is conducted on a case-by-case basis (9). The mechanisms of the adverse pharmacodynamics effects are also investigated in these studies.

According to ICH Guideline S7A, three types of safety pharmacology studies are described (42).

1. Standard battery of tests: these involve the assessment of effects on especially central nervous (alteration in body temperature, motor activity, behavioral alteration, coordination, and sensory/motor reflexes), the respiratory system (changes in respiratory function) and circulatory systems. These should generally be completed before clinical trials (46,54-57).
2. Supplemental studies: These focus on more complicated systems such as gastrointestinal, renal and immune systems (55). Especially, vaccine adjuvants or adjuvanted vaccines have the potential to influence physiological functions beyond the immune system (29).
3. following up studies of standard tests (42): These studies are carried out for the characterization of adverse effects observed in previous studies, because these adverse effects on organ function are not readily determined by standard toxicological testing (55).

Research the any effects of the vaccine formulation on vital functions are not generally essential unless suggested by the authorities (57). There is a discrepancy between guidance documents about safety pharmacology studies. These studies are routinely performed according to the European guidance (47), while they may not be required according to the WHO guidance (15).

Safety pharmacology studies are conducted on intact animals, isolated organs or other test systems with relatively low costs. The implementation of in vitro, ex vivo, and in vivo preliminary tests within the scope of these studies helps with the decision on whether continuing vaccine development phase or not (55). In these studies, GLP compliance is recommended but not strictly necessary. In vivo studies should be carried out in the same animal species used for primary pharmacodynamics or other nonclinical pharmacology studies. The reasons for the selection of animal species used in pharmacology and safety assessment studies should be explained. To reduce animal use, conception should be given to inclusion of any in vivo evaluations as additions to general toxicity studies (29, 57, 58). Due to ethical reasons, 3R rules should be applied for the use of animals and further in vitro techniques should be developed (28, 59). Generally, there is a tendency to combine safety pharmacology studies with toxicology assessment (47). This incorporation provides advantages such as increasing sensitivity with the large number of animals used in toxicological studies, reducing the number of animals needed for safety evaluation and cost reduction (42,55).

Pharmacokinetic Studies

Pharmacokinetic studies that are performed during the nonclinical stage support the pharmacology studies are an integral part of pharmaceutical product development process (60, 61). While pharmacodynamic studies are conducted to determine the immune response of the organism to a vaccine or vaccine antigen, pharmacokinetics assays involve the quantitative evaluation of the time course of absorption, distribution, metabolism, and excretion of the vaccine (55, 62). These studies also play a critical role in explanation of efficacy and toxicology of vaccines as well as determining optimal dosage and formulation (63).

Pharmacokinetic studies ensure a mathematical basis to assess the time course of pharmaceutical products and their effects in the body. It is supported to perform pharmacokinetic studies on vaccines for improve their development and reduce the chances of negative health effects resulting from vaccination (50). However, vaccines do not establish a pharmacodynamics and pharmacokinetic profile except for non-antigen components of vaccines such as excipients (55,62). Since kinetic properties of antigens do not provide beneficial data for determining of the vaccine dose, pharmacokinetic studies (determining serum concentration of antigens) for vaccines are generally not required (16). However, these studies might be applicable if the vaccine contains adjuvants or excipients, because adjuvants might be distributed over the body. Pharmacokinetic studies for alone adjuvant and an adjuvant/antigen combination should be taken in consideration (14).

Seroconversion is the production of detectable specific antibodies in blood serum against the infectious agent (62). The presence of an antibody response after administration of vaccine to the organism demonstrates that an immune response has been initiated and a specific antibody becomes dominant in the serum (21). The original antigen that caused the seroconversion is no longer detectable in the blood, but the antigen-antibody immune complex is detectable. Seroprotectivity refers to the protective effect gained after immunization or after infection, measured as the percentage of vaccinated subjects who achieve seroconversion (62). After vaccination (or infection) there is no direct correlation between the magnitude of the antibody response and the rate of protection (21). Absence of the antibodies after vaccination does not mean zero protection effect. In vaccines, this effect may be mediated by cellular immunity. When determining the vaccination schedule for vaccines, seroconversion and seroprotectivity of the vaccine need to be investigated (62). Antibody production in response to vaccination is an indicator of immunogenicity, not efficacy. Nonetheless, experience on vaccines has proved that the linkage between immune response and vaccine effect is so robust even though the antibody is only part of the protective immune response. Mature antibody response is accepted for licensure (21).

Preclinical Safety Testing

Vaccines are applied to healthy people for prophylactic purposes; therefore, it is important to demonstrate the safety of them. Vaccine safety is subsequently monitored and evaluated by multiple aspects and at many levels during vaccine development process (12). For safe use of a vaccine on human, they are assessed with a number of nonclinical safety assessment studies. Safety pharmacology and toxicity assessment in vitro or in relevant animal species are required before the human clinical trials with a candidate vaccine (11). The aim of these studies is to identify the potential toxic effects (16,64). These assessments play significant roles in providing vaccine safety. The nonclinical safety studies allow the identification of potential toxicities expected in humans and eliminate vaccine candidates that have intolerable risks for human clinical trials (5, 16, 64). These evaluations include safety pharmacology studies, nonclinical pharmacokinetic studies, and general and/or special toxicity studies in animal models (44). In addition to this, in vitro and in vivo assays involve the identity, purity, and potency of the vaccine play an important role in assessing of vaccine safety (65). Seroconversion, seroprotectivity and efficacy of the active component of the vaccine are the basis for determination of the schedule for vaccination (48) (See Section 2.3.).

Toxicity Assessment

There are different nonclinical toxicology studies for the evaluation of new vaccine safety. These are basic toxicity assessments (single and repeat dose toxicity studies, local tolerance assessment) and additional toxicity assessments (reproductive and developmental toxicity study, mutagenicity/genotoxicity and carcinogenicity studies) (3). Before the human clinical studies, basic toxicity studies are considered as the minimum requirement for safety evaluations (15). These studies can ensure information to support the conclusion that it is rationally safe to continue clinical studies (52). The nonclinical toxicology studies should also allow for evaluation of local tolerance (15, 45). According to WHO Guideline, the toxicity evaluation studies of the vaccine formulation may be combined with immunogenicity or safety pharmacology studies or performed as stand-alone toxicity studies (15). However, according to the EU guidance, stand-alone toxicity studies are not generally demanded, and these studies should either be

integrated into safety studies or be performed as repeat-dose toxicity studies (16). In addition to this, nonclinical toxicity assessments are not required in the vaccine combinations with known antigens (16,66). Although the content of the EMEA guideline in the scope of toxicity assessments is somewhat different from that described in the WHO guideline and the other guidelines, all guidelines suggest a case-by-case approach to nonclinical safety evaluations of vaccines (52, 66).

In nonclinical safety studies of a vaccine, it is significant to determine both immunogenic and a safe dose in animals and to define potential target organs for toxicity studies (52). The aim of the nonclinical toxicity evaluation is to characterize the potential toxic effects of a vaccine before the human clinical trials. Achievement of the nonclinical toxicology studies depends on a lot of factors such as appropriate experimental study designs, relevant animal model, and eliciting an effective immune response (65, 67). The parameters such as the right animal species and strain, dose level and immunization schedule, the route of administration, duration and frequency of treatment and assessment of endpoints (e.g. clinical blood chemistry, antibody response and necropsy evaluations) should be considered in animal toxicology studies (15).

The use of up-to-date animal models to detect rare or particular toxicities that may appear in a specific human subpopulation is limited. However, to evaluate the nonclinical safety of the vaccine, toxicology studies using these animal models play a crucial role (52). In these studies, a single animal model is satisfactory provided that it showed a proper antibody response to the vaccine antigen. The study design should include a clinical vaccine formulation group, an antigen-alone group, an adjuvant-alone group, and a negative control group (injected with saline) (3).

The antigen concentration of the vaccine in nonclinical toxicology studies is a significant factor. For this, human equivalent dose based on the projected clinical dose should be experimented to allow the generation of dose-response curves to obtain higher safety margins (3). The number of doses planned to be administered to test animals should be equal to or exceed the recommended number of doses in humans (15). The intervals between dosing depends on species and the expected immune response, such as the antibody response profile induced by the vaccine antigens (3). Dosing intervals in the toxicity studies may be shorter (15). Although the standard application is 2–3-week intervals, more studies should be made to evaluate antibody levels in progress of time to assure inclusion of the minimal interval in study plan (3). If any adverse effects are observed during these studies, this information is used to estimate an initial safe dose and dose range for the human clinical studies (3). It is recommended that the lots of antigen and adjuvant in ultimate vaccine formulation used in the human clinical studies should be same with the lots tested in non-clinical toxicology studies. According to WHO, these lots should be produced in accordance with the GMPs (68).

Administration route of the vaccine in the toxicity studies must be same route of administration with that in the clinical studies. If the vaccine will be implemented in human using a particular device such as aerosol vaccines, the same device should also be used in the animal study (15). If this is not possible, another application route may be used with proper justification (16). If any toxic findings are obtained from the safety studies using a particular administration route, to understand of toxicity spectrum of the vaccine, using a different administration route in toxicity studies may be useful (15). The common administration routes are intramuscular, subcutaneous or intradermal routes. Although vaccines can be administered to experimental animals in these routes, there are limitations to large amounts of applications to rodents (9).

The toxicokinetic research conducted for vaccine adjuvants are one of the nonclinical tests advised by the regulatory agencies (63, 69, 70). These studies are mostly typically conducted in conjunction with toxicology studies and should comply with GLP standards (56). The systemic exposure of an adjuvant is determined by the toxicokinetic assays in animals. The assays determine the relationship between the administered dose and the time course of the adjuvant. These studies also evaluate the potential of the adjuvant to accumulate in a specific organ or tissue. In the toxicokinetic studies besides blood, other biological samples should also be collected (71). Selection of the test protocol and the plan of the study should be described according to circumstances (55, 63, 69).

Single Dose (Acute Toxicity) Studies

A single-dose toxicity study is a crucial part of nonclinical study data. According to WHO Guideline (15), in cases where the vaccine-induced antibodies are expected to neutralize a live viral vector, a single-dose study should be performed. In contrast to WHO Guideline (15), the EMEA Guideline (47) makes mention of single dose toxicity studies. This guideline indicates that data from at least one animal species should be obtained, and these studies should be performed with a dose that provides an adequate safety margin relative to human dose.

In many situations, data from the single dose toxicity studies is available from the repeat-dose toxicity studies. These data are also available from animal immune response studies or safety pharmacology studies on the condition that histopathology of target organs is included (16). Therefore, generally when a repeated dose toxicity study will be available, stand-alone single dose toxicity study is not performed (9). Notwithstanding, single-dose toxicity studies are valuable in many situations. These studies can ensure safety and preliminary tolerability of the vaccine formulation and evaluate the acute effects of the vaccine (3,16). These studies may be important where antigens may have significant pharmacological effect and where the immune response induced by the first vaccination significantly changes reactions to subsequent vaccination (9).

Rodents are usually used in vaccine single dose studies (9). In these studies, the administration route and dose should reflect the clinical use. If toxicity findings are determined in these studies, the dose-response relationship should be characterized (72).

Repeated Dose (General Toxicity) Studies

The main studies supporting the safety profile of vaccines are repeated dose toxicity studies (9). The repeated dose toxicity studies are very important to assess multiple-doses vaccinations suggested for immunizations of humans (52). For vaccines that with require multiple doses application in the clinical use, a repeated dose toxicity study is generally required in one animal species. Although some vaccines are administered only single dose in clinical use, repeated dose toxicity studies are strongly recommended for these vaccines (16,73).

The design of these studies was defined in WHO Guidelines (15), and it was planned to use of the repeated dose toxicity study design for pharmaceutical products as experimental model for these products. However, vaccine specific issues such as in determination of experimental design, selection of dose levels, treatment period, pharmacodynamics, monitorization, follow-up period and a list of histopathology tissues should be considered.

Appropriate control groups should be included in this study design in order to assess the reversibility of possible adverse events and to investigate possible delayed adverse effects. In these studies, it must be taken into consideration whether the need for placebo or vehicle groups, solely adjuvant and antigen groups, etc. (9). According to the US, EU and Japan regulations, at least one additional dose, relative to the clinical trial should be added into this type of study design because the number of administrations in the toxicity study should exceed the number planned for human administration to provide the safety of the dosing schedule (52). This is called to as the (n+1) rule and this means that at least one more application is required as in the recommended clinical scheme (9, 52). The selection of animal species in these studies should be carefully evaluated on a case-by-case basis. The administration routes and doses of vaccines should reflect the clinical use. The dose administered to animals depends on the planned clinical dose and the expected immune response induced by the vaccine. Vaccines should be administered as 2–3 weeks' interval, rather than daily, doses according to WHO guideline (15). The EMEA Guideline also accepts the proposed episodic dosing using 2–3-week intervals (47). Thus, a clinical immunization schedule is simulated in animals with 4 administrations at intervals of 3 weeks. Repeated vaccination protocol may result in an increasingly immune response.

If applicable, single human dose (mL or mg/bw) should be administered to animals. When it is not possible, the maximum applicable dose that exceeds the human dose that induces immunogenicity in the animal should be administered. Instead of this, it may be feasible to apply the total volume to multiple sites using the same administration route. However, in certain situations that are poorly known of antibody levels or other intended immune responses, to justify the minimal interval in study design, the primary and secondary immune responses may

be evaluated over an extended period with further studies (52).

According to WHO Guideline, a wide range of information such as systemic and toxic effects on the immune system and local inflammatory reactions (See Section 2.4.2.), may be obtained from the repeated dose toxicity studies. Clinical monitorization should include general health, weekly body weights, weekly feed consumption and body temperature (52). After the dose administration, interim data analysis of serum biochemistry and hematology parameters should be carried out. Local toxicity should be evaluated prior to the vaccination and routinely day-to-day following the vaccination until the local reaction is resolved (74). At the end of the study, a gross necropsy and complete tissue histopathology are recommended. Histopathological assessment should be done on especially immune system organs and target organs. Also, other organs that may be affected due to the administration route and organs on the site of vaccine administration should be assessed histopathological. In case of the new vaccine products, whole tissue examination is required (15, 52).

Local Tolerance Assessment

Local tolerance assessment could be carried out either as a part of the repeated dose toxicity study or as a stand-alone study according to WHO Guideline (15). The aim of the local tolerance studies is to observe tissue reactions at the administration site and to evaluate with histopathology (9). Local tolerance should be evaluated at sites into contact with the vaccine antigen due to the route of administration and at sites incorrectly exposed to the vaccine (e.g. eye exposure during administration by aerosol vaccine) (15, 74).

Vaccines are commonly administered by intramuscular, subcutaneous or intradermal routes, and local reactions at injection sites are not all that infrequent in clinical use. Local toxicity should be assessed using a quantitative grading system such as Draize test as a toxicological standardization method to study irritation and toxicity of substances applied to the skin or the eye (9, 52, 75). If significant reactions are observed, follow-up studies may be conducted to examine the persistence of vaccine antigen or adjuvant at the administration site. In case of a new vaccine product, this assessment can be included in the repeat-dose toxicity studies (9). In some instances, a stand-alone study may be preferable. For example, if the repeated dose toxicity study was carried out in the mice; local tolerance assessment may be performed in rabbits as a stand-alone study.

Adjuvant frequently produce local reactions, and therefore, the adjuvant-only group should be included in the study design to assess the contribution of the adjuvant to local reactions. Symptoms and local reactions such as redness, pain, swelling, granuloma formation, abscess, necrosis and regional lymphadenopathy can be seen in local tolerance studies depending on the severity of the tissue reactions (9). The pathologist should differentiate healthy tissue responses and undesired pathological changes in the tissue as response to the injection of the vaccine. C-reactive protein (CRP) which is a sensitive marker of inflammatory changes in humans and some animal models is an acute phase response protein involved in complement activation. After immunization with vaccines that produce local reactogenicity in clinical studies, it was shown that CRP levels were raised. Therefore, it is thought to be a useful biomarker in nonclinical local tolerance studies (76).

Reproductive and Developmental Toxicity Studies

Data on reproductive function for vaccines are generally not necessary. Histopathological findings obtained from toxicity studies can provide sufficient information about the integrity of the reproductive organs (16). Besides, for prophylactic vaccines, reproductive toxicity evaluations are usually limited to pre and postnatal developmental studies to detect any potential undesirable effect on the developing embryo, fetus or newborn (15, 52). In order to verify exposure of the embryo or fetus to maternal antibody in the animal model chosen, maternal antibody transfer should be evaluated by measuring vaccine-induced antibody in cord or fetal blood. The administration route of vaccine should be same route within the clinical use and the maximal human dose should be applied to the experimental animals. If it is not possible, a dose that exceeds the human dose on mg/kg basis and is able to induce an immune response in the animal should be used (69, 77).

According to ICH S5 R2 (77), the gestating animals should be exposed to the vaccine until the end of their gestation period to assess any potential adverse effects of the vaccine during the period of organogenesis. In order to assure

maximal exposure of the embryo or fetus to the vaccine-induced immune response, due to the relatively short gestation period of most test animals used, pre-mating exposure is required. The number of doses applied depends on the time of onset and duration of the response. Booster immunizations may be essential at particular times during the gestation period to expose the developing embryo to the components of the vaccine formulation and to maintain a high level of antibody throughout the gestation period. In this study design, end-points include viability, fetal body weight and morphology, resorptions and abortions but are not limited to them. In addition to this, it is also suggested that a period of postnatal follow-up of pups from birth to the end of breastfeeding be included in the study design to evaluate especially normality of growth and viability. Therefore, these studies should be planned with experimental groups divided into appropriate subgroups (69, 77).

Mutagenicity/genotoxicity and Carcinogenicity Studies

Genotoxicity and carcinogenicity studies for the final vaccine formulation are not needed according to the EMEA Guideline (47), however these tests are required for new adjuvants and additives. If needed, prior to human clinical trials in vitro tests for identification of vaccine-induced mutations and chromosomal damage should be carried out. Whole genotoxicity tests may be performed in parallel with clinical studies (78).

CONCLUSION

One century after Spanish flu killed millions all over the globe, Covid 19 respiratory virus from the same family as some smaller epidemics from last 20 years spread quickly and caused pandemic. It is known that as of today, 17 COVID-19 vaccines have been developed and 13 marketed (79). When examined the EMA reports for market authorization of COVID-19 vaccine, it is observed that EMA assessment of the nonclinical studies consisted most frequently of comments related to study design, species selection and missing data (80). However, it appears that all steps of the vaccine development process, including their nonclinical evaluations, are also valid for these vaccines. Analysis of historical data connected to epidemics, pandemics, and vaccine development process showed three main components connected to science and society: the start of pandemic, vaccine development process including the supply process, and post pandemic challenges. Developing pandemic emergency plans against such pandemic situations in the future should be a top priority.

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References

1. Ochmann S, Roser M. Polio. Published online at OurWorldInData.org. https://ourworldindata.org/polio 2018
2. Wolf JJ, Plitnick LM, Herzyk DJ. Strategies for the Nonclinical Safety Assessment of Vaccines. In Novel Immune Potentiators and Delivery Technologies for Next Generation. Vaccines. 2012; 323-349
3. Van der Laan JW, Forster R, Ledwith B, Gruber M, Gould S, Segal L, Penninks A. Nonclinical testing of vaccines: Report from a workshop. Drug Information Journal 2009; 43: 97–107.
4. Nascimento IP, Leite LCC. Recombinant vaccines and the development of new vaccine strategies, Braz J Med Biol Res 2012; 45(12): 1102–1111.
5. Novicki DL, Wolf JJ, Plitnick LM, Hartsough M. Vaccines: Preventive and Therapeutic Product Studies, Chapter 25, in the “The Role of the Study Director in Nonclinical Studies Pharmaceuticals, Chemicals, Medical Devices, and Pesticides” 1st Edition, Ed by William J. Brock, Barbara Mounho and Lijie Fu. John Wiley & Sons, Inc. 2014; 439.
6. Pliaka V, Kyriakopoulou Z, Markoulatos P. Risks associated with the use of live-attenuated vaccine poliovirus strains and the strategies for control and eradication of paralytic poliomyelitis. Expert Rev Vaccines 2012; 11(5): 609-628.
7. FDA-CBER US Food and Drug Administration: Points to Consider in the Production and Testing of New Drugs and Biologicals Produced by Recombinant DNA Technology, 1985.
8. Glick BR, Pasternak JJ, Patten CL. Molecular Biotechnology: Principles and Applications of Recombinant DNA, ASM Press. 2010.
9. Forster R. Study designs for the nonclinical safety testing of new vaccine products. Journal of Pharmacological and Toxicological Methods 2012; 66: 1–7.

10. Khan KH. DNA vaccines: roles against diseases. Germs 2013; 3(1): 26-33.
11. Da Silva FT, Di Pasquale A, Yarzabal JP, Garçon N. Safety assessment of adjuvanted vaccines: Methodological considerations, Hum Vaccin Immunother. 2015; 11(7): 1814–1824.
12. Di Pasquale A, Bonanni P, Garçon N, Stanberry LR., El-Hodhod M, Da Silva FT. Vaccine safety evaluation: Practical aspects in assessing benefits and risks. Vaccine 2016; 34: 6672–6680.
13. Di Pasquale A, Preiss S, Da Silva FT, Garçon N. Vaccine Adjuvants: from 1920 to 2015 and beyond. Vaccines (Basel). 2015; 3(2): 320–343.
14. Guideline on adjuvants in vaccines for human use. 2005. EMEA, European Agency for the Evaluation of Medicinal Products. EMEA/CHMP/VEG/134716/2004
15. WHO Guidelines on nonclinical evaluation of vaccines. 2005. WHO/BS/03.1969. (WHO Technical Report Series No 927, Annex 1).
16. Wolf JJ, Kaplanski CV, Lebron JA. Nonclinical Safety Assessment of Vaccines and Adjuvants; In Vaccine Adjuvants Methods and Protocols. Part of the Methods in Molecular Biology book series (MIMB) Springer Inc. 2010; 626:29-40. doi: 10.1007/978-1-60761-585-9_3.
17. Guideline on clinical evaluation of vaccines, Committee for Medicinal Products for Human Use (CHMP) EMEA/CHMP/VWP/164653/05 Rev. 1.2023
18. WHO Expert Committee on Biological Standardization. Guidelines on clinical evaluation of vaccines: regulatory expectations (WHO Technical Report Series 1004, Annex 9, 2017 Sixty-seventh report)
19. Questions and answers on the withdrawal of the CPMP, Note for guidance on preclinical pharmacological and toxicological testing of vaccines. 2016 (CPMP/SWP/465), EMEA, European Agency for the Evaluation of Medicinal Products. EMA/CHMP/SWP/242917/2016.
20. Han S. Clinical vaccine development, Clin Exp Vaccine Res 2015; 4(1): 46–53.
21. Cunningham AL, Garçon N, Leo O, Friedland LR, Strugnell R, Laupèze B, Doherty M, Stern P. Vaccine development: From concept to early clinical testing. Vaccine 2016; (34): 6655–6664.
22. OECD Principles on Good Laboratory Practice, Organization for Economic Co-operation and Development, Paris, 1998. ENV/MC/CHEM (98)17.
23. WHO Vaccine Supply and Quality Unit. Manual of laboratory methods for testing of vaccines used in the WHO Expanded Programme on Immunization. 1997. https://iris.who.int/handle/10665/63576
24. WHO Laboratory Biosafety Manual. 4th edition: Biosafety programme management World Health Organization, Geneva, Switzerland. 2020.
25. Code of Federal Regulations, Title 21, Part 58 (21 CFR 58). Good Laboratory Practice for Nonclinical Laboratory Studies. Washington, DCUS Government Printing Office. 2024.
26. Shanks N, Greek R, Greek J. Are animal models predictive for humans? Philos Ethics Humanit Med 2009; 4: 2.
27. Dey AK, Malyala P, Singh M. Physicochemical and functional characterization of vaccine antigens and adjuvants, Journal Expert Review of Vaccines 2014; 13(5): 671-685.
28. WHO Guidelines for Independent Lot Release of Vaccines by Regulatory Authorities. WHO Expert Committee on Biological Standardization, Sixty-first Report 2013a (WHO Technical Report Series, No. 978, Annex 2, 2014)
29. WHO Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines, 2013b (WHO Technical Report Series, TRS 987, Annex 2, 2014)
30. ICH Q6B Guidance, Specifications: Test Procedures and Acceptance Criteria for Biotechnological/ Biological Products, 1999. EMEA, European Agency for the Evaluation of Medicinal Products. CPMP/ICH/365/96.
31. Rodríguez-Ortega MJ, Norais N, Bensi G, Liberatori S, Capo S, Mora M, Scarselli M, Doro F, Ferrari G, Garaguso I, Maggi T, Neumann A, Covre A, Telford JL, Grandi G. Characterization and identification of vaccine candidate proteins through analysis of the group A Streptococcus surface proteome, Nature Biotechnology 2006; 24: 191–197.
32. Becht S, Ding X, Gu X. Vaccine Characterization Using Advanced Technology. Mass spectrometry offers the potential for an unprecedented understanding of vaccines and why they fail. BioPharm International 2007; 2007 (Suppl 5): 40–45. http://www.genalysis.com.au/pharmaceutical/vaccine/viral/
33. Mason PW, Shustov AV, Frolova I. Production and characterization of vaccines based on flaviviruses defective in replication, Virology. 2006; 351(2): 432–443.
34. WHO Guidelines on stability evaluation of vaccines, WHO Expert Committee on Biological Standardization Fifty-seventh report. 2006. (WHO Technical Report Series TRS 962. Annex 3) WHO/BS/06.2049.

35. Dumpa N, Goel K, Guo Y, McFall H, Pillai AR, Shukla A, Repka MA, Murthy SN. Stability of Vaccines. AAPS Pharm Sci Tech 2019; 20(2):42.
36. Knezevic I. Stability evaluation of vaccines: WHO approach. Biologicals 2009;37(6):357-9; discussion 421-3. doi: 10.1016/j.biologicals.2009.08.004.
37. Schofield T, Krause PR. Stability evaluation of vaccines. Biologicals 2009;37(6):355. doi: 10.1016/j.biologicals.2009.09.001.
38. Galazka A., Milstien J., Zaffran M. Thermostability of vaccines, World Health Organization Global Programme for Vaccines and Immunization 1998.
39. Code of Federal Regulations, Title 21, sec 600 (21 CFR 600) Biological products: general provisions. Washington, DCUS Government Printing Office. 2024.
40. McVey DS, Galvin JE, Olson SC. A review of the effectiveness of vaccine potency control testing. Int J Parasitol 2003; 33(5-6):507-16.
41. Taffs RE. Potency Tests of Combination Vaccines. Clinical Infectious Diseases 2001; 33(Suppl 4): S362–S366.
42. ICH S7A Harmonised Tripartite Guideline, Safety Pharmacology Studies for Human Pharmaceuticals. International Conference on Harmonization, Geneva, Switzerland, 2001. CPMP/ICH/539/00
43. Arrigoni C, Perego V. Chapter 5 Safety Pharmacology; In Pharmaceutical Toxicology in Practice: A Guide for Non-Clinical Development. Editor(s): Alberto Lodola, Jeanne Stadler. 2011. doi: 10.1002/9780470909911.ch5
44. ICH M3(R2) Harmonised Tripartite Guideline, Guidance on Nonclinical Safety Studies for The Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals. International Conference on Harmonization, Geneva, Switzerland, 2009.
45. Wolf JJ. 11.38 - Immunopharmacology and Immunotoxicology Assessment of Vaccines and Adjuvants. Reference Module in Biomedical Sciences. Comprehensive Toxicology (Third Edition) 11: 852-872. Zaitseva M, Romantseva T, Blinova K, Beren J, Sirota L, Drane D, Golding H. (2012) Use of human MonoMac6 cells for development of in vitro assay predictive of adjuvant safety in vivo. Vaccine, 2018; 30:4859–4865.
46. Klug B, Celis P, Ruepp R, Robertson JS. EU regulatory guidelines for the clinical evaluation of adjuvants. Clinical Res Regulatory Affairs 2015; 32(2):55-60. doi: 10.3109/10601333.2015.1001899.
47. Note for guidance on Preclinical pharmacological and toxicological testing of vaccines, 1997. EMEA, European Agency for the Evaluation of Medicinal Products. EMA/CPMP/SWP/465/95.
48. Vladimir O, Zuzana K, Štefkovičová M. How Do We Evaluate and Manage Many Different Vaccination Schedules in the EU? Cent Eur J Public Health 2015; 23(3):218-22.
49. Granath B. Development of immunogenicity models in mice for improved risk assessment of biopharmaceuticals. University of Gothenburg, 2013.
50. Gómez-Mantilla JD, Trocóniz IF, Garrido MJ. ADME Processes in Vaccines and PK/PD Approaches for Vaccination Optimization, Pharmaceutical Sciences Encyclopedia: Drug Discovery, Development, and Manufacturing, 2015.
51. Guideline on dossier structure and content for pandemic influenza vaccine marketing authorization application, 2008b. EMEA, European Agency for the Evaluation of Medicinal Products.
52. Sun Y, Gruber M, Matsumoto M. Overview of global regulatory toxicology requirements for vaccines and adjuvants. J Pharmacol Toxicol Methods 2012; 65(2), 49-57.
53. Zaitseva M, Romantseva T, Blinova K, Beren J, Sirota L, Drane D, Golding H. Use of human MonoMac6 cells for development of in vitro assay predictive of adjuvant safety in vivo. Vaccine 2012; 30:4859–65.
54. Pugsley MK, Authier S, Curtis MJ. (2008) Principles of safety pharmacology. Br J Pharmacol. 154: 1382–99, doi: 10.1038/bjp.2008.280.
55. Andrade EL, Bento AF, Cavalli J, Oliveira SK, Freitas CS, Marcon R, Schwanke RC, Siqueira JM, Calixto JB. Non-clinical studies required for new drug development - Part I: early in silico and in vitro studies, new target discovery and validation, proof of principles and robustness of animal studies. Brazilian J Med and Biological Research 2016; 49(11): e5644. doi: 10.1590/1414-431X20165644.
56. Hentz KL. 3.03 - Safety Assessment of Pharmaceuticals in Comprehensive Toxicology. 2010; 3:17-28.
57. Amouzadeh HR, Engwall MJ, Vargas HM. Safety Pharmacology Evaluation of Biopharmaceuticals. In Principles of Safety Pharmacology. Ed by Michael K. Pugsley, Michael J Curtis. 2015.
58. ICH S6(R1) Harmonised Tripartite Guideline, Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals. International Conference on Harmonization, Geneva, Switzerland, 1997. Addendum June 2011.
59. Shin J, Lei D, Conrad C, Knezevic I, Wood D. International regulatory requirements for vaccine safety and potency testing: a WHO perspective. Procedia in Vaccinology, 2011; 5:164–170.

60. Ducharme J, Dudley AJ, Thompson RA. Pharmacokinetic issue in drug discovery. In: Rang HP (Editor), Drug discovery and development. Philadelphia: Churchill Livingstone Elsevier. 2006: 141–161.
61. Tuntland T, Ethell B, Kosaka T, Blasco F, Zang RX, Jain M, Gould T, Hoffmaster K. Implementation of pharmacokinetic and pharmacodynamics strategies in early research phases of drug discovery and development at Novartis Institute of Biomedical Research. Frontiers in Pharmacology 2014; 5:174. doi: 10.3389/fphar.2014.00174. eCollection 2014.
62. Oleár V, Krištúfková Z, Štefkovičová M. How Do We Evaluate and Manage Many Different Vaccination Schedules in the EU? Cent Eur J Public Health 2015; 23(3): 218–22.
63. Singh SS. Preclinical pharmacokinetics: an approach towards safer and efficacious drugs. Curr Drug Metab 2006; 7: 165-182, doi: 10.2174/138920006775541552.
64. Valentin JP, Hammond T. Safety and secondary pharmacology: successes, threats, challenges and opportunities. J Pharm Toxicol Methods 2008; 58: 77–87. doi: 10.1016/j.vascn.2008.05.007.
65. Wolf JJ. Chapter 10: Special Considerations for the Nonclinical Safety Assessment of Vaccines, In Nonclinical Development of Novel Biologics, Biosimilars, Vaccines and Specialty Biologics, Ed by Lisa M. Plitnick and Danuta J. Herzyk. Elsevier Inc. 2013; 243-255
66. Note for Guidance on Pharmaceutical and Biological Aspects of Combined Vaccines, 1998. EMEA, European Agency for the Evaluation of Medicinal Products. CPMP/BWP/477/98.
67. Green MD, Al-Humadi NH. Chapter 27 - Preclinical Toxicology of Vaccines, A Comprehensive Guide to Toxicology in Nonclinical Drug Development (Second Edition) 2017; 709-735.
68. WHO Expert Committee on Specifications for Pharmaceutical Preparations: Good manufacturing practices: supplementary guidelines for the manufacture of investigational pharmaceutical products for clinical trials in humans, 1996. (WHO Technical Report Series, No. 863, Annex 7, 1996 - Thirty-fourth Report)
69. ICH S3A Note for guidance on toxicokinetics: the assessment of systemic exposure in toxicity studies. International Conference on Harmonization, Geneva, Switzerland, 2018.
70. Masson JD, Crépeaux G, Authier FJ, Exley C, Gherardi RK. Critical analysis of reference studies on the toxicokinetics of aluminum-based adjuvants. J Inorg Biochem 2018; 181:87-95. doi: 10.1016/j.jinorgbio.2017.12.015.
71. NIH Guidelines for survival bleeding of mice and rats. National Institutes of Health, 2021. https://research.umd.edu/sites/default/files/2021-12/D19a_Survival_Bleeding_10-26-08.pdf
72. Nuffield Council on Bioethics. Chapter 9 Animal Use in Toxicity Studies; In the Ethics of Research Involving Animals. London. 2005.
73. Note for guidance on repeated dose toxicity. EMEA, European Agency for the Evaluation of Medicinal Products. London, Committee for Proprietary Medicinal Products 2010. CPMP/SWP/1042/99 Rev1.
74. Note for guidance on non-clinical local tolerance testing of medicinal products. EMEA, European Agency for the Evaluation of Medicinal Products. London, Committee for Proprietary Medicinal Products, 2014. CPMP/SWP/2145/2000 Rev1.
75. Draize JH, Woodard G, Calvery HO. Methods for The Study of Irritation and Toxicity of Substances Applied Topically to The Skin and Mucous Membranes. J Pharmacol Experimental Therapeutics 1944; 82(3): 377-390.
76. Watterson C, Lanevschi A, Horner J, Louden C. A comparative analysis of acute-phase proteins as inflammatory biomarkers in preclinical toxicology studies: implications for preclinical to clinical translation. Toxicologic Pathology 2009; 37(1):28-33. doi:10.1177/ 0192623308329286.
77. ICH S5(R2) Detection of Toxicity to Reproduction for Medicinal Products & Toxicity to Male Fertility. International Conference on Harmonization, Geneva, Switzerland. 2005. CPMP/ICH/386/95.
78. ICH guideline S2(R1) on genotoxicity testing and data interpretation for pharmaceuticals intended for human use - Step 5, 2012. EMEA, European Agency for the Evaluation of Medicinal Products. EMA/CHMP/ICH/126642/2008
79. Injac R. Global pandemic vaccine development, production and distribution challenges for the world population. Int J Risk Safety Med 2022; 33(3): 235-248. doi: 10.3233/JRS-227019.
80. Schilder NKM, Tiesjema B, Theunissen PT, Rengerink KO, van der Laan JW. Evaluation of non-clinical toxicity studies of COVID-19 vaccines. Regul Toxicol Pharmacol. 2023; 142:105438. doi: 10.1016/j.yrtph.2023.105438.

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Peripheral nerve injuries: non-surgical treatment approaches

Periferik sinir yaralanmaları: cerrahi olmayan tedavi yaklaşımları

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Dear Editor

I read with great interest the review titled 'Peripheral nerve injuries: current surgical management strategies' written by Mr Orif et al. in Volume 63, Issue 3 of your journal dated 09 September 2024. I would like to thank the authors for their valuable contribution to this topic, which is of great interest to physicians interested in the musculoskeletal system and whose diagnosis and treatment is very important (1). Although the review focuses on surgical approaches, I would like to contribute and share my knowledge on non-surgical treatment methods from the perspective of a physiatrist.

In peripheral nerve injuries, non-surgical treatment approaches, especially physical therapy applications, can play an effective and supportive role in many cases.

Physical Exercise

Even if there is no loss of muscle strength, pain and sensory disturbances may lead the patient to use the affected limb less. This may lead to the development of motor loss over time. In this process, the following exercise approaches can be applied effectively, with or without motor loss:

Range of motion exercises,

Assistive exercises,

Proprioceptive neuromuscular facilitation techniques,

Strengthening exercises

Nerve mobilization techniques.

These methods may play an important role in the preservation of muscle functions and prevention of motor losses (2).

Electrical Stimulation

Electrical stimulation is an important physical therapy method that should be included in the treatment plan in order to prevent atrophy due to muscle immobilization, to prevent muscle loss due to denervation in the early period and to accelerate nerve regeneration (2).

Clinical studies have demonstrated that electrical stimulation applied after peripheral nerve injuries increases axon growth and accelerates the sensorimotor recovery process (3). This application plays a supportive role in nerve repair, contributing to improved functional outcomes.

Low Level Laser Therapy

Low-level laser therapy (LLLT) plays an important role in scar tissue remodeling by mechanisms such as reducing inflammation and oedema, providing analgesic effect, promoting collagen synthesis and accelerating tissue repair. In addition, it has been shown to promote axon regeneration in peripheral nerve injuries, increase the secretion of neurotrophic factors, support revascularization and angiogenesis, and reduce Wallerian degeneration (4).

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Studies conducted with various intensities, irradiation points, application numbers and treatment durations have reported that LLLT supports improvement in axon myelination and acceleration of regeneration process (2). With these features, LLLT offers an effective complementary treatment option for peripheral nerve injuries.

Therapeutic Ultrasound

Studies have shown that low intensity ultrasound (LIU) improves nerve conduction velocity and muscle action potentials by increasing axon number, diameter and myelination. (2, 5). LIU should be applied in the intensity range of 200-500 mW/cm² to promote nerve regeneration. It has been reported that it is not effective at lower intensities (≤100 mW/cm²) and the effects decrease or disappear at higher intensities (≥1 W/cm²) (5). These findings reveal that LIU plays an important role in nerve healing and offers a valuable treatment option in clinical applications.

Extracorporeal Shock Wave Therapy

Extracorporeal shock wave therapy (ESWT) has been observed to significantly increase functional recovery by supporting regeneration of injured nerves. In addition, ESWT has been found to prevent denervation atrophy and to have positive effects on neuro-reorganization and nerve redistribution (2). This treatment method provides an important supportive mechanism in nerve healing and accelerates the functional recovery process.

CONCLUSION

Non-surgical treatment modalities in peripheral nerve injuries offer effective results, especially when initiated early. These approaches should be applied within the framework of a multidisciplinary treatment plan to optimize nerve regeneration, control pain and improve the quality of life of patients. These approaches, which can be considered as alternative or complementary to surgical methods, should be planned according to patient selection and individualized rehabilitation goals, which will increase the success of clinical outcomes.

Best regards

Keywords: Peripheral nerve, injuries, physical medicine and rehabilitation.

References

1.Orif SE, Uyanıkgil Y. Peripheral nerve injuries: the recent surgical management strategies. Ege Tıp Dergisi. 2024;63(3):487-500.

2.Pop NL, Mitrea DR, Urdă-Cîmpean AE, Filip A, Clichici S, Orăsan R. Peripheral nerve injury rehabilitation. Health, Sports & Rehabilitation Medicine. 2020;21(4).

3.Ni L, Yao Z, Zhao Y, Zhang T, Wang J, Li S, Chen Z. Electrical stimulation therapy for peripheral nerve injury. Frontiers in Neurology. 2023;14:1081458.

4.Muniz XC, de Assis ACC, de Oliveira BSA, Ferreira LFR, Bilal M, Iqbal HM, Soriano RN. Efficacy of low-level laser therapy in nerve injury repair—A new era in therapeutic agents and regenerative treatments. Neurological Sciences. 2021;42:4029-4043.

5.Acheta J, Stephens SB, Belin S, Poitelon Y. Therapeutic low-intensity ultrasound for peripheral nerve regeneration–a schwann cell perspective. Frontiers in Cellular Neuroscience. 2022;15:812588.

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EGE TIP DERGİSİ
Yazar Bilgi Formu

Ege Tıp Dergisi, Ege Üniversitesi Tıp Fakültesi'nin resmi yayın organı olup üç ayda bir yayımlanır ve Mart, Haziran, Eylül ve Aralık aylarında olmak üzere, dört sayı ile bir cilt tamamlanır. Dergi tüm tıp alanıyla ilgili güncel, nitelikli ve özgün çalışmaları yayımlamayı amaçlamaktadır.

Dergi sayfasına yüklenmiş olan başvurular dergi editörü veya onun belirlemiş olduğu bir alan editörü tarafında ön değerlendirmeye tabi tutulur. Ön değerlendirme sürecinde, uygun bulunan yazılar değerlendirme aşamasına geçirilirken, yayın koşullarına uymayan yazılar düzeltilmek üzere sorumlu yazara geri gönderilebilir, biçimce düzenlenebilir veya reddedilebilir. Değerlendirme aşamasında editör ya da alan editörü, yazıyı uygun gördüğü danışmanlara (hakemlere) incelenmek üzere gönderir. Hakemlik süreci çift kör olarak yürütülmektedir. Gerekli durumlarda, hakem ve editör görüşleri doğrultusunda sorumlu yazardan düzeltme/düzenleme yapması istenebilir. Yazardan düzeltme istenmesi, yazının yayımlanacağı anlamına gelmez. Bu düzeltmelerin en geç 21 gün içinde tamamlanıp dergiye gönderilmesi gereklidir. Sorumlu yazara yazının kabul veya reddedildiğine dair bilgi verilir.

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Yazar(lar)dan yazılarının yayımı için herhangi bir ücret talep edilmez.

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Yazarlık Kriterleri

Makalenin dergi sayfasına yüklenmesi sırasında, tüm yazarların adı, soyadı, ORCID numaraları ve tarih bilgisi ile ıslak imzalarının bulunduğu "Yayın Hakkı Devir Formu" ile yazarlık kriterlerinin

açıklandığı ve yazar katkılarının belirtildiği "Yazar Katkı Formu"nun doldurularak yüklenmesi zorunludur.

Ege Tıp Dergisi, Uluslararası Tıp Dergileri Editörleri Kurulu'nun (*International Committee of Medical Journal Editors*) standartlarını uygulamayı kabul etmiştir. Yazarlar "Biyomedikal Dergilere Gönderilen Makalelerin Uyması Gereken Standartlar: Biyomedikal Yayınların Yazımı ve Baskıya Hazırlanması (*Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication*)'daki yazarlık kriterlerini karşılamalıdır. Bu konudaki bilgiye www.icjme.org adresinden ulaşılabilir.

Etik Sorumluluk

Ege Tıp Dergisi, etik ve bilimsel standartlara uygun yazıları yayımlar. Dergide yayımlanan yazıların etik, bilimsel ve hukuki sorumluluğu yazar(lar)a ait olup editör ve yayın kurulu üyelerinin görüşlerini yansıtmaz.

Deney hayvanları ile yapılan çalışmalar dahil, tüm prospektif ve gerek görülen retrospektif çalışmalar için Etik Kurul Onayı alınmalı ve yazının "Gereç ve Yöntem" bölümünde Etik Kurul Onayının numarası ile birlikte alındığı tarih (gün-ay-yıl) belirtilmelidir. Hastanın mahremiyetinin korunmasının gerektiği tüm yazılarda etik ve yasal kurallar gereği, hastaların kimliğini tanımlayıcı bilgiler ve fotoğraflar, hastanın (ya da yasal vasisinin) yazılı bilgilendirilmiş onamı olmadan basılamadığından, ***"Hastadan (ya da yasal vasisinden) tıbbi verilerinin yayınlanabileceğine ilişkin yazılı onam belgesi alındı"*** cümlesinin "Gereç ve Yöntem" bölümünde (Gereç ve Yöntem bölümü olmayan yazılarda Giriş bölümünün sonuna) belirtilmesi gereklidir. Hayvanlar üzerinde yapılan çalışmalarda uluslararası etik kurallara uygunluğu gösteren komite onayı ilgili hayvan etik kurulundan alınmalıdır. Etik kurul onayı yanı sıra hayvanlara ağrı, acı ve rahatsızlık verilmemesi için yapılanlar açık olarak makalede belirtilmelidir (Bilgi için: www.nap.edu/catalog/5140.html*)*.

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Araştırma Makalesi, yeni bilgiler içeren ve güncel konularda yapılmış olan orijinal çalışmaları tanımlar. Bu çalışmalar randomize kontrollü, gözlemsel, tanımlayıcı, teşhis veya tedavi doğrulayıcı, klinik, deneysel veya deney hayvanları ile yapılmış olabilirler. Kaynaklar, Öz-Abstract bölümleri ve Tablo/Şekil açıklamaları hariç, ana metin 3000 sözcük sayısını aşmamalıdır.

Olgu Sunumu**,** okuyucular için önemli olabilecek yeni bir bulgu veya nadir ve ilginç vaka veya durumları, tanı veya tedavi ile ilgili bir yaklaşımı içermelidir. En fazla beş yazar, Kaynaklar listesi hariç, 1000 sözcük ve 10 kaynak ile sınırlıdır. Sadece bir tablo ya da bir şekil ile desteklenebilir.

Klinik Görüntü, eğitsel önemi olduğu düşünülen, orijinal, ilginç ve yüksek kaliteli görüntü içermelidir. En fazla beş yazar, beş kaynak ve bir şekil (fotoğraf, görüntü, çizim, grafik vb.) içerebilir. Kaynaklar listesi hariç 500 kelimeyi geçmemeli, şekil alt yazısı 100 kelimeyi aşmamalıdır.

Teknik Not, eğitim, araştırma, tanı veya tedavi amaçlı gerçekleştirilmiş olan yeni ve orijinal bir uygulamayı, tekniği, alet veya cihazı tarif etmelidir. En fazla beş yazar, beş kaynak ve bir şekil (fotoğraf, görüntü, çizim, grafik vb.) veya tablo içerebilir. Kaynaklar listesi hariç 500 kelimeyi geçmemeli, şekil (varsa) alt yazısı veya tablo (varsa) açıklaması 50 kelimeyi aşmamalıdır.

Editöre Mektup**,** yayımlanan metinlerle veya mesleki konularla ilgili olarak 500 sözcüğü aşmayan ve beş kaynak ile bir tablo veya şekil içerecek şekilde yazılabilir. Ayrıca daha önce dergide yayınlanmış metinlerle ilişkili mektuplara cevap hakkı verilir.

Davetli Derleme Yazıları, Yayın Kurulunun daveti üzerine, tıpta özellikli konuların kapsamlı değerlendirmelerini içeren, konusunda deneyimli ve yetkin yazarların yazdığı derlemelerdir. Derleme yazıları da derginin değerlendirme sürecinden geçirilir. Kaynaklar, tablo ve şekil alt yazıları hariç 5000 kelimeyi geçmemelidir. En fazla beş yazar ve 80 kaynak ile sınırlıdır. Davetli yazılar dışında derleme yazıları kabul edilmez.

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Ege Tıp Dergisine gönderilen tüm yazılar aşağıdaki kurallara uygun olarak hazırlanmalıdır.

Genel biçim

a- Metin iki satır aralıklı olarak Arial 10 punto ile yazılmalıdır,

b- Sayfa kenar boşlukları 2,5 cm olmalıdır,

c- Sayfalar başlık sayfasından başlamak üzere, sağ üst köşesinden numaralandırılmalı ve satır numaraları eklenmelidir (Microsoft Office Word™ - Düzen - Satır numaraları - Sürekli)

d- Kısaltmalar, metinde ilk olarak açık şekliyle yazılmış olanı takiben, yuvarlak parantez içinde yazılmalı ve tüm metin boyunca kısaltma aynı şekilde kullanılmalıdır. Başlık ve Öz bölümünde kısaltma kullanmaktan kaçınılmalı, metin içinde de gereksiz kısaltma kullanılmamasına özen gösterilmelidir. Cümleler kısaltma ile başlatılmamalıdır.

e- Ana metin içerisinde belirtilen ürün (ilaç, cihaz, donanım veya yazılım vb.), ürünün adını takiben, üretici şirketin adı, şehri ve ülkesi parantez içinde yazılmalıdır. Örnek: Discovery St PET / CT tarayıcı (General Electric, Milwaukee, WI, ABD).

f- Tüm ölçümlerin birimleri metrik sisteme (Uluslararası Birimler Sistemi, SI) göre yazılmalıdır. Örnek: mg/kg, µg/kg, mL/min, µL/h, mmHg, vb. Ölçümler ve istatistiksel veriler, cümle başında olmadıkları sürece rakamla belirtilmelidir.

g- Eğer varsa, uygulanan istatistiksel yöntem, Gereç ve Yöntem bölümünde belirtilmelidir.

h- Herhangi bir birimi ifade etmeyen ve 10'dan küçük sayılar ile cümle başında yer verilen sayılar yazı ile yazılmalıdır. Ondalık sayılar tam sayıdan Türkçe metinlerde virgül ile, İngilizce metinlerde nokta ile ayrılmalıdır.

i- İlgili yazı, yazı türüne göre tarif edilmiş olan bölümler şeklinde hazırlanmış olmalıdır.

Ön Yazı

Editöre hitaben yazının başlığı, yazı türü, ilgili yazının neden Ege Tıp Dergisinde yayımlanması gerektiğini özetleyen kısa bir açıklama ile sorumlu yazar belirtilerek tüm yazarların adı-soyadı, ORCID numarası, kurum ve iletişim bilgileri (telefon, e-posta ve posta adresleri) yazılmalıdır. Yazının daha önce başka bir yerde yayımlanmadığına veya yayımlanmak üzere gönderilmediğine dair yazılı ifade içermelidir. Ege Tıp Dergisi başka bir dilde dahi olsa daha önce yayımlanmış, kabul edilmiş veya değerlendirme aşamasında olan hiçbir yazıyı yayımlamayı kabul etmemektedir. Yazı yazar(lar)ın daha

önce yayımlanmış bir yazısındaki konuların bir kısmını içeriyorsa, bu durumun da ön yazıda belirtilmelidir.

Daha önce bilimsel bir toplantıda sözlü veya poster bildiri şeklinde sunulmuş olan yazılar, sunumun gerçekleştirildiği toplantı ile ilgili bilgiler (tarih, yer, toplantının ismi) olacak şekilde Ön Yazıda belirtilmeli, Öz bölümünün sonuna da not olarak yazılmalıdır.

Ana Metin

Sisteme yüklenen Microsoft Office Word™ formatındaki ana metin dosyasında yazarlara ait isim ve kurum bilgileri yer almamalıdır. Ana metin yazı türüne göre aşağıdaki bölümlerden oluşmalıdır:

- Araştırma Makalesi: Türkçe başlık, Öz ve Anahtar Sözcükler / İngilizce başlık, *Abstract* ve *Keywords* / Giriş / Gereç ve Yöntem / Bulgular / Tartışma / Sonuç / Çıkar Çatışması / Teşekkür (varsa) / Kaynaklar / Tablolar (başlıkları ve açıklamalarıyla beraber) / Şekil Alt Yazıları.

- Olgu Sunumu: Türkçe başlık, Öz ve Anahtar Sözcükler / İngilizce başlık, *Abstract* ve *Keywords* / Giriş / Olgu Sunumu / Tartışma / Sonuç / Çıkar Çatışması / Kaynaklar / Tablo (başlıkları ve açıklamalarıyla beraber) / Şekil Alt Yazısı.

- Klinik Görüntü: Türkçe başlık / İngilizce başlık / Olgu / Çıkar Çatışması / Teşekkür (varsa) / Kaynaklar / Şekil Alt Yazısı.

- Teknik Not: Türkçe başlık / İngilizce başlık / Teknik not / Çıkar Çatışması / Teşekkür (varsa) / Kaynaklar / Tablo (başlıkları ve açıklamalarıyla beraber) (varsa) / Şekil Alt Yazısı (varsa).

Yazının Başlığı

Kısa, kolay anlaşılır ve yazının içeriğini tanımlar özellikte, kısaltma içermeyecek şekilde Türkçe ve İngilizce olarak yazılmalıdır.

Özler

Türkçe (Öz) ve İngilizce (*Abstract*) başlığı altında yazılmalıdır. Araştırma Makalelerinde Amaç, Gereç ve Yöntem, Bulgular ve Sonuç (*Aim, Materials and Methods, Results, Conclusion*) olmak üzere dört bölümden oluşmalı, en fazla 250 sözcük içermelidir. Araştırmanın amacı, yapılan işlemler, gözlemsel ve analitik yöntemler, temel bulgular ve ana sonuçlar belirtilmelidir. Öz metninde kaynak numarası ve mümkün olduğunca kısaltma kullanılmamalıdır. Olgu Sunumlarında bölümlere ayrılmamalı ve 200 sözcüğü aşmamalıdır. Klinik Görüntü, Teknik Not ve Editöre Mektup için öz gerekmemektedir.

Anahtar Sözcükler

Öz (*Abstract)* bölümünün sonunda, Anahtar Sözcükler (*Keywords)* başlığı altında, bilimsel yazının ana başlıklarını yakalayan, *Index Medicus Medical Subject Headings (MeSH)*'e uygun olarak yazılmış en az üç, en fazla beş anahtar sözcük olmalıdır. Türkçe anahtar sözcüklerin, Türkiye Bilim Terimlerinden (*www.bilimterimleri.com*) seçilmesine özen gösterilmelidir.

Metin

Yazı metni, yazının türüne göre yukarıda tanımlanan bölümlerden oluşmalıdır.

Kaynaklar

Ege Tıp Dergisi, ulusal kaynaklardan yararlanmaya özel önem verdiğini belirtir ve yazarların bu konuda duyarlı olmasını bekler.

Kaynaklar metinde, tablo açıklamaları ve şekil alt yazılarında yer aldıkları sırayla, cümle içinde atıfta bulunulan ad ya da cümle bitiminde, noktadan önce yuvarlak parantez "()" içinde, Arabik rakamlarla numaralandırılmalıdır. Birden fazla kaynak numarasının belirtilmesi durumunda rakamlar birbirlerinden virgül ve bir boşluk bırakılarak ayrılmalı ardışık ikiden fazla rakam olması durumunda en küçük ve en büyük rakamlar arasına tire işareti konarak yazılmalıdır. Örnekler: (2, 5, 7); (3-7).

Dergi isimleri, *Index Medicus (PUBMED)*'de kullanıldığı şekilde kısaltılmalıdır. Kısaltılmış yazar ve dergi adlarından sonra nokta olmamalıdır. Yazar sayısı altı veya daha az olan kaynaklarda tüm

yazarların adı yazılmalı, yedi veya daha fazla olan kaynaklarda ise üç yazar adından sonra "*et al.*" veya *"ve ark."* yazılmalıdır. Kaynak gösterilen derginin sayı ve cilt numarası mutlaka yazılmalıdır. Sayfa numaraları yazılırken başlangıç ve bitiş sayfa sayılarının sadece değişen basamakları yazılmalıdır. Örnekler: 45-48 yerine 45-8, 219-222 yerine 219-22.

Kaynaklar, yazının alındığı dilde ve aşağıdaki örneklerde görüldüğü şekilde düzenlenmelidir:

Dergilerdeki yazılar

Tkacova R, Toth S, Sin DD. Inhaled corticosteroids and survival in COPD patients receiving long-term home oxygen therapy. Respir Med 2006;100(3):385-92.

Ek sayı (Supplement)

Solca M. Acute pain management: Unmet needs and new advances in pain management. Eur J Anaesthesiol 2002;19(Suppl 25):3-10.

Erken görünümde (E-pub) makale

Butterly SJ, Pillans P, Horn B, Miles R, Sturtevant J. Off-label use of rituximab in a tertiary Queensland hospital. Intern Med J doi: 10.1111/j.1445-5994.2009.01988.x

Kitap

Bilgehan H. Klinik Mikrobiyoloji. 2. Baskı. İzmir: Bilgehan Basımevi; 1986:137-40.

Kitap bölümü

McEwen WK, Goodner IK. Secretion of tears and blinking. In: Davson H (ed). The Eye. Vol. 3, 2nd ed. New York: Academic Press; 1969:34-78.

İnternet makalesi

Abood S. Quality improvement initiative in nursing homes: The ANA acts in an advisory role. Am J Nurs [serial on the Internet] 2002 [cited 12 Aug 2002]. Available from: *www.nursingworld.org/AJN/2002/june/wawatch.htm*

Web sitesi

Cancer-pain.org [homepage on the Internet]. New York: Association of Cancer Online Resources [updated 16 May 2002; cited 9 July 2002]. Available from: *www.cancer-pain.org*

Tablolar

Tablolar metni tamamlayıcı olmalı, metin içerisinde tekrarlanan bilgiler içermemelidir. Metinde yer alma sıralarına göre Arabik sayılarla numaralandırılıp isimlendirilmelidir (örnek: Tablo-1). Tablonun üstüne tablo ismini takip eden kısa ve açıklayıcı bir başlık yazılmalıdır. Tabloda yer alan kısaltmalar, tablonun hemen altında açıklanmalıdır. Dipnotlarda sırasıyla şu semboller kullanılabilir: *, †, ‡, §, ¶.

Şekiller

Çizim, resim, grafik ve fotoğrafların tümü "Şekil" olarak adlandırılmalı ve ayrı birer dosya olarak (.jpg, .png, .tif vb., en az 300 dpi çözünürlükte) sisteme eklenmelidir. Şekil dosyaları yüksek çözünürlükte ve iyi kalitede olmalıdır. Şekiller metin içinde kullanım sıralarına göre parantez içinde Arabik rakamla numaralandırılmalıdır (örnek: Şekil-1).

Şekil Alt Yazıları

Şekil alt yazıları, şekillere karşılık gelen Arabik rakamlarla çift aralıklı olarak yazılmalıdır. Şeklin belirli bölümlerini işaret eden sembol, ok veya harfler kullanıldığında bunlar alt yazıda açıklanmalıdır. Başka yerde yayınlanmış olan şekiller kullanıldığında, yazarın bu konuda izin almış olması, bunu belgelemesi ve alt yazıda belirtmesi gerekir.

Ölçümler ve Kısaltmalar

Yazının hazırlanması bölümünde "Genel biçim" başlığı altında açıklanmıştır.

Başvuruda Yüklenecek Belgeler

- **Ön Yazı**
- **Ana Metin**
- **Yayın Hakkı Devir Formu**
- **Yazar Katkı Formu**
- **Çıkar Çatışması Formu**
- **Şekil(ler)**

REVİZYONLAR

Yazarlar makalelerinin revizyon dosyalarını gönderirken ana metin üzerindeki değişiklikleri işaretlemeli, ek olarak hakemler tarafından belirtilen önerilerle ilgili notlarını "Hakemlere Yanıt" dosyasından göndermelidir. Bu dosyada her hakemin yorumunun ardından yazarın yanıtı gelmeli ve makalede değişikliklerin yapıldığı yer de belirtilmelidir. Revize makaleler karar yazısını takip eden 21 gün içinde dergiye gönderilmelidir.

Editör Yazışmaları

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Original Articles comprise original research reporting new information on contemporary issues. These studies might be randomized controlled, observational, descriptive, methodological, clinical, experimental or animal studies. They should not exceed 3000 words excluding the abstracts and table/figure captions.

Case Reports should present an important finding, or rare, interesting case or condition or a novel approach to diagnosis or treatment. They should have a maximum of five authors and should not exceed 1000 words excluding the references and have at most 10 references. It may be accompanied by one figure or one table.

Clinical Image should include a high quality original and interesting image considered as valuable for education. It should have at most five authors, five references and a figure (photography, image,

drawing, graphic, etc.). It should not exceed 500 words excluding the references and the figure caption should not exceed 100 words.

Technical Note should describe a new and original application, technique, tool or device developed for educational, research, diagnostic or curative purposes. It should have at most five authors, five references and a figure (photography, image, drawing, graphic, etc.) or a table. It should not exceed 500 words excluding the references and if present, the figure or table caption should not exceed 50 words.

Letter to the Editor should not exceed 500 words. Short relevant comments on published articles, medical and scientific issues, particularly controversies, having no more than five references and one table or figure are encouraged. Where letters refer to an earlier published paper, authors will be offered the right to reply.

Invited Reviews are written on the invitation of the Editorial Board, on comprehensive evaluations of specific medical topics. Invitations are sent to experienced and competent experts of the field. Reviews will also be evaluated through the peer review procedure of the journal. They should not exceed 5000 words excluding the references, table and figure captions. They might have at most five authors and 80 references. Reviews other than invited papers are not accepted.

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All articles submitted to the Journal must comply with the following instructions:

General format

a- The text should be doubled-spaced and typed in Arial 10 points,

b- Page margin width should be 2.5 cm,

c- All pages should be numbered consecutively in the top right-hand corner and line numbers must be added beginning with the title page.

d- Abbreviations should first be stated openly, followed by the abbreviation in () brackets and the same abbreviation should be used throughout the text. Abbreviations should be avoided in the Title and Abstract and care should be given to prevent unnecessary abbreviations. Sentences should not start with abbreviations.

e- Products (drug, device, hardware or software etc.) mentioned in the main text should be presented as product name followed by manufacturer, city and country in brackets. E.g. Discovery St PET / CT scanner (General Electric, Milwaukee, WI, ABD).

f- All measure units should be according to the metric system (International System of Units, SI). E.g. mg/kg, µg/kg, mL/min, µL/h, mmHg etc. Measures and statistical data should be presented with numbers unless at the beginning of the sentence.

g- If applied, the statistical methods should be stated in the Materials and Methods section.

h- All numbers smaller than 10 not representing a unit should be written as words. Decimals should be separated by points in English texts and by commas in Turkish texts.

i- The text should be organized under the headings described for the relevant types of manuscript.

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The editor should be informed with the title of the manuscript, type of the manuscript, a brief description on the reasons why the relevant submission should be published in Ege Journal of Medicine, along with the responsible author stated, and all authors' names, ORCID numbers, institutions and contact information (phone numbers, e-mail and s-mail addresses) provided. A statement assuring that the manuscript is not submitted, accepted or published elsewhere should be written. Even though its language is might be different, Ege Journal of Medicine does not accept manuscripts published or accepted elsewhere.

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The title page of the main text (comprising only the Turkish and English titles of the manuscript) submitted in Microsoft Office Word™ format should not include the names and institutions of the authors. The main text should have the following sections, according to the type of the manuscript:

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Keywords

At least three and at most five keywords in order of importance for indexing purposes should be supplied below the abstract and should be selected from, *Index Medicus Medical Subject Headings* (MeSH), available at *https://www.nlm.nih.gov/mesh/MBrowser.html*

Text

Authors should use subheadings to divide sections regarding the type of the manuscript as described above.

References

In the text, references should be cited using Arabic numerals in parenthesis in the order in which they appear. If cited only in tables or figure legends, they should be numbered according to the first identification of the table or figure in the text. Names of the journals should be abbreviated in the style used in Index Medicus. The names of all authors should be cited when there are six or fewer; when seven or more, the first three should be followed by *et al*. The issue and volume numbers of the referenced journal should be added.

References should be listed in the following form:

Journal article

Tkacova R, Toth S, Sin DD. Inhaled corticosteroids and survival in COPD patients receiving long-term home oxygen therapy. Respir Med 2006;100(3):385-92.

Supplement

Solca M. Acute pain management: Unmet needs and new advances in pain management. Eur J Anaesthesiol 2002; 19(Suppl 25):3-10.

Online article not yet published in an issue

Butterly SJ, Pillans P, Horn B, Miles R, Sturtevant J. Off-label use of rituximab in a tertiary Queensland hospital. Intern Med J doi: 10.1111/j.1445-5994.2009.01988.x

Book

Kaufmann HE, Baron BA, McDonald MB, Waltman SR (eds). The Cornea. New York: Churchill Livingstone; 1988:115-20.

Chapter in a book

McEwen WK, Goodner IK. Secretion of tears and blinking. In: Davson H (ed). The Eye. Vol. 3, 2nd ed. New York: Academic Press; 1969:34-78.

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Abood S. Quality improvement initiative in nursing homes: The ANA acts in an advisory role. Am J Nurs [serial on the Internet] 2002 [cited 12 Aug 2002]. Available from: *www.nursingworld.org/AJN/2002/june/wawatch.htm*

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Cancer-pain.org [homepage on the Internet]. New York: Association of Cancer Online Resources [updated 16 May 2002; cited 9 Jul 2002]. Available from: *www.cancer-pain.org*

Acknowledgements

The source of financial grants and the contribution of colleagues or institutions should be acknowledged.

Tables

Tables should be complementary, but not duplicate information contained in the text. Tables should be numbered consecutively in Arabic numbers, with a descriptive, self-explanatory title above the table. All abbreviations should be explained in a footnote. Footnotes should be designated by symbols in the following order: *,†, ‡, §, ¶.

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All illustrations (including line drawings and photographs) are classified as figures. Figures must be added to the system as separate files (.jpg, .png, .tif etc., at least 300 dpi resolution). Figures should be numbered consecutively in Arabic numbers and should be cited in parenthesis in consecutive order in the text.

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All measurements must be given in metric system (*Système International d'Unités, SI*). Example: mg/kg, µg/kg, mL, mL/kg, mL/kg/h, mL/kg/min, L/min, mmHg, etc. Statistics and measurements should always be given in numerals, except where the number begins a sentence. When a number does not refer to a unit of measurement, it is spelt out, except where the number is greater than nine.

Abbreviations that are used should be defined in parenthesis where the full word is first mentioned. Some common abbreviations can be used, such as *iv*, *im*, *po,* and *sc.*

Drugs should be referred to by their generic names, rather than brand names.

Documents to be uploaded:

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Authors should mark the changes in the main text when submitting revision files of their manuscripts. A separate text (Response to Reviewers) including the author's response for each reviewer's comment and indications where the changes were made should be written. Revised articles should be sent to the journal within 21 days following the decision.

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