Ege Journal of Medicine

Ege Tıp Dergisi

Cilt : 63
Sayı: 3
Eylül- 2024

ARAŞTIRMA MAKALELERİ

OLGU SUNUMU

DERLEME

EDİTÖRE MEKTUP

egetipdergisi.com.tr

ISSN 1016 - 9113

ISSN 1016 - 9113

EGE TIP DERGİSİ / *EGE JOURNAL OF MEDICINE*

Ege Üniversitesi Tıp Fakültesi Resmi Yayın Organıdır/ *The Official Publication of Ege University Faculty of Medicine.*

İmtiyaz Sahibi/ Publisher Ege Üniversitesi Tıp Fakültesi Adına/ *On behalf of Ege University Faculty of Medicine* Rüçhan SERTÖZ Dekan Vekili / *Deputy Dean*

Cilt / *Volume:* 63 Sayı / *Issue:* 3 Eylül / *September* 2024 Sayfa / *Pages:* 323-504

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T.C. Kültür ve Turizm Bakanlığı / *Republic of Türkiye Ministry of Culture and Tourism*
Sertifika / *Certificate* No. 52149

Yayın Tarihi / *Release Date*
09.09.2024

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Nörofizyoloji laboratuvarına düşük ayak tanısıyla yönlendirilen olguların retrospektif değerlendirilmesi

Retrospective evaluation of cases referred to neurophysiology laboratory with drop foot diagnosis

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ÖZ

Amaç: Bu çalışmamızda düşük ayak ön tanısı düşünülerek nörofizyoloji laboratuvarına yönlendirilen olguların etiyolojik ve elektrofizyolojik özelliklerini ortaya koymayı amaçladık.

Gereç ve Yöntem: Ocak 2019 - Eylül 2022 arasında düşük ayak kliniği nedeniyle elektromiyografi (EMG) laboratuvarına yönlendirilen 127 olgunun klinik ve elektrofizyolojik bulguları retrospektif olarak değerlendirildi.

Bulgular: Çalışmaya 114 olgu dahil edildi. Olguların %31 i kadın, %69 u erkekti. Olguların yaşları 18-85 arasında değişmekteydi. 79 olgu dahili, 35 olgu ise cerrahi branşlardan yönlendirilmişti. Düşük ayak etiyolojisi olarak en sık fibuler sinir hasarı saptanmakla birlikte, sıklık sırasına göre radikülopati, siyatik sinir hasarı, polinöropati, lumbosakral pleksopati, ön boynuz motor nöron hastalığı saptanan diğer etiyolojilerdi. %83 olguda tek taraflı, %17 olguda ise bilateral düşük ayak mevcuttu. Bilateral düşük ayak olgularında en sık neden olarak polinöropati saptandı. Elektrofizyolojik bulgular, olguların %85'inde aksonal, %11'inde demiyelinizan özellik göstermekteyken, %4 olguda demyelinizan veya aksonal hasar ayırt edilemedi. Fibular sinir hasarı dahili ve cerrahi branşlardan yönlendirilen olgularda en sık etiyolojik etken olmakla birlikte, dahili branşlarda polinöropati cerrahi branşlara göre daha sıktı. Tüm olgularda klinik olarak etkilenen bölge ile patolojik elektrofizyolojik bulguların elde edildiği bölge birbiri ile uyumluydu.

Sonuç: Elektrofizyolojik testler düşük ayak kliniği ile yönlendirilen olgularda farklı periferik patolojilerin belirlenmesinde yol göstericidir. Bu nedenle bu olgularda lezyon lokalizasyonunun belirlenmesinde, etiyolojiye yönelik yapılması gereken tetkiklerin planlanmasında, nörolojik muayene ile birlikte elde edilen elektrofizyolojik bulgular mutlaka göz önünde bulundurulmalıdır.

Anahtar Sözcükler: Düşük ayak, elektromiyografi, etiyoloji.

Not: *Bu çalışma 38.Ulusal Klinik Nörofizyoloji EEG-EMG Kongresi'nde (26-30 Ekim 2022) sözel bildiri şeklinde sunulmuştur.*

ABSTRACT

Aim: In this study, we aimed to reveal the etiologic and electrophysiologic characteristics of patients with foot drop referred to the neurophysiology laboratory.

Materials and Methods: The clinical and electrophysiologic findings of 127 patients referred to the electromyography (EMG) laboratory between January 2019 and September 2022 were retrospectively evaluated.

Sorumlu yazar: Şeyma Aykaç
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Başvuru tarihi: 11.12.2023 Kabul tarihi: 10.01.2024

Results: The ages of the 114 patients included in the study ranged between 18-85 years. 31% of the patients were female and 69% were male. 79 cases were referred from internal medicine and 35 cases were referred from surgery. The most common etiology of foot drop was fibular nerve injury, followed by radiculopathy, sciatic nerve injury, polyneuropathy, lumbosacral plexopathy, anterior horn motor neuron disease. Unilateral and bilateral foot drop was present in 83% and 17% of the cases, respectively. Polyneuropathy was the most common cause in the patients with bilateral foot drop. Electrophysiologic findings were axonal in 85% of cases and demyelinating in 11%, while demyelinating or axonal damage could not be differentiated in 4% of cases. Fibular nerve injury was the most common etiologic factor in cases referred from internal and surgical branches, but polyneuropathy was more common in internal branches than in surgical branches. In all cases, the clinically affected area and the area of pathologic electrophysiologic findings were consistent with each other.

Conclusion: Electrophysiologic tests are guiding in the determination of different peripheral pathologies in cases referred by foot drop. Therefore, electrophysiological findings obtained together with neurological examination should be taken into consideration in determining the lesion localization and planning the investigations to be performed for the etiology in patients with foot drop.

Keywords: *Foot drop, electromyography, etiology.*

Note: *This study was presented as an oral presentation at the 38th National Clinical Neurophysiology EEG-EMG Congress (26-30 October 2022).*

GİRİŞ

Sinir iletim çalışmaları ve iğne EMG, nörojenik hastalıkların değerlendirilmesinde önemli rol oynamaktadır. Elektrofizyolojik incelemeler, nörolojik muayenenin devamı gibidir ve lezyon lokalizasyonunu sağlar. Düşük ayak, en sık fibuler sinir hasarı nedeniyle gelişmektedir. Bununla birlikte siyatik sinir hasarı, L5 radikülopati, pleksopati, polinöropati ve ön boynuz motor nöron hastalığı diğer etiyolojik nedenlerdir. Bu olgularda elde edilen elektrofizyolojik bulgular lezyon lokalizasyonunu sağlar ve uygun rehabilitasyon planının düzenlenmesinde yol göstericidir.

Biz de bu çalışmada düşük ayak olgularını retrospektif olarak değerlendirerek, laboratuvarımızda saptadığımız düşük ayak etiyolojilerini karakterize etmeyi ve düşük ayak değerlendirilmesinde elektrofizyolojik incelemelerin tanısal katkısını değerlendirmeyi amaçladık.

GEREÇ ve YÖNTEM

Ocak 2019 - Eylül 2022 arasında düşük ayak kliniği ile etiyoloji araştırılması için Ege Üniversitesi Tıp Fakültesi Hastanesi Nöroloji Anabilim Dalı, Nörofizyoloji Bilim Dalı Elektromiyografi (EMG) Laboratuvarına yönlendirilen 127 olgununun klinik ve elektrofizyolojik bulguları retrospektif olarak incelendi. Çalışmaya düşük ayak kliniğinin akut dönemi sonrasında (1 ay sonrası) değerlendirilen olgular dahil edildi. Olguların yaş, cinsiyet, yönlendiren klinik, ön tanı, öykü ve nörolojik muayene bulguları, EMG bulguları ve var ise görüntüleme bulguları dosyaları taranarak kaydedildi. 13 olgu, EMG incelemesinin normal olması ve klinik bilgi eksikliği nedenli çalışmadan çıkarıldı. Tüm hastalarda elektrofizyolojik olarak sinir iletim çalışmaları ve iğne EMG yapıldı. Sinir iletim çalışmalarında yüzeyel elektrotlarla kayıtlama yapılırken, iğne EMG de konsantrik iğne elektrotlar kullanıldı. Sinir iletim çalışmalarında; incelenen duysal sinirlerde amplitüd, distal latans ve iletim hızı, motor sinirlerde ise distal-proksimal latans, birleşik kas aksiyon potansiyeli (BKAP) amplitüdü ve iletim hızı değerlendirildi. Standart sinir iletim çalışması, tek taraflı düşük ayak saptanan olgularda, alt ekstremite ekstansör digitorum brevisden ayak bileği, fibula başı altı ve üstü uyarımlarla kayıtlanan fibular motor sinir iletim çalışmalarını, abduktor hallusis kasından kayıtlanan tibial motor sinir iletim çalışmalarını, fibular duysal ve sural sinir iletim çalışmalarını içermekle birlikte bilateral düşük ayak olan olgularda sinir iletim çalışmaları her iki alt ekstremitede yapıldı. İğne EMG'de, incelenen kas istirahatte iken spontan potansiyelin varlığı, hafif kas kasılmasında izlenen motor ünite potansiyellerinin (MUP) süresi ve maksimal kasılmada motor ünitelerin maksimal kasılmaya katılım paterni değerlendirildi. İğne EMG'de; fibular nöropati düşünülen olgularda tibialis anterior, peroneous longus ve biseps femoris kısa başı, siyatik sinir hasarı düşünülen hastalarda, tibialis anterior, gastrokınemius, biceps femoris uzun veya kısa

başı, pleksus etkilenimi olan olgularda da siyatik sinirden innerve olan kaslara ek olarak gluteus medius ve gluteus maksimus kasları değerlendirildi. Radikülopati düşünülen olgularda L5-S1 ve L3-4'ten innerve olan tibialis anterior ve posterior, vastus medialis, adduktor magnus kasları incelendi. Aksonal hasar; sinir iletim çalışmalarında sinir iletim hızının normalin %75 inden hızlı olması ve distal latansın normalin %130 undan düşük olması olarak kabul edildi. Demyelinizan etkilenim ise sinir iletim hızının normal alt değerinin %75 inden yavaş, distal latansın da normalin %130 undan uzun olması olarak tanımlandı. İğne EMG de demyelinizan hasarda, istirahatte denervasyon potansiyelleri gözlenmemesi, normal MUP süresi ve maksimal kasılmada motor ünitelerin tam katılımının olmaması; aksonal hasarda ise denervasyon potansiyellerinin gözlenmesi, MUP süresinin uzaması (>15msn) ve maksimal kas kasılmasında motor ünite katılımının tam olmaması değerlendirildi. Fibula başı fibular sinir nöropatisinde; motor iletim çalışmalarında fibula başı segmentinde motor iletim hızında ≥10 m/s hız yavaşlaması veya fokal iletim bloğunu gösteren BKAP amplitüdünde fibula başı bölgesinde ≥%50 amplitüd düşüklüğü ile desteklendi.

Çalışmada elde edilen bulgular tanımlayıcı istatiksel verilerle (sayı, yüzde, ortalama) belirtildi.

BULGULAR

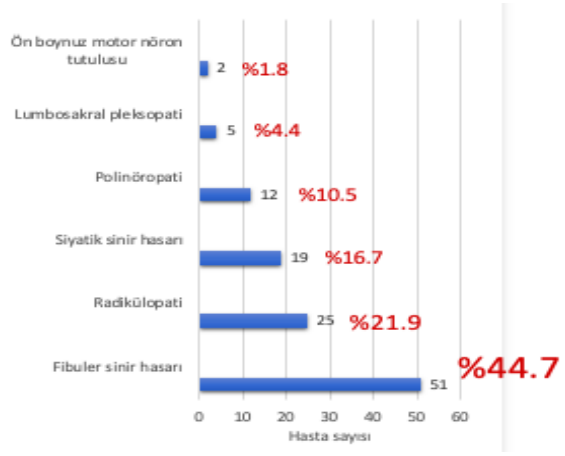
Çalışmaya dahil edilen 114 olgunun yaşları 18-85 (ort. 49,6) aralığında değişmekteydi. Olguların %31 si kadın, %69 ü erkekti. 79 olgu dahili, 35 olgu cerrahi branşlardan tarafımıza yönlendirilmişti. Klinik olarak 95 olguda tek taraf, 19 olguda ise bilateral düşük ayak mevcuttu (Tablo-1). Bilateral düşük ayak saptanan olguların 12 sinde polinöropati, üçünde radikülopati, birinde lumbosakral pleksopati, birinde ön boynuz motor nöron hastalığı ve ikisinde fibula başı nöropatisi gözlendi. Tüm olgularda klinik olarak etkilenen bölge ile patolojik elektrofizyolojik (EF) bulguların elde edildiği bölge birbiri ile uyumluydu. EF bulgular, tüm olguların %85'inde aksonal, %11'inde demiyelinizan özellik göstermekteyken; %4 olguda demyelinizan veya aksonal hasar net ayırdedilemedi.

Düşük ayak etiolojisi açısından tüm olgular değerlendirildiğinde en sık fibular sinir hasarı saptandı (%44,7). Bununla birlikte sıklık sırasına göre radikülopati (%21,9), siyatik sinir hasarı (%16,7), polinöropati (%10,5), lumbosakral pleksopati (%4,4), ön boynuz motor nöron hastalığı (%1,8) saptanan diğer nedenlerdendi (Tablo-2). Etiyolojik nedenler dahili ve cerrahi branşlarda ayrı ayrı değerlendirildiğinde; dahili branşlardan gönderilen olguların %47'sinde fibuler sinir hasarı, %23'ünde radikülopati, %13 ünde polinöropati, %11'inde siyatik sinir hasarı, %5'inde lumbosakral pleksopati ve %1'inde ön boynuz motor nöron hastalığı saptandı. Cerrahi branşlardan yönlendirilen olgularda ise %46'sında fibular sinir hasarı, %20'sinde radikülopati, %5'inde polinöropati, %23'ünde siyatik sinir hasarı, %3'ünde lumbosakral pleksopati ve %3'ünde ön boynuz motor nöron hastalığı tespit edildi (Tablo-3).

Tablo-1. Olguların cinsiyet, klinik bulgusu ve yönlendiren branşlar

CİNSİYET		
Kadın	**35**	**%31**
Erkek	**79**	**%69**
KLİNİK		
Bilateral düşük ayak	19	%17
Unilateral düşük ayak	95	%83
YÖNLENDİREN KLİNİK		
Dahili	79	%69
Cerrahi	35	%31

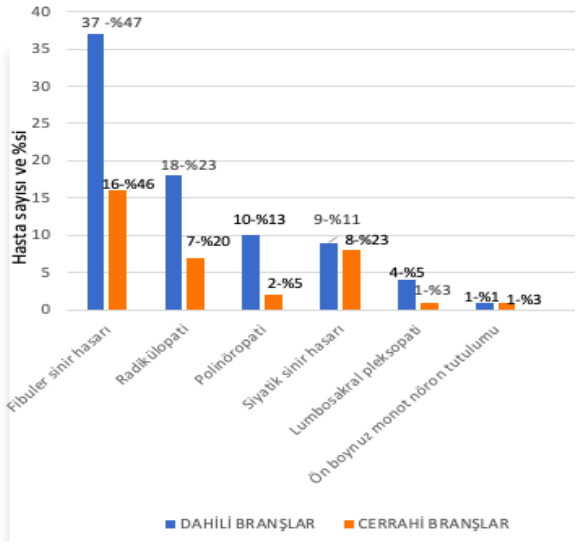
Tablo-2. Düşük ayak olgularında saptanan etiyolojik nedenler ve oranları



Fibular sinir hasarı saptanan olguların %45 inde travma ve/veya cerrahi operasyon öyküsü mevcuttu. Olguların %47 sinde fibular sinirin fibula başı nöropatisi saptandı. Fibula başı fibular

sinir nöropatisi saptanan olguların elektrofizyolojik bulguları incelendiğinde %42 olguda demyelinizan, %38 inde aksonal etkilenim izlenirken, %22 olguda aksonal veya demyelinizan hasar ayırt edilemedi.

Tablo-3. Dahili ve cerrahi branşlardan yönlendirilen düşük ayak olgularında saptanan etiyolojik nedenler ve oranları



Radikülopati tespit edilen olguların tümünde L5 kök etkilenimi saptanmakla birlikte olguların %68 inde diğer köklerin etkilenimi (L3,L4,S1) de eşlik etmekteydi. Bu olguların %56'sında spinal görüntüleme yapıldı. Spinal görüntülemede elde edilen kök etkilenim düzeyi EMG bulgularıyla uyumluydu.

Siyatik sinir hasarı saptanan olgularda ise %26'sında fibular dalın, %74'ünde ise hem fibular hem de tibial sinir dalının etkilendiği gözlendi. Olguların %79'unda travma veya operasyon öyküsü, %11'inde ise gluteal bölgeye enjeksiyon öyküsü mevcuttu.

Polinöropati saptanan olgularda polinöropati nedeni olarak diyabet, kronik böbrek yetmezliği ve kemoterapi öyküsü bulunmaktaydı.

Ön boynuz motor nöron tutumu saptanan iki olguda diğer miyotomlarda da yaygın ön boynuz motor nöron etkilenimi gözlendi. Lumbosakral pleksus tutulumu saptanan beş olgunun ikisinde travma nedeniyle pelvik fraktür öyküsü, ikisinde ise malinite öyküsü (akciğer kanseri ve lumbosakral pleksus tutuluşu gösteren sinir kılıfı tümörü) mevcuttu.

Bilateral düşük ayak saptanan olgulara bakıldığında, toplamda 19 olguda bilateral düşük ayak mevcuttu. Bu olgularda elektrofizyolojik olarak, 12'sinde polinöropati, üçünde radikülopati (L4-5, S1 kök), ikisinde fibula başı nöropatisi, birinde lumbosakral pleksopati, birinde ön boynuz motor nöron hastalığı saptandı.

TARTIŞMA

Düşük ayak, ayağın ve ayak parmaklarının dorsifleksiyon yapamamasıdır. Bu durum olgularda önemli düşme ve yaralanmalara neden olabilir. Düşük ayak, klinik sonuçları aciliyet ve prognoz açısından farklılık gösterebilen, çok çeşitli etiyolojiler nedeniyle gelişebilen önemli bir klinik bulgudur. Söz konusu patolojinin anatomik lokalizasyonu dikkate alındığında düşük ayak; beyin, omurilik, ön boynuz motor nöron hücresi, lumbosakral sinir kökleri-pleksus, siyatik sinir, peroneal sinir lezyonlarında veya polinöropati ve miyopati gibi diğer periferik tutulum gösteren hastalıklar nedeniyle gelişebilir.

Düşük ayak gelişimine neden olan periferik etkenlere bakıldığında birinci sıklıkta fibular nöropati bildirilmektedir (1). Fibular nöropati yetişkinlerde görülen tüm mononöropatilerin %15 ini oluşturur ve alt ekstremitede en sık sinir basısına bağlı gelişen mononöropatidir (2). Fibular sinir, L4-L5,S1 köklerinden oluşur, bu sinir lifleri önce lumbosakral pleksus, sonra da siyatik sinir içinde seyreder (3). Siyatik sinir, popliteada tibial ve fibular sinir dallarına ayrılır ve fibuler sinir, fibula başının altından geçerek yüzeyel ve derin dallarına ayrılır (4). Fibular sinirin en sık basıya uğradığı nokta, sinirin yüzeyelleştiği fibula başı bölgesidir. Bu olgularda genellikle kilo kaybı, diyabet, travma, diz bölgesine dışardan bası ve/veya cerrahi operasyon öyküsü bulunmaktadır (5,6). Bacak bacak üstüne atma gibi bazı pozisyonlar da fibula başı nöropatisini kolaylaştırır. Fibular sinir motor BKAP amplitüdünde düşüklük, iletim hızında yavaşlama, fibular duysal yanıtın alınmaması fibular nöropatiyi düşündüren elektrofizyolojik bulgulardır. Aksonal etkilenim varlığında iğne EMG'de fibular sinir inerve kaslarda, denervayon ve nörojenik MUP'ler saptanır. Fibular sinirin fibula başının proksimalinde etkileniminin belirlenmesinde iğne EMG de biseps femoris kasının kısa başı değerlendirilmesi yol göstericidir (7). Fibula başı demyelinzan fibular sinir nöropatisinde, fibular motor iletiminde fibula bölgesinin proksimalinden uyarımla BKAP amplitüdünde düşme veya bu bölgede motor iletim hızında yavaşlama saptanır. Bununla birlikte distalden elde edilen fibular duysal

yanıtlar korunabilir. Demyelinizan etkilenimde iğne EMG'de, fibular sinir inerve kaslarda normal MUP morfolojisinde maksimal kası seyrelme paterni izlenir. Bizim çalışmamızda, literatürde belirtildiği gibi, düşük ayak nedeni olarak en sık fibular sinir nöropatisi saptandı. Dahili ve cerrahi kliniklerden yönlendirilen olgular şeklinde ayrım yapıldığında, 2 grupta da fibular sinir nöropatisi en sık düşük ayak nedeniydi. Bu olgularda literatürde bildirildiği gibi travma ve cerrahi öyküsü mevcuttu. Fibular sinirin en sık fibula başı bölgesinde etkilenimini gösteren elektrofizyolojik bulgular saptanmakla birlikte, demyelinizan ve aksonal fibular sinir hasarı sıklığı birbirine benzerdi. Olgularda takip EMG yapılmadığı için demyelinizan veya aksonal hasar varlığının prognoza etkisi değerlendirilmedi.

İkinci en sık gözlenen düşük ayak nedeni olarak disk herniasyonu ve spinal stenoz bildirilmektedir (1). Aono ve arkadaşlarının düşük ayak saptanan 46 hastayı inceledikleri bir çalışmada, neden olarak hastaların %57'sinde disk herniasyonu ve %35'inde spinal stenoz olduğu görülmüştür (8). Wang ve Nataraj (1) tarafından yapılan sistematik bir derlemede tanımlandığı üzere, L5 sinir kökü vakaların çoğunda L4-L5 seviyesindeki lomber intervertebral diskin protrüzyonunda basıya uğramaktadır. Olgularda genellikle unilateral düşük ayak gelişmekle birlikte, santral disk protruzyonunda veya spinal dar kanal varlığında bilateral düşük ayak gelişebilir. Düşük ayak tipik olarak lomber disk herniasyonunda monoradikülopati sonucu ortaya çıksa da (9), spinal stenozda birden fazla spinal seviyenin tutulumu yaygındır. Yapılan bir çalışmada, lomber spinal stenoza bağlı düşük ayak hastalarının %64'ünde iki veya daha fazla spinal seviyenin tutulduğu bildirilmektedir (10). Lumbosakral radikülopatili olguların elektrofizyolojik incelemesinde, sinir iletim çalışmalarında duysal yanıtlar korunmuştur, fakat alt ekstremitede fibular ve/veya posterior motor sinir BKAP amplitüdlerinde düşme saptanabilir. Bu durumda iğne EMG ile belirli köklerden inerve kaslarda saptanan nörojenik bulgular etkilenen kök hakkında bilgi verir. Alt ekstremitede tek taraflı radikülopati saptandığında karşı ekstremite de radiküler etkilenim açısından değerlendirilmelidir. Elektrofizyolojik olarak bilateral radiküler etkilenim bulguları varlığında dar kanal akla gelmelidir. Bizim çalışmamızda da düşük ayak nedeni olarak radikülopati ikinci en sık neden olarak saptandı. Radikülopati saptanan olguların %88'inde (22 olgu) L5 kök etkilenimi sonucu tek taraflı düşük ayak izlendi. Radikülopati saptanan olguların çoğunda L5 kök etkilenimi yanında çoklu radiküler etkilenim (L2-3-4 ve S1) de mevcuttu. Bu olguların yaklaşık yarısında lumbosakral spinal görüntüleme yapılmıştı ve görüntülemelerde çoklu radiküler tutulumun olduğu olgularda, dar kanal veya diskopatiye bağlı çoklu kök basısı izlendi. Radikülopati, dahili branşlardan yönlendirilen hasta grubunda cerrahi branşlardan gönderilenlere göre daha sıktı.

Siyatik sinir hasarı alt ekstremitelerde en sık görülen ikinci nöropati olarak bilinmektedir (11). Siyatik sinir L4-S3 sinir köklerinden köken alır ve pelvisi gluteal kaslar tarafından örtülen büyük siyatik foramenden terk eder. Popliteal fossada, fibular ve tibial bileşenlere ayrılır ve sonunda bacak ve ayaktaki kasları innerve eder (12). Uzun anatomik seyri ve posterolateral konumu, çeşitli yaralanma türlerine zemin hazırlar. En yaygın yaralanma mekanizmaları arasında kalçaya yapılan intramüsküler enjeksiyonlar, travma ve pelvik bölgenin cerrahi prosedürleri yer almaktadır (13). Klinik olarak siyatik sinir yaralanması olan hastalar, fibuler ve tibial innervasyonlu kaslarda güçsüzlük ve lateral baldır, ayak sırtı ve ayak tabanında hipoestezi tariflerler (14,15). Düşük ayak, tibialden ziyade fibular bölümün yaralanmasıyla uyumludur. Siyatik sinirin fibular komponentinin yaralanmaya karşı daha hassas olmasının aşağıdaki özellikleriyle ilişkili olduğu düşünülmektedir: (1) daha az destekleyici epidural bağ dokuları ile daha büyük ancak daha az sayıda fasyalar; (2) siyatik çentikte ve fibular boyunda daha sabit ve açılı bir seyir ve bu nedenle gerilme yaralanmasına daha yatkın; ve (3) kalça ve proksimal uyluk bölgelerinde daha yüzeysel konum (15,16,17). Femur fraktürü, uyluk bölgesi yaralanmalarında genellikle tibial ve fibular divizyonun birlikte hasarlandığı (17) gluteal bölge enjeksiyonlarında bağlı siyatik sinir nöropatisinde ise fibular dalın daha sık ve ağır etkilendiği bildirilmektedir (18). Elektrofizyolojik incelemede, asimetrik tek ekstremitede fibular sinir iletiminde patoloji ile birlikte alt ekstremitede posterior tibial ve sural sinir iletim çalışmalarında da patoloji saptandığında olgularda siyatik sinir hasarı akla gelmelidir. Siyatik sinir hasarında iğne EMG'de, siyatik sinirden innerve olan uyluk arka grubu kaslarında, siyatik sinirin distal dalları olan fibular ve tibial sinirden innerve olan kaslarda izlendiği gibi patolojik bulgular saptanır. Bizim çalışmamızda düşük ayak nedeniyle yönlendirilen ve siyatik sinir hasarı saptanan olguların

tamamında siyatik sinirin fibular dalı etkilenmişti. Bununla birlikte %74 olguda fibular sinir etkilenimine tibial dal etkilenimi de eşlik etmekteydi. Olgularda en sık etiyolojik etken femur, pelvis bölgesinde travma veya cerrahi operasyon öyküsüyken, gluteal bölge enjeksiyonu 2. sıklıktaydı. Etiyoloji ve elektrofizyolojik bulgular birlikte değerlendirildiğinde literatürle uyumlu olarak femur ve uyluk bölgesi travma, cerrahi operasyon öyküsü olan olgularda fibular- tibial dal birlikte etkilenimini sıktı. Gluteal bölge enjeksiyon öyküsü olgularda ise fibular dal etkilenimi gözlendi. Olguların hepsinde aksonal hasar saptandı. Cerrahi branşlardan yönlendirilen olgularda daha yüksek oranda siyatik sinir hasarı mevcuttu.

Lumbosakral pleksus, alt ekstremitelere duysal ve motor innervasyon sağlar, L1-S4 sinir köklerinden oluşur. Üst lumbar pleksustan femoral, obturator ve safen; alt lumbosakral pleksustan ise siyatik, superior-inferior gluteal sinirler ve pudental sinir köken alır (19). Travma, malignite, enfeksiyon (Lyme, HIV, HSV) metabolik veya inflamatuar nedenler lumbosakral pleksus hasarına neden olabilir. Malignite nedenli etkilenimde özellikle L4-S1 bölgesinde sık etkilenim görülür (20). Sistemik malignitenin pleksusa metastazı nedenli etkilenim olduğu gibi, nadir olarak primer sinir veya sinir kılıfı tümörleri de lumbosakral pleksus etkilenimine neden olabilir. Elektrofizyolojik olarak da lumbosakral pleksusun etkilenen divizyonuna göre femoral ve siyatiğin dalı olan fibular, tibial ve sural sinir iletim çalışmalarında ve iğne EMG'de de, femoral, obturator, siyatik ve superior-inferior gluteal sinirler tarafından innerve kaslarda nörojenik etkilenimi gösteren bulgular saptanır. Bizim çalışmamızda beş olguda (olguların %4'ü) düşük ayak nedeni olarak lumbosakral pleksus etkilenimi mevcuttu. Bu olguların üçünde lumbosakral pleksus alt divizyon, ikisinde üst ve alt divizyon etkilenimi izlendi. Olguların hepsinde alt divizyon etkilenimi nedeniyle düşük ayak gelişmişti. Etiyolojide iki olguda pelvik fraktür nedeniyle gelişen travma, bir olguda da primer sinir kılıfı tümörü nedenli pleksus tutulumu saptandı. Bir olguda akciğer kanseri öyküsü olmakla birlikte pleksus görüntülemesi yapılmadığı için pleksus hasarının etiyolojisi netleştirilemedi, bir olguda da etiyolojiye yönelik bilgi elde edilemedi.

Amyotrofik lateral skleroz (ALS), üst ve alt motor nöron hasarı sonucu gelişen, ekstremite güçsüzlüğü, kas atrofisi ve fasikülasyon ile karakterize yıkıcı bir hastalıktır (21). Ön boynuz motor nöronların etkilendiği, alt motor nöron hasarında, başlangıç bölgesine bağlı olarak bulber ve ekstremite başlangıçlı olmak üzere iki gruba ayrılır. Bu olguların yaklaşık %75'i ekstremite başlangıçlıdır (22). Klinik olarak, ekstremite başlangıçlı ALS hastalarında kas güçsüzlüğü ve atrofi tipik olarak fokaldir. Ayak bileği dorsifleksiyon zayıflığı, yaygın bir klinik bulgu olan düşük ayak gelişmesine neden olabilir ve bu bulgu erken evrelerde alt ekstremite başlangıçlı ALS hastalarının belirgin bir özelliği olabilir. Poliomyelit, polio virüsünün neden olduğu, enfeksiyöz, ön boynuz motor nöron hasarı ile giden bir hastalıktır. Spinal kordun servikal, torakal veya lomber segmentlerini etkileyebilir. Bu olgularda genellikle alt ekstremite güçsüzlüğü sık olmakla birlikte tutulan spinal seviyeye göre tek kol, her iki kol ve her iki bacakta veya kol ve bacakta güçsüzlüğe neden olabilir. Genellikle asimetrik pareziye neden olur. Kronik dönemde bu olgularda parezi gelişen ekstremitede atrofi ve ekstremite kısalığı gözlenir. Ön boynuz motor nöron hasarının geliştiği bu iki hastalıkta, elektrofizyolojik olarak sinir iletim çalışmalarında motor BKAP amplitüdlerinde düşüklükle birlikte duysal iletimler normaldir. İğne EMG de Polio olgularında kronik dönemde farklı miyotomlarda denervasyon izlenmeksizin büyük boylu, geniş süreli nörojenik MUP'ler izlenir. ALS olgularında ise farklı miyotomlara ait kaslarda denervasyon, fasikülasyon ve nörojenik MUP'leri saptanır. Alt ekstremite başlangıçlı ALS formlarında L5-ağırlıklı L5-S1 bölgesindeki ön boynuz motor nöronların etkilenmesi nedeniyle bu durum lomber disk patolojileriyle karıştırılabilir. Yapılan bir çalışmada bu olgularda klinik olarak ayak bileği dorsifleksiyonun plantar fleksiyona göre daha güçsüz olduğu belirtilmektedir. Elektrofizyolojik olarak da sinir iletim çalışmalarında ekstansör digitorum brevis kasından elde edilen fibular sinir BKAP amplitüdleri, abduktor hallusis kasından elde edilen tibial sinir BKAP amplitüdünden düşük bulunmuştur. İğne EMG'de ise fibular sinirin innerve ettiği tibialis anterior kasında, tibial sinirin innerve ettiği gastroknemius kasına göre yoğun denervasyon potansiyelleri izlenmiştir (23). Sonuç olarak bu olgularda ayak bileği dorsifleksiyon zaafı hem klinik hem de elektrofizyolojik olarak daha belirgindir ve bu nedenle ALS tanısı da düşük ayak nedeni olan fibular nöropati, radikülopati, siyatik sinir hasarı gibi diğer nedenlerin arasında yer almaktadır.

Bizim olgularımızda da, iki olguda düşük ayak etiyolojisi olarak ön boynuz motor nöron hastalığı saptandı. Bir olguda ALS, diğer olguda ise polio tanısı düşünüldü. ALS olgusunda bilateral düşük ayak mevcutken, polio tanısı olan olguda tek taraflı düşük ayak saptanmıştı. 2 olguda fibular sinir-L5 miyotomu klinik ve elektrofizyolojik olarak S1-tibial sinire göre daha ağır etkilenmişti, bu olgularda ayrıca üst ekstremitelerde ve karşı alt ekstremitede de farklı miyotomlara ait nörojenik etkilenimi gösteren elektrofizyolojik bulgular mevcuttu.

Çalışmamızda 19 olguda bilateral düşük ayak izlendi. Bu olguların %63 ünde sensorimotor polinöropati saptandı. Diffüz polinöropati veya miyopatide, dorsifleksiyon güçsüzlüğü plantar fleksiyon güçsüzlüğünden daha belirgin olabilir ve bilateral düşük ayak nedenleri arasında bu nedenler de akla gelmelidir (24). Biz olgularımız arasında miyopati saptamadık. Bununla birlikte radikülopati, bilateral fibula başı nöropatisi, pleksopati ve ön boynuz motor nöron hastalığı bizim olgularımızda bilateral düşük ayağa neden olan diğer etiyolojilerdi. Polinöropati, bilateral ve simetrik, yaygın bir periferik sinir bozukluğudur. Miyelin disfonksiyonu veya aksonopatiye ikincil olabilir. Aksonal polinöropatinin nedenleri geniştir ve diyabet, üremi, alkol, B12 vitamini, bakır, tiamin, folat vb. beslenme eksiklikleri, kemoterapötik ajanlar, lepra, lyme hastalığı ve HIV gibi enfeksiyöz nedenler dahil çok çeşitli nedenlere gelişir (25). Çalışmamızda tüm polinöropati olgularında elektrofizyolojik olarak aksonal sensörimotor polinöropati saptandı. Bu olgularda sinir iletim çalışmalarında motor ve duysal sinir amplitüdlerinde düşüklük ve iletim hızlarında yavaşlama gözlenmekle birlikte, iğne EMG de aksonal etkilenimi destekler şekilde distal kaslarda nörojenik MUP leri izlendi. Olgularda polinöropatiyle ilişkilendirilebilecek diyabet, kronik böbrek yetmezliği ve kemoterapi öyküsü mevcuttu.

Düşük ayak, parasentral alandan alt torasik spinal korda kadar kortikospinal yolağı etkileyen lezyonlar nedeniyle de gelişebilir. Serebral infarkt veya parasagital alanı etkileyen patolojiler periferik lezyon benzeri izole düşük ayağa neden olabilir (26). Bu olgularda nörolojik muayene yol göstericidir ve tipik olarak olgularda etkilenen ekstremitede artmış kas tonusu, hiperrefleksi, ayak bileği klonusu ve Babinski pozitifliği saptanır (26,27,28). Elektrofizyolojik olarak ise sinir iletim çalışmaları normalken, iğne EMG de normal MUP morfolojisiyle birlikte maksimal kasılmada tibialis anterior ve ekstansör digitorum brevis kaslarında maksimal kasılmada seyrelme paterni izlenir (26). Bizim çalışmamızda da 13 olguda düşük ayak etiyolojisine yönelik yapılan elektrofizyolojik testlerde periferik patoloji izlenmemişti. Olguların nörolojik muayeneleri ve radyolojik görüntülemelerine ait elimizde veri olmadığı için, bu olgularda düşük ayak etiyolojisinin santral nedenlere bağlı gelişebileceği konusunda yorum yapamadık. Fakat normal elektrofizyolojik bulgular varlığında düşük ayak gelişen olgularda mutlaka nörolojik muayenede üst motor nöron bulguları da değerlendirilmeli ve olgular bu açıdan tetkik edilmelidir.

Çalışmamızda elektrofizyoloji laboratuvarına hasta yönlendiren klinik branşlar göz önünde bulundurulduğunda, fibular nöropati dahili ve cerrahi branşlarda en sık düşük ayak nedeniydi. Bununla birlikte polinöropati ve lumbosakral pleksopati, dahili branşlardan yönlendirilen olgularda cerrahi branşlardan yönlendirilen olgulara göre daha sık gözlendi. Siyatik sinir nöropatisi, cerrahi branşlardan yönlendirilen olgularda 2.en sık düşük ayak nedeni olarak izlendi. Radikülopati, cerrahi ve dahili branşlarda sık saptanmakla birlikte, her iki branş olgularında da nadir olarak motor nöron hastalığı tespit edildi. Bu bulgular ışığında özellikle tek taraflı düşük ayak olgularında yönlendiren klinik fark etmeksizin ön tanı olarak fibular nöropati, bilateral düşük ayak varlığında da özellikle dahili branşlardan yönlendirilen olgularda polinöropati akla gelmelidir. Yine travma, operasyon öyküsü olup, cerrahi branşlardan yönlendirilen olgularda fibular nöropatiden sonra siyatik sinir tutuluşu da düşünülmelidir.

Sonuç olarak, düşük ayak olgularında fibular nöropati, siyatik sinir hasarı, lumbosakral pleksus tutuluşu, radikülopati-ön boynuz motor nöron hastalığı ve yaygın tutuluş varlığında da polinöropati düşünülmesi gereken etiyolojilerdir. Elektrofizyolojik testler düşük ayak kliniği olan olgularda bu periferik patolojilerin ayrımını sağlar. Elektrofizyolojik olarak periferik patoloji saptanmayan olgularda da mutlaka nörolojik muayene ile santral patolojinin varlığına yönelik bulgulara dikkat edilmeli ve bu yönde radyolojik incelemeler planlanmalıdır.

Çıkar çatışması: Yazarlar, bu çalışma ile ilgili çıkar çatışması bildirmemiştir.

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Research Article / *Araştırma Makalesi*

Ege Journal of Medicine / *Ege Tıp Dergisi* 2024; 63 (3): 332-338

Birth statistics of adolescent pregnancies; evaluation of maternal and fetal outcomes

Adölesan gebeliklerin doğum istatistikleri; maternal ve fetal sonuçlarının değerlendirilmesi

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ABSTRACT

Aim: This study aims to evaluate the maternal and fetal outcomes of adolescent pregnant women who gave birth in the Gynecology and Obstetrics Clinic of Ağrı Training and Research Hospital.

Materials and Methods: The study was conducted as a retrospective study. The automation system of Ağrı Training and Research Hospital was used to conduct the study, and the data of 1560 patients who gave birth between January 1, 2018, and December 31, 2022, were examined retrospectively. 263 patients were excluded from the study due to missing data. The patients were divided into two groups: early adolescence and late adolescence. 84 patients in the early adolescence group and 1213 patients in the late adolescence group were included.

Results: A statistical difference was detected between the groups regarding age, gravida, and parity ($p<0.05$). There was no statistically significant difference between the groups regarding birth weight, birth weeks, first and fifth-minute Apgar values, type of birth, and stillbirth or preterm birth rates. Nulliparity rates were significantly higher in the early adolescent group.

Conclusion: Adolescent pregnancies are high-risk pregnancies with increased risks of pregnancy complications, including maternal and infant death. Therefore, studies to reduce adolescent pregnancy rates are essential for all societies.

Keywords: Adolescent pregnancies, stillbirth, preterm labor.

ÖZ

Amaç: *Bu çalışmanın amacı, Ağrı Eğitim ve Araştırma Hastanesi Kadın Hastalıkları ve Doğum Kliniğinde doğum yapan adölesan gebelerin maternal ve fetal sonuçlarını değerlendirmektir.*

Gereç ve Yöntem: *Çalışma retrospektif bir çalışma olarak yapıldı. Çalışmanın yapılması için Ağrı Eğitim ve Araştırma Hastanesi otomasyon sisteminden faydalanıldı ve 01 ocak 2018 ile 31 aralık 2022 tarihleri arasında doğum yapan 1560 hastanın verileri retrospektif olarak incelendi. Eksik veri nedeniyle 263 hasta çalışma dışı bırakıldı. Hastalar, erken adölesan ve geç adölesan dönem olmak üzere 2 gruba ayrıldı. Erken adölesan dönem grubunda 84, geç adölesan dönem grubuna 1213 hasta dahil edildi.*

Bulgular: *Gruplar arasında yaş, gravida ve parite açısından değerlendirildiğinde istatistiksel fark saptandı ($p<0.05$). Gruplar arasında doğum kiloları, doğum haftaları, birinci ve beşinci dakika Apgar değerleri, doğum türü, ölü doğum veya erken doğum oranları açısından istatistiksel anlamlı fark saptanmadı. Nulliparite oranları anlamlı derecede erken adölesan grubunda daha fazlaydı.*

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Sonuç: *Adölesan gebelikler, anne ve bebek ölümü de dahil olmak üzere gebelik komplikasyonları risklerinin arttığı riskli gebeliklerdir. Bu yüzden, adölesan gebelik oranlarının azaltılması için yapılacak olan çalışmalar tüm toplumlar için zaruridir.*

Anahtar Sözcükler: *Adölesan gebelikler, ölü doğum, erken doğum.*

INTRODUCTION

According to the World Health Organization (WHO) data, 21 million women worldwide are between the ages of 15-19 and 2.5 million women under 16 give birth every year. Adolescent pregnancies cause an increase in pregnancy-related complications such as endometritis and systemic infections in women compared to older age pregnancies, and these complications cause severe deaths in women in this age group (1).

Adolescence is the period between the ages of 10 and 19. This is the transition period from childhood to adulthood, with its characteristics (2). Adolescent pregnancies are defined as pregnancies occurring in women in this age period (2). Physiological and psychological changes that occur during this period may cause these women to be interested in sexuality, and these women who do not have sufficient information about sexuality face the risk of sexually transmitted diseases and pregnancy.

Although it varies by country, adolescent pregnancies constitute approximately 11% of all pregnancies (2). Adolescent pregnancies are also related to the development level of countries. According to WHO data, the adolescent pregnancy rate was 28.8% in Nicaragua in 2014, while this rate was 0.7% in Japan in the same year (3). In recent years, adolescent pregnancy rates have been reported to be close to 20% in African countries (4), and even up to 50% in sub-Saharan regions (5).

Studies have shown that 60% of adolescent pregnancies result in birth (6). There is a significant increase in both maternal and fetal complication rates in pregnancies in this age group. Examples of these include complications such as premature birth, low birth weight, and increased rates of newborns needing intensive care (7). In addition, data regarding the increase in preeclampsia rates in adolescent pregnancies vary between studies (8), (9). In the studies conducted, there are contradictions in the findings regarding the Apgar scores of the newborns of adolescent women (10), (11).

In our study, we aimed to evaluate adolescent pregnant women's maternal and fetal outcomes. As maternal outcomes, we evaluated maternal age, gestational age, cesarean and normal birth rates, cesarean section indications, and premature birth rates, and as fetal outcomes, we evaluated results such as live and stillbirth rates, birth weight, and first and fifth minute Apgar scores.

MATERIALS and METHODS

The study was carried out in Ağrı province, which has a low development level in the Eastern Anatolia region of Turkey. For the study, the data of patients who gave birth at the Ağrı Training and Research Hospital Gynecology and Obstetrics Clinic between January 1, 2018 and December 31, 2022 were examined retrospectively. To conduct the study, ethical approval was received from Ağrı İbrahim Çeçen University Scientific Research Ethics Committee with number 292, and permission was received from Ağrı Provincial Health Directorate with number 107.

At the beginning of the study, the data of 1560 adolescent patients who gave birth between the dates mentioned above were examined. 263 patients were not included in the study due to missing data. The study was conducted with data from 1297 patients. Hospital automation system was used to collect data.

The study was designed in two groups, early adolescence (<15 years) and late adolescence (15-19 years), in accordance with the WHO definition, and the data of the two groups were compared. Age, gravity, parity, number of abortions and week of birth were evaluated as demographic data. As fetal outcomes, gender, birth weight, and first- and fifth-minute Apgar scores were evaluated. As maternal outcomes, type of birth, cesarean section indications, live-stillbirth rates were evaluated. Pregnancies completed after 20 weeks were considered birth. Pregnancies completed before 20 weeks, pregnancies with chromosomal anomalies, and ectopic pregnancies were excluded from the

study. Pregnancies completed before the 37th week were considered preterm birth.

Spss 28.0 program was used to analyze the data. Categorical measurements are summarized as number percentages. Mean and standard deviation values were used in the descriptive statistics of the data. Shapiro Wilk test was used to determine whether the variables met the assumption of normal distribution. Independent samples t test was applied to find out which group caused the difference between continuous variables in terms of means between groups, and Chi-square test was applied to find out which group caused the difference between nominal variables. P< 0.05 was accepted for the results to be considered statistically significant.

RESULTS

Our study was conducted by examining the data of 1297 patients. 84 patients were included in the early adolescence (<15 years) group, and 1213 patients were included in the late adolescence (15-19 years) group.

When demographic data were examined, a statistically significant difference was found between the groups in terms of age, gravida and parity (p<0.05) (Table-1). When the groups were compared according to the number of abortions, number of stillbirths and average week of birth, no statistically significant difference was detected (p>0.05) (Table-1).

When we look at the fetal results, the groups were compared according to the first and, fifth minute Apgar values and the baby's birth weight, and no statistically significant difference was observed between the groups (p>0.05) (Table-2). When the babies of patients in both groups were examined according to gender, no statistically significant difference was found between the groups (p>0.05) (Table-2). While 49 of the babies born in the early adolescence group were boys (58.3%) and 35 were girls (41.7%), this rate was 626 boys (51.6%) and 587 girls (48.4%) in the late adolescence group (Table-2).

When the groups were examined according to live and stillbirth rates within the scope of maternal results, it was seen that 84 patients in the early adolescence group had a live birth, while there was no patient with a stillbirth. In the late adolescence group, 1202 (99.1%) patients had a live birth, while 11 (0.9%) patients had a stillbirth, but this was not statistically significant (p>0.05) (Table-3).

When the groups were compared according to birth types, no statistically significant difference was found between the groups (p>0.05). While the number of normal births was 71 (84.5%) and the number of cesarean births was 13 (15.5%) in the early adolescence group, the number of normal births was 958 (79%) and the number of cesarean births was 255 (21%) in the late adolescence group (Table-3).

The groups were examined according to premature birth rates and no statistically significant difference was found between the groups (p>0.05). While 13 patients (15.5%) in the early adolescence group had premature birth, 202 patients (16.7%) in the late adolescence group had premature birth (Table-3).

Additionally, when the nulliparity rates are evaluated between the groups, we see that the nulliparity rates are higher in the early adolescence group and this is statistically significant (p<0.05) (Table-3). In addition, when we examined the indications for cesarean section, it was seen that there was no statistically significant difference between the groups (p>0.005) (Table-3).

Table-1. Demographic data.

Demographic Data	Early Adolescence ≤15 years old	Late Adolescence 16-19 years old	T Value	P Value
	Average±SD	Average±SD		
Age	14.80 ± 0.460	18.10 ± 0.712	-42.469	**0.000***
Gravida	1.10±0.334	1.21±0.481	-2.996	**0.003***
Parity	0.04±0.187	0.14±0.372	-4.506	**0.000***
Number of Abortions	0.06±0.238	0.07±0.281	-0.415	0.678*
Number of Stillbirths	0.00±0.000	0.00±0.041	-0.372	0.710*
Birth Week	38.10±2.290	38.02±2.202	0.283	0.777*

* Independent Samples T-test

Table-2. Fetal outcomes.

Fetal Outcomes		Early Adolescence ≤15 years old		Late Adolescence 16-19 years old		T Value	P Value
		Average±SD		Average±SD			
Apgar 1.minute		7.89±0.581		7.81±0.856		0.834	0.404*
Apgar 5.minute		8.90±0.670		8.85±0.893		0.527	0.598*
Birth weight		3008.99±552.42		2966.73±483.33		0.767	0.443*
		n	%	n	%		
Gender	Male	49	58.3	626	51.6	1,424	0.233**
	Girl	35	41.7	587	48.4		

* Independent Samples T-test

** Chi-Square analysis test

Table-3. Maternal outcomes.

Maternal Outcomes		Early Adolescence ≤15 years old		Late Adolescence 16-19 years old		TOTAL		x^2	P.
		n	%	n	%	n	%		
Birth	**Live birth**	84	100.0	1202	99.1	1286	99.2	0.768	0.381**
	Stillbirth	0	0.0	11th	0.9	11th	0.8		
Birth Type	**Vaginal Birth**	71	84.5	958	79.0	1029	79.3	1,474	0.225**
	Cesarean Birth	13	15.5	255	21.0	268	20.7		
Mature and Premature Birth Rates	**37 weeks and above**	71	84.5	1011	83.3	1082	83.4	0.079	0.779**
	36 weeks and below	13	15.5	202	16.7	215	16.6		
Nulliparity Rates	**Nulliparous**	81	96.4	1055	87.0	1136	87.6	**6,458**	**0.011****
	Multiparous	3	3.6	158	13.0	161	12.4		
Indications for Caesarean Section	**Breech Presentation**	one	1.2	35	2.9	36	2.8	7,609	0.815**
	Previous Uterine Surgery	0	0.0	42	3.5	42	3.2		
	Fetal Distress	9	10.7	97	8.0	106	8.2		
	Other Presentation anomalies	0	0.0	11th	0.9	11th	0.8		
	CPD	0	0.0	5	0.4	5	0.4		
	Multiple pregnancies	0	0.0	11th	0.9	11th	0.8		
	IUGR	0	0.0	one	0.1	one	0.1		
	Hypertensive Diseases of Pregnancy	one	1.2	12	1.0	13	1.0		
	Placental Abruption	0	0.0	7	0.6	7	0.5		
	Non-Progressive Labor	2	2.4	28	2.3	30	2.3		
	Macrosomic Fetus	0	0.0	4	0.3	4	0.3		
	Cord Prolapse	0	0.0	2	0.2	2	0.2		

** Chi-Square Analysis

DISCUSSION

Adolescent pregnancy rates vary by country. When WHO data is examined, it will be seen that 11% of all pregnancies consist of pregnant adolescents (1). Additionally, adolescent pregnancies are also related to the development level of countries. For example, while the average adolescent pregnancy rate in African countries is 141/1000, this rate is 25/1000 in Europe (2). In our study, we examined 5-year birth data and found the adolescent pregnancy rate to be 9.41%. Considering the low development level of the province where the study was conducted, this rate is surprisingly close to WHO data. The main reason for this is that the age and birth data of women giving birth may be incompletely recorded in the hospital automation system.

Studies have reported that stillbirth rates are high in adolescent pregnant women (12). Lewis et al. In their study, they reported that the risk of stillbirth was higher in late-term adolescent pregnant women (aged 17-18) and that this situation may also be related to low socio-economic status (12). Again, Zhang et al. also found in their study that adolescent pregnancies were associated with a higher risk of stillbirth and neonatal death, and that the risk was even higher, especially in young adolescents (10-17 years old) (13). However, Karataşlı et al. In their study, they found that adolescent pregnancies were not associated with stillbirth (14). In our study, we investigated the relationship between early and late-term adolescent pregnancies and stillbirth. While there was no stillbirth in the early-term adolescent pregnancy group, we found that 11 patients had stillbirth in the late-term adolescent pregnancy group, but this was not statistically significant.

Studies in the literature show that 5th minute Apgar values vary according to maternal age. Vieira et al. They reported that Apgar values in babies of adolescent pregnant women were lower than in babies of older pregnant women (10). Karataşlı et al. also obtained similar results in their study (14). Unlike other studies, we compared adolescent pregnant women in two groups, early and late term, and did not detect any statistical difference between the groups in terms of Apgar scores.

Agbor et al. In their study with Cameroonian adolescent pregnant women, they found that adolescent pregnancies were associated with low birth weight (SGA) (15). Zhang et al. They also obtained similar results in their study (13). In our study, unlike others, we compared adolescents in two groups, and when we considered the results of both groups, we did not find that adolescent pregnancies were associated with SGA.

In a study, it was reported that 3.4% of women became pregnant for the first time before the age of 15 and 39.5% had their first birth between the ages of 15-19 (16). According to the results of our study, 6.5% of adolescents gave birth before the age of 15, and 93.5% gave birth between the ages of 15-19. In addition, in our study, the groups were compared in terms of normal and cesarean birth rates and it was observed that there was no statistical difference between the groups. In another study, it was observed that the majority of adolescent pregnant women were nulliparous (17). In our study, similar to other studies in the literature, we found that the rates of nulliparous patients were higher in both groups.

When we examine cesarean section rates, we encounter very different results between countries. Özdemirci et al. In their study, they found that cesarean delivery rates were higher in adolescent pregnant women (18). In addition, Medhi and colleagues compared the cesarean delivery rates between adolescent pregnant women and older women in their study and found that there was no difference (9). Zhang et al., in their study, found that the cesarean section rates in adolescent pregnant women were lower than in adult pregnant women. They attributed this to the higher rates of premature birth and low birth weight babies in adolescent pregnant women compared to adults (13). In our study, we compared the cesarean section rates of pregnant adolescents among themselves and found that there was no statistical difference between the groups. Additionally, the groups were compared in terms of cesarean section indications and no statistical difference was found.

In the literature, many studies have been conducted on premature birth rates in adolescent pregnant women (7). In some of these studies, it was found that adolescent pregnant women were at a higher risk of premature birth compared to adult pregnant women (18). Karataşlı et al. In

their study, similar to other studies , they found that (14). Zhang et al. Similar to others, they found higher rates of preterm birth in adolescents (13). Contrary to all these results, Althabe et al., in their study on African-American adolescent pregnant women, found that premature birth rates were lower in adolescents than in adults, and they emphasized that the reason for this may be due to ethnic differences. Premature birth is defined as a multifactorial pregnancy complication. Factors such as the fact that adolescent women are not fully mature both anatomically, physiologically and psychologically, as well as low education and socio-economic levels, suggest that they may be associated with premature birth (7), (19). In our study, we compared early adolescence pregnancies with late adolescence pregnancies in terms of preterm birth and found that contrary to expectations, preterm births were not higher in the early adolescence period and the results were statistically similar.

The limitation of our study is that it was conducted retrospectively. Additionally, data from 263 patients were not included in the study due to lack of hospital data. If these data had been included in the study, perhaps the results would have been different. In addition, since the hospital where the study was conducted was a tertiary hospital, it may have resulted in fewer negative outcomes due to the good prenatal care of adolescent pregnant women who applied to the hospital.

CONCLUSION

As a result, adolescent pregnancies are a common problem of countries and societies all over the world. Since adolescent women have not completed their maturation both anatomically and physiologically, the pregnancies of these women carry both maternal and fetal risks. Preventing adolescent pregnancies should be the common goal of all societies. For this, the most important thing to do is to increase the education levels of adolescents and integrate them into socio-economic life.

Conflict of interest: All authors participating in the study declare that there is no conflict of interest regarding the study.

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Araştırma Makalesi / *Research Article*

Ege Tıp Dergisi / *Ege Journal of Medicine* 2024; 63 (3): 339-349

Madde kullanım bozukluğu olan bireylere yönelik karar verme becerileri eğitim programının geliştirilmesi ve etkililiğinin değerlendirilmesi

Development and evaluation of the effectiveness of a decision-making skills training program for individuals with substance use disorder

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ÖZ

Amaç: Çalışmanın amacı madde kullanım bozukluğu olan bireylere yönelik karar verme becerileri müdahale programının geliştirilmesi ve etkililiğinin değerlendirilmesidir.

Gereç ve Yöntem: Katılımcılara Psikolojik Belirti Tarama Envanteri, Hamilton Depresyon Ölçeği, Hamilton Anksiyete Değerlendirme Ölçeği ve DSM-IV'e Dayalı Erişkin DEB/DEHB Tanı ve Değerlendirme Envanteri uygulanmıştır. Bu ölçeklerden üst sınırların üzerinde puan alan katılımcılar çalışmaya dahil edilmemiştir. Bu çalışmaya, deney grubu (n=36) ve kontrol grubu (n=36) kişi olmak üzere 72 kişi katılmıştır. Grup çalışmaları, karar verme becerileri, dürtüsellik ve stresle başa çıkma alt başlıkları ile altı oturumda gerçekleştirilmiştir. Katılımcılara müdahale öncesi ve sonrası Melbourne Karar Verme Ölçeği, Barratt Dürtüsellik Ölçeği ve Stresle Başa Çıkma Tarzları Ölçeği uygulanmıştır. Çalışmada, deney ve kontrol grubu ölçek puanları Tekrarlı Ölçümler Anova ile karşılaştırılmıştır.

Bulgular: Melbourne Karar Verme Ölçeğinden alınan puanlara göre, deney ve kontrol grubu katılımcılarının karar vermede özsaygı düzeylerinin müdahale öncesinden sonrasına istatistiksel olarak anlamlı farklılık gösterdiği bulunmuştur ($p<0.05$). Gruplar arasında karar verme stilleri açısından ise istatistiksel düzeyde anlamlı farklılık saptanmamıştır ($p>0.05$). Deney grubu ve kontrol grubu stresle başa çıkma ve dürtüsellik toplam puanları açısından istatistiksel olarak anlamlı düzeyde değişim göstermiştir ($p<0.05$).

Sonuç: Çalışmanın bulguları, karar verme becerisi eğitim programının madde kullanımı olan bireylerin karar vermede özsaygı, dürtüsellik ve stresle başa çıkma becerilerini olumlu yönde etkilediğini göstermektedir.

Anahtar Sözcükler: Madde kullanımı, karar verme, müdahale programı, bağımlılık.

ABSTRACT

Aim: *The aim of the study is to develop decision-making skills intervention program for individuals with substance use disorder and evaluate its effectiveness.*

Materials and Methods: *Symptom Checklist, Hamilton Depression Rating Scale, Hamilton Anxiety Rating Scale and Adult ADD/ADHD DSM IV-Based Diagnostic Screening and Rating Scale were administered to the participants. Participants with scores above the upper limits on these scales were not included.*

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Başvuru tarihi: 21.09.2023 Kabul tarihi: 07.02.2024

This study was conducted with 72 people, experimental group (n=36) and control group (n=36). Group studies were carried out in six sessions, including the subheadings of decision making skills, impulsivity and coping with stress. Melbourne Decision-Making Scale, Barratt Impulsivity Scale, and Coping Style Scale were administered to the participants before and after the intervention. The scores of the experimental group and the control group were analyzed using Repeated Measurements Anova.

Results: *According to the scores obtained from the Melbourne Decision Making Scale, it was found that the self-esteem levels of the experimental and control group participants in decision making showed a statistically significant difference from before to after the intervention (p <0.05). There was no statistically significant difference between the groups in terms of decision-making styles (p>0.05). The experimental group and the control group showed statistically significant changes in terms of coping with stress and impulsivity total scores (p <0.05).*

Conclusion: *The findings of the study show that the decision-making skill training program positively affects the self-esteem in decision-making, impulsivity and stress coping skills of individuals with substance use.*

Keywords: *Substance use, decision making, intervention program, addiction.*

GİRİŞ

Karar verme, yaşamın her alanında karşımıza çıkan ve araştırmacılar tarafından sıklıkla ele alınan bir konudur. Karar vermenin farklı biçimlerde tanımlamaları yapılmıştır. Karar verme; bir gereksinim anında, o gereksinime yanıt verebilmek adına mevcut olasılıklar içerisinden en uygun olanın tespit edilmesi olarak tanımlanabilir. Karar verme sürecinde, tercih yapılırken belirsizlik ve karışıklığı en aza indirgemek gerekmektedir (1). Karar verme; zamana yayılan, emek gerektiren bir davranıştır. Bu davranış kalıbı geliştirilebilir ve değişime uğrayabilir. Karar alma sürecinde bireylere özgü farklılıkların söz konusu olduğu ve bu farklılıkların bir sebebinin de karar verme tarzları olduğu düşünülmektedir (2). Connor ve Becker (2003)'a göre; bireylerin öğrenmiş olduğu alışkanlıklar karar alma stillerinin belirleyicisidir (3). Bireylerin karar alma biçimlerini tanıması, karar verme becerilerini geliştirmesi açısından kritik öneme sahiptir.

Madde kullanım bozukluğu, yineleyici biçimde madde kullanımı ile kendisini gösteren, maddenin kullanılması ve sınırlamasında kişisel kontrolün kaybolduğu, madde kullanımının sonlandırılması durumunda ise disfori, anksiyete, irritabilite gibi yoksunluk semptomları yaratabilen bir bozukluktur. Madde kullanım bozukluğu; bireysel, çevresel ve toplumsal açıdan olumsuz sonuçlar yaratan çok yönlü bir halk sağlığı sorunu olarak kabul edilmektedir (4,5). Madde kullanımının farklı alanlarda birçok olumsuz etkileri olmakla birlikte, özellikle bireylerin karar verme süreçlerindeki olumsuz etkileri oldukça dikkat çekicidir (6). Kullanılan maddelerin çoğu bilinçte değişikliklere neden olarak bireylerin yanlış tercihlerde bulunmasına yol açmaktadır. Örneğin; esrar maddesinin yeni şeyleri öğrenme, bunları hatırlama, dikkat ve odaklanma becerilerinde yavaşlamaya yol açtığını bildiren çalışmalar mevcuttur (7). Kronik madde kullanıcılarında beynin frontal lob bölgesinde ve limbik sistemde hasarlar oluştuğu, bilişsel süreçlerin olumsuz etkilendiği görülmüştür. Madde kullanımının yarattığı bu bilişsel tahribatlar, bireylerin karar verme süreçlerini de olumsuz yönde etkilemektedir (8).

Karar verme süreçleri, madde kullanımının yanında birçok farklı içsel ve dışsal faktörden etkilenmektedir. Dürtüsellik, bu faktörler arasında önemli bir yer tutmaktadır. Dürtüsellik, olumsuz sonuçlarına rağmen hızlı, plansızca tepkilerde bulunma ve dürtülere karşı koymakta başarılı olamama hali şeklinde tanımlanabilir. Dürtüsellik, bireylerin yaşamdaki seçimleri ve karar verme süreçleri üzerinde oldukça etkilidir. Dürtüsellik seviyesi yüksek bireyler, bir karar vermeden önce dikkatli incelemelerde bulunmakta zorlanırlar (9). Yapılan çalışmalar, yüksek dürtüsellik düzeyi ile madde kullanımı arasında anlamlı bir ilişki olduğunu göstermektedir (10). Stres, karar verme süreçlerini etkileyen bir diğer önemli faktördür. Stres düzeyindeki artış ile bilgi işleme süreci olumsuz etkilenmekte ve bilişsel işlevler bozulmaktadır. (11). Bireyler stres altında, panik haline geçmekte ve dürtüsel karar vermeye yönelmektedir. Stres altında daha riskli davranışların sergilendiği, yaşamsal açıdan daha dezavantajlı kararların alındığı görülmüştür (12).

Bağımlılık açısından karar verme süreçlerindeki bozulmaların önemi oldukça açıktır. Yapılan çalışmalar, karar verme süreçlerinde yaşanan bozulmalar ile madde kullanımı, bağımlılığın gelişmesi ve tedavi sonrası yüksek nüks riskleri arasındaki anlamlı ilişkileri göstermektedir (13, 14). Bağımlılık sürecinde davranışların ve hastalığın sorumluluğunun alınması ile iyileşme sürecinde sağlıklı kararlar vermenin önemi göz önüne alındığında, karar verme becerilerini hedefleyen psikolojik müdahaleler, özellikle bağımlılık tedavileri bağlamında önem kazanmaktadır. Karar verme süreçlerinin bağımlılık tedavisindeki önemine rağmen, bu alanda oluşturulan müdahale programlarının sayısı kısıtlıdır (15). Dizin incelendiğinde, özellikle ülkemizde madde kullanım bozukluğu olan bireylere yönelik oluşturulan karar verme becerileri müdahale programlarına ihtiyaç olduğu görülmektedir. Madde kullanımı olan bireylerin karar verme konusunda yaşadıkları sıkıntıların çok dikkat çekici olduğu düşünülerek, bu çalışmada bağımlı bireylerin karar verme becerilerini artırmaya yönelik müdahalede bulunulması hedeflenmektedir.

Çalışmanın amacı, madde kullanım bozukluğu olan bireylerin karar verme becerileri eğitimi sonrası panik ve dürtüsel hareket etmeksizin daha etkin ve dikkatli nasıl karar verebilecekleri konusunda farkındalık kazanmaları ve karar verme mekanizmaları üzerinde etkili olabilecek diğer faktörlere ilişkin bilgi sahibi olmalarıdır. Bu çalışmada, dürtüsellik hakkında farkındalık kazanımının madde kullanım sorunu olan bireylerin daha dikkatli kararlar vvermesine olan etkisi değerlendirilmiştir. Stres ve karar verme becerileri arasındaki yakın ilişki göz önünde tutularak, bu çalışma kapsamında oluşturulan müdahale programında stresle baş etme becerileri ile ilişkili bilgilere de yer verilmiştir. Kullanılan maddelerle birlikte karar verme süreçlerinde bozulmalar olan bu bireylerin, karar verme becerileri eğitimi sonrası kazandıkları yeni bilgi ve beceriler ile tedavi süreçlerine katkı sağlamak çalışmanın temel hedefleri arasındadır.

YÖNTEM

Araştırmanın örneklem yeri; farklı türde ve şiddette madde kullanımının söz konusu olması nedeniyle Karşıyaka Denetimli Serbestlik Müdürlüğü olarak belirlenmiştir. Örneklem Adalet Bakanlığının da izni ile dosyalarının infazına devam edilen ve madde kullanım bozukluğu olan bireylerden oluşturulmuştur. Olguların okur yazar olmaması, sınır ya da altı zeka düzeyinde olması, Hamilton Depresyon Ölçeğinden 29 puan ve üstü alması, Hamilton Anksiyete Ölçeğinden 15 puan ve üstü alması, madde etkisinde olması, Psikolojik Belirti Tarama Envanterinde (SCL-90) psikotik bulguların olması, DSM-IV 'e Dayalı Erişkin DEB/DEHB Tanı ve Değerlendirme Envanterinden 59 puan ve üzeri alması dışlama kriteri olarak kabul edilmiştir. Araştırmaya katılmayı kabul eden 36'sı deney grubu ve 36'sı kontrol grubunda olacak şekilde 72 kişiyle çalışma yürütülmüştür. Çalışma, kontrol grubu ve deney grubuna ön test ve son testlerin uygulandığı deneysel desende gerçekleştirilmiştir. Olgular, seçkisiz olarak kontrol ve deney grubuna ayrılmıştır. Bu çalışmanın etik kurul onayı Ege Üniversitesi Tıp Fakültesi Klinik Araştırmalar Etik Kurulu'ndan (20.12.2018 tarih 18-2.1/36 no'lu karar) alınmıştır. Etik kurul izninin ardından Ceza ve Tevkif Evleri Genel Müdürlüğü'nden gerekli resmi izin alınmıştır

Araştırma, İzmir ili Karşıyaka ilçesinde bulunan Karşıyaka Denetimli Serbestlik Müdürlüğünde Nisan 2019 - Eylül 2019 tarih aralığında gerçekleştirilmiştir. Bu çalışmaya, Karşıyaka Denetimli Serbestlik Müdürlüğünde madde kullanımı nedeniyle denetimli serbestlik alan ve dosyalarının infazına başlanan bireyler alınmıştır. Olgulara çalışma hakkında ayrıntılı bilgi verilmiş ve çalışmaya katılmayı kabul eden olgular ile çalışma gerçekleştirilmiştir. Grup oturumları başlamadan önce; Kişisel bilgi formu, Psikolojik Belirti Tarama Envanteri (SCL-90-R), Hamilton Depresyon Ölçeği, Hamilton Anksiyete Değerlendirme Ölçeği ve DSM-IV'e Dayalı Erişkin DEB/DEHB Tanı ve Değerlendirme Envanteri uygulanmıştır. Araştırmanın kriterlerine uygun olan 18-55 yaş aralığındaki 72 erkek birey çalışmaya dahil edilmiştir. Çalışmaya katılmayı kabul eden kişilerden bilgilendirilmiş onam formu alınmıştır ve seçkisiz atama yönetimi ile deney ve kontrol grupları oluşturulmuştur. Araştırma ön test-müdahale-son test aşamaları ile gerçekleştirilmiştir.

Deney grubuna 3 ay boyunca karar verme becerileri eğitim programı uygulanırken toplamda 36 kişiden oluşan kontrol grubu ise denetimli serbestlik prosedürü kapsamında zorunlu olarak gerçekleştirilen farklı konu başlıklarından oluşan seminerlere (1-İletişim becerileri--2-Hayır Diyebilme--3-Sigaranın zararları--4-Motivasyon--5-Güven ve özgüven) katılmıştır. İlk

seminerlerinde kontrol grubuna ön test formları ve 3 ay sonrasında son test formları uygulanmıştır.

Karar Verme Becerileri eğitim programın geliştirilme sürecinde; karar vermenin kuramsal temelleri, karar vermeyi etkileyen faktörler, karar verme süreçleri ile bağımlılık ilişkisi ve karar verme becerileri programları incelenmiştir. Program içeriğini oluşturmak için madde kullanımı olan bireylere yönelik karar verme becerilerini geliştirecek etkinlikler planlanmıştır. Son olarak, etkinlik süreleri de göz önünde bulundurularak; oturum süreleri ve sıklıkları belirlenmiştir. Karar verme becerileri programının oluşturulmasında araştırma ekibinin öncesinde eğitimini almış olduğu bilişsel davranışçı yaklaşımdan; uygulanma kısmında ise rehberlik modelleri ve grup çalışması modellerinden yararlanılmıştır. Grup teknikleri arasında yer alan ve grup çalışmalarında sıklıkla kullanılan, bilgilendirme, tartışma, oyun oynama ve ev ödevi teknikleri oturumlarda kullanılmıştır (16). Yapılacak olan eğitim programı, grubun öncesinden yöntem ve süre açısından bilgilendirildiği kapalı bir grup çalışması olarak planlanmıştır. Araştırmacı bu çalışmada grup lideri olarak belirli bir amaca yönelik olarak oturumları yönetmiştir.

Karar Verme Becerileri Eğitim Programı; psikoeğitim, ev ödevlerinin verilmesi ve izlenmesini içerecek şekilde altı oturum olarak oluşturulmuştur. Karar Verme Becerileri Eğitim Programı; "1. Oturum: Tanışma ve Karar Verme Becerisi Eğitim Programına Giriş; 2. Oturum: Karar Verme Basamakları ve Karar Alma Tarzları; 3. Oturum: Madde Kullanımı ve Karar Vermeyi Etkileyen Diğer Etmenler; 4. Oturum: Dürtüsellik ve Karar Verme; 5. Oturum: Stres ve Karar Verme; 6. Oturum: Kapanış ve Genel Değerlendirme"

Programın 1. oturumunda; tanışma, grup kurallarının belirlenmesi, eğitim programının tanıtımı, programın amacı, ısınma alıştırması, ev ödevinin verilmesi ile ön test formlarının uygulanması gerçekleşmiştir. İkinci oturumda; birinci oturum özetlenmiş, ev ödevleriyle ilgili geribildirimler alınmış, katılımcılara karar verme basamakları örnek senaryolar eşliğinde anlatılmış, karar verme stillerine ilişkin bilgilendirme yapılmış, ev ödevlerinin verilmesi ile oturum tamamlanmıştır. Üçüncü oturumda; özet gerçekleştirilmiş ve ev ödevleriyle ilgili geribildirim alınmış, sonrasında karar vermeyi etkileyen faktörlere ilişkin bilgi verilmiştir. Madde kullanımının karar verme üzerindeki etkisi alıştırmalar ve video gösterimi aracılığı ile aktarılmıştır. Dördüncü oturumda; özet ve ev ödevleri sonrası, dürtüsellik kavramıyla ilgili ayrıntılı bilgilendirme yapılmıştır. Dürtüselliğin karar verme süreçlerindeki etkisi üzerinde durulmuştur. Dürtüsellikle ilgili video gösterimi yapılmıştır. Ev ödevleri dağıtılarak oturum sonlandırılmıştır. Beşinci oturumda; stres kavramı anlatılmış, stresin nedenleri ve sonuçları üzerinde durulmuş, stresle baş etme yöntemlerine ilişkin bilgilendirme yapılmıştır. Stresin karar verme süreçleri üzerindeki etkisi uygulamalar ile aktarılmıştır, en son olarak gevşeme egzersizi ile sonlandırılmıştır. Altıncı oturumda ise; genel bir eğitim özeti yapılarak, tüm katılımcıların eğitim programıyla ilgili değerlendirmeleri alınmıştır. Bu oturumda, son test formları uygulanmıştır ve eğitim programı sonlandırılmıştır. 3 ay sonra eğitim programının etkinliğinin değerlendirilmesine yönelik deney grubuna tekrar ön test son test formları uygulanmış ve izlem gerçekleştirilmiştir.

Uygulamalar için 12 kişilik 3 farklı deney grubu oluşturulmuştur. Deney grupları, 3 ay boyunca 2 haftada bir defa olmak üzere 60 dakikalık oturumlara katılım sağlamıştır. Karar verme becerileri eğitim programı, "U" çalışma düzeni ile grup çalışmaları şeklinde bilgisayar ve projeksiyon eşliğinde gerçekleştirilmiştir.

Karar verme becerileri eğitim programı öncesi; Kişisel Bilgi Formu, Hamilton Depresyon Ölçeği (HAM-D), Hamilton Anksiyete Değerlendirme Ölçeği (HAM-A), Psikolojik Belirti Tarama Envanteri (SCL-90-R), DSM-IV'e Dayalı Erişkin DEB/DEHB Tanı ve Değerlendirme Envanteri uygulanmıştır.

Programın etkinliğinin değerlendirilmesi için ön test son test formları olarak; Melbourne Karar Verme Ölçeği, Barratt Dürtüsellik Ölçeği, Stresle Başa Çıkma Tarzları Ölçeği uygulanmıştır.

Kişisel Bilgi Formu

Araştırmacılar tarafından hazırlanan kişisel bilgi formunda, katılımcıların demografik bilgileri, fiziksel sağlık ve psikolojik durumları ve madde kullanım bilgileri yer almaktadır.

Hamilton Depresyon Ölçeği (HAM-D)

HAM-D; Max Hamilton'un 1960 yılında depresyon düzeyini belirlemek amacıyla geliştirdiği 17 soruluk bir ölçektir (17). Ölçekten 0-53 arasında değişen puan alınmaktadır. 7 puan ve altı;

depresyon yok, 8 -15 puan; hafif depresyon, 16 - 28 puan; orta düzey depresyon, 29 puan ve üzeri ise; şiddetli düzeyde depresyonu ifade etmektedir. Akdemir ve arkadaşları tarafından ölçeğin Türkçe geçerlik ve güvenirlik çalışması 1996 yılında gerçekleştirilmiştir (18).

Hamilton Anksiyete Değerlendirme Ölçeği (HAM-A)

HAM-A, anksiyetenin fiziksel ve psikolojik belirtilerini ölçmek amacıyla 1959 yılında Max Hamilton tarafından geliştirmiştir. Ölçekte, 0-4 arasında puanlanan 14 madde yer almaktadır. Ölçekten alınan puanlar 0 ile 56 arasında değişmektedir (19). Ölçeğin Türkçe geçerlik ve güvenirlik çalışması Yazıcı ve ark. tarafından 1998'de yapılmıştır (20).

Psikolojik Belirti Tarama Envanteri (SCL-90)

SCL-90; Derogatis tarafından 1977 yılında geliştirilmiş, çok boyutlu psikolojik semptomları içeren bir öz değerlendirme ölçeğidir. 90 soruyu içermektedir ve beşli likert tipidir. Her bir soru "Hiç" ile "İleri derecede" arasında işaretlenmektedir. Dokuz belirti alanı ve üç global indeksten oluşmaktadır. Dokuz belirti alanı; Somatizasyon, Obsesif-Kompulsif Bozukluk, Kişiler arası İlişkilerde Duyarlılık, Depresyon, Anksiyete, Öfke, Paranoid Düşünce, Fobik Anksiyete, Psikotik belirtilerden oluşmaktadır (21). 90 soruya verilen yanıtların toplanması ve elde edilen sonucun toplam soru sayısına bölünmesi ile genel toplam puana ulaşılmaktadır. Elde edilen puan 1'in üzerinde olması durumunda psikiyatrik bir sorunun varlığına işaret etmektedir. Ölçeğin Türkçe geçerlik ve güvenirlik çalışmasını 1991 yılında Dağ tarafından yapılmıştır (22).

DSM-IV 'e Dayalı Erişkin DEB/DEHB Tanı ve Değerlendirme Envanteri

Ölçek, 1995 yılında Turgay tarafından geliştirmiştir. Ölçek; 5'li likert tipidir ve 3 bölümden oluşmaktadır. Birinci bölüm; 9 sorudur ve dikkat eksikliğini değerlendirmektedir. 9 soruluk ikinci bölüm ise aşırı hareketlilik ve dürtüselliği değerlendirmektedir, Üçüncü bölümde; dikkat eksikliği ve hiperaktiviteyi ölçen 30 soru bulunmaktadır. Ölçekten alınan toplam puan 20'nin altında ise düşük, 20 - 59 arasında orta ve 59 puanın üzerinde ise yüksek düzeyde DEHB semptomlarına işaret etmektedir. Günay ve arkadaşları ölçeğin geçerlilik ve güvenirlik çalışmasını 2006 yılında yapmışlardır (23).

Melbourne Karar Verme Ölçeği (MKVÖ)

MKVÖ, Mann ve arkadaşları tarafından 1998 yılında geliştirilmiştir. Ölçek iki bölümden oluşmaktadır. 6 sorudan oluşan birinci bölüm "karar vermede özsaygıyı" ölçmektedir. Ölçeğin ikinci bölümünde ise; "karar verme stilleri" değerlendirmektedir. Bu bölümde 22 madde ve 4 alt boyut yer almaktadır. Karar verme stilleri; dikkatli, kaçıngan, erteleyici ve panik olmak üzere sınıflandırılmıştır. Hangi alt boyuttan yüksek puan alınmış ise bireyin o karar verme stilini daha çok kullanıldığı şeklinde yorumlanmaktadır (24). Deniz tarafından 2004 yılında ölçeğin geçerlik ve güvenirlik çalışması yapılmıştır (25).

Barrat Dürtüsellik Ölçeği (BDÖ)

BDÖ, dürtüselliği ölçmek amacıyla geliştirilmiş ve araştırmalarda yaygın olarak kullanılan bir ölçektir. Ölçek; motor dürtüsellik, dikkatte dürtüsellik ve plansızlık şeklinde 3 alt boyutu kapsayacak şekilde 30 sorudan oluşmaktadır. Dörtlü likert tipi bir ölçektir. Genel toplam puanının yüksek çıkması dürtüsellik seviyesinin yüksek olduğu şeklinde yorumlanmaktadır (26). Güleç ve arkadaşları geçerlilik ve güvenirlik çalışmasını gerçekleştirmişlerdir (27).

Stresle Başa Çıkma Tarzları Ölçeği (SBTÖ)

Folkman ve Lazarus tarafından geliştirilen ölçek, 4'lü likert tipindedir. Özbildirime dayalı ölçek, stres altında bireyin düşünce ve davranışlarını değerlendirmeye yönelik tasarlanmıştır. Geçerlilik ve güvenirlik çalışması 1995 yılında Şahin ve Durak tarafından yapılmıştır. 7 soru kendine güvenli olmayı, 5 soru iyimser olmayı, 8 soru çaresizce yaklaşmayı, 6 soru boyun eğici olmayı ve son olarak 4 soru ise sosyal desteğe yönelimi ölçen toplam 30 soruluk ve 5 alt boyutlu bir ölçektir. Alt boyutlardan alınan yüksek puan bireyin söz konusu başa çıkma tarzını daha sık kullandığı şeklinde yorumlanmaktadır (28).

İstatistiksel Analiz

Çalışmadan elde edilen verilerin istatistiksel analizi SPSS 21 paket programı ile gerçekleştirilmiştir. İstatistik çözümlemeler için, 0,05 anlamlılık düzeyi temel alınmıştır. Grup özelliklerinin değerlendirilmesinde, tanımlayıcı istatistik analizleri (ortalama, standart sapma, frekans, yüzde) gerçekleştirilmiştir. Verilerin analiz türünü belirlemek için öncelikle veri dağılımının normalliği Kolmogorov Smirnov normallik testi aracılığı ile kontrol edilmiştir. Katılımcıların sosyodemografik özelliklerinin gruplara göre dağılımını belirlemek amacıyla Ki-kare testi yapılmıştır. Deney ve kontrol grubundan elde edilen verilerin analizinde, tekrarlı ölçümler için ANOVA (repeated measures) testi kullanılmıştır.

BULGULAR

Örneklemin Sosyodemografik Özelliklerine İlişkin Bulgular

Çalışmaya katılan olguların (n=72) sosyodemografik özellikleri deney ve kontrol grubu şeklinde iki grup için ayrı değerlendirilmiştir. Gruplara ait sosyodemografik bilgiler Tablo-1'de özetlenmiştir. Deney grubunun yaş ortalaması 32.19 ± 9.62'dir, Kontrol grubunun yaş ortalaması 33.77 ve ± 9.06'dır. Gruplar yaş ortalamaları açısından benzerdir (U=579.000; p> 0.05). Medeni durum, eğitim durumları, çalışma durumları, meslek ve aylık gelirleri yönünden deney ve kontrol grupları benzerdir.(p> 0.05) (Tablo-1).

Örneklemin Madde Kullanım Özelliklerine İlişkin Bulgular

Grupların madde kullanım özellikleri değerlendirildiğinde, deney grubundaki katılımcıların maddeye başlama yaş ortalamaları 20.47±6.50 olarak bulunmuştur. Kontrol grubundaki katılımcıların maddeye başlama yaş ortalamaları 22.36±6.73'tür. Deney grubundaki katılımcıların madde kullanım süreleri ortalama 8.05±7.04 yıldır. Kontrol grubundaki katılımcıların madde kullanım süreleri ortalama 8.05 ±7.78 yıldır. Deney grubundaki kişilerin maddeden uzak kalabildikleri süre ortalaması 5.22 ± 1.94 aydır. Kontrol grubundaki kişilerin maddeden uzak kalabildikleri süre ortalaması 4.91±1.2 aydır. Madde kullanım sebepleri değerlendirildiğinde deney grubundaki katılımcıların 9'u (%25) merak, 3'ü (%8.3) özenti, 14'ü (%38.9) arkadaş etkisi ve 10'u (%27.8) ailevi sorunlar nedeniyle madde kullanmaya başladığını belirtmiştir. Kontrol grubundaki katılımcıların 7'si (%19.4) merak, 7'si (%19.4) özenti, 15'i (%41.7) arkadaş etkisi ve 7'si ise (%19.4) ailevi sorunlardan madde kullanmaya başladığını bildirmiştir. Grupların madde kullanım özellikleri karşılaştırıldığında maddeye başlama yaşları, madde kullanım süreleri, ayıklık süreleri, maddeye başlama nedenleri bakımından anlamlı bir farklılaşma yoktur (p> 0.05) (Tablo-2).

Tablo-1. Deney ve Kontrol Gruplarının Sosyodemografik Özelliklerine Göre Dağılımı**.**

Demografik Özellikler	**Deney Grubu (n=36)**		**Kontrol Grubu (n=36)**		**P değeri**	**Test Değeri**
Yaş Ortalaması	32.1 ± 9.62		33.7 ±9.06		0.437	U=579.000
Medeni durum	**N**	**%**	**N**	**%**	**P**	**X²**
Evli	19	52.8	15	41.7		
Bekar	15	41.7	20	55.6	0.468	1.518
Boşanmış	2	5.6	1	2.8		
Eğitim Durumu	**N**	**%**	**N**	**%**	**P**	**X²**
İlkokul mezunu	8	22.2	10	27.8		
Ortaokul mezunu	15	41.7	14	38.9	0.706	1.397
Lise mezunu	11	30.6	8	22.2		
Üniversite ve üstü	2	5.6	4	11.1		
Çalışma Durumu	**N**	**%**	**N**	**%**	**P**	**X²**
Evet	28	77.8	27	75.0	0.781	0.077
Hayır	8	22.2	9	25.0		
Meslek	**N**	**%**	**N**	**%**	**P**	**X²**
İşçi	28	77.8	28	77.8		
Esnaf	7	19.4	6	16.7	0.815	0.410
Emekli	1	2,8	2	5.6		
Aylık Gelir	**N**	**%**	**N**	**%**	**P**	**X²**
500 - 1000 TL	5	13.9	6	16.7		
1001 - 2000 TL	9	25.0	10	27.8	0.4222	2.810
2001 - 3000 TL	10	27.8	14	38.9		
3000 TL ve üzeri	12	33.3	6	16.7		

Deney ve kontrol gruplarının yaşam boyu kullandıkları maddeler belirlenmiştir. Deney ve kontrol grubundakilerin tamamı (%100) esrar kullandığını bildirmiştir. Deney grubunda kullanılan maddeler sıklık sırasına göre; ekstazi (n=9), sentetik kannabinoidler (n=7), kokain (n=7), uçucu (n=5), amfetamin (n=5), çeşitli haplar (n=4), rohipnol ve rivotril (n=3), eroin (n=1) şeklindedir. Kontrol grubunda kullanılan maddeler sıklık sırasına göre; ekstazi (n=6), kokain (n=6), sentetik kannabinoidler (n=2), uçucu (n=3) ve çeşitli haplar (n=1) şeklindedir.

Örneklemin Psikolojik Belirtilerine İlişkin Bulgular

Örneklemin psikiyatrik ek tanı ve psikolojik belirtilerinin değerlendirilmesi açısından uygulanan Hamilton Depresyon Ölçeği (HAM-D), Hamilton Anksiyete Değerlendirme Ölçeği (HAM-A), DSM-IV'e Dayalı Erişkin DEB/DEHB Tanı ve Değerlendirme Envanteri puanları Tablo-3'te sunulmuştur. Deney ve kontrol grupları ölçeklerden alınan puanlar açısından istatistiksel olarak anlamlı biçimde farklılaşma olmamıştır. Gruplar depresyon, anksiyete, DEHB ve psikolojik belirtiler açısından benzerlik göstermektedir (Tablo-3).

Karar Verme Becerileri Eğitim Programının Etkililiğine İlişkin Bulgular

Bu bölümde karar verme becerileri eğitim programının etkileri, deney ve kontrol grubu katılımcılarının ölçeklerden aldığı puanların gruplara, ölçümlere (ön test ve son test) ve ortak etkiye göre gösterdiği farklılıklara göre değerlendirilmiştir.

Katılımcıların Melbourne Karar Verme Ölçeğinden aldıkları ön test ve son test ortalama puanlarına ve grup karşılaştırmalarına yönelik bulgular Tablo-4'te yer almaktadır. Melbourne Karar Verme Ölçeğinden alınan puanlara göre, deney ve kontrol grubu katılımcılarının karar vermede özsaygı düzeylerinin müdahale öncesinden sonrasına istatistiksel olarak anlamlı farklılık gösterdiği bulunmuştur ($p<0.05$).

Deney ve kontrol grubunun Barrat Dürtüsellik Ölçeğinden aldıkları ön test ve son test ortalama puanlarına ve grup karşılaştırmalarına yönelik bulgular Tablo-5'te özetlenmiştir. ANOVA analizi sonuçlarına göre, deney ve kontrol grubunun ön test ve son test dürtüsellik toplam puan ve motor dürtüsellik puanlarında istatistiksel olarak anlamlı düzeyde değişim vardır ($p<0.05$).

Deney grubu ve kontrol grubunun, Stresle Başa Çıkma Tarzları Ölçeği ön test ve son test toplam puanları ile çaresiz yaklaşım, boyun eğici yaklaşım ve pasif başa çıkma tarzı alt boyutlarındaki değişim istatistiksel olarak anlamlı farklılık göstermiştir. Grupların Stresle Başa Çıkma Tarzları Ölçeğinden aldıkları ortalama puanlar ve ANOVA sonuçları tabloda yer almaktadır (Tablo-6).

Tablo-2. Grupların Madde Kullanım Özelliklerine Göre Dağılımı.

Madde kullanım özellikleri	**Deney Grubu (n=36)**		**Kontrol Grubu (n=36)**		**P**	**U**
Maddeye başlama yaşı ortalama	20.47	± 6.50	22.36	± 6.73	0.083	494.500
Madde kullanım süresi (yıl)	8.05	±7.04	8.05	±7.78	0.914	638.500
Ayıklık süresi (ay)	5.22	± 1.94	4.91	±1.20	0.133	517.500
Maddeye başlama nedenleri	**N**	**%**	**N**	**%**	**P**	**X^2**
Merak	9	25	7	19.4		
Özenti	3	8.3	7	19.4	0.491	2.414
Arkadaş etkisi	14	38.9	15	41.7		
Ailevi sorunlar	10	27.8	7	19.4		

Tablo-3. Grupların HAM-D, HAM-A ve DEHB Puanlarına Göre Dağılımı**.**

ÖLÇEKLER	**Deney Grubu (n=36)**	**Kontrol Grubu (n=36)**	**P**	**U**
Hamilton Anksiyete Toplam	6.30 ± 3.42	6.05 ± 2.68	0.861	632.500
Hamilton Depresyon Toplam	4.30 ± 3.51	3.41 ± 3.12	0.246	546.000
DEHB Toplam	22.47 ± 15.16	23.38 ± 13.45	0.710	615.000
SCL-90 Toplam	0.36 ± 0.17	0.32 ± 0.13	0.344	564.000

Tablo-4. Deney ve Kontrol Grubu Melbourne Karar Verme Ölçeği Ön Test – Son Test Ortalama Puanları ve ANOVA Sonuçları.

			Ön Test		Son Test			
Melbourne Karar Verme Ölçeği	**Grup**	**n**	**Ortalama**	**SS**	**Ortalama**	**SS**	**F**	**P**
Karar Vermede Özsaygı	Deney	36	9.16	2.33	10.08	1.40	5.816	0.019
	Kontrol	36	9.83	1.73	9.97	1.84		
Dikkatli karar stili	Deney	36	9.38	2.51	10.52	1.87	3.394	0.070
	Kontrol	36	10.13	1.97	10.50	1.61		
Kaçıngan Karar Stili	Deney	36	3.80	1.75	2.41	1.64	3.064	0.084
	Kontrol	36	4.13	2.23	3.66	2.08		
Erteleyici Karar Stili	Deney	36	3.52	2.31	2.19	1.56	4.010	0.552
	Kontrol	36	3.33	1.92	2.86	1.74		
Panik Karar Stili	Deney	36	3.36	1.82	3.02	1.90	1.538	0.219
	Kontrol	36	3.52	1.85	3.77	1.77		

Tablo-5. Deney ve Kontrol Grubu Barrat Dürtüsellik Ölçeği Ön Test – Son Test Ortalama Puanları ve ANOVA Sonuçları

			Ön Test		Son Test			
Barrat Dürtüsellik Ölçeği	**Grup**	**n**	**Ortalama**	**SS**	**Ortalama**	**SS**	**F**	**P**
Dürtüsellik Toplam	Deney	36	62.05	10.65	57.02	8.98	5.779	0.019
	Kontrol	36	61.83	9.88	61.00	9.07		
Dikkatte Dürtüsellik	Deney	36	15.52	3.23	14.58	2.78	2.390	0.127
	Kontrol	36	15.38	3.27	15.55	3.27		
Motor Dürtüsellik	Deney	36	21.27	3.88	17.75	3.03	5.874	0.018
	Kontrol	36	22.25	5.18	21.16	4.52		
Plansızlık	Deney	36	25.25	5.10	24.69	4.83	0.367	0.547
	Kontrol	36	24.19	4.54	24.27	4.57		

Tablo-6. Deney ve Kontrol Grubu Stresle Başa Çıkma Tarzları Ölçeği Ön Test – Son Test Ortalama Puanları ve ANOVA Sonuçları

			Ön Test		Son Test			
Stresle Başa Çıkma Tarzları Ölçeği	**Grup**	**n**	**Ortalama**	**SS**	**Ortalama**	**SS**	**F**	**P**
Başa Çıkma Toplam	Deney	36	47.05	8.32	42.61	6.12	7.087	0.010
	Kontrol	36	48.63	5.83	49.02	6.22		
Kendine güvenli yaklaşım	Deney	36	16.25	3.52	15.66	3.07	0.457	0.501
	Kontrol	36	17.19	2.99	17.08	3.08		
İyimser yaklaşım	Deney	36	10.05	2.75	10.13	2.03	0.239	0.626
	Kontrol	36	10.22	2.71	10.61	2.78		
Çaresiz yaklaşım	Deney	36	8.61	3.97	5.83	2.70	6.137	0.016
	Kontrol	36	9.00	3.94	8.50	4.03		
Boyun eğici yaklaşım	Deney	36	5.61	2.49	4.19	2.50	5.712	0.020
	Kontrol	36	5.02	2.99	4.97	3.18		
Sosyal desteğe başvurma	Deney	36	6.52	2.00	6.77	1.70	0.494	0.484
	Kontrol	36	7.19	2.18	7.86	2.07		
Aktif başa çıkma	Deney	36	32.83	6.78	32.58	4.63	0.896	0.347
	Kontrol	36	34.61	5.75	35.55	5.66		
Pasif başa çıkma	Deney	36	14.22	5.86	10.02	4.64	8.004	0.006
	Kontrol	36	14.02	5.69	13.47	6.01		

TARTIŞMA

Madde kullanımı, karar verme süreçleri üzerinde olumsuz etkisi olduğu bilinen önemli faktörlerden biridir. (15, 29). Bir diğer yandan ise, madde kullanımı olan bireylere özgü karar verme becerilerini iyileştirmeye yönelik uygulamaların sonuçlarına ilişkin veriler sınırlıdır. Bu çalışmada madde kullanım bozukluğu olan bireyler için geliştirilmiş olan karar verme becerileri eğitiminin etkililiği değerlendirilmiştir. Çalışmadan elde edilen bulgular bu programın madde kullanım bozukluğu olan bireylerin karar verme becerilerine olumlu yönde etkisi olduğu yönündedir.

Karar verme becerileri eğitimi, karar verme becerilerini geliştirmeyi hedefleyen altı haftalık bir müdahale programı olarak oluşturulmuştur. Çalışma içerisinde programın etkililiğinin değerlendirilmesi adına dört aşamalı bir süreç izlenmiştir. Süreçler; ön değerlendirme, müdahale ve son değerlendirme şeklindedir. Çalışmadan elde edilen bulgulara göre, uygulanan müdahale ile gruplar arasında karar vermede özsaygı, dürtüsellik ve stresle başa çıkma tarzları açısından oluşan değişimin anlamlı bir farklılık gösterdiği belirlenmiştir. Müdahale sonrası ortaya çıkan bu farklılıklar, müdahale programının etkili olduğuna işaret etmektedir.

Madde kullanım bozukluğuna özgü hazırlanmış karar verme becerileri programlarına rastlanmaması nedeniyle çalışmadan elde edilen bulgular farklı gruplara uygulanan karar vermeye yönelik müdahaleler ile kıyaslanmıştır. Dizin incelendiğinde ilköğretim, lise ve üniversite öğrencilerinde karar verme beceri programlarının etkinliğini araştırmaya yönelik çalışmalar vardır. Öğrencilerin bir karar alma beceri programı sonrası, karar vermede kendilerine olan güvenlerinin arttığı ve öğrencilerin karar verme stillerini olumlu yönde kullanmaya başladıkları yönünde sonuçlar bildirmiştir (30). Bu çalışmada da benzer şekilde uygulanan müdahale programı, madde kullanım bozukluğu olan bireylerin karar vermede özsaygılarını artırmıştır. Bununla birlikte, gruplar arasında karar verme stilleri açısından bir anlamlı düzeyde bir değişim yaşanmamıştır. Bu bulgu, katılımcılar tarafından kullanılan karar verme tarzlarının değişimi için daha uzun süreli bir müdahale programının gerektiğini düşündürmektedir.

Yeterli düzeyde düşünmeden ve yargılamadan ani biçimde harekete geçme eğilimi olarak tanımlanan dürtüselliğin artması ile sağlıklı kararlar alınması zorlaşmaktadır (31). Madde kullanımı olan bireylerin karar verme becerilerinde zayıflıklar olduğu ve dürtüsel davranışları sergilemeye daha yatkın oldukları öne sürülmektedir. (32). Madde kullanım bozukluğu olan bireylere yönelik hazırlanmış olan bu karar verme becerileri eğitim programında dürtüsellik oturumuna da yer verilmiştir. Çalışmamızda deney grubunun ön test ve son testleri arasındaki dürtüsellik toplam puanı ve motor dürtüsellik alt boyut farklılıkları, deney grubunu oluşturan katılımcıların program sonrası daha az dürtüsel davranışlar sergilediklerini göstermektedir. Karar verme becerileri eğitimi ile madde kullanımı olan bireylerin dürtüsellikle ilgili farkındalığının arttığı ve dürtüsellik düzeyinde düşüş olduğu söylenebilir.

Stres, yaşamı birçok yönden olumsuz yönde etkileyebildiği gibi karar verme davranışını da olumsuz olarak etkilemektedir. Karar verme süreci karmaşık yapısı nedeniyle kendisi de bir stres etkenine dönüşebilmektedir. Bireyler stres altındayken sağlıklı kararlardan uzaklaşabilmektedir. Van den Bos ve arkadaşlarının (2009) yaptığı bir çalışmada strese maruz kalan gruptaki kişilerin karar verme performanslarının olumsuz etkilendiğini ve riskli davranışlar sergilediğini göstermiştir (33). Stres ve karar verme arasındaki bu yakın ilişki nedeniyle, karar verme becerileri eğitim programına stresle baş etme modülü de eklenmiştir. Çalışmadan elde edilen bulgulara göre, deney grubunda stresle başa çıkma tarzları toplam puanı, çaresiz ve boyun eğici yaklaşım alt boyutlarında, stresle pasif başa çıkma tarzı açısından olumlu yönde değişimler olduğu belirlenmiştir. Uygulanan eğitim programının, bireylerin stresle baş edebilme becerilerini iyileştirici nitelikte olduğunu söylemek mümkündür. Madde kullanım bozukluğu olan bireylere yönelik geliştirilen karar verme becerileri eğitim programının stres üzerindeki etkisini inceleyen benzer bir çalışmaya dizinde rastlanmaması nedeniyle elde edilen bulguları ancak farklı müdahale programları ile karşılaştırmak mümkün olmaktadır. Yapılan çalışmalar, çalışmamıza benzer şekilde etkin müdahale programlarının stresle baş etme becerilerine katkı sağladığını ve bireyleri aktif planlama becerilerine yönelttiğini göstermektedir (34).

SONUÇ

Çalışmadan elde edilen bulgular, oluşturulan karar verme becerileri eğitim programının madde kullanımı olan bireylerin karar verme sürecindeki özsaygılarına, dürtüsellik düzeylerinde düşüşe ve stresle başa çıkma tarzlarına olumlu yönde katkı sağladığını göstermektedir. Madde kullanımının karar verme süreçlerindeki olumsuz etkisi ve uygulanan müdahale programının olumlu yönde yarattığı değişim dikkate alındığında, özellikle madde kullanımı olan gruplara uygulanan müdahale programlarına karar verme becerileri eğitim programının modüllerinin eklenmesinin etkili olacağı düşünülmektedir.

Sonuçlar, programın etkinliği açısından ümit vadeden biçimde olsa da bu araştırmanın bazı sınırlılıkları bulunmaktadır. Araştırma, yalnızca denetimli serbestlik tedbiri almış olan erkek madde kullanım bozukluğu olguları ile yürütülmüştür. Bu nedenle bu araştırmadan elde edilen sonuçların madde kullanım bozukluğu olgularının oluşturduğu evrene genellenmesi güçtür. Çalışmanın bu temel sınırlılığının yanında, eğitim programının etkililiğini değerlendirmek için sadece öz bildirime dayalı ölçeklerden yararlanılması da bir sınırlılık olarak görülebilir. Araştırmadan elde edilen sonuçlar ışığında gelecekte oluşturulacak uygulama ve araştırmalarda bu sınırlılıklar göz önünde bulundurulmalıdır. Madde kullanımı olan bireylerin karar verme stillerinin değişimine yönelik hazırlanacak müdahaleler için ileriki çalışmalara ihtiyaç duyulmaktadır. Gelecekte gerçekleştirilecek benzer çalışmaların, programın etkililiğini artırmak adına geniş örneklem ve uzun dönem yapılması faydalı olabilir.

Çıkar çatışması: Yazarlar, bu çalışma ile ilgili çıkar çatışması bildirmemiştir.

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Araştırma Makalesi / *Research Article*

Ege Tıp Dergisi / *Ege Journal of Medicine* 2024; 63 (3): 350-355

İskemik inmeli hastalarda karotis intima-media kalınlığının vasküler risk faktörleri ile korelasyonu ve inme tipleri arasındaki dağılımı

Correlation of carotid intima-media thickness with vascular risk factors in ischemic stroke patients and distribution among stroke types

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ÖZ

Amaç: Nörolojik acillerin başında gelen, önemli bir mortalite ve morbidite nedeni olan iskemik inmeden korunmada altta yatan vasküler risk faktörlerinin ve etiyolojik inme tipinin belirlenmesi önem taşımaktadır. Bu çalışmada aterosklerozun erken bulgusu olan karotis intima-media kalınlık artışının iskemik inmeli hastalarda vasküler risk faktörleri ile korelasyonu ve inme tipi ile ilişkisini araştırmak amaçlanmıştır.

Gereç ve Yöntem: Çalışmaya iskemik inme nedenli takip edilmiş 40-90 yaş arası hastalar dahil edilmiş olup veriler hasta dosyalarından taranarak retrospektif olarak değerlendirilmiştir.

Bulgular: Çalışmaya 161 kadın, 112 erkek olmak üzere toplam 273 iskemik inmeli hasta dahil edildi. Karotis intima-media kalınlık artışının vasküler risk faktörleri ile korelasyonuna bakıldığında diyabet varlığı ile anlamlı ilişki saptanırken diğer risk faktörleri ile istatistiksel olarak anlamlı bir ilişki saptanmadı (p:0,03). İnme tipleri ile ilişkisi değerlendirildiğinde büyük arter aterosklerozuna bağlı inmelerde karotis intima-media kalınlık artışının anlamlı olarak daha fazla olduğu gösterildi.

Sonuç: Bu çalışmada karotis intima-media kalınlığının vasküler risk faktörleri ile birlikte değerlendirilmesinin inme etiyolojisini aydınlatmada ve dolayısıyla inmeden korunmada önemli katkısı olabileceği vurgulanmaktadır.

Anahtar Sözcükler: İskemik inme, karotis intima-media kalınlığı, inme tipleri.

ABSTRACT

Aim: *Stroke is one of the leading neurological emergencies and a significant cause of mortality and morbidity. Determining the underlying vascular risk factors and etiological stroke type is important in preventing ischemic stroke. This study aimed to investigate the correlation of carotid intima-media thickness increase with vascular risk factors and stroke type in patients with ischemic stroke.*

Materials and Methods: *Ischemic stroke patients between the ages of 40 and 90 were included in the study, and the data were evaluated retrospectively from patient files.*

Results: *A total of 273 ischemic stroke patients, 161 women and 112 men, were included in the study. A significant relationship was found between the presence of diabetes and increased carotid intima-media thickness, but no relationship was found with other risk factors (p:0,03). And, it has been shown that the increase in carotid intima-media thickness is significantly higher in strokes due to large artery atheroclerosis.*

Conclusion: *This study emphasizes that evaluation of carotid intima-media thickness with vascular risk factors may contribute significantly to clarifying the etiology of stroke and therefore preventing stroke.*

Keywords: *Ischemic stroke, carotid intima-media thickness, stroke subtypes.*

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Başvuru tarihi: 20.01.2024 Kabul tarihi: 07.02.2024

GİRİŞ

İnme, ülkemizde ve dünyada en önemli engellilik ve mortalite nedenlerinden biridir (1). İnmelerin çoğunluğunu oluşturan iskemik inmeye neden olan vasküler risk faktörleri ve etiyolojik araştırma inmeden ikincil korunmada nörologların başlıca uğraş alanı olmaktadır. Ekstrakraniyal karotid arter hastalığının ayrıntılı değerlendirmesi, uygun risk sınıflandırması ve iskemik inme ile başvuran bireylerin yönetimi için kritik öneme sahiptir (2). Bilgisayarlı tomografi anjiografi (BTA), manyetik rezonans anjiografi (MRA), dijital substraksiyon anjiografi (DSA), pozisyon emisyon tomografi (PET-BT) ve hatta PET-MR gibi görüntüleme teknikleri ile gösterilebilen vasküler stenozun derecesi yanı sıra karotis ve vertebral doppler ultrasonografi (KVDUSG) ile karotis intima-media kalınlığı (KİMK), plak kalınlığı ve hacmi, plak içi bileşenleri gibi tedavi yaklaşımını değiştirebilecek birçok veri elde edilebilir (3). Tüm bu görüntüleme teknikleri arasında KVDUSG ucuz, ulaşılabilir, non-invaziv, sensitif ve tekrar edilebilir olması ile öne çıkmaktadır. Atheresklerozun erken bir belirteci olduğu kabul edilen KİMK artışının, karotis arter duvarının orta katmanını temsil ettiği ve arteriyopatinin göstergesi olduğu da bildirilmiştir (4). Yüksek çözünürlüklü KVDUSG ile ölçülen KİMK'nin rutin vasküler risk faktörlerinden bağımsız olarak kardiyovasküler hastalıkların ön görücüsü olduğu gösterilmiştir (5). Yapılan çalışmalarla vasküler risk faktörlerinin KİMK artışı ile ilişkisi ortaya konmuştur. Çin'den 2500'ün üzerinde hasta ile yapılan bir çalışmada KİMK'nin ileri yaş, erkek cinsiyet, hipertansiyon (HT), diabetes mellitus (DM), düşük HDL (yüksek yoğunluklu lipoprotein) ve yüksek LDL (düşük yoğunluklu lipoprotein) ile arttığı bildirilmiştir (6). Yapılan başka bir çalışmada ise hipertansiyonlu hastaların başlangıç KİMK ile inme riski arasında pozitif bir ilişki olduğu ve bu ilişkinin ortalama arteriyel basınç veya diyastolik kan basıncı daha yüksek olanlarda daha güçlü olduğu gösterilmiştir (7). Bu çalışma ile iskemik inmeli hastalarda KİMK artışının vasküler risk faktörleri korelasyonu ve TOAST'a (Trial of ORG 10172 in Acute Stroke Treatment System) göre inme tipi ile ilişkisinin araştırılması amaçlanmıştır.

GEREÇ ve YÖNTEM

Çalışmaya Ege Üniversitesi Hastanesi Nöroloji Kliniğinde izlenmiş 40-90 yaş arası, iskemik inme tanılı hastalar dahil edildi. Hastaların demografik verileri, vasküler risk faktörlerine ait tetkikleri, TOAST'a göre inme tipleri ve KVDUSG sonuçları hasta dosyasından retrospektif olarak değerlendirildi. Hemorajik inme geçiren, vasküler risk faktörlerine ait verileri eksik olan ve KVDUSG yapılmamış hastalar dışlandı. Hastaların KİMK ölçümü nöroloji kliniği bünyesindeki doppler ultrasonografi laboratuvarında bu konuda deneyimli ve yeterliliği olan bir nörolog tarafından, yüksek çözünürlüklü B-mod ultrasonografi (Siemens Ultramark 9, Advanced Technology Laboratories, Signal Hill, CA, ABD) cihazı ile sırt üstü pozisyonda başları 45° eğimli olacak şekilde yatırılarak sağ ve sol ana karotid arter görüntülenerek yapıldı. Ölçümler Mannhiem konsensusuna uygun olarak her iki tarafta, ana karotis bifürkasyonunun 1 cm proksimalinden plaksız bir alandan yapıldı (8). Hesaplanma her iki ana karotid arterden yapılan, arka duvara ait KİMK değerlerinin ortalaması alınarak yapıldı. İstatistik değerlendirme için SPSS 16 programı kullanıldı. Sayısal değişkenler için tanımlayıcı istatistikler (ortalama, ortanca, standart sapma, minimum, maksimum) kullanıldı. Sayısal değişkenler normal dağılım göstermediği için bağımsız gruplar arasındaki karşılaştırmalar Kruskal-Wallis ve Mann-Whitney U testi ile, sayısal değişkenler arasındaki ilişkiler Spearmen's rho korelasyon için Ki-kare testi ile yapıldı. İstatistiksel olarak $p< 0,05$ olması anlamlı olarak kabul edildi.

BULGULAR

Çalışmaya 161'i (%59,0) erkek, 112'si (%41,0) kadın olmak üzere dahil edilme kriterlerini karşılayan 273 hasta alındı. Ortalama yaş 64,6 ±12,6 saptandı. Hastalar; yaş dağılımını göstermek amacıyla 40-49, 50-59, 60-69, 70-79, 80 ve üzeri olarak gruplandırıldı. Hastaların vasküler risk faktörlerinin sıklığı Tablo-1.'de gösterilmiştir. Hastaların TOAST'a göre inme sınıflaması yapıldığında 88 (%32,2) hastada küçük arter hastalığı (laküner inme), 67 (%24,5) hastada büyük arter aterosklerozu, 59 (%21,6) hastada kardiyoembolizm, 34 (%12,5) hastada diğer etiyolojiler, 25 (%9,2) hastada ise etiyoloji bulunamadığı saptandı. Hastaların bakılan KİMK ortalaması 0,78 mm (min: 0,32 mm maks: 1,35 mm) olarak hesaplandı. Yaş gruplarına ve cinsiyete göre KİMK'ye bakıldığında gruplar arasında anlamlı fark olmadığı görüldü (sırasıyla p:0,46, p:0,78). KİMK artışı ile vasküler risk faktörleri arasındaki ilişkiyi göstermek amacıyla hastalar KİMK'lerine göre grup 1: 0,37-0,56 mm,

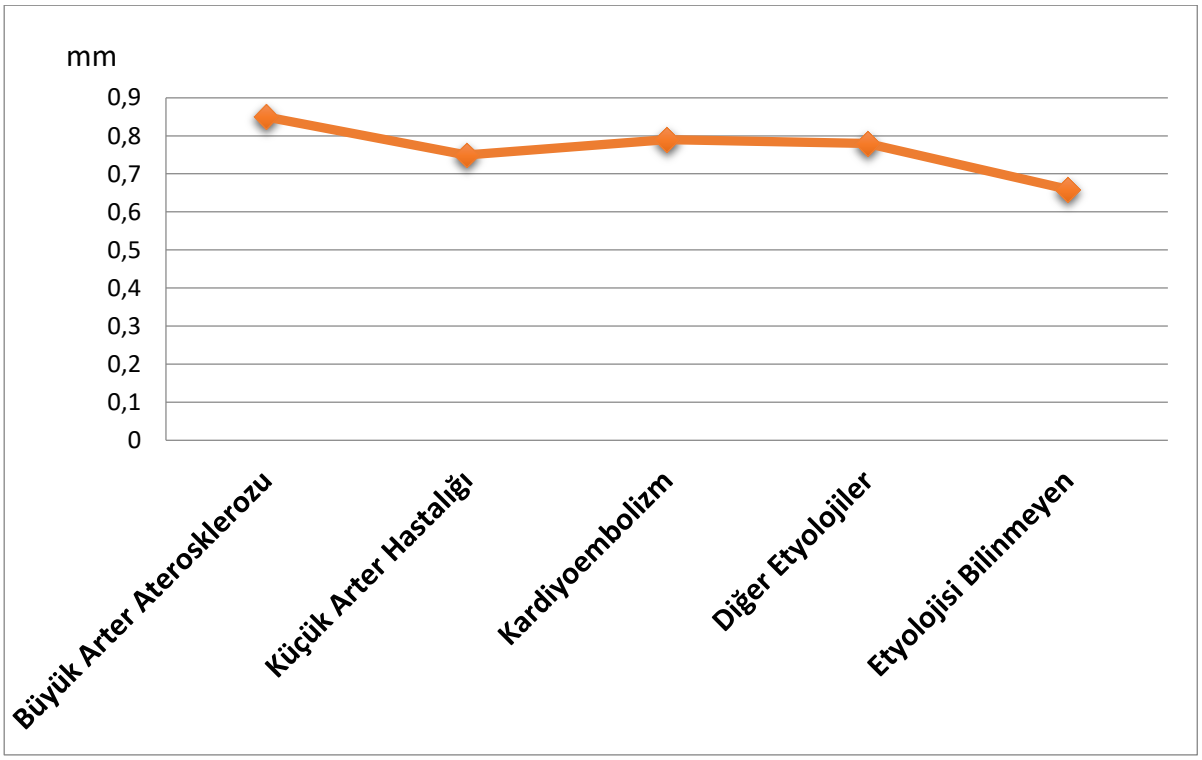
grup 2: 0,57-0,77 mm, grup 3: 0,78-0,97 mm, grup 4: 0,98-1,35 mm olmak üzere dört gruba ayrıldı. Vasküler risk faktörlerinin KİMK artışına olan etkisine bakıldığında DM sıklığının KİMK artışı ile anlamlı olarak artış gösterdiği saptandı (p:0,03). Fakat diğer vasküler risk faktörlerinin varlığı ile anlamlı ilişki olmadığı görüldü (Tablo-2). KİMK'nin TOAST sınıflamasına göre dağılımında ise büyük arter aterosklerozuna bağlı inmede; etyolojisi bilinmeyen gruba (p:0,02) ve küçük arter hastalığına göre (p:0,011) KİMK'nin anlamlı olarak daha yüksek olduğu saptanmıştır (Şekil-1).

Tablo-1. İnme risk faktörlerinin sıklığı.

İnme risk faktörleri	**n**	**%**
Hipertansiyon	225	82,4
Diabetes mellitus	104	38,1
Hiperlipidemi	114	41,8
Sigara	109	39,9
Obezite	113	41,4
Kardiyak aritmi	71	26,0
Geçici iskemik atak öyküsü	26	9,5
İnme rekürrensi	45	16,5

Tablo-2. KİMK'nin vasküler risk faktörleri ile ilişkisi.

	Karotis İntima-Media Kalınlıkları (mm)				
	1	**2**	**3**	**4**	**p**
N	70	69	67	67	
KİMK aralığı (mm)	0,37-0,56	0,57-0,77	0,78-0,97	0,98-1,35	
Yaş (ort.±SD)	63±14,2	63±11,2	66,2±11,7	64,6±12,6	
Erkek cins (%)	70	60,8	53,7	61,1	0,79
Hipertansiyon (%)	78,5	88,4	82,1	80,6	0,46
Diyabet (%)	38,5	42	47,8	53,9	**0,03**
Hiperlipidemi (%)	32,8	44,9	47,8	41,8	0,31
Sigara (%)	37,1	47,8	37,3	37,3	0,95
Obezite (%)	42,8	40,6	38,8	43,3	0,94
Aritmi (%)	31,4	20,3	26,9	25,4	0,45



Şekil-1. TOAST sınıflamasına göre KİMK ort. Dağılımı.

TARTIŞMA

İskemik inmeden sekonder korunmada altta yatan nedenin ve risk faktörlerinin tespiti hayati önem taşımaktadır. Günümüzde tüm ileri tetkik imkanlarına rağmen iskemik inmelerin yaklaşık üçte biri kriptojeniktir. Yapılan çalışmalar KİMK artışının iskemik inmelerin %15-20'sinin nedeni olan karotid arter aterosklerozunun preklinik bulgusu olmasının yanı sıra kriptojenik inme etiyolojisini ortaya koymada katkı sağlayabileceğini göstermiştir (9, 10). Bu retrospektif çalışmada iskemik inmeli hastalarda KİMK artışının vasküler risk faktörleri ilişkisine bakılmış, yalnızca DM varlığının KİMK artışı ile ilişkisi anlamlı bulunmuştur. KİMK artışının kümülatif endotel hücre hasarının bir sonucu olduğu ve DM'nin de erken dönemden itibaren sistemik inflamasyona neden olan vasküler endotelyal disfonksiyon yaptığı bilinmektedir (4, 11). Endotel disfonksiyonu sonucu lipitler köpük hücreleri içinde lokalize olmak üzere toplanır, kalın yağlı çizgilerin oluşumu uyarılarak İMK'yi arttırır, böylece zamanla lokal kan akışını kısıtlayarak iskemik inme riski oluşturur (12). Çin'de 314 non-kardiyojenik iskemik inmeli hastada yapılan bir çalışmada KİMK artışının tip 2 DM'si olanlarda 3 aylık modifiye Rankin Skorunun (mRS) ≥3 olması olarak tanımlanan kötü prognoz ile ilişkili olduğu gösterilmiştir (13). İsveç'ten 1400'ün üzerinde iskemik inmeli hastanın KVDUSG'si ile yapılan bir çalışmada yaş, inme şiddeti ve TOAST'a göre inme tipinde iki grup arasında anlamlı farklılık olmadığı halde diyabetiklerin diyabetik olmayanlara göre artmış KİMK'ye sahip olduğu bildirilmiştir (14). KİMK artışının inme tipleri ile ilişkisi uzun yıllardır araştırma konusu olmuş olup yapılan çalışmalarda çelişkili sonuçlar mevcuttur. Çalışmamızda KİMK'nin TOAST sınıflamasına göre inme tipleri ile ilişkisine bakıldığında büyük arter aterosklerozuna bağlı inmede; etyolojisi bilinmeyen gruba (p:0,02) ve küçük arter hastalığına göre (p:0,011) KİMK'nin anlamlı olarak daha yüksek olduğu saptanmıştır. İskemik inmeli hastaların normal kontrollerle karşılaştırıldığı bir çalışmada tüm inme tiplerinde normallere göre KİMK artışı gösterilmiş olup büyük arter aterosklerozuna bağlı inmelerde bu ilişkinin daha güçlü olduğu vurgulanmıştır (15). Başka bir çalışmada ise büyük arter aterosklerozu ve küçük arter hastalığına bağlı inmelerle KİMK artışının anlamlı olarak ilişkili olduğu, kardiyoembolik inmelerle ilişkili olmadığı gösterilmiştir (16). Çin'den binin üzerinde hasta ile yapılan bir çalışmada bizim çalışmamızla benzer olarak artmış KİMK'nin büyük arter

aterosklerozu ile ilişkili olup küçük damar hastalığı ile ilişki olmadığı bildirilmiştir (17). Hemorajik inmelerin de dahil edildiği başka bir çalışmada KİMK artışının laküner olmayan ve kardiyoembolik inmelerde anlamlı olarak daha fazla olduğu, hemorajik ve laküner inmelerde anlamlı bir ilişki olmadığı gösterilmiştir. Bu sonuç, hemorajik ve laküner inmelerin patofizyolojisinin farklılığı ile açıklanmıştır (18). Bunun aksine, Japonya'da toplum tabanlı yapılan bir çalışmada hemorajik, laküner ve laküner olmayan inme tipleri ile KİMK artışının ilişkisi değerlendirilmiş olup laküner iskemik inmelerde KİMK'nin anlamlı olarak arttığı, hemorajik ve laküner olmayan inmelerde anlamlı bir ilişki olmadığı gösterilmiştir (19). KİMK'nin inme riski ve inme tipleri ile ilişkisini araştıran çalışmaların gözden geçirildiği bir meta-analizde çalışmamızdakine benzer şekilde KİMK artışının küçük damar hastalığına göre büyük damar aterosklerozu varlığı ile anlamlı olarak ilişkili olduğu desteklenmiştir (20). Çalışmamızın bazı kısıtlılıkları bulunmaktadır. Kliniğimizde çalışmanın yapıldığı dönemde yatan her iskemik inme hastasına bu konuda deneyimli ve yeterliliği olan bir nörolog tarafından rutin olarak uygulanan KVDUSG sonuçları retrospektif olarak değerlendirilmiş olup radyolog tarafından teyit edilmemiştir. Her ne kadar dahil etme kriterlerine göre 40-90 yaş arası hastalar değerlendirilmiş olsa da yaş dağılımında 60-79 yaş arasındaki yığılma nedeniyle yaşın KİMK'ye olan etkisi gösterilememiş olabileceği düşünülmüştür.

SONUÇ

Bu çalışmada iskemik inmeli hastalarda DM varlığı KİMK artışı ile ilişkili bulunmuştur. KVDUSG gibi hızlı, yatak başı uygulanabilen, zararsız ve görece ucuz bir tetkik ile elde edilebilen KİMK artışının diyabeti olan iskemik inme hastalarında gösterilmesi inmeden korunmada diyabet kontrolünün önemine vurgu yapmaktadır. KİMK artışının TOAST inme tiplerinden büyük damar aterosklerozunda anlamlı olarak fazla olması yapılan çalışmalardaki çelişkili sonuçlara rağmen KİMK artışının aterosklerozun subklinik kanıtı olduğu düşünülürse akla yatkın görünmektedir. Sonuç olarak, KİMK ölçümünün inmeden korunmada elzem olan etiyolojik araştırma ve inme tipinin belirlenmesine önemli katkısı olabileceği düşünülmektedir.

Çıkar çatışması: Yazarların tümünün herhangi bir çıkar çatışması bulunmamaktadır.

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Research Article / *Araştırma Makalesi*

Ege Journal of Medicine / *Ege Tıp Dergisi* 2024; 63 (3): 356-361

Is circular-stapled gastrojejunostomy anastomosis appropriate for pancreaticoduodenectomy?

Pankreatikoduodenektomi prosedüründe sirküler stapler ile gastrojejunostomi yapılması uygun mudur?

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ABSTRACT

Aim: Pancreaticoduodenectomy is a highly complex procedure that requires surgical experience. Among these is the use of a stapler in the construction of the gastrojejunostomy anastomosis during the procedure. Our study compares the patient outcomes of gastrojejunostomy anastomosis procedures performed manually and with a circular stapler.

Materials and Methods: Our study retrospectively evaluated the data of 44 patients who had undergone pancreaticoduodenectomy performed by the same surgical team between May 2015 and December 2019. The manual gastrojejunostomy anastomosis (n = 32) and stapled (circular stapler 25 millimeter) anastomosis (n=12) patient groups were compared for anastomotic stricture.

Results: Of the 44 patients undergoing pancreaticoduodenectomy, 68.2% were male, the mean age was 62.9±12.1 years and the mean follow-up was 28.2±21.2 months. The rate of gastrojejunostomy stricture was significantly higher in the circular stapler group ($p = 0.017; p < 0.05$).

Conclusion: The increased risk of postoperative pancreatic fistula and anastomotic stricture prevents us from recommending the use of a circular-stapler in the creation of the gastrojejunostomy anastomosis in pancreaticoduodenectomy procedures, as it increases the risk of postoperative pancreatic fistula and anastomotic stricture, and provides no operative time advantage.

Keywords: Anastomotic stricture, gastrojejunostomy, pancreaticoduodenectomy.

ÖZ

Amaç: *Pankreatikoduodenektomi, cerrahi deneyim gerektiren oldukça karmaşık bir işlemdir. İşlem sırasında gastrojejunostomi anastomozunun yapımında stapler kullanılması bunların arasında yer almaktadır. Çalışmamız elle ve sirküler stapler ile yapılan gastrojejunostomi anastomoz işlemlerinin hasta sonuçlarını karşılaştırmaktadır.*

Gereç ve Yöntem: *Çalışmamızda Mayıs 2015 ile Aralık 2019 tarihleri arasında aynı cerrahi ekip tarafından pankreatikoduodenektomi yapılan 44 hastanın verileri geriye dönük olarak değerlendirildi. Gastrojejunostomi anastomozu el ile yapılan hasta grubu ile (n=32) ve anastomozu stapler (25 milimetre sirküler stapler) ile yapılan hasta grubu(n=12) anastomoz darlığı açısından araştırıldı.*

Bulgular: *Pankreatikoduodenektomi yapılan 44 hastanın %68,2'si erkekti, yaş ortalaması 62,9±12,1 yıl ve ortalama takip süresi 28,2±21,2 aydı. Gastrojejunostomi darlığı oranı sirküler stapler grubunda anlamlı derecede yüksekti ($p = 0,017; p < 0,05$).*

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Application date: 04.10.2023 Accepted: 12.02.2024

Sonuç: *Pankreatikoduodenektomi prosedüründe gastrojejunostomi anastomozunun sirküler stapler ile yapılmasını, postoperatif pankreas fistülü ve anastomoz darlığı riskini yükseltmesi, ayrıca operasyon süresi açısından da avantaj sağlamaması nedeniyle önermemekteyiz.*

Anahtar Sözcükler: *Anastomoz darlığı, gastrojejunostomi, pankreatikoduodenektomi.*

INTRODUCTION

Pancreaticoduodenectomy (PD) is a procedure that is usually performed on periampullary tumors and more rarely for benign conditions such as chronic pancreatitis and trauma. PD requires time-consuming reconstruction after a major resection that involves the reconstruction of the pancreas, the bile duct, and the gastrointestinal tract, significantly prolonging the operative time (1-2). PD is still associated with high mortality and morbidity rates, and a prolonged operative time is among the reported risk factors (3-4). Various techniques have been suggested to shorten the operative time, among which is the use of a stapler for the creation of the gastrojejunostomy (GJ) anastomosis during the procedure (5). The present study compares the patient outcomes of group who underwent circular-stapled GJ anastomosis (Ethicon Curved İntraluminal Stapler, circular 25 millimeter) (CSA) and the group who underwent manual GJ anastomosis (MA).

MATERIALS and METHODS

Upon the receipt of ethics committee approval (No: HNEAH-KAEK 2021/KK/268), patients who underwent PD in our hospital between June 2015 and December 2019 were identified, and their medical records were evaluated retrospectively. Of the 107 patients identified, 44 patients whose surgeries were performed by the same experienced team [the surgeon-in-chief (MAU) with more than 20 years of surgical experience and his/her team] and with sufficiently complete medical records and follow-ups were included in the study. Accordingly, 32 patients who underwent manual GJ anastomosis (MA) (Group 1) and 12 patients who underwent circular-stapled (circular 25 millimeter) GJ anastomosis (Group 2) were included in the study. Demographic data, complaints, preoperative and perioperative findings, operative times, pancreaticojejunostomy (PJ) techniques, presence of postoperative pancreatic fistula (POPF), pathological staging, complications, recurrence, and the need for adjuvant chemotherapy and radiotherapy were recorded.

Surgical Procedure

The patients were administered prophylactic antibiotic therapy with Ceftriaxone + Metronidazole 30 minutes as routine before the operation. A bilateral subcostal incision was made through which the perioperative pancreatic tissue and pancreatic duct width were assessed, and the most appropriate type of anastomosis was determined. At the reconstruction stage, end-to-end or end-to-side dunking, duct-to-mucosa or simple invagination techniques were used for PJ. Hepaticojejunostomy (HJ) anastomosis was performed by placing single-layer end-to-side and interrupted 4/0 or 5/0 polydioxanone (PDS) sutures. The GJ anastomosis was performed 40–50 cm distal to the HJ anastomosis using the antecolic technique. MA was performed manually in two layers, with the first layer using 3/0 Vicryl and the second layer using 4/0 PDS continuous sutures. CSA was performed using a circular stapler No. 25, which was inserted through the tip of the jejunum that had not yet been anastomosed, the bowel perforator apparatus was brought out 50 cm behind, and the anvil was placed on the posterior surface of the stomach, 3 cm away from the gastric linear incision line. A second layer was fixed on the stapler line using 4/0 PDS continuous sutures. The patients were subcutaneously administered routine octreotide 0.1 mg ampoule (3x2) for seven days postoperatively. The 2016 revision of the International Study Group of Pancreatic Surgery (ISGPS) was used to define and grade POPF (6). GJ anastomotic stricture was diagnosed using the failure of the endoscope to pass through the GJ anastomosis as a criterion during the esophagogastroscopy of symptomatic patients.

Statistical method: Descriptive statistics included mean, standard deviation, median, minimum and maximum variables, frequency and ratio. A Kolmogorov-Smirnov test was used for the assessment of the normality of the variables. An Independent Samples t-test and a Mann-Whitney U test were used to analyze quantitative data. A Chi-square test was used for the analysis of qualitative variables and a Fisher's exact test if the conditions were not met for a Chi-square test.

The analyses were performed using IBM SPSS Statistics (Version 28.0. Armonk, NY: IBM Corp.).

RESULTS

Of the patients, 68.2% were male and the mean age was 62.9±12.1 years. The demographic and clinicopathological characteristics of the groups are presented in Table-1. There were no significant differences between-group differences in terms of age, gender, ASA (American Society of Anesthesiologists) score, mass localization and adjuvant therapy, while the incidence of advanced disease was statistically significantly higher in Group 1. The operative and postoperative characteristics of the groups are presented in Table-2, in which it can be seen that the groups did not differ significantly in terms of PJ technique, operative time, amount of perioperative bleeding and the incidence of postoperative biliary leak, while the incidences of POPF and GJ stricture were statistically significantly higher in Group 2. No significant difference was found in other postoperative complications. The mean follow-up of the patients was 28.2±21.2 months.

Table 1. Demographic and clinicopathological characteristics of groups.

	Group 1 (MA) n: 32 n (%) or mean (±SD)	**Group 2 (CSA) n: 12 n (%) or mean (±SD)**	**p-value**
Age	64,6 ±11,7	58,3 ±12,3	0,127^{t}
Gender			0,113$^{X^2}$
Male	24 (75.0%)	6 (50.0%)	
Female	8 (25.0%)	6 (50.0%)	
ASA Score			0,516$^{X^2}$
I	0 (0.0%)	1 (8.3%)	
II	10 (31.3%)	4 (33.3%)	
III	19 (59.4%)	7 (58.3%)	
IV	3 (9.4%)	0 (0.0%)	
Mass settlement area			
Pancreas	20 (62,5%)	7 (58,3%)	0,800 $^{X^2}$
Ampulla of Vater	8 (25,0%)	1 (8,3%)	0,222$^{X^2}$
Distal common bile duct	3 (9.4%)	2 (16,7%)	0,603$^{X^2}$
Duodenum	0 (0.0%)	1 (8,3%)	0,273$^{X^2}$
Chronic pancreatitis	1 (3.1%)	1 (8,3%)	0,476$^{X^2}$
Pathological Stage			**0,021**$^{X^{2*}}$
0	2 (6,3%)	3 (25,0%)	
I	5 (15,6%)	4 (33,3%)	
II	15 (46,9%)	3 (25,0%)	
III	10 (31,3%)	2 (16,7%)	
Adjuvant therapy			0,095$^{X^2}$
None	15 (46,9%)	9 (75,0%)	
Chemotherapy	11 (34,4%)	2 (16,7%)	
Chemoradiotherapy	6 (18,8%)	1 (8,3%)	

MA:Manual gastrojejunostomy anastomosis;

CSA:circular-stapled gastrojejunostomy anastomosis

ASA: American Society of Anesthesiologists SD:Standard deviation ^{t}: t test; $^{X^2}$: Chi-square test (Fischer test) * :p<0.5

Table-2. Operative and postoperative characteristics of groups.

	Group 1 (MA) n: 32 n (%) or mean (±SD)	Group 2 (CSA) n: 12 n (%) or mean (±SD)	p-value
PJ Technique			
Duct to Mucosa	4 (12,5%)	0 (0.0%)	0,562 $^{X^2}$
End to End Dunking	25(78,1%)	9 (75.0%)	0,826 $^{X^2}$
End to Side dunking	1 (3,1%)	1 (8.3%)	0,476 $^{X^2}$
Simple Invagination	2 (6,3%)	2 (16,7%)	0,297 $^{X^2}$
Operation Duration	370.3 ±96,3	368.3 ±88,3	0,951 ^{t}
Peroperative Bleeding Volume (milliliter)	566,3 ±270,5	487,5 ±357,5	0,436 ^{t}
POPF			**0,015** $^{X^{2*}}$
Biochemical Leak	1 (3,1%)	2 (16,7%)	
Clinically Significant POPF	0 (0.0%)	2 (16,7%)	
POPF B	0 (0.0%)	2 (16,7%)	
POPF C	0 (0.0%)	0 (0.0%)	
Postoperative Biliary Leakage	0 (0.0%)	1 (8.3%)	0,273 $^{X^2}$
GJ anastomotic stenosis	0 (0.0%)	3 (25,0%)	**0,017** $^{X^{2*}}$
Other Postoperative Complications	5 (15,6%)	3 (25,0%)	
Wound Site Infection	2 (6,3%)	1 (8.3%)	1.000 $^{X^2}$
Delayed Gastric Emptying	1 (3,1%)	1 (8.3%)	0.476 ^{X}
Marginal ulcer	2 (6,3%)	0 (0.0%)	1.000 $^{X^2}$
Mortality	1 (3,1%)	0 (0.0%)	1.000 $^{X^2}$

MA:Manual gastrojejunostomy anastomosis;

CSA: circular-stapled gastrojejunostomy anastomosis

PJ: Pancreaticojejunostomy; POPF: postoperative pancreatic fistula; GJ: Gastrojejunostomy; SD: Standard deviation; ^{t}: t test; $^{X^2}$: Chi-square test (Fischer test); * :p<0.5

DISCUSSION

The reconstruction of the gastrointestinal tract using a stapler is a widely accepted technique during gastric and colorectal surgery, although few studies to date have evaluated the use of a stapler in PD (7-8). The first studies of this subject identified in literature belong to a Japanese group, who reported a lower incidence of delayed gastric emptying (DGE) in the stapled gastro/duodenojejunostomy anastomosis (linear or circular) group than the group who underwent manual anastomosis during a Roux-en-Y reconstruction (9-10). A subsequent prospective randomized controlled study by Sakamoto et al. compared the incidence of DGE in circular-stapled anastomosis and manual anastomosis patient groups with pyloric preserving PD but found no significant difference (11). A meta-analysis by Hajibandeh, S et al. reported the incidence of DGE to be lower in the stapled anastomosis group than in the manual anastomosis group, but the incidence of anastomotic bleeding to be higher. The same meta-analysis revealed no statistically significant difference in the rates of POPF, anastomotic leak or mortality (12). Our study, in turn, found no statistical difference in the incidence of DGE, while the rates of POPF and anastomotic stricture were significantly higher in the SA group. These differences in the data in literature may be attributed to other technical differences in the reconstruction. The study by Sato N. et al., which was the most similar to ours in terms of the reconstruction technique, compared 19 SA cases and 19 manual anastomosis cases, and revealed a shorter reconstruction time and less perioperative bleeding in the SA group, but no difference in the incidences of DGE, POPF and other complications (5). The same study reported no difference in the total operative time between the groups, and contrary to expectations, our study also established no significant difference in the total operative time between the groups.

The most remarkable finding of our study was the high incidence of anastomotic stricture in the SA group. This aspect of stapled anastomosis has not been evaluated or reported on before in PD-related literature. Studies evaluating stapled anastomoses usually focus on surgery for morbid obesity, and according to such studies, the incidence of stricture increases after manual anastomosis, linear-stapled and circular-stapled anastomoses, respectively. The reported incidence of stricture ranged from 0% and 31% for circular-stapled anastomosis (13-16).

Anastomotic tension, anastomotic leak, damage from exposure to acid and submucosal hematoma are blamed for the development of anastomotic stricture (17-18). No anastomotic stricture occurred in the manual anastomosis group in the present study, while the rate was 25% in the SA group, which can be considered a significant disadvantage of SA. The fact that the stage was significantly higher in patients in the MA group who underwent manual anastomosis in the postoperative pathological staging was considered coincidental.

A success rate of up to 75% has been reported for endoscopic dilatation for the treatment of anastomotic stricture, and very few patients may require surgery due to failure or complications of endoscopic dilatation treatment (19). In the present study, one of the three patients with a GJ stricture was treated with repeated endoscopic dilatation and did not require surgery, while the other two patients were surgically treated with anastomosis revision due to endoscopic treatment failure. The primary limitations of the study include its retrospective design, the low number of patients and the lack of endoscopically recorded anastomosis diameters.

CONCLUSION

We do not recommend circular-stapled gastrojejunostomy anastomosis over manual anastomosis for PD due to the high risk of postoperative pancreatic fistula and anastomotic stricture, and the lack of any operative time advantage.

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

Informed Consent: Retrospective study.

Financial Disclosure: The authors declared that this study received no financial support.

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Research Article / *Araştırma Makalesi*

Ege Journal of Medicine / *Ege Tıp Dergisi* 2024; 63 (3): 362-368

The relationship of gros anomalies of the umbilical cord with placental pathologies

Göbek kordonu gros anomalilerinin plasenta patolojileri ile ilişkisi

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ABSTRACT

Aim: Within the scope of this research, we aimed to elucidate and compare placental histological features and perinatal outcomes in all deliveries with or without umbilical cord anomaly.

Materials and Methods: Regarding patient groups, 270 cases with cord abnormalities were included in the study group and 835 cases in the control group. Umbilical cord abnormalities: The presence of a true or false knot in the umbilical cord was determined based on the umbilical cord wrapping around the fetal neck and the presence of stenosis. The cases without any umbilical cord abnormalities mentioned in the study group were determined as the control group. All patients' demographic data, prenatal information, intrapartum information, postpartum information, postpartum period, and newborn follow-up were recorded. After delivery, umbilical cord abnormalities and placenta macroscopic and microscopy results were evaluated.

Results: No placental pathology was detected in the control group, but there was a statistical significance between the study and control groups, including fetal vascular thrombosis and ectasia pathology and fetal vasculopathy or avascular villi pathology. There was no difference between the study and control groups regarding preeclampsia, ablatio placenta, and intrauterine fetal demise. Intrauterine growth restriction was detected at a higher rate in the study group, and the difference was significant. No difference was observed between the two groups regarding Apgar scores *1st* and *5th*min of newborns and the requirement for hospitalization in the neonatal intensive care unit. **Conclusion:** Gros cord anomalies, fetal vascular ectasia and thrombosis, and fetal thrombotic vasculopathy lead to pathologies associated with placental insufficiency, suggesting that it is an independent risk factor for intrauterine growth restriction.

Keywords: Cord anomaly, intrauterine maternal loss, intrauterine growth restriction, apgar score, newborn.

ÖZ

Amaç: *Bu araştırma kapsamında göbek kordonu anomalisi olan ve olmayan tüm doğumlarda plasentanın histolojik özelliklerin ve perinatal sonuçların aydınlatılması ve karşılaştırılması amaçlandı.*

Gereçler ve Yöntem: *Hasta gruplarına bakıldığında çalışma grubunda 270, kontrol grubunda 835 olgu yer aldı. Kordon anormalliği olanlar çalışma grubuna dahil edildi. Göbek kordonu anormallikleri; Kordonda gerçek ya da yalancı düğüm olması, göbek kordonunun fetal boyun çevresine dolanması ve kordonda darlık varlığı olarak belirlendi. Çalışma grubunda adı geçen herhangi bir göbek kordonu anormalliği olmayan olgular kontrol grubu olarak belirlendi. Tüm hastaların demografik verileri, prenatal bilgileri, intrapartum bilgileri, postpartum bilgileri, postpartum dönemleri ve yenidoğan izlemleri kaydedildi. Doğumdan sonra göbek kordonu anormallikleri ve plasenta makroskobik ve mikroskopi sonuçları prospektif olarak izlendi.*

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Application date: 09.07.2023 Accepted: 13.02.2024

Bulgular: *Kontrol grubunda plasenta patolojisi saptanmadı, ancak çalışma grubunda fetal vasküler tromboz ve ektazi patolojisi ve fetal vaskülopati veya avasküler villus patolojisi gibi anormalikler daha fazla izlendi. Çalışma ve kontrol grupları arasında preeklampsi, plasenta dekolmanı ve intrauterin fetal kayıp açısından fark yoktu. Çalışma grubunda intrauterin gelişme geriliği daha yüksek saptandı ve aradaki fark anlamlıydı. Yenidoğanların 1. ve 5. dakika Apgar skorları ve yenidoğan yoğun bakım ünitesinde yatış gerekliliği açısından iki grup arasında fark izlenmedi.*

Sonuç: *Gros kordon anomalileri, fetal vasküler ektazi ve tromboz ve fetal trombotik vaskülopati plasenta yetmezliği ile ilişkili patolojilere yol açarak intrauterin gelişme geriliği için bağımsız bir risk faktörü olduğunu düşündürmektedir.*

Anahtar Sözcükler: *Kordon anomalisi, intrauterin fetal kayıp, intrauterin gelişme geriliği, apgar skoru, yenidoğan.*

INTRODUCTION

The umbilical cord is an important structure that plays a critical role in the life of the developing fetus, both structurally and functionally, that provides the relationship between the fetus and the placenta. Generally, ultrasonographic examinations of the umbilical cord were based on the number of vessels and Doppler blood flow. There is limited information about the effects of prenatal umbilical cord morphology on the fetus in the prenatal period and the newborn in the postnatal period. The results of a limited number of studies have shown that umbilical cord morphology and its components affect the pregnancy process, mode of delivery, and outcome (1, 2).

Although the umbilical cord is the only organ that disappears afterlife begins, it is the most important component of the fetoplacental unit. It plays a decisive role in the onset of extrauterine life. Fetal growth and development are characterized by differentiation, maturation, and growth of fetal tissues and organs (3). The main factors affecting fetal growth and development are genetic structure, uteroplacental function, and maternal environment. Under conditions where all these factors are favorable, a healthy fetus completes its intrauterine somatic growth. If the conditions are not suitable, fetal growth and development may be adversely affected and limited. Abnormal maternal, fetal, and placental factors may adversely affect fetal growth and development individually or together (4, 5).

Clinical experience and experimental evidence have shown that the morphology and components of the umbilical cord affect the pregnancy process, mode of delivery, and outcome (6). Many researchers reported that altered umbilical cord morphology is associated with hypertensive disorders, fetal distress, gestational diabetes, fetal growth restriction, and intrapartum complications. They altered umbilical vein blood flow in the second and third trimesters (2). The genetic and physiological factors predisposing to complications related to the umbilical cord are not yet clearly understood. However, the localization of some maternal factors, abnormal cord insertion and morphology, and cord length can be considered among the possible causes. Vital dysfunction of the umbilical circulation is suspected in at least 20% of autopsy examinations of stillbirths (7). Any force that compresses the cord may reduce blood flow to the umbilical vessels and cause fetal hypoxia and circulatory dysfunction. Mechanical cord compression or 'cord accident' can be caused by cord entanglement or prolapse, as well as abnormal cord structures such as true knots, hyper coiling, abnormally long cord, abnormal cord insertion, and cord stenosis (8).

Parast et al. (9) demonstrated that non-acute umbilical cord occlusion is associated with congestion and stasis resulting from umbilical vein compression and also included thrombosis, ectasia of large vessels in the placenta, and avascularity in terminal chorionic villi. The condition formerly called fetal thrombotic vasculopathy (FTV) has been associated with poor perinatal outcomes, including stillbirth and neurological damage (10). Tantbirojn et al. (11) reported that thrombosis and fetal thrombotic vasculopathy were specific for stillbirths originating from the umbilical cord.

Within the scope of this research, we aimed to elucidate and compare placental histological features and perinatal outcomes in all deliveries with or without umbilical cord anomaly.

MATERIALS and METHODS

A total of 1105 females who had a delivery in our institution have been enrolled in this prospective study. Regarding patient groups, 270 cases with cord abnormalities were included in the study

group and 835 cases in the control group. Umbilical cord abnormalities: The presence of a true or false knot in the umbilical cord was determined based on the umbilical cord wrapping around the fetal neck and the presence of stenosis. The cases without any umbilical cord abnormalities mentioned in the study group were determined as the control group. All patients' demographic data, prenatal information, intrapartum information, postpartum information, postpartum period, and newborn follow-up were recorded. After delivery, umbilical cord abnormalities and placenta macroscopic and microscopy results were evaluated. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Ethics committee approval has been granted from our institution, and informed consent has been obtained from all participants.

Age, height, weight, arterial blood pressure, gestational week values, and previous pregnancy status of 1105 cases participating in the study were evaluated regarding complications with preeclampsia, ablation placenta, placenta previa, intrauterine growth restriction (IUGR), intrauterine fetal loss (IUFL). As intrapartum period records the week of delivery, the amount of amniotic fluid in the last stage of pregnancy, the birth weight of the baby, the weight of the placenta, the status of the amniotic fluid or placenta stained with meconium during delivery, the last non-stress test (NST) reactivity before delivery and the mode of delivery data were recorded. but there was a statistical significance between the study and control groups

During birth, anomalies detected such as length or shortness of the umbilical cord, presence of stenosis, presence of real or false knot, coil index, and wrapping of the umbilical cord around the fetal neck were recorded.

As postpartum fetal information, 1st and 5th-minute APGAR scores have been obtained. Babies with low Apgar results were transported to the neonatal intensive care unit (NICU).

The pathological examination of the two samples taken from the placental parenchyma has been transferred to the pathology department for investigation. The samples were taken from a region close to the umbilical cord insertion and placental membranes.

Statistical Analysis

SPSS (Statistical Package for Social Sciences) 13 program was used for the data analysis. The mean value was calculated for those with normal distribution among the numerical variables, and a t-test was performed for statistical analysis. The chi-square test was utilized to compare the meaning of qualitative data between groups. The results were evaluated at the 95% confidence interval and the significance level of $p<0.05$.

RESULTS

A total of 1105 pregnant women (835 in the control group and 270 in the study group) were included in this prospective research. In the study group, fetal neck cord entanglement was observed in 120 cases, coil index anomaly in 112 cases, false knot anomaly in 60 cases, long umbilical cord abnormality in 18 cases, true knot anomaly in 15 cases, and no umbilical cord stricture abnormality was observed in any individual.

The median birth week of the cases in the study group was 37.4, and the control group was 37.9 weeks. Birth median weight in the study group was 2938 gr. And 3035 g in the control group. The median placenta weight was 564 g in the study group. 564 gr in the control group. A statistically significant difference was observed between the study and control groups regarding the birth week (p:0.017). In contrast, no statistically significant difference was found in terms of newborn birth weight and placental weight.

During the pathological examination, the normal placenta was detected in 804 (72.8%) cases, chorangiosis in 168 (15.2%) cases, fetal vascular thrombosis and ectasia in 126 (11.4%) cases, fetal vasculopathy, and avascular villi in 7 (0.6%) cases. There were 193 (71.5%) cases in the study group with normal pathology results and 611 (73.2%) cases in the control group. The normal placenta detection rate was higher in the control group than in the study group, and the difference was statistically significant. There were 33 cases in the study group regarding fetal vascular thrombosis and ectasia pathology and 93 cases in the control group, and the difference was statistically significant (Figure-1 & Figure-2).

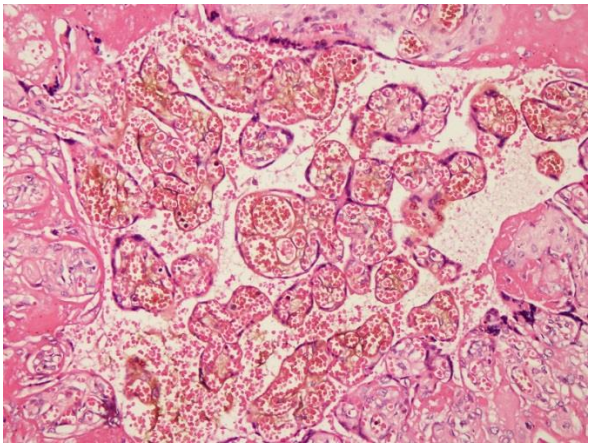

Figure-1. *Chorangiosis.*
Terminal chorionic villi containing ten or more capillary vessels are seen in at least ten terminal villi in the hematoxylin-eosin-stained preparation (x40 magnification).

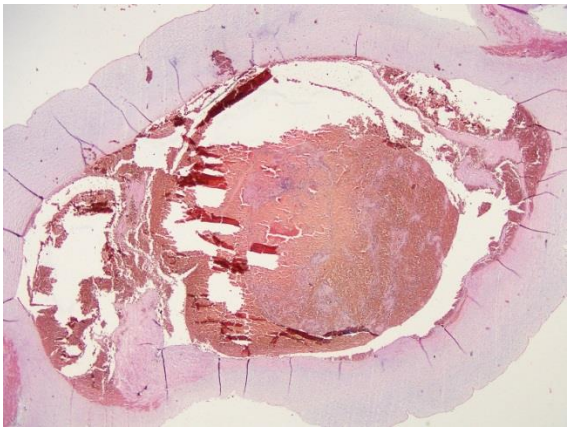

Figure-2. *Vascular thrombosis and ectasia.*
In the hematoxylin-eosin-stained preparation (in x10 magnification), a vessel with dilatation at least four times the diameter of the surrounding umbilical artery and/or vein, and the endothelial integrity of the tunica intima layer is partially disrupted by the organized thrombus.

It was determined that 70 (6.3%) of the 1105 cases included in the study were complicated by IUGR. Of these, 39 cases were diagnosed as early IUGR, and 31 were diagnosed as late IUGR. It was revealed that there was a statistically significant difference between the study and control groups in terms of complicating pregnancy with IUGR(p<0.02). It was observed that the cases in the study group were more complicated with IUGR (Table-1).

When the Apgar scores and the requirement for NICU were interpreted at the 1st and 5th minutes, the 1st minute Apgar score was ≥7 in 874 (79.1%) of the cases, within normal limits. The Apgar score was between 4 – 7 in 203 (18.4%) cases and 4≥ in 28 (2.5%) cases. There was no statistical difference between the study and control groups regarding the Apgar score (p=0.320). The 5th minute Apgar score was ≥7 in 1062 (96.1%) cases, between 4 – 7in 38 cases, and 4≥ in 5 cases, with no difference between the groups (p=0.353) (Table-2).

Table-1. Comparison of clinical conditions complicating pregnancy.

	Study Group	Control Group	P-value
Preeclampsia	15 (5.5&)	38 (4.5+)	0.756
IUGR	25 (9.3%)	45 (5.4%)	0.020*
IUML	1 (0.4%)	2 (0.2%)	0.567
Ablatio plasenta	3 (1.1%)	9 (1.1%)	1.000

(*p<0.05)

Table-2. Comparison of newborn findings.

	Study Group	Control Group	P-value
1. Minute Apgar score	56(20.7%)	175(21.0%)	0.320
5. Minute Apgar score	14(5.2%)	29 (3.5%)	0.353
Newborn Hospitalization	40 (14.8%)	107 (12.8%)	0.701
Exitus	1 (0.4%)	3 (0.4%)	1.000

(*p<0.05)

We evaluated the intrapartum variables regarding amniotic fluid at the time of delivery, whether the amniotic fluid was stained with meconium at birth, NST reactivity at the time of delivery, and mode of delivery. The amniotic fluid was within normal limits according to the gestational week in 983 (89.0%) cases, oligohydramnios was found in 105 (9.5%) cases, and polyhydramnios was in 17 (1.5%) cases. There was no statistically significant difference between the study and control groups in terms of normal amniotic fluid at the time of delivery, polyhydramnios, or oligohydramnios (p=0.200).

The rate of cesarean sections was higher in the study group than controls, but the difference was insignificant.

DISCUSSION

In parallel with the increasing use of ultrasonography in perinatal processes, clinical practice and research focus more on the fetus.

Ultrasonographic applications related to the umbilical cord are mostly limited to determining the number of vessels and evaluation of umbilical artery and vein Doppler blood flow (12). However, the important role of the placenta and umbilical cord in regulating fetal development cannot be denied. Studies on the umbilical cord have shown that it and its components are effective in pregnancy and neonatal outcomes. Current studies draw attention to intrauterine loss, gestational diabetes, preeclampsia, intrauterine growth restriction, fetal distress during delivery, and the relationship between fetuses with meconium and umbilical cord (13).

Benirschke emphasized the association with umbilical cord pathologies such as long and short umbilical cord, velamentous insertion, umbilical cord knot, and poor newborn outcomes (14). Collins stated that there may be a relationship between umbilical cord abnormalities and unexpected neonatal deaths (15). Naeye mentioned the association of short umbilical cord abnormality with low Apgar score, low IQ, and neurological abnormalities (16). Peng et al. stated that umbilical cord stricture and hyper coiling abnormalities cause fetal death (17). In our study, a statistically significant difference was detected in the pathological findings in terms of fetal vascular thrombosis and ectasia pathology in the control group and the study group. For this reason, In our study investigating non-acute obstruction of the umbilical cord as a predisposing cause for poor pregnancy outcomes, findings related to vascular congestion and stasis were associated with neonatal morbidity and mortality.

Previous literature reported that decreased fetal movements and changes in fetal cardiac rate may be signs of intrauterine asphyxia in the advanced stages of pregnancy. Umbilical cord-related complications accompany fetal asphyxia with a rate of 5 – 18% and still births of 10 – 12% (18). Morrison et al. reported cord complications in 48% of asphyxia newborns at term (19).

The cord pathologies and neonatal pathologies have been comprehensively elaborated. Machin et al. emphasized the connection between abnormal umbilical coil index and intrauterine fetal death and intrauterine growth restriction (20), Naeye et al. stated that umbilical cord length could be affected by many factors such as maternal weight, pre-pregnancy weight, socioeconomic status, and infant weight, and that umbilical cord abnormality is associated with psychomotor restriction. It has been reported that it may be related (16). In another study, a relationship was found between the long umbilical cord and infant development, and an increased risk of fetuses with abnormal neurologic development was reported in subsequent pregnancies (21). Airas et al. conducted a study of 23.315 cases and found a significant relationship between true knots in the umbilical cord and stillbirth and low 1st-minuteApgar score (22). Rhoades et al. stated that cord entanglement is an independent risk factor for delivery. Their study revealed that although cord entanglement to the fetal neck poses a risk for perinatal problems, it does not affect the length of hospital stay (23).

Tantbirojn et al. (11) focused on fetal blood flow-limiting vascular changes other than cord entanglement. They evaluated histological features in their study, in which fetal vascular ectasia, fetal vascular thrombosis, fetal thrombotic vasculopathy (avascular villi, villous stromal choriorexis, and their combinations) were found in cases of a bad pregnancy, especially IUGR. The fetal thrombotic vasculopathy was associated with gross umbilical cord anomaly in stillbirth cases. A close relationship has been reported between fetal thrombotic vasculopathy and stillbirth (24). A massive fetal thrombotic vasculopathy is detected in approximately 50% of stillbirth placentas. On the other hand, Larson et al. (25) examined 13.895 pregnant women and found the rate of cord entanglement in the 20th week to be 5.8% and 29% in the 42nd week.

Chorangiosis is defined by numerous enlarged, highly vascular villi throughout the placenta. However, chorangiosis is a nonspecific change associated with maternal diabetes, hypertension, infections, anomalies, intrauterine fetal death, and growth restriction.

In our present study, while a significant relationship was found between cord pathologies and IUGR, no significant difference was found for preeclampsia, intrauterine fetal loss, and ablatio placenta. Cord pathologies were found to be an independent risk factor for IUGG rather than other poor pregnancy outcomes. When the placental pathology results were examined, we found the frequency of chorangiosis to be similar. However, placental pathology results evaluated as fetal vascular ectasia and thrombosis, fetal thrombotic vasculopathy, and avascular villi were

found to be statistically significant in the study group.

In our study, the relationship between umbilical cord entanglement in the fetal neck, umbilical cord coil index abnormality, and false node abnormalities with placental pathologies was more evident in the cases in the study group.

When the study and control groups were evaluated regarding newborn outcomes, no significant difference was found in terms of 1st and 5th-minuteApgar scores and the requirement for hospitalization in the neonatal intensive care unit. In addition to perinatal outcomes such as intrauterine death, prematurity, the tendency to neonatal complications, neuromotor developmental defects, and IUGR also paves the way for diseases such as diabetes, cardiovascular diseases, and depressive disorders that are reflected in adult life (26).

These results are associated with impaired fetal programming and delayed fetal life maturation due to IUGR. Therefore, although the 1st and 5th-minute Apgar scores of the newborn in cases with gross umbilical cord anomalies are similar to the cases in the control group, the significantly higher neonatal morbidity and mortality in the study group can be explained by chronic placental insufficiency.

Gross cord anomalies such as fetal neck cord entanglement, long or short cord, hyper coiling or hypo coiling false or true knot, and cord stricture are associated with stillbirth IUGR, intrapartum and postpartum complications. Stasis-related changes in fetal vessels and chorionic villi in placental microscopic evaluations were significantly increased in cases with gross cord anomaly, and the increase in poor perinatal outcomes, especially in IUGR, is remarkable. Gross cord anomalies are an independent risk factor for IUGR by causing pathologies associated with placental insufficiency, such as fetal vascular ectasia and thrombosis, and fetal thrombotic vasculopathy. In addition, no significant difference was observed between the 1st and 5th minute Apgar scores of the postpartum newborn, while high morbidity and mortality were detected in the follow-up of the newborn.

CONCLUSION

In order to evaluate the presence of thrombosis and fetal thrombotic vasculopathy, especially in gross anomalies predisposing to vascular occlusion, it seems necessary to send all placentas for pathological examination, including adequate sampling from large fetal vessels and placental parenchyma. For this, giving the placenta and cord the value they deserve after birth is necessary to carry out their macroscopic examination meticulously. Even if the placenta is macroscopically normal after birth, it should be routinely subjected to histopathological examination, especially when conditions that are thought to be associated with poor obstetric outcomes are detected during antenatal follow-up. In this way, placental histopathology can contribute to the planning and management of subsequent pregnancies by revealing the causes of poor pregnancy outcomes.

Conflict of interest: All authors participating in the study declare that there is no conflict of interest regarding the study.

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Research Article / *Araştırma Makalesi*

Ege Journal of Medicine / *Ege Tıp Dergisi* 2024; 63 (3): 369-377

Olea europaea L.'den elde edilen triterpenoid ve polifenol bileşiklerinin antimikrobiyal ve yaşlanma karşıtı etkilerinin değerlendirilmesi: Ekstraksiyon, Tanılama ve *in vitro* Testler

Assessment of antimicrobial and anti-aging effects of triterpenoid and polyphenol compounds from olea europaea L: extraction, identification and in vitro tests

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ABSTRACT

Aim: This study investigated the antimicrobial and anti-aging effects of bioactive compounds derived from Olea europaea L. leaves and flowers, widely used in traditional treatments in European and Mediterranean countries.

Materials and Methods: Following solid-liquid extraction, the control of purification processes was conducted using thin-layer chromatography. Identification of the obtained molecules was performed through high-performance liquid chromatography (HPLC) and nuclear magnetic resonance (NMR) analyses. For determining antimicrobial activity, Gram-positive and Gram-negative bacteria, *Bacillus cereus*, and *Escherichia coli* O15:H7, were respectively used for minimum inhibitory concentration (MIC) tests. In the assessment of Oleuropein's *in vitro* cytotoxicity on adult human dermal fibroblasts (HDFa), the MTT assay was employed using HDFa cell lines, while an ELISA Test kit was utilized to determine changes in collagen type-I levels.

Results: As a result of the study, oleanolic acid (L1), oleuropein (L2), and ursolic acid (L3) were isolated from olive leaves, whereas oleuropein aglycone (F1) molecule was isolated from olive flowers. According to the results of the MIC tests, compounds L1, L2, and L3 isolated from the leaves exhibited an inhibitory effect against *B.cereus* within the concentration range of 5-250 µg/mL, whereas Oleuropein aglycone (F1) did not demonstrate any activity. Furthermore, except for the Oleuropein (L2) molecule, no other compound was effective against *E.coli.* In the evaluation of Oleuropein's *in vitro* cytotoxicity, a dose-dependent effect on HDFa cell viability was observed, and collagen type-I levels were significantly higher than levels obtained with vitamin C.

Conclusion: Based on the results, it is believed that the active molecules derived from olive plant's leaves and flowers exhibit antimicrobial effects, potentially serving as natural preservatives in the cosmetics industry. Moreover, their contribution to cell regeneration suggests potential use in wound treatments.

Keywords: Oleuropein, antimicrobial effect, *olea europaea L.,* MIC, anti-aging.

ÖZ

Amaç: *Bu çalışmada geleneksel tedavilerde Avrupa ve Akdeniz ülkelerinde kullanılmakta olan Olea europaea L. yaprakları ve çiçeklerinden elde edilen biyoaktif bileşiklerin antimikrobiyal ve yaşlanma karşıtı etkileri araştırılmıştır.*

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Application date: 10.01.2024 Accepted: 13.02.2024

Gereç ve Yöntem: *Katı-sıvı ekstraksiyonu ve saflaştırma adımlarını takiben, saflaştırma işlemlerinin kontrolü ince tabaka kromatografisi (ITK) ile yapılmıştır. Elde edilen moleküllerin tanımlanması yüksek basınçlı sıvı kromatografisi (HPLC) ve nükleer manyetik rezonans (NMR) analizleri ile gerçekleştirilmiştir. Antimikrobiyal aktivitenin belirlenmesinde minimum inhibisyon konsantrasyonu (MIC) testleri için gram pozitif ve gram negatif bakterilerin temsilcisi olarak sırasıyla Bacillus cereus ve Escherichia coli O15:H7 kullanılmıştır. Oleuropein'in insan dermal fibroblastları üzerindeki in vitro sitotoksisite değerlendirmesinde, HDFa hücre hatları kullanılarak MTT testi, kollajen tip-I seviyesindeki değişimin belirlenebilmesi için ise ELISA Test kiti kullanılmıştır.*

Bulgular: *Çalışma sonucunda zeytin yaprağından Oleanolik asit (L1), oleouropein (L2) ve Ursolik asit (L3) molekülleri, zeytin çiçeğinden ise oleuropein aglikon (F1) molekülü izole edilerek tanımlanmıştır. Minimal inhibisyon konsantrasyonu (MIK) testi sonuçlarına göre, yapraklardan izole edilen L1, L2 ve L3 bileşikleri, Bacillus cereus'a karşı 5-250 µg / mL konsantrasyon aralığında inhibisyon etkisi göstermiştir, ancak Oleuropein aglikon (F1) herhangi bir aktivite göstermemiştir. Ayrıca, Escherichia coli'ye karşı Oleuropein (L2) molekülünün dışında hiçbir bileşiğin etkili olmadığı bulunmuştur. Oleuropein'in insan dermal fibroblastları üzerindeki in vitro değerlendirmesinde, HDFa hücrelerinin hücre canlılığı üzerinde doza bağlı bir etki gözlenmiş ve kollajen tip-I seviyeleri vitamin C ile elde edilen seviyelerden önemli ölçüde yüksek bulunmuştur.*

Sonuç: *Sonuçlara dayanarak, zeytin bitkisinin yaprakları ve çiçeklerinden elde edilen aktif moleküllerin antimikrobiyal etkiler sergilediği ve kozmetik endüstrisinde doğal koruyucu olarak hizmet edebileceği düşünülmektedir. Dahası, hücre yenilenmesine olan katkıları, yara tedavilerinde potansiyel kullanımı önermektedir.*

Anahtar Sözcükler*: Oleuropein, antimikrobiyal etki, olea europaea L., MIC, yaşlanma karşıtı.*

INTRODUCTION

Olea europaea L. (olive) is known as one of the oldest cultivated trees and healthiest natural vegetable oil sources in the world (1). According to one theory, the homeland of the olive tree is Southwestern Asia and Upper Mesopotamia, which includes Syria and Southeast Anatolia (2). Encompassing economical, agricultural, nutritional, and environmental aspects, *Olea europaea L.* type is represented by two varieties in Türkiye; *Olea europaea* L. var. *europaea* Zhukovsky and *Olea europaea* L. var. *Sylvestris* (Miller) Lehr (3). In Mediterranean countries olive groves are spread over large areas and olive products have wide usage areas like table oils, cosmetic and medical ingredients.

As the number of studies on olives increases, it has been revealed that not only olive oils but also all parts of the olive plant (leaf, flower, seed) contain important bioactive compounds. For example; olive leaves and olive flowers are very rich in phenols (oleuropein), flavanols (rutin), catechin (flavan-3-ols), oleoside, flavones and secoiridoid glycoside (4-7) compounds that extensively valuable and widely used in pharmaceutical industry. Amounts and varieties of bioactive compounds of olives are depending to their species, the climate, and the geographical location (8). Literature findings showed that oleuropein is the main phenolic compound from the leaves and has an increasing interest in recent years due to its beneficial contributions in health such as antitumoral, blood pressure-lowering, hypertension, antimicrobial, cardioprotective, anti-inflammatory, antioxidant, anti-cancer, anti-angiogenic and neuroprotective functions (9-11).

Although various extraction methods such as maceration, percolation, solid-liquid, ultrasound, microwave and Soxhlet extraction can be used, there is no optimized method for all types of polyphenols because of their complex structures. Moreover, the recent studies have shown that the variation and quality of the phenolic contents can be affected by the extraction, purification and separation methods (12). For this purpose, in many studies Soxhlet extraction is preferred as an extraction method that because of higher purification efficiency than other methods (13).

Herein this study, we aimed to investigate the antimicrobial and anti-aging effects of triterpenoid and polyphenolic compounds that extracted from *Olea europaea L*. leaves and flowers collected from Türkiye and extracted with hexane, chloroform, ethyl acetate, methanol and distilled water by Soxhlet extraction method.

MATERIALS and METHODS

Plant material, extraction, separation and identification methods

Olea europaea L. leaves and flowers have been collected from Culhalar village (Aydın, Türkiye) and dried under shade and open-air conditions.

The dried samples were ground via industrial type grinder into a particle size of 0.5 mm.

In order to reach the maximum efficacy of liquid-solid extraction; hexane, chloroform, ethyl acetate, methanol and distilled water have been tested. Extraction procedure was carried out with an automatic Soxhlet apparatus at 80°C. According to the thin layer chromatography results (data not shown), methanol extract showed the highest band diversity and thus selected for further separation and purification steps. The extracted compounds stored in dry and dark conditions until use. Stock solutions were prepared in DMSO (dimethyl sulfoxide) and filtered with 0.45 µM filter (EMD Millipore, Bedford, MA, USA).

For purification 4.4 g methanol extract of flower was subjected to the silica-gel column using a mixture of chloroform-methanol (95:5, 80:20, 70:30 v/v) solvent system and 160 fractions were collected into flasks. A total of 7 combining processes were carried out and purification processes were continued. At the end of the purification procedure, one molecule was separated and identified as Oleuropein aglycon (F1).

On the other hand, 5,64 gr leaf extract was subjected to the silica-gel column using a mixture of hexane-ethyl acetate (50:50, 40:60, 30:70, 20:80 v/v) solvent system and 50 fractions collected into flasks. A total of 8 combining processes were carried out and purification processes were continued. At the end of the purification procedure, three molecules were identified as Oleanolic acid (L1), Oleuropein (L2) and Ursolic acid (L3).

Oleuropein molecules were identified with the standard molecules by High Pressure Liquid Chromatography (HPLC) method. For this aim, the standard calibration curve of pure oleuropein molecule was conducted and samples were analysed at the same method. Briefly; HPLC analysis was performed on a Thermo Scientific Ultimate 3000 (ThermoFisher Scientific, Massachusetts, USA) plus photo diode array apparatus using and Hypersil™ ODS C18 (Thermo Scientific™) column. Isocratic elution was performed, and the mobile phase comprised 0.01% trifluoroacetic acid in water (60%) and methanol (40%). 20µl samples were injected with a flow rate of 1.2 mL/min and detected at wavelength of 223nm.

For identification the other molecules, Nuclear magnetic resonance (NMR) method was used with ^{1}H (proton) analysis which performed and identified in the Ege University EBILTEM NMR Satellite Laboratory.

Antimicrobial activity

Minimum Inhibitory Concentration (MIC)

Antimicrobial effects of the compounds were tested against to gram negative *Escherichia coli* O157:H7 (RSKK 234) and gram-positive *Bacillus cereus ATCC 10876* obtained from the Microbiology Department Culture Collection of Ege University, Faculty of Science.

Broth microdilution method was carried on 96-well plate system according to the guideline of Clinical and Laboratory Standard Institute (14). For this purpose, bacteria strains were growth on Muller Hinton Broth (MHB) for 24h at 37° C. After incubation, 0.1 mL growth medium transferred on Muller Hinton Agar (MHA) plates and incubated for overnight at 37° C. Isolated colonies were picked by sterile pipette tips and suspended in 0.85% saline solutions. Optical density of the bacterial solution was adjusted to 0.3-0.5 optical density (approximately 10^6 cfu / mL) by spectrophotometer. 5 µL bacterial suspension was added to each well contains 195 µL MHB with a different final concentration of pure compounds listed in Table-1.

Table-1. Purified compounds from *Olea europaea* and final concentrations tested for antimicrobial activity (L1, L2 and L3; leaf, F; Flower).

Molecule Code	Final Concentrations
L1	5- 25- 50 µg/mL
L2	5- 25- 50 µg/mL
L3	50- 250- 500 µg/mL
F1	50- 250- 500 µg/mL

A set of wells containing only bacteria suspension served as positive control. Furthermore, 0.5%, 2.5% and 5% of DMSO plus bacteria containing wells checked for the potential inhibitory effects of DMSO. Plates were incubated for 18h at 37 °C under aerobic condition. At the end of the incubation period, 20 µL of 1% TTC (2,3,5-Triphenyltetrazolium chloride) solution was added to the wells for the determination of microbial activity.

In vitro cell viability testing and collagen type-I level assessment

Adult human dermal fibroblasts (HDFa, Gibco, C-013-5C) were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % (v/v) FBS, 1 % (v/v) L-glutamine and 0.5 % (v/v) penicillin/streptomycin (100U/100 mg/ml) at 37 °C and 5% CO_2. *In vitro* cytotoxicity and IC_{50} of oleuropein were evaluated via 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma-Aldrich, USA) assay. In brief, HDFa cells were seeded in 96-well plates ($5x10^5$ cells/well) and cultured overnight in the cell culture medium at 37 °C and 5% CO_2. Followed, cells were treated with 1- 1000 µg/ml of oleuropein for 7-days. At the end of the incubation, 100 µL of 10% (v/v) MTT solution was added to each well and incubated for 4 h at 37 °C. Finally, 100 µL DMSO added to each well, and absorbance measurement was performed to measure cell viability at 570 nm via microplate reader (Multiskan™ GO, Thermo Scientific). Vitamin C, of which effectiveness in collagen production and cell proliferation has been proven in the literature, and DMSO were used as a positive control and negative controls in the MTT test, respectively. To assess the effect of oleuropein on collagen type-I levels, ELISA Kit (E-EL-H0869, Elabscience) was used by following the manufacturer's instructions.

Statistical analysis

The statistical analyses were conducted using Two-way analysis of variance (ANOVA) with Tukey's Multiple Comparison Test using Prism 8.3 software (GraphPad, San Diego, CA, USA), with a confidence interval of ±95%.

RESULTS and DISCUSSION

Purification and identification of polyphenolic compounds

After the extraction process the extracted compounds stored in dry and dark conditions until use. Stock solutions were prepared in DMSO and filtered with 0.45 µM filter (EMD Millipore, Bedford, MA, USA).

Oleuropein aglycon (F1), Oleanolic acid (L1) and Ursolic acid (L3) were detected by NMR with ^1H (proton) analysis which performed and identified in the Ege University EBILTEM NMR Satellite Laboratory.

Oleuropein molecule (sample L2) was detected with standard molecule by HPLC method which described above. The standard calibration curve of oleuropein was established between 28.4-1000 µg/mL concentration (Figure-1). Isolated oleuropein molecule was prepared with a concentration of 1000 µg/mL dissolved in HPLC grade methanol (Figure-2).

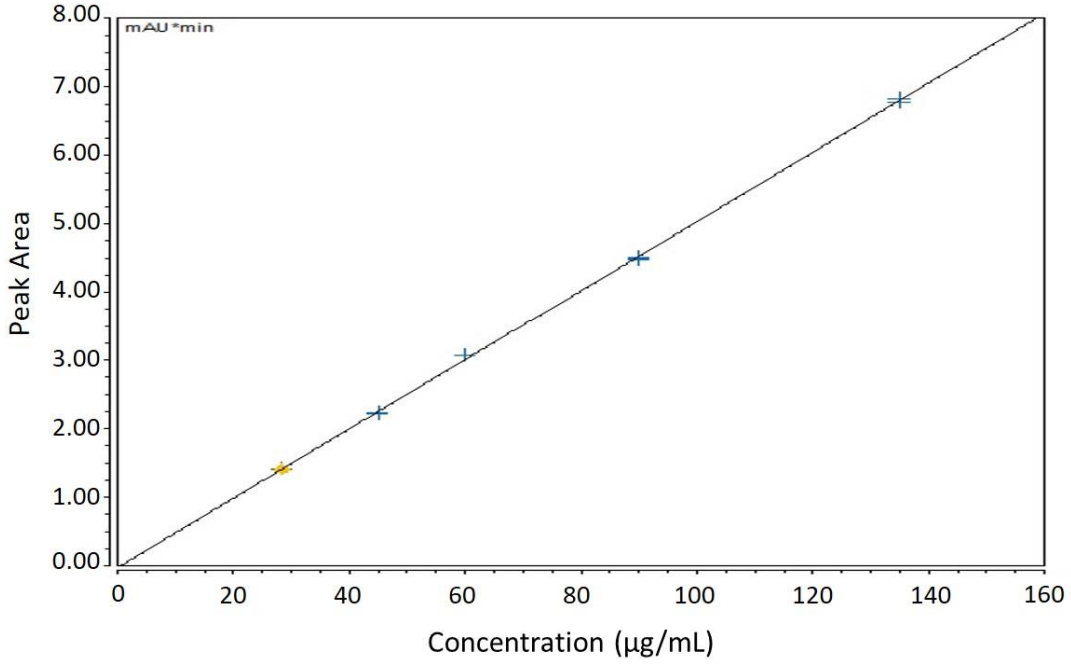
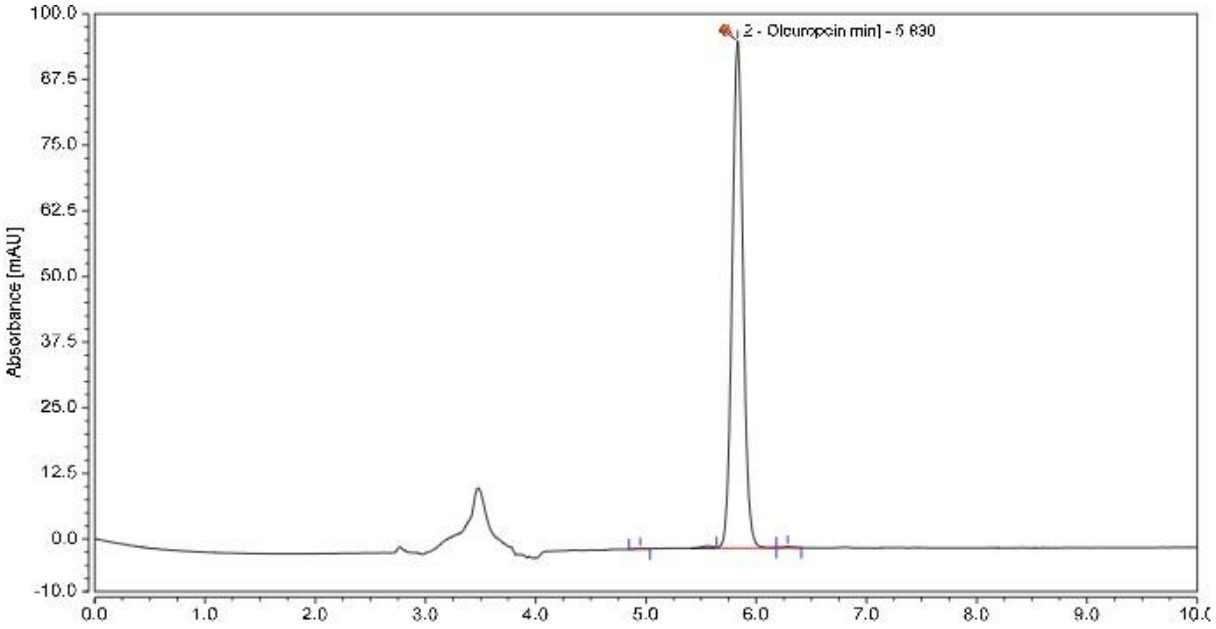


Figure-1. Standard calibration curve of pure oleuropein standard molecule. (R^2:0.9996)



No.	Peak Name	Retention Time	Area	Height	Relative Area
		min	mAU*min	mAU	%
UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1
1		4.947	0.01	0.113	0.09
2	Oleuropein	5.83	11.07	96.502	99.67

Figure-2. HPLC analysis results of the L1 sample with oleuropein standard calibration curve.

Table-2. The viability of the microorganisms after TTC solution addition. (nt: not tested) (+: positive results for formazan formation; -: negative results for formazan formation)

Molecule Names	***E.coli* O157:H7**					***B.cereus***				
Concentrations (µg/ml)	5	25	50	250	500	5	25	50	250	500
L1- Oleanolic acid	+	+	+	nt	nt	+	-	-	nt	nt
L2- Oleuropein	+	+	+	+	-	-	-	-	nt	nt
L3- Ursolic acid	nt	nt	+	+	+	nt	nt	+	-	-
F1- Oleuropein aglycon	nt	nt	+	+	+	nt	nt	+	+	+

Anti-Microbial Activity

According to the MIC results, none of molecules and concentrations showed antimicrobial activity against *E.coli* O157:H7 except Oleuropein (L2). As listed in Table-2, L2 sample showed an inhibition effect at 500 µg/mL concentration. In addition, Ursolic acid (L3) molecules reduced the viability and could show antimicrobial activity at concentrations higher than 500 µg/ml.

Topuz and Bayram (15) tested the antimicrobial activity of crude extract (CE), pure oleuropein (PO) and particularly purified oleuropein (PPO) molecules of olive leaves which collected from different location of Türkiye against *Escherichia coli* O157:H7, *Listeria monocytogenes, Salmonella typhimurium* and *Staphylococcus aureus* bacteria. According to their results, PO and PPO are more effective then CE samples. MIC values show differences between the microorganism groups and extraction methods. In summary, they identified the MIC value of PPO and PO as 12.5 mg/ml and 0.781 mg/ml against to *E.coli* and *S.aureus*, respectively. When compared with our results, L2 and L3 samples have a higher antimicrobial effect against *E. coli* O15:H7.

On the other hand, it has been determined that the Oleuropein (L2) molecule has an

antimicrobial effect on *B.cereus* even at a concentration of 5 µg/ml. While the Oleanolic acid (L1) molecule acts at 25 - 50 µg/ml, the L3 molecule appears to be effective at around 250 and 500 µg/ml. Moreover, it was observed that the molecules obtained from the olive flower did not show antimicrobial activity at the concentrations tested against both groups of microorganisms. Therefore, it was seen that the molecules obtained from the olive leaf are more effective against gr (+) *B.cereus*. In another study (16) olive leaf extract was added into pasteurized milk and tested for using as a potential natural preservative agent. They analyzed the extract with HPLC analyses and found out that oleuropein was the dominant compound. According to the agar well diffusion assay results, inhibition halos width of 6.75 ± 0.31 and 5.33 ± 0.17 mm were achieved with the concentrations of undiluted (at 1.44 mg/mL oleuropein) and diluted (1:2 v/v, at 0.72 mg/mL oleuropein) extracts against to *B.cereus*, respectively.

In order to control the inhibition effect of DMSO, pure DMSO was added to the wells with a final concentration of 0.5%-2.5% and 5% without active molecules. According to the results, it was observed that DMSO did not have an inhibitory effect against both organisms at the concentrations used.

Assessment of *in vitro* cytotoxicity and collagen level

Locating at the dermis layer of skin, fibroblasts are constantly exposed to various environmental insults. As they are responsible for the recovering and generating process of connective tissues, they were selected as main target for *in vitro* viability assessments in this study. Cytotoxicity assay was performed to evaluate the effect of various concentration of oleuropein on cell viability. Notably, although *in vitro* anticancer activity of oleuropein has been well characterized through various studies subjected breast cancer (17), hepatocarcinoma (18) or neuroblastoma (19) cancer cells, there are limited information about the effect of oleuropein on healthy cell lines. Herein this study, a dose-dependent effect on cell viability of HDFa was observed (Figure-3A) which was also in agreement with the 7-days of proliferation profile (Figure-3B).

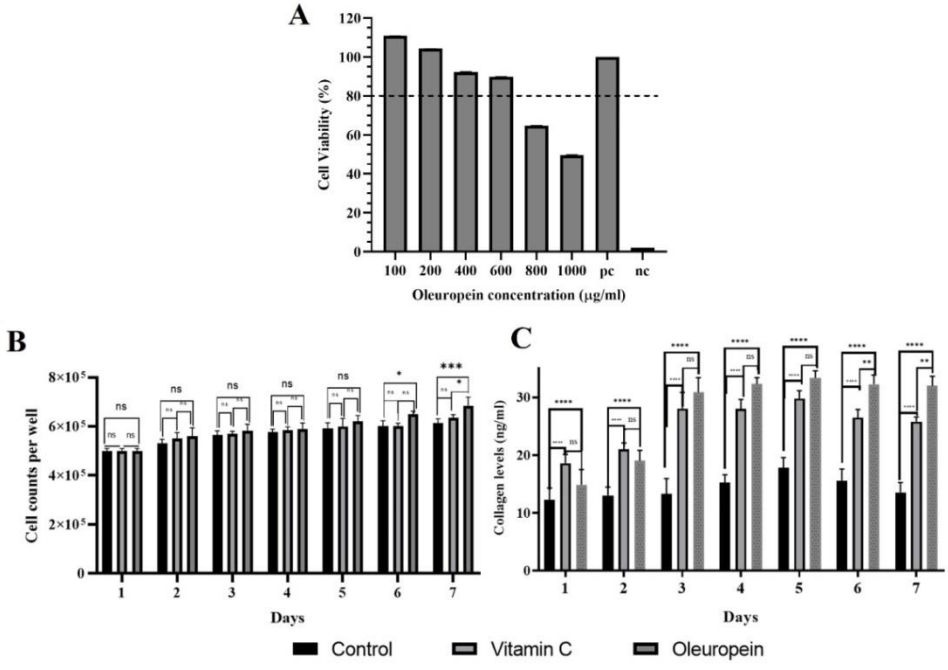


Figure-3. *In vitro* cytotoxicity and collagen type-I level assessment.

A) Cytotoxicity results of oleuropein (200 µg/ml).

B) Effects of oleuropein on proliferation of HDFa.

C) ELISA results representing the collagen type I levels in control (non-treated), vitamin C (200 µg/ml) and oleuropein (200 µg/ml) added groups. pc: positive control; nc: negative control; ns: $p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$; Two-Way ANOVA, Multiple Comparison Test.

Bal et al. (20) also reported a dose-dependent cytotoxicity and proliferation phenomenon in healthy human bronchial epithelium cell lines, where they observed proliferation up to 1000 µg/ml. Moreover, on day 7, a higher rate of cell proliferation ($p<0.05$) was observed in the group treated with oleuropein (200 µg/ml) compared to the group treated with vitamin C (200 µg/ml), which is well-known for its function in stimulating collagen synthesis and cell proliferation (21, 22). Consisted with the cytotoxicity data, Goldsmith et al. (23), demonstrated that the application of oleuropein showed no effect on non-tumorigenic cells. Katsiki et al. (24), on the other hand, observed that the use of oleuropein resulted in a postponement of senescence-related characteristics, leading to an extension of the life span of human fibroblasts by around 15%.

Consistent with the cell proliferation and cytotoxicity data, ELISA results revealed that the collagen type-I levels were significantly higher than that of achieved with vitamin C treatment at day 6 ($p < 0.01$) and day 7 ($p < 0.01$**)** (Figure-3C). Overall, the increase in collagen type-I levels is thought to be associated with the tendency of oleuropeins to act as inhibitors of collagenase (25). Notably, besides the antioxidant, anti-inflammatory, and anticancer activities, the effectiveness of oleuropein has also been reported for wound healing studies considering such parameters like delaying senescence, reducing ROS levels, and showing increased proteasome activity (22). Moreover, the enhancement of wound healing through oleuropein treatment has been observed to entail a reduction in cell infiltration and improvement in the deposition of collagen fibers and re-epithelialization (26). Herein, the obtained data also indicates that oleuropein activates fibroblasts, leading to proliferation with a subsequent increase in collagen type-I expression. This achievement is attributed to the prevention of the deterioration of dermal skin due to aging and wrinkles, as tightly and well-organized collagen proteins support the mechanical interaction between fibroblasts. Thus, it has been concluded that the oleuropein molecule can be considered an effective stimulator for collagen expression and has great potential in anti-aging and tissue recovery studies. It has been emphasized that oleuropein, which exhibited a supportive effect on the proliferation of healthy fibroblasts and collagen synthesis in this study, also demonstrates inhibitory properties in studies conducted with cancer cells. This is shown to be associated with the excellent antioxidant and anti-inflammatory properties of oleuropein.

CONCLUSION

This work showed the antimicrobial effects of extracted molecules from *Olea europaea L.* leaves and flowers collected from Culhalar village (Aydin, Türkiye). In parallel with literature but at lower concentrations, especially for oleuropein molecule, showed inhibition effects on both gram positive and negative bacteria. Furthermore, oleuropein, which has been reported in the literature to selectively inhibit proliferation in cancer cell types, was found in the study to exhibit non-toxic properties on healthy dermal fibroblasts. Moreover, contrary to its effects on fibrotic tissues, it was demonstrated that oleuropein positively influences collagen type- I production in healthy cells. The observed contrasting mechanisms in cancer and fibrotic cells was attributed to oleuropein's sensitivity to ROS levels, selective toxicity on cell types and their collagenase inhibition capacities. Particularly, the presence of collagen type-I protein as a significant connective tissue fibril in the dermal layer of the skin, contributing to the structure of elastic fibrils and playing crucial roles in skin tightness and wrinkle formation, makes this finding noteworthy. The loss of elasticity in aging and wrinkled skin, parallel to the diminishing proliferative properties of fibroblasts and the decrease in the synthesis of collagen and other dermal matrix proteins, leads to adverse effects on the skin. In this context, it is possible to suggest that the application of the oleuropein molecule to human dermal fibroblasts under *in vitro* conditions has the potential to mitigate existing negative impacts, and this can be attributed to its ability to increase cell proliferation and stimulate collagen synthesis. Hence, these results support the usage of *Olea europaea L.* extracts as natural preservative agents in many application areas like cosmetic and by products through the selective activity of oleuropein molecules among healthy and damaged cells provide an excellent applicability property. The results of this study can be used for further studies to identify the mechanism of multifaceted effects of oleuropein and other phenolic compounds of *Olea europaea L.*

Acknowledgements: The authors declare no conflict of interest, no ethical approval required, and no informed consent required.

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Araştırma Makalesi / *Research Article*

Ege Tıp Dergisi / *Ege Journal of Medicine* 2024; 63 (3): 378-382

Pandemi döneminde subakut granülomatöz tiroidit

Subacute granulomatous thyroiditis during the SARS-CoV-2 pandemic

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ÖZ

Amaç: Tiroidit, tiroit bezinin enflamasyonu ile karakterize, tiroit fonksiyon bozukluğuna yol açabilen, çoğunlukla benign seyreden bir klinik tablodur. Enfeksiyonlar akut tiroidit ve subakut granülomatöz tiroidit (SAT) etiyolojisinde yer alır. Ancak enfeksiyon etkenlerini saptamak her zaman mümkün olmamaktadır.

Çalışmamızda Ege Üniversitesi Tıp Fakültesi Hastanesi'nde Endokrinoloji ve Metabolizma veya Enfeksiyon Hastalıkları Polikliniği'nde tiroidit tanısı ile takip edilen hastalar arasında SAT tanısı alan hastaların geriye dönük incelenmesi, SARS-CoV-2 enfeksiyonu veya SARS-CoV-2 aşısının etiyolojideki rolünün incelenmesi amaçlanmıştır.

Gereç ve Yöntem: Çalışmanın evrenini Mart 2020 ve Temmuz 2023 tarihleri arasında ICD tanı kodu tiroidit olarak girilen hastalar oluşturmaktadır. Bu hastalar arasında SAT tanısı alan hastaların yaşı, cinsiyeti, eşlik eden hastalıkları, SARS-CoV-2' ye karşı aşılanma öyküleri, tiroidit ile ilişkili klinik yakınmaları, yakınmalar öncesinde üst solunum yolu enfeksiyonu (ÜSYE), gastroenterit veya SARS-CoV-2 enfeksiyonu geçirme öyküsü, SAT tanısı aldığı tarih, laboratuvar ve görüntüleme bulguları, hastalara uygulanan tedaviler retrospektif olarak değerlendirilmiştir.

Bulgular: Çalışmaya belirlenen tarihler arasında SAT tanısı alan 9 hasta dahil edilmiştir. Olguların yaş ortalaması 47,6 ± 7,3 (min. 39, maks. 58) ve hastaların altısı kadındır. En sık klinik yakınma boyun ve boğaz ağrısı, boğazda şişlik hissi ve ateş yüksekliği olarak saptanmıştır. Hastalardan ikisinde SAT tanısı almadan üç ay içerisinde SARS-CoV-2 aşısı öyküsü mevcuttur. ÜSYE öyküsü olan hastalardan birinde hem SARS-CoV-2 enfeksiyonu hem de aşı öyküsü vardır.

Sonuç: Çalışmamızda sadece iki hastada SAT etiyolojisinde SARS-CoV-2 enfeksiyonu veya aşısı sorumlu olabileceği düşünülmüştür ancak diğer hastalarda ilişki bulunamamıştır. Hasta sayımız az olmakla birlikte elimizdeki veriler SARS-CoV-2 enfeksiyonu ve aşışı ile SAT ilişkisini ortaya koymak için yeterli değildir.

Anahtar Sözcükler: Tiroidit, viral enfeksiyon, SARS-CoV-2

Not: *Poster bildirisi şeklinde 11. Türkiye EKMUD bilimsel kongresinde (3-7 Mayıs 2023, Girne, Kıbrıs) sunulmuştur.*

ABSTRACT

Aim: *Thyroiditis is a mostly benign clinical entity that is characterized by inflammation of the thyroid gland and can lead to thyroid dysfunction. Infections are involved in the etiology of acute thyroiditis and subacute granulomatous thyroiditis. However, it is not always possible to identify the infectious agents.*

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In our study, we aimed to retrospectively examine patients diagnosed with subacute thyroiditis among those who were followed up with a diagnosis of thyroiditis in the Endocrinology and Metabolism or Infectious Diseases Outpatient Clinics of Ege University Medical Faculty Hospital. We also aimed to investigate the role of SARS-CoV-2 infection or SARS-CoV-2 vaccine in the etiology of SAT.

Materials and Methods: *The study population includes patients whose ICD diagnosis code was recorded as thyroiditis between March 2020 and July 2023.*

Age, gender, comorbidities, history of vaccination against SARS-CoV-2, clinical complaints related to thyroiditis, history of upper respiratory tract infection, gastroenteritis or previous SARS-CoV-2 infection prior to the onset of symptoms, date of diagnosis of subacute thyroiditis, laboratory and imaging findings, and treatments administered to the patients were retrospectively assessed.

Results: *The study included nine patients diagnosed with SAT within the specified dates. The mean age was 47.6 ± 7.3 years (min. 39, max. 58), and six patients were female. The most common clinical complaints were neck and throat pain, a feeling of swelling in the throat, and a high fever. Two of the patients had received a SARS-CoV-2 vaccination within three months prior to being diagnosed with subacute thyroiditis. One of the patients with a history of upper respiratory tract infection had both a history of SARS-CoV-2 infection and vaccination.*

Conclusion: *In our study, SARS-CoV-2 infection or vaccine was involved in the etiology of SAT in only two patients, but no association was found in other patients. Although the number of patients is small, our data are not sufficient to demonstrate the association of SAT with SARS-CoV-2 infection and vaccination.*

Keywords: *Thyroiditis, viral infection, SARS-CoV-2.*

Note: *Presented as a poster presentation at the 11th Scientific Congress of Turkish EKMUD (May 3-7, 2023, Kyrenia, Cyprus).*

GİRİŞ

Tiroidit, tiroit bezinin inflamasyonu ile karakterize, tiroit fonksiyon bozukluğuna yol açabilen, çoğunlukla benign seyreden bir klinik tablodur. Klinik özelliklerine göre akut tiroidit, subakut granülomatöz tiroidit (De Quervain tiroiditi), kronik tiroidit (Hashimoto), riedel tiroiditi (Ig G4 aracılıklı), sessiz tiroidit (ağrısız, postpartum) ve diğer tiroiditler olarak sınıflandırılmaktadır (1). Özellikle akut tiroidit ve subakut granülomatöz tiroidit (SAT) etiyolojisinde enfeksiyöz nedenler rol oynamaktadır (2). Ayrıca nedeni bilinmeyen ateş etiyolojisinde de tiroiditler akılda tutulmalıdır (3).

Subakut granülomatöz tiroidit kadınlarda daha sık görülen, 30-50 yaş arasında sıklığı artan, yaz aylarında daha sık görülen ve çoğunlukla viral üst solunum yolu enfeksiyonu sonrasında ortaya çıkan bir klinik tablodur. Etiyolojisinde ise genellikle viral ajanlar (Adenovirüsler, Coxsackie Virüs, İnfluenza Virüs, Ebstein-Barr Virüs ve kabakulak virüsü vs) yer almaktadır (1). Pandemi ile birlikte etiyolojide sorumlu olan viral etkenlere SARS-CoV-2'de eklenmiştir (4). Bununla birlikte SARS-CoV-2 aşılarının da SAT ile ilişkili olabileceğini bildiren çalışmalar mevcuttur (5).

Hastalık seyri sırasında ateş yüksekliği, özellikle kulağa veya göğüs bölgesine doğru yayılan, yutkunmakla veya baş hareketleri ile artabilen boyun bölgesinde ağrı, kızarıklık ve hassasiyet görülebilir. Eritrosit sedimantasyon hızı (ESH) ve beyaz küre sayısı artmış olarak bulunur; tiroit fonksiyon testleri ise genellikle normaldir (1,6).

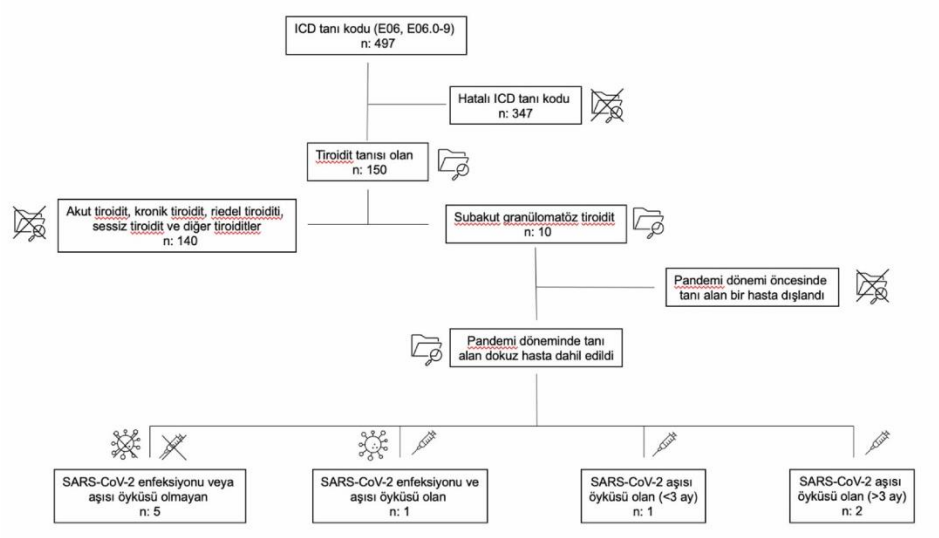
Çalışmamızda pandemi süresince tiroidit tanısı alan olgular arasında subakut granülomatöz tiroidit olgularının retrospektif olarak değerlendirilmesi ve SARS-CoV-2 enfeksiyonu ve aşısının etiyolojideki rolünün irdelenmesi amaçlanmıştır.

GEREÇ ve YÖNTEM

Ege Üniversitesi Tıp Fakültesi Endokrinoloji ve Metabolizma veya Enfeksiyon Hastalıkları Polikliniği'ne Mart 2020 ve Temmuz 2023 yılları arası başvuran ve elektronik hasta dosyasına tiroidit ve tiroidit ilişkili ICD tanı kodu (E06, E06.0-9) girilen 497 hastanın dosyaları geriye dönük incelenmiştir. Bu hastaların 10'unda subakut granülomatöz tiroidit tespit edilmiştir. Hastaların biri SAT nedeni ile takiplerine devam etmekte olup pandemi döneminden önce tanı aldığı için çalışma dışında bırakılmıştır. (Şekil-1)

Dahil edilen dokuz hastanın yaşı, cinsiyeti, eşlik eden hastalıkları, SARS-CoV-2 aşılanma öyküleri, tiroidit ile ilişkili klinik yakınmaları, SAT tanısı aldığı tarih, yakınmalar öncesinde üst solunum yolu enfeksiyonu, SARS-CoV-2

enfeksiyonu veya gastroenterit öyküsü, ESH, C-reaktif protein (CRP), hemogram, tiroit fonksiyon testleri (TFT), tiroit oto antikorları (anti-TPO, anti-TG ve TRAb), tiroit ultrasonografisi (USG), tiroit sintigrafisi bulguları ve hastalara uygulanan tedaviler kayıt altına alınmıştır. Tüm veriler Microsoft Excel'e aktarılmıştır. Çalışma için EÜTF araştırma etik kurulundan onay alınmıştır. (Onay Kararı 22-12.1T/20, Onay Tarihi: 20.12.2022)



Şekil-1. Çalışma tasarımı.

BULGULAR

Subakut granülomatöz tiroidit tanısı alan dokuz hasta (altı hasta kadın) çalışmaya dahil edilmiştir. Hastaların yaş ortalaması 47,6 ± 7,3 (min. 39, maks. 58) yıldır. Olguların beşinde SAT ile ilişkili şikayetler yaz aylarında görülmüştür. En sık klinik yakınma boyun ve boğaz ağrısı (n:8), boğazda şişlik hissi (n:4) ve ateş yüksekliği (n:2) olarak saptanmıştır.

Sekiz hastada ESH, CRP tetkik edilmiş olup ortalama ESH 79±23,1 mm (min.37 max.101) ve ortalama CRP 93,5 mg/L (min.12, max.236; normal değeri: 0.5 mg/L) olarak bulunmuştur. Hastaların ortalama lökosit sayısı 9.232 10^3/µL (min.4.170, max.12.690) olarak saptanmıştır. (Tablo-1) Sekiz hastaya tiroit USG yapılmış olup hepsinde tiroidit uyumlu bulgular saptanmıştır.

Hastaların dördünde tiroidit teşhisi öncesinde üst solunum yolu enfeksiyonu, bir hastada akut gastroenterit öyküsü mevcuttu. ÜSYE öyküsü olan hastaların birinde SARS-CoV-2 polimeraz zincir reaksiyonu (PZR) pozitif saptanmıştır.

Beş hasta ülkemizde SARS-CoV-2 aşısının henüz uygulanmadığı dönemde SAT tanısı almıştır. Dört hastada SARS-CoV-2 aşı öyküsü mevcuttur. SARS-CoV-2 aşısı (BNT162b2, Biontech, Pfizer) ve SAT teşhisi koyulması arasında geçen süre hastalarda 1, 2, 10 ve 11 aydı. Nedeni bilinmeyen ateş nedeni ile hastaneye yatırılarak takip edilen hastamızın (olgu no: 9) SAT teşhisi öncesinde hem SARS-CoV-2 enfeksiyonu hem de SARS-CoV-2 aşısı öyküsü vardı.

Sekiz hastada tiroit peroksidaz antikoru (anti-TPO), beş hastada anti-tiroglobulin antikoru (anti-TG) ve iki hastada TSH reseptör antikoru (TRAb) bakılmış olup normal aralıkta saptanmıştır. Hastaların eşlik eden kronik hastalıkları, laboratuvar-görüntüleme sonuçları ve uygulanan tedaviler ise tabloda sunulmuştur (Tablo-2).

Tablo-1. Olguların tiroid fonksiyonları ve akut faz reaktanları.

Test	Normal değer	Ortalama (±)
TSH	0,27 – 4,2 mU/L	0,097 (± 0,22) mU/L
FT4	0,89 – 1,76 ng/dL	2,9 (± 1,87) ng/dL
CRP	0-5 mg/L	93,5 (± 72) mg/L
ESH	<20 mm	79 ± 23,1 mm

Tablo–2. Hastaların demografik özellikleri, komorbiditeleri, tetkik sonuçları ve tedaviler.

Olgu No.	Cinsiyet - Yaş	Komorbidite	Semptom	ESH (mm)	CRP (mg/L)	Lökosit - Nötrofil (10^3/µL)	TSH (mU/L) - fT4 (ng/dL)	Tiroidit uyumlu görüntüleme	Tedavi
1	Erkek - 58	Yok	Boğaz ağrısı, ateş	93	106	12.690 - 8.700	0,008 - 7,13	Yok	Metilprednisolon
2	Kadın - 56	Meme kanseri	Boğaz ve boyunda ağrı, şişlik	37	12	4.170 - 2.550	0,014 - 1,8	USG	NSAİİ
3	Kadın - 49	Yok	Boyunda ağrı, kızarıklık ve şişlik	85	68	8.850 - 6.560	0,01 - 2,19	USG	NSAİİ, metilprednisolon
4	Kadın - 41	Yok	Boğaz ağrısı, şişlik ve yutma güçlüğü	76	83	9.070 - 5.820	0,019 - 2,31	USG	NSAİİ, metilprednisolon
5	Kadın - 41	Yok	Boyunda ağrı, halsizlik	51	36	12.130 - 8.110	0,014 - 3,33	USG	NSAİİ, metilprednisolon
6	Kadın - 42	Tip I Diyabetes Mellitus	Boğaz ağrısı, çarpıntı	92	155	9.800 - 7.400	0,01 - 4,45	USG	NSAİİ, metilprednisolon, propranolol
7	Kadın - 48	Alerjik Rinit	Senkop	97	52	9.330 - 5.690	0,01 - 2,29	USG	Metilprednisolon
8	Erkek - 39	Yok	Boğaz ağrısı			5.480 - 3.660	0,68 - 1,06	USG	NSAİİ
9	Erkek - 55	Hipertansiyon, Ankilozan spondilit	Boğaz ağrısı, şişlik, ateş	101	236	11.570 - 9.410	0,11 - 1,57	USG	NSAİİ

TARTIŞMA

Subakut granülomatöz tiroidit, tiroit bezinde ağrının en sık nedenidir (1). Hastaların başvuru nedenleri genellikle boyun ağrısı, şişlik ve ateş yüksekliğidir. Akut dönemde tiroit foliküllerinin harabiyetine bağlı yaklaşık %50 hastada tirotoksikoz ve buna bağlı çarpıntı, senkop, kilo kaybı, titreme, halsizlik gibi semptomlar görülebilir (6). Ülkemizde yapılan bir çalışmada hastaların tamamında tiroit bölgesinde ağrı ve %45,9 hastada ise ateş yüksekliği saptanmıştır (7). Çalışmamızda sekiz hastada boyun bölgesinde ağrı, dört hastada ise şişlik mevcut idi. Ağrı şikayeti olmayan bir hasta ise tirotoksikoz ilişkili olabilecek senkop şikayeti ile başvurmuştu. Sadece iki hastada ateş yüksekliği saptanmıştır. Daha önce ülkemizden bir çalışmada ateş yüksekliği %46,2 hastada saptanmıştır (8). Çalışmamızda ateş yüksekliğinin az saptanmasının nedeni hasta sayısının az olması, çalışmanın retrospektif olarak yapılması ve poliklinik hastalarında başvuru sırasında ateş yüksekliği saptanmaması veya epikrize kaydedilmemesi ile ilişkili olabilir.

Sedimantasyon ve CRP inflamasyon durumunda da artan biyobelirteçler olup hastalarımızda da yüksek saptanmıştır. Hastaların başvuru şikayetleri olan ateş yüksekliği ve boğaz ağrısı dikkate alındığında artmış CRP ve sedimantasyon değerleri hastalara gereksiz antibiyotik reçetelenmesine ve gereksiz tetkiklere yol açabilmektedir. Yine sedimantasyon yüksekliği romatolojik ve hematolojik hastalıkları da akla getirmektedir. Çalışmamızda da ateş yüksekliği ile başvuran iki hastaya ek serolojik tetkikler ve bilgisayarlı tomografi çekimi yapılmıştır. Her iki hasta da teşhis süreci uzamış ve hastalar antibiyotik tedavisi almıştır. Bu hastalarda SAT tanısının ilk aşamada akla gelmemesinin nedeni tirotoksikoz ilişkili şikayetlerinin olmaması olabilir. Ek olarak subakut tiroidit için kesin tanı kriterlerinin olmaması da hastalığın geç teşhisine yol açıyor olabilir (9).

SARS-CoV-2 aşısı ile SAT tanısı arasında 10 ve 11 ay olan hastalarda aşı ile SAT arasında bağlantı kurulamamıştır. SARS-CoV-2 enfeksiyonu ve aşı öyküsü olan hastada ise SAT tanısının her iki durumla da ilişkili olabileceği düşünülmüş fakat ayrım yapılamamıştır. Literatürde SARS-CoV-2 enfeksiyonu ve aşıları sonrasında subakut tiroidit bildirilen yayınlar mevcuttur (4, 5). Yapılan çalışmalarda SARS-CoV-2 enfeksiyonu sonrasında tiroidit gelişmesinin doğrudan viral hasar kaynaklı olabileceği, SARS-CoV-2'nin hücreye girişi için gerekli olan ACE-2 reseptörünün tiroid hücrelerinde de bulunduğu ve bunun viral hasarın mekanizmasını açıklayabileceği öne sürülmüştür (10). Aşılama sonrasında bu kliniğe yol açan mekanizmalardan biri ASIA (Autoimmune/inflammatory syndrome induced by

adjuvants) sendromudur. Bir diğer mekanizma ise moleküler benzerlik olup SARS-CoV-2'ye karşı oluşan antikorların farklı doku antijenleri ile çapraz reaksiyona girmesinden kaynaklanabilir (5).

Geçmiş çalışmalarda antikor yokluğu subakut tiroidit için destekleyici olarak kabul edilmekle birlikte yakın zamanlı çalışmalarda antikorların artabileceği gösterilmiştir (11). Hastalarımızdan sekizinde tiroid peroksidaz antikoru (anti-TPO), beşinde antitiroglobulin antikoru (anti-TG) ve ikisinde TSH reseptör antikoru (TRAb) bakılmış olup normal aralıkta saptanmıştır.

Çalışmamızda veri taraması ICD tanı kodları üzerinden yapılmıştır. Çalışmamızın yapıldığı tarihler arasında 497 hastaya tiroidit ve tiroidit ilişkili tanı kodları girildiği ancak bu hastaların sadece 150'sinde (%30) tirodit (akut tiroidit, subakut granülomatöz tiroidit, kronik tiroidit, Riedel tiroiditi, sessiz tiroidit ve diğer tiroiditler) olduğu saptanmıştır. ICD kodlarının doğru girilmemesi sebebi ile subakut tiroidit tanısı alan birçok hastaya ulaşamamış olabiliriz. Ayrıca çalışmanın retrospektif olarak tasarlanması, pandemi döneminde ayaktan hizmetlerin ve poliklinik başvurularının azalmış olması, subakut tiroidit ile ilgili tanı kriterlerinin olmaması çalışmamızın diğer kısıtlılıklarıdır.

SONUÇ

Pandemi döneminde hem COVİD-19 hem aşılara bağlı SAT olguları bildirilmiştir. Çalışmamızda sadece bir hastada SAT tanısı öncesinde COVİD-19 aşısı ve bir hastada hem COVİD-19 enfeksiyonu hem de aşı öyküsü mevcuttur. Diğer yedi hastada ise ilişki kurulamamıştır. Hasta sayımız az olmakla birlikte elimizdeki verilen COVİD-19 enfeksiyonu ve aşışı ile SAT ilişkisini ortaya koymak için yeterli değildir.

Çıkar çatışması: Bu makale ile ilgili herhangi bir çıkar çatışması bulunmamaktadır.

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Research Article / *Araştırma Makalesi*

Ege Journal of Medicine / *Ege Tıp Dergisi* 2024; 63 (3): 383-389

Comparison of the effect of candida score and candida colonization index on decrease in candidemia incidence in our intensive care unit

Yoğun bakım ünitemizde kandida skoru ve kandida kolonizasyon indeksinin kandidemi insidansındaki azalmaya etkisinin karşılaştırılması

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ABSTRACT

Aim: In this retrospective study, the effect of starting empiric treatment on the incidence of candidemia according to the candida score (CS) and candida colonization index (CCI) in patients followed in the intensive care unit (ICU) was investigated.

Materials and Methods: One hundred non-neutropenic adult patients aged 18-80 years old, hospitalized in the intensive care unit of our hospital, were included in the study. Cultures taken from patients hospitalized in the ICU between 01.06.2018 and 01.08.2021 were examined retrospectively. Swab samples were routinely taken from five main areas: mouth, nose, skin, perineum, and catheter, on the 7th day of each patient's hospitalization, to determine the CCI and CS. These samples were plated on Sabouraud dextrose agar (SDA) plates and the plates were incubated at 35 °C for 48 hours. The resulting yeast colonies were identified according to their microscopic appearance and biochemical properties. Fluconazole prophylaxis was initiated in patients with CS ≥3 or CCI ≥0.5. **Results**: A total of 500 culture samples from 100 non-neutropenic adult patients were analyzed (Average 5 cultures/patient). Seventy of the patients were male (70%), 30 (30%) were female and the average age was 71.5. While no growth was detected in any sample in 32 of a hundred patients (32%), growth was detected in at least one of the samples taken from 68 patients (68%), for a total of 118 samples. Of the yeasts, 104 were identified as Candida albicans, 10 as Candida glabrata, and 4 as Candida inconspicua. CS≥ 3 and CCI ≥0.5 were found in 11 (11%) patients, and CS≥3 and CCI<0.5 were found in 12 (12%) patients. Fluconazole prophylaxis was started in a total of 23 (23%) patients. No patient developed candidemia during their follow-up

Conclusion: These findings suggest that the evaluation of patients followed in the ICU with CCI and CS, and initiation of prophylactic treatment in patients who are found to be at risk may be effective in preventing possible fungal infections.

Keywords: Candida colonization index, candida score, incidence of candidemia, anti-fungal prophylaxis

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Application date: 24.05.2023 Accepted: 04.03.2024

ÖZ

***Amaç**: Bu retrospektif çalışmada, yoğun bakım ünitesinde (YBÜ) takip edilen hastalarda kandida skoru (KS) ve kandida kolonizasyon indeksine (KKİ) göre ampirik tedavi başlanmasının, kandidemi insidansına olan etkisi araştırıldı.*

***Gereç ve Yöntem**: Hastanemiz yoğun bakım ünitesinde yatan 18-80 yaş arası nötropenik olmayan yüz erişkin hasta çalışmaya dahil edildi. YBÜ'de, 01.06.2018 ile 01.08.2021 tarihleri arasında yatmış olan hastalardan alınan kültürler retrospektif olarak incelenmiştir. KKİ ve KS belirlemek için her hastanın hastaneye yatışının 7. gününde ağız, burun, deri, perine ve kateter olmak üzere beş ana bölgeden rutin olarak sürüntü örnekleri alındı. Bu numuneler Sabouraud dekstroz agar (SDA) plakalarına ekildi ve plakalar 35 °C'de 48 saat inkübe edildi. Elde edilen maya kolonileri mikroskobik görünümlerine ve biyokimyasal özelliklerine göre tanımlandı. KS ≥3 veya KKİ ≥0,5 olan hastalara flukonazol profilaksisi başlandı.*

***Bulgular:** Nötropenik olmayan yüz erişkin hastadan alınan toplam 500 kültür örneği incelenmiştir (Ortalama 5 kültür/hasta). Hastaların 70'si erkek (70%), 30'u (30%) kadın hasta olup yaş ortalaması 71,5 idi. Yüz hastanın 32'inde (%32) hiçbir örnekte üreme saptanmazken, 68 hastadan alınan (%68) örneklerden ise en az birinde olmak üzere toplam 118 numunede üreme oldu. Üreyen mayaların 104 tanesi Candida albicans, 10 tanesi Candida glabrata ve 4 tanesi Candida inconspicua olarak tanımlanmıştır. Onbir (%11) hastada KS≥ 3 ve KKİ ≥0,5, 12 (%12) hastada ise KS≥3 ve KKİ<0,5 saptandığı için toplam 23 (%23) hastaya flukonazol profilaksisi başlanmıştır. Takiplerinde kandidemi gelişen hasta olmamıştır.*

***Sonuç:** Bu bulgular, YBÜ'de takip edilen hastaların KKİ ve KS ile değerlendirilip riskli hastalarda profilaktik tedavi başlanmasının oluşabilecek fungal infeksiyonları engellemede etkili olabileceğini düşündürmektedir.*

***Anahtar Sözcükler:** Kandida kolonizasyon indeksi, kandida skoru, kandidemi insidansı, anti-fungal profilaksi*

INTRODUCTION

Candidas take fourth place as an infectious agent in intensive care units. Candida are found in the normal flora of the oropharynx and gastrointestinal tract. Many risk factors play a role in infections caused by candida. The most common among these risk factors is the patient's flora (1-3). Studies have shown that 90% of intensive care patients are colonized with Candida species (4). Invasive interventions in intensive care units, use of broad-spectrum antibiotics, advanced age, and immunosuppression or malignant diseases increase the incidence of candidemia (5, 6).

Candidemia is an important cause of mortality and morbidity in patients hospitalized in the intensive care unit (ICU). Therefore, various methods are used to identify high-risk patients and empirical anti-fungal therapy is recommended for these patients (3). Since the most important factor in the development of candidemia is the patient's flora, candida colonization should be accurately demonstrated. For this purpose, Candida colonization index (CCI) and Candida score (CS) scoring are recommended (7).

Empirical anti-fungal therapy is recommended according to the results of serological tests such as candida colonization index, candida score, and beta-glucan, especially in patients undergoing abdominal surgery and undergoing invasive intervention (8).

The aim of our study is to evaluate the effectiveness of the candida colonization index and candida score to prevent the development of invasive candidiasis in patients with risk factors for candidemia.

MATERIALS and METHODS

Ethics committee approval was received from Istinye University clinical research ethics committee on 23.06.2021, with decision number 2/2021.K-47. One hundred non-neutropenic adult patients aged 18-80 years old, hospitalized in the intensive care unit of our hospital, were included in the study. Patients with comorbidities at high risk of candidemia were included in the study. These risk factors were determined as central catheter application, total parenteral nutrition, malignancy, use of broad-spectrum antibiotics, and steroid use. Patients with at least 2 of these were included in the study. Patients with fewer

than two risk factors, patients younger than 18 years of age and older than 80 years of age, patients with a procalcitonin value>0.5, and growth detected in blood cultures were not included in the study. APACHE 2 and SOFA scores were used as scoring tools in all patients.

CCI is determined by dividing the number of anatomical regions sampled by the total number of samples taken (9). Candida score (CS), another scoring method, was determined by Leon et al. suggested by. CS is based on the scoring of 4 previously known independent risk factors (10). Sepsis was determined as 2 points, abdominal surgery 1 point, total parenteral nutrition 1 point and multifocal candida colonization 1 point and a value of ≥3 was accepted as a cut-off. In this way, the sensitivity was found to be 81% and the specificity to be 74%.

In the symposiums named "Advances in Antifungal Therapy" and "Transatlantic Controversies in the Management of Serious Fungal Infections" presented at the 11th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), it was recommended that surveillance cultures be made from five anatomical regions (11). Accordingly, in the intensive care unit of our hospital, swab samples are routinely taken from five main areas: mouth, nose, skin, perineum, and catheter, on the 7th day of each patient's hospitalization, to determine the CCI and CS (12-15). These samples are plated on SDA plates and the plates are incubated at 35 °C for 48 hours. The resulting yeast colonies are identified according to their microscopic appearance and biochemical properties. The CCI and CS of the patients are evaluated, and values of ≥3 for the CS and ≥0.5 for the CCI are accepted as the cut-off value.

In our ICU, prophylactic fluconazole treatment is started in patients who are found to be at risk. During the follow-up of all patients, it is monitored whether or not candida infection developed. Our study was conducted by retrospectively examining these samples.

Statistical Analysis

Data were analyzed by using SPSS version 20.0 for Windows. Results were given as percentages, mean and standard deviations, or median and ranges. Quantitative and qualitative variables were compared with Student's t-test and chi-squared (Pearson's or Fisher's exact) test, respectively. A P value of <0.05 was considered significant.

To calculate the CCI and CS, swab cultures were taken with sterile swabs from five areas of each patient's mouth, nose, skin, perineum and catheter, and then inoculated on SDA plates and incubated at 35°C for 48 hours. Yeast colonies formed on SDA were identified according to their microscopic and biochemical properties. The CCI and CS of the patients were evaluated, and values of ≥3 for the CS and ≥0.5 for the CCI were accepted as the cut-off value (10). Prophylactic fluconazole treatment was started in patients who were found to be at risk. During the follow-up of all patients, it was monitored whether or not candida infection developed.

RESULTS

Cultures taken from 100 adult patients followed in the intensive care unit between 01.06.2018 and 01.08.2021 were analyzed retrospectively. The APACHE 2 score of 100 patients was at least 14 and at most 25, and the average APACHE 2 score was 18.2. According to APACHE 2, the expected mortality rate was 29.13% and the actual mortality rate was 18.4%. The average SOFA score was found to be 2.2. Procalcitonin value was found to be <0.5 in all patients and there was no growth in blood cultures. In other words, there was no sepsis in the patients.

A total of 500 culture samples were taken from 100 non-neutropenic patients and analyzed (5 cultures/patient). There was no growth in any sample in 32 patients (32%), growth was detected in at least one of the samples taken from 68 patients (68%). Yeast colonies were detected in 118 (23.6%) of 500 samples. Of the 118 detected yeasts, 104 were identified as Candida albicans, 10 as Candida glabrata, and 4 as Candida inconspicua.

CCI and CS of the patients were evaluated. Values of ≥3 for CS and ≥0.5 for CCI were accepted as cut-off values (11).

In 11 patients (11%), CCI was higher than 0.5 and CS higher than 3. Although CCI was <0.5 in 12 patients, CS ≥3 was detected. The candida colonization indices and candida scores of all patients are given collectively (Table-1).

Prophylactic fluconazole treatment was given to 23 (23%) patients with CCI ≥0.5 or CS ≥3. The comparison of the CCI and CS cut-off values of the patients is also shown in Table-2.

During the follow-up, none of the patients developed candidemia.

Table-1. Collected results from patients.

Number of patients/Total number of cultures	CCI		CS	Fluconazole prophylaxis	Number of patients who developed IC
32/160	0/5	0	<3 (32 patients)	-	0
30/150	1/5=0.2	<0.5	<3 (30 patients)	-	0
27/135	2/5=0.4	<0.5	<3 (15 patients)	-	0
			>3 (12 patients)	+	0
10/50	3/5=0.6	>0.5	>3 (10 patients)	+	0
1/5	4/5=0.8	>0.5	>3 (1 patients)	+	0
0	5/5=1.0	0	0	-	0
100/500					

Candida Colonization Index (CCI): *Number of anatomical regions sampled / total number of samples taken*

Candida Score (CS): *Sepsis: 2 points, abdominal surgery: 1 point, total parenteral nutrition: 1 point, multifocal candida colonization: 1 point*

IC: Invasive Candidiasis

0.5 *is the cut-off value of CCI*

3 *is the cut-off value of CS.*

Table-2. Comparison of patients' CCI and CS cut-off values

	CS> 3		CS<3	
CCI> 0.5	11	patients	0	patient
CCI < 0.5	12	patients	77	patient

DISCUSSION

Candida are found in the flora of the gastrointestinal tract and oropharynx (2). Although there are many risk factors in infections caused by Candida species, it is known that the risk increases very much if there is Candida colonization in the endogenous flora (3, 16, 17). Candidemia occurs when Candida crosses the mucosal barrier and enters the blood. Therefore, it is recommended to start prophylactic antifungal therapy in patients with high colonization rates (18, 19).

In some studies, conducted in our country, it has been reported that candida colonization rates are high in intensive care units (20).

More than half of candidemia develop in ICUs. In a study conducted by Yapar et al., the incidence of candidemia in our country between 2000 and 2003 was found to be 0.24 per 1000 hospitalizations, and it was reported that 53% of these cases developed in intensive care units (2).

In a study by Çolak et al. (21), candida colonization was detected in 37 (92.5%) of 40 patients in the intensive care unit. In our study, candida colonization was detected in 68% of the patients.

In the EPIC II study, in which 1265 ICUs from 75 countries participated, 17% of nosocomial agents were found to be due to Candida, and the prevalence of candidemia was reported as 6.87 in 1000 ICU patients (6, 22).

In our study, we evaluated intensive care patients, the group in which candida infections are most common.

Delays in the diagnosis of candidemia and inadequate initial treatment are associated with high mortality (3). In a study, growth in blood cultures occurred after death in 41.2% of fatal candidemia cases (16). Therefore, early prophylactic anti-fungal therapy can be lifesaving in high-risk patients. Clinical scoring procedures and serological tests can be used to detect these high-risk patients (10).

In this approach, known as preemptive treatment, treatment is initiated if the CCI is ≥0.5, the CS is ≥3, or in the presence of fungal antigens such as 1-3-beta-D-glucan (23).

In our study, 1-3-beta-D-glucan and galactomannan antigens were not evaluated because they could not be studied in our hospital. Because serological tests such as 1-3 B D glucan, galactomannan and anti-mannan cannot be performed everywhere, it is not possible to study every patient because the results are late or expensive. Instead, it seems more appropriate in practice to study scoring systems such as CCI and CS because of their very low cost and quick results. In our study, we started prophylactic treatment in patients who were found to have CS ≥3 or CCI ≥0.5 by evaluating the CCI and CS of the patients.

In a study by Posteraro et al., they used the candida score due to the inability to perform serological tests such as beta-glucan and stated that it is an easy and effective method to be applied in patients (24).

Colonization means the risk of infection for many microorganisms (25). A CCI of ≥ 0.5 indicates a high risk of developing candidemia. The CCI reaches ≥0.5 on average 6 days before the development of candidemia. Therefore, CCI is valuable in identifying patients at risk for candidemia, initiating prophylactic anti-fungal therapy, and preventing the overuse of antifungals (26). In the CS, when the value of ≥3 is taken as a cut-off, its sensitivity was reported as 81% and specificity as 74%. It has been reported that the risk of candidemia increases 7.75 times when the CS is ≥3 (10).

In a prospective multicenter study to demonstrate the value of Candida score in distinguishing between colonization and candidemia in ICU patients, 1107 patients in 36 ICUs were included in the study. In this study, by evaluating the CCI and CS of the patients; CS ≥3, CCI was accepted as ≥0.5 cut-off value. Candida colonization was detected in 892 patients, and it was reported that ICU developed in 45 (13.8%) of 327 patients with CS ≥3 and 13 (2.3%) of 565 patients with a CS <3. The difference was found to be statistically significant. When evaluated according to CCI, it was reported that IC developed in 3.9% of those with a CCI of <0.5 and 8.7% of those with a CCI of ≥0.5. As a result, it has been reported that CS is better than CCI in predicting IC (27).

In our study, candida colonization was detected in 68% of 100 patients. Both CS ≥3 and CCI ≥0.5 were detected in 11 (11%) patients; Although CCI was <0.5, CS≥3 was detected in 12 (12%) patients and prophylactic treatment was given. This suggests that CS may be a more sensitive parameter than CCI.

We think that studying CS in all patients with CCI 2/5 will also be useful in deciding to start fluconazole prophylaxis. Invasive candidiasis did not develop in all of our patients. The lack of development of candidemia was attributed to the fact that the patients were not neutropenic and necessary hygienic precautions were taken and fluconazole treatment. The major limitations of our study are its retrospective nature and the absence of a control group that did not receive prophylactic treatment. There is a need for randomized controlled studies with a larger number of cases, including a control group.

CONCLUSION

As a result, it is possible to identify high-risk patients by evaluating with CS and CCI in non-neutropenic patients followed up in the intensive care unit, and to reduce the risk of candidemia and related mortality with prophylactic anti-fungal treatment in these patients. We think that CS is more sensitive in identifying more risky patients, and therefore, it may be more reliable to decide by calculating CS when starting prophylactic anti-fungal therapy. In addition, it is not possible to study every patient since serological tests such as 1-3 Beta D-glucan and anti-mannan, galactomannan cannot be performed everywhere, the results are delayed and expensive. Instead, it seems more appropriate in practice to study scoring systems such as CCI and KS in terms of very low cost and quick results.

Conflict of interest: The authors declare that they have no conflict of interest.

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Research Article / *Araştırma Makalesi*

Ege Journal of Medicine / *Ege Tıp Dergisi* 2024; 63 (3): 390-395

A novel challenge in elderly people with hemophilia: Cancer

Yaşlanan hemofilik bireylerde yeni bir problem: Kanser

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ABSTRACT

Aim: Our study is aim to evaluate the prevalence of cancer in patient with hemophilia (PWH) and describe the demographic and clinical features of hemophilia patients with cancer.

Materials and Methods: The data of PWH who were followed our center between 2010-2020 were analyzed retrospectively. Among them, patients who were diagnosed with any type of cancer were evaluated. Clinical characteristics such as hemophilia type (A and B) and severity (severe, moderate and mild), inhibitor status, factor replacement strategy (prophylaxis or on-demand) of patients with and without cancer were recorded.

Results: Totally, 447 patients with hemophilia A (n =355) and B (n =92) were included and median age was 40 years (range, 19-86). Fifteen (3.4%) patients had a history of cancer. There is no significant difference between patients with and without cancer about hemophilia type, hemophilia severity, factor replacement therapy and inhibitor status. Hemophilia patients with cancer were older than without cancer. Colorectal carcinoma (n=3) and lung cancer (n=3) were most common types of cancer among patients. Two patients infected with hepatitis C had virus related cancer, hepatocellular carcinoma. All the patients treated with adequate treatment modality for their malignancies and hemostasis was effectively established during the chemotherapy and/or radiotherapy and/or surgery.

Conclusion: Nowadays cancer is expected to be commonly seen in elderly PWH. The adequate treatment strategies like as general population and personalized comprehensive hemophilia care with factor replacement should be provided for them.

Keywords: Hemophilia, cancer, prevalence, comorbidity.

ÖZ

Amaç: *Çalışmamızda hemofilik bireylerde kanser prevalansının değerlendirilmesi ve kanserli hemofili hastalarının demografik ve klinik özelliklerinin tanımlanması amaçlanmıştır.*

Gereç ve Yöntem: *Merkezimizde 2010-2020 yılları arasında takip edilen hemofilik bireylerin verileri geriye dönük olarak analiz edildi. Bunlar arasında herhangi bir tipte kanser tanısı konulan hastalar ayrıca değerlendirildi. Kanser tanısı olan ve olmayan tüm hemofilik bireylerin hemofili tipi (A ve B) ve hastalık şiddeti (ciddi, orta ve hafif), inhibitör durumu ve faktör tedavisi stratejisi (profilaksi veya kanadıkça) gibi klinik özellikleri kaydedildi.*

Bulgular: *Ortanca yaşı 40 (aralık, 19-86) olan toplamda 447 hasta (Hemofili A =355 ve B =92) çalışmaya dahil edildi. On beş (%3,4) hastanın en az bir kanser tanısı vardı. Kanser tanısı olan ve olmayan hastalar hemofili tipi, hemofili şiddeti, faktör replasman tedavisi ve inhibitör durumu açısından karşılaştırıldığında hastalar arasında anlamlı bir fark yoktu.*

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Application date: 17.01.2024 Accepted: 06.03.2024

Kanser tanısı olan hemofili hastaları kanser olmayanlara göre daha yaşlıydı. Hastalarda en sık görülen kanser türleri kolorektal karsinom (n=3) ve akciğer kanseri (n=3) idi. Hepatit C ile enfekte olan iki hastada virüsle ilişkili kanser, hepatoselüler karsinom vardı. Tüm hastalar kanserleri için yeterli ve uygun olan tedavi yöntemleriyle tedavi edildi. Hastaların tamamında kemoterapi ve/veya radyoterapi ve/veya cerrahi sırasında hemostaz etkin bir şekilde sağlandı.

Sonuç: *Günümüzde, yaşlı hemofilik bireylerde kanser tanısının daha sık görülmesi beklenmektedir. Bu hastalara kişiselleştirilmiş kapsamlı hemofili bakımı ve faktör replasmanları ile birlikte genel popülasyona benzer şekilde uygun kanser tedavilerinin verilmesi sağlanmalıdır.*

Anahtar Sözcükler: *Hemofili, kanser, prevalans, komorbidite.*

INTRODUCTION

There has been significant improvement in life expectancy in patient with hemophilia (PWH) over the past few decades as a result of increased safety and accessibility of recombinant and plasma-derived coagulation factor products, highly effective antiviral medications, establishment of comprehensive hemophilia care centers and specialized treatment strategy (1, 2). Today, longevity is becoming the most important issue in PWH because of the age-related chronic diseases in addition to the comorbidities of hemophilia (arthropathy, complications of viral infections). Most common age-related comorbidities are cardiovascular diseases (such as coronary artery and cerebrovascular atherothrombotic disease), arrhythmia, heart failure, diabetes, chronic kidney disease, degenerative arthritis, dementia and cancer (3). Besides, there is still limited data about the prevalence and management of age-related comorbidities in PWH. The subject of malignancies in hemophilia is especially noteworthy due to the hypothesis of protective effect of impairment of coagulation factors from the progression of cancer (4). Activated coagulation factors can activate endothelial cells and/or platelets, leading to the release of many mediators including growth factors and proliferation of tumor cells. The tumor cell can evade the immune system because of the tumor cell-platelet-fibrin complex that is created by this mechanism. Additionally, the complex sticks to the endothelium in the vascular wall and leads to tumor-associated angiogenesis (5). Based on in vitro research, factor VIII deficiency and reduced thrombin activity inhibit angiogenesis and prevent metastases of solid tumors (6). However, currently there is no clinical evidence endorsing this hypothesis.

Between early 1970s and mid-1980s, the substitution of plasma-derived factors resulted in a significant increase in the spread of human immunodeficiency virus (HIV) and hepatitis C virus (HCV) infections (7). In this regard, virus-associated malignancies such as non-Hodgkin's lymphomas, Kaposi sarcoma and hepatocellular carcinomas (HCC) have been seen more common in PWH (8, 9). Either HCV infection alone and concomitant HCV and HIV infections increased risk of end-stage liver disease and HCC (10). After improvement of the effective viricidal techniques and development recombinant factor concentrates, the mortality rates because of virus-associated malignancies have been decreased significantly (11). Nevertheless, epidemiologic data about non-virus-associated malignancies in PWH is very limited and controversial. Therefore, we aim to determine the prevalence of cancer in patients with hemophilia who were followed in our center. Our secondary objective is to define our experience of cancer management in PWH.

MATERIAL and METHODS

Study Design

This was an observational retrospective study conducted with hemophilia patients treated at *European* Hemophilia Comprehensive *Care* Centre (EHCCC) certificated Ege Adults Hemophilia and Thrombosis Center. A total of 447 patients with a previous diagnosis of hemophilia A (*n* =355) and B (*n* =92) aged higher than 18 years were included. Clinical and socio-demographic information was collected from the hospital's electronic databases included hemophilia type and severity [hemophilia is classified into severe (<0.01 IU/ml FVIII or FIX), moderate (0.01–0.05 IU/ml FVIII or FIX) or mild (0.05–0.40 IU/ml FVIII or FIX) hemophilia], inhibitor status, viral infections [hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV)]. Type of cancer, the date of diagnosis and management

(chemotherapy, radiotherapy, or invasive surgery), hemophilia treatment strategy (prophylaxis and on-demand, both at diagnosis and during cancer management), bleeding complications, cancer progression or recurrence, and outcome (alive or dead) were documented for each case.

Statistical Analysis

Statistical data analysis was carried out using SPSS version 16.0 (2007, SPSS for Windows, SPSS Inc., Chicago, IL, USA) package. Exploratory analysis was carried out to describe the study population where categorical variables were summarized using frequency tables and continuous variables were summarized using measures of central tendency and dispersion such as mean ± standard deviation (SD) and median (range). Qualitative or categorical variables were described as frequencies and proportions. Chi-square test and Fisher's exact test were used to determine association between categorical variables. Mann–Whitney U test was used to analyze quantitative data with skewed distribution. A level of p-value < 0.05 was considered statistically significant.

Ethical Considerations

During the planning of the study, necessary permissions were obtained from a Ege University Department of Hematology where the study was carried out. The study was approved by Ege University, Clinical Research Ethics Committee (Date: 08.07.2021, Decision No: 21-7T/47). Written and verbal consent of the individuals included in the study was taken.

RESULTS

A total of 447 PWH (355 with hemophilia A and 92 with hemophilia B) were evaluated and the median age 40 (range 19-86) years. Severe hemophilia was seen in 51.9% (n=232) patients and moderate and mild hemophilia was seen in 29.3% (n=131) and %18.3 (n=32) patients, respectively. Most of the patients (64.7%, n=289) were on prophylactic factor replacement therapy. Inhibitor positivity was detected in 4.3% (n=19) patients. Among 447 patients, 15 (3.4%) PWH had a history of cancer. Comparison of the characteristics of patients with and without cancer were summarized in Table-1. There was no difference of hemophilia type, severity and factor replacement strategy between two groups. Nevertheless, hemophilia patients with cancer were significantly older than without cancer group [63 (range, 44-72) vs 40 (range, 19-86) years, respectively and p=0.002]. Inhibitor status was negative in all PWH with cancer group however the prevalence of inhibitor was 4.4% (19/432) in PWH without cancer.

Table-1. Characteristics of patients.

Variables	Total (n= 447, 100%)	Patients without cancer (n= 432, 96.6%)	Patients with cancer (n= 15, 3.4%)	P value
Age in years, median (range)	40 (19-86)	40 (19-86)	63 (44-72)	**0.002***
Hemophilia type				
Hemophilia A	355 (79.4)	343 (79.3)	12 (80)	0.62**
Hemophilia B	92 (20.6)	89 (20.6)	3 (20)	
Disease severity, n (%)				
Severe	232 (51.9)	223 (51.6)	9 (60)	0.77**
Moderate	131 (29.3)	128 (29.6)	3 (20)	
Mild	82 (18.3)	79 (18.3)	3 (20)	
Factor VIII treatment, n (%)				
Prophylactic treatment	289 (64.7)	281 (65)	8 (53.3)	0.48**
On-demand treatment	155 (34.7)	148 (34.3)	7 (46.7)	
Presence of inhibitor, n (%)				
Yes	19 (4.3)	19 (4.4)	0	0.51**
No	427 (95.5)	412 (95.4)	15 (100)	

*Mann–Whitney U test; **Chi-square test

Table-2. Clinical features and cancer types of hemophilia patients with cancer.

Case No	Age, years	Hemophilia type, severity	Factor therapy	Comorbidity	Viral serology	Cancer type	Treatment	Outcomes
1	63	B, Mild	On- demand	DM	Negative	Colorectal ca	Surgery CT	Alive
2	50	B, Mild	On- demand	None	Negative	Nasopharynx ca	Surgery CT	Alive
3	52	A, Severe	On- demand	None	Negative	Acute myeloid leukemia	CT	Alive
4	64	A, Severe	Prophylactic	Cirrhosis	HCV	HCC	None	Dead
5	51	A, Mild	On- demand	None	Negative	Colorectal ca	Surgery CT	Alive
6	67	A, Severe	Prophylactic	DM, Hypertension	Negative	Gastric ca	Surgery	Alive
7	67	A, Mild	On- demand	None	Negative	Lung ca brain metastasis	Surgery	Dead
8	71	A, Moderate	Prophylactic	DM, Hypertension	HCV	HCC	None	Alive
9	63	A, Severe	Prophylactic	None	Negative	Lung ca	CT/ RT	Alive
10	72	B, Moderate	On- demand	Hypertension	Negative	Prostate ca	Surgery	Alive
11	44	A, Severe	Prophylactic	None	Negative	Pancreas ca	CT	Alive
12	47	A, Severe	Prophylactic	None	HCV	Nasopharynx ca	CT/ RT	Alive
13	68	A, Mild	On- demand	None	Negative	Colorectal ca	Surgery CT/ RT	Alive
14	48	A, Severe	Prophylactic	None	HCV	Bone tumor	CT	Alive
15	60	A, Severe	On- demand	None	Negative	Lung ca	CT	Dead

CA, cancer; HA, hemophilia A; HB, hemophilia B; HCV, hepatitis C; CT, Chemotherapy; RT, Radiotherapy; DM, diabetes mellitus; HCC, hepatocellular carcinoma

Table-2 summarizes the characteristics of fifteen hemophilia patients with cancer. In patients with cancer group 12 patients were hemophilia and 3 were hemophilia B. When eight patients of 15 had severe disease, moderate and mild hemophilia were seen in 2 and 5 patients, respectively. Other than one patient, all patients with severe hemophilia were treated with prophylactic factor replacement 3 times in a week. In total, 7 patients with cancer were treated with prophylactic factor replacement therapy. Lung (n=3) and colorectal cancers (n=3) are the most common types of malignancy. However, four patients were HCV infected, only 2 patients had virus related cancer and both of them had end stage liver disease and HCC. The patients infected with HCV were on antiviral therapy. Nasopharynx carcinoma was seen in 2 patients and acute myeloid leukemia (AML), bone tumor, gastric, prostate and pancreas cancer were detected in one patient each. Diabetes and hypertension are commonly seen comorbidities in PWH with cancer group and they were seen in 4 patients each.

All patients received the same recommended treatment modality as patients who do not have coagulation disorder for their cancer**.** Other than 2 patients with HCC, all patients treated with chemotherapy and/or radiotherapy and/or surgery. The patient (number #3) with AML were diagnosed when he was 7 years old. The hemostasis was successfully controlled even he had severe thrombocytopenia during the induction and consolidation chemotherapy. Major surgery was performed in 7 patients and hemostasis was effectively established in every invasive procedure. Twenty percent of patients with cancer (n=3) were dead. One of them (number #4) had HCC and he died from liver failure. One patient (number #7) had cranial surgery for the resection of brain mass and diagnosed with metastasis of non-small cell lung cancer. Although he had a successful surgery without any bleeding complication, he died from sepsis 2 weeks after the operation. The other one (number #15) died because of pneumonia.

DISCUSSION

Hemophilia was thought to provide a barrier of cancer spread due to lower thrombin generation (4, 6) however, observational studies could not offer conclusive evidence that hemophilia patients had decreased incidence or mortality rates from non-virus-related cancers compared to general population (12-15). On the other hand, extended life expectancy in PWH, typical aging-related diseases such as lung, colon, and prostate cancer have become more prevalent and cause morbidity and death in this population (16). To our knowledge, this is the first and largest study which investigated the cancer prevalence in adult patients with hemophilia.

We reported that cancer prevalence is 3.4% in our study group. As expected, hemophilia patients with cancer were older than patients without cancer in our cohort (median age was 63 and 40 years, respectively). However, this prevalence rate is very low when compare to a previous study from Germany (17). It was reported that five times higher prevalence of cancer in PWH than in the age-matched general population (28% vs. 5.2%). In this study, patients who were at least 60 years of age at their last visit were included and the median age was 64 years (range, 60-85) (17). We thought that this finding is resulted from relatively younger median age of our cohort (median age was 40 years for all patients). Median age was higher in the studies from in industrialized countries since the expected life time for PWH is probably longer in developed countries. So far, increased incidence of virus-related malignancies such as HCC and non-Hodgkin's lymphoma due to HCV and/or HIV in PWH are well known. However, there have been few studies comparing PWH's incidence or prevalence of cancer to that of the general population, with inconsistent findings (12, 18, 19). It is anticipated that PWH will experience a rise in virally linked tumors, but not other cancer types, such as lymphoma and hepatocellular carcinoma. In comparison to controls, a study from Canada showed a significantly increased occurrence of malignancies in hemophilia (20). Additionally, Miesbach et al. reported PWH had a greater cancer prevalence than the overall population in both virus-related and non-virus-related cancers (14, 17). Lung and colorectal cancers were common cancer types in our study group like as the distribution of cancer type in general population. It is noteworthy that conclusions could not be made due to the small number of patients. Virus-related cancers were detected in only 2 patients and both of them had HCC.

The management of cancer in PWH is very complex because they are supposed to receive the same chemotherapy, examinations and invasive procedures as general population without hemophilia (21). All the patients in our study received the appropriate treatment for their cancer without any bleeding complication. Death from bleeding- related complications in hemophilia patients with cancer was not commonly reported in some of the studies so far (12, 22), somehow the survival rate was not longer than in general population with cancer (12). The cancer management of PWH should be individualized in order to balance the bleeding risk with the chemoradiotherapy, the severity of factor deficiency and/or bleeding phenotype and increased cancer related thromboembolism. Especially severe hemophilia patients should get factor prophylaxis during the cancer treatment due to increased bleeding risks other than factor deficiency such as thrombocytopenia and/or frequent invasive procedures (21). Prior to invasive operations such prostate biopsies and colonoscopies, patients should get factor replacement up to 80–100 U/dL, with a trough above 50 U/dL. Therefore, in order to enable early detection and management, older PWH should continue to get routine, age-appropriate screenings. For example, PSA screening, digital rectal examination and transrectal prostate biopsy are necessary for the diagnosis of prostate cancer and fecal occult blood testing and colonoscopy should be done for colorectal carcinoma.

Even though our study has some limitations. It is a retrospective analysis with a relatively small number of patients. We could not compare the prevalence rate of cancer with general population since we do not have any control group. Our study highlights that cancer is a growing problem in adult PWH. Besides, most substantial epidemiological studies document cancer mortality retrospectively but not incidence. Regarding potential links between cancer and hemophilia, surveillance of cancer occurrences and metastases appears to provide more accurate data than mortality rates. We need more prospective data to evaluate the relationship between hemophilia and cancer incidence.

CONCLUSION

The aging of PWH has revealed the need for more prospective research to help with the appropriate delivery of care to this unique population, as well as the necessity for data regarding their treatment and age-related comorbidities. There is still a debate about the incidence and prevalence rate of cancer in PWH comparing to general population. According to our research, PWH who are older have a higher risk of developing any type of cancer. Although longevity is one of the most important issues in PWH even in developing countries, any consensus and guidelines on the management of cancer in PWH have not been established yet.

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

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Araştırma Makalesi / *Research Article*

Ege Tıp Dergisi / *Ege Journal of Medicine* 2024; 63 (3): 396-403

Meme kanseri hücresi tanımlayan biyosensör

Breast cancer detecting biosensor

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ÖZ

Amaç: Bu çalışmada meme kanseri hücrelerini membran reseptörleri aracıyla tanımlayabilen yüksek hassasiyet ve özgüllükle çalışan hızlı bir tanı aracı oluşturmak hedeflendi. Kuvars kristal mikrodenge (QCM) sistemi hücrelerin reseptörlerine özgü ligantlarla işlevselleştirilerek etkin bir biyosensör geliştirmek amaçlandı.

Gereç ve Yöntem: Biyosensörler afinite prensibiyle çalışan biyoreseptör ve dönüştürücü bölümden oluşan hassas tanı araçlarıdır. Kuvars kristal mikrodenge sistemi kuvars kristal rezonatörünün frekansındaki minimal kütle artışlarına bağlı değişimi saptar. QCM çipi öncelikle hazırladığımız polimerik nanopartiküllerle kaplandı. Nanopartikül tabakasının üzerine transferrin, noç 4 ve her2/neu monoklonal antikoru gibi ligantlar bağlanarak yüzey işlevselleştirildi. Modifiye edilen QCM çip yüzeyinin kimyasal ve fiziksel özellikleri incelendi. Üçlü negatif olarak bilinen meme kanseri hücre hattı MDA-MB 231 ve SKBR3 ile kontrol fibroblast hücreleri L929, kültürde çoğaltılarak deneylere hazırlandı. Hücreler PBS içinde çip yüzeyinden geçirildi ve QCM sisteminde hücre tutunmasına bağlı olarak oluşan frekans değişimleri saptandı. Geliştirilen biyosensör sisteminin bağlanma kinetiği, hassasiyeti ve tekrar kullanılabilirliği belirlendi.

Bulgular: QCM çipi kaplamak için hazırlanan nanopartiküllerin çapı 73,22 nm ve polidispersitesi 0, 229 olarak bulundu ve yüzeyi homojen bir şekilde kapladıkları gözlendi. Transferrin, noç 4 ve her2/neu monoklonal antikoru ile işlevselleştirilen QCM'in saptama limiti 4-10 hücre/ml olarak saptandı. Bağlanmanın Langmuir tipinde olduğu hesaplandı.

Sonuç: Geliştirilen QCM temelli biyosensör meme kanseri hücrelerini reseptörleri aracılığıyla hızlı, hassas ve seçici biçimde tanımladı. Biyosensör tekrarlı kullanımda etkinliğini korudu. Bu hızlı tanı aracının klinik uygulamalarda yer alabileceği sonucuna varıldı.

Anahtar Sözcükler: Meme kanseri, biyosensör, QCM, reseptör.

ABSTRACT

Aim: *This study aims to develop a rapid system to detect breast cancer cells, which is highly sensitive and selective. An efficient biosensor is aimed to be formed by functionalizing quartz crystal microbalance (QCM) system with ligands those are specific for breast cancer cell membrane receptors.*

Materials and Methods: *Biosensors are sensitive diagnostic devices based on affinity principle are composed of a bioreceptor and a transducer. Quartz crystal microbalance (QCM) system detects the changes in the frequency of crystal resonator created by minimal changes in the mass. QCM chip was first covered with polymeric nanoparticles that we prepared. Its surface is functionalized by attaching ligands like transferrin, notch 4 and her2/neu monoclonal antibodies on the nanoparticle layer.*

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The chemical and physical features of modified QCM chip surface is analyzed. The human breast cancer cells MCF 7, MDA-MB 231, SKBR3 and control fibroblast cells L929 are prepared for experiments by growing in culture. Cells suspended in PBS are passed over the QCM surface and frequency changes resulting from cell binding are recorded. The binding kinetics, affinity and reusability of the biosensor is determined.

***Results**: The nanoparticles for coating the QCM chip had a diameter of 73, 22 nm and the polydispersity was 0, 229. It is observed that they covered the surface homogeneously. The detection limit of transferrin, notch 4 and her2/neu functionalized QCM was 4-10 cells/ml. Binding kinetics best fitted to Langmuir type binding.*

***Conclusion:** The QCM based biosensor detected breast cancer cells through their membrane receptor rapidly with high affinity and selectivity. The biosensor retained its efficiency in repeated usage. It is concluded that this rapid detection system may find a place in clinical applications.*

***Keywords:** Breast cancer, biosensor, QCM, receptor.*

Bu çalışma 20-22 Ekim 2023 tarihinde İzmirde düzenlenen 27. Ulusal Biyomedikal Bilim ve Teknoloji Sempozyumunda özet bildiri olarak sunulmuştur

GİRİŞ

Kanser günümüzdeki başlıca sağlık problemlerinden birisidir. Meme kanseri heterojen moleküler özellikleri olan ve kadınlarda en sık gözlenen kanser türüdür. Meme kanseri için kullanılan tanı yöntemleri pahalı, zaman alıcı ve bazan invaziv yapıdadır (1). Meme kanseri hücresinin moleküler özelliklerini saptayarak tanıya destek olacak hassas ve hızlı sistemlere gereksinim vardır (2). Biyosensörler meme kanserinin moleküler belirteçlerini etkin biçimde saptayabilecek aygıtlardır. Temel olarak biyoreseptör ve fizikokimyasal bir dönüştürücü olmak üzere iki bölümden oluşurlar. Dönüştürücü elektrokimyasal, optik, kalorimetrik veya kütle değişimlerine dayalı olabilir (3, 4). Kuvars kristal mikrodenge (QCM) sensörler piazoelektrik yapıda olup iki elektrot arasına yerleştirilmiş ince bir kuvars kristal içerirler. Özel çiplerinin yüzeyine nanogram düzeyinde bir kütle eklenmesiyle oluşan kuvars kristalin rezonansındaki değişimi saptayabilen çok hassas aygıtlardır. Bu sistemde etiketleme gerekmemekte ve opak bir çözelti içinde bile olsa hedef molekülü tanımlayabilmektedir (5, 6). QCM sistemi DNA, aptamer, enzim, antikor veya mikroorganizma gibi biyotanıma elemanı içeren çok çeşitli formlarda hazırlanabilmektedir (7). QCM gibi piazzoelektrik sensörlerin antijen-antikor veya ligant- reseptör etkileşimlerine olan duyarlılığı kanser hücrelerine karşı yüksek afinite oluşturmaktadır. Meme kanseri hücrelerinin normal eşdeğer hücrelerine göre farklı ya da fazlaca eksprese ettikleri reseptörleri vardır. Örneğin, demir gereksinimi artmış olan meme kanseri hücreleri membranlarında çokça transferrin reseptörü bulundururlar (8. Farklılaşma sonucunda meme hücrelerinde azalan noç 4 reseptörü de farklılaşma düzeyi gerilemiş olan meme kanseri hücrelerinde tanımlanan bir reseptördür (9). HER2/neu ise epidermal büyüme faktörü reseptörünün (EGFR) mutant formudur ve meme kanserlerinin yaklaşık dörtte birinde saptanmaktadır (10).

Bu çalışmada MDA-MB-231 ve SKBR3 insan meme kanseri hücrelerini her biri hücreleri tanımlamada aracı olmuş bu üç reseptörü birlikte hedefleyen etkin bir biyosensör hazırlanması ve etkinliğinin araştırılması amaçlanmıştır. MDA-MB 231 yüksek metaztaz niteliği olan ancak her2/neu eksprese etmeyen, SKBR3 ise bu reseptörü taşıyan meme kanseri hücreleridir. QCM çipinin yüzeyini hidrofilikleştirmek, protein bağlama özelliği sağlamak ve yüzey alanını genişletmek amacıyla p(HEMA) gibi polimerik nanopartiküllerle kaplanması planlanmıştır. QCM çipi nanopartikül kaplandıktan sonra transferrin, noç4 ve her2/neu antikorlarıyla işlevselleştirilerek etkin bir meme kanseri tanı aracı geliştirilmesi amaçlanmıştır.

GEREÇ ve YÖNTEM

Nanopartiküllerin Hazırlanması

Poli(HEMA) nanopartiküller iki çözelti karıştırılarak hazırlandı. Bunun için 93,7 mg polivinil alkol (mw:100, 000) 50 ml suda çözülerek hazırlanan sürekli faza 14,4 mg Sodyum Dodesil Sülfat (SDS) ve 11,7 mg NaHCO3 eklendi. Diğer çözelti de 50 miligram (mg) Polivinil Alkol (PVA), 100 mililitre (mL) iyonize edilmiş suda çözülüp içine 50 mg SDS, 0,45 mL Hidroksietil Metakrilat ile 1,05 mL Etilen

Glikol Dimetakrilat eklendi. İki çözelti karıştırılarak 50,000 rmp de 30 dakika santrifüj edilerek miniemülsiyon hazırlandı. Miniemülsiyona polimerizasyonu başlatmak için 0,44 mg/mL potasyum persülfat eklendi. Polimerizasyon reaktöründe (Radleys Carousel 6, Essex, UK), 600 rpm de 40°Cde 24 saat çalkalanarak bekletildi. Alkol ve distile su ile yıkamalardan sonra 15 dakika sonikasyonla partiküller elde edildi). Nanopartiküller sırasıyla %70'lik alkol, deiyonize su ve piranha çözeltisi ile yıkanıp 200 mmHg vakumlu fırında kurutulan altın QCM çipinin üzerine damlatılıp UV ışığında 37°C de 30 dakika bekletilerek bağlandı. QCM çipi Maxtek (New York, ABD) firmasından temin edildi.

Hücreler

Araştırmada kullanılan tüm hücreler Sigma Chem. Co., St. Louis, ABD'den temin edildi. HER2/neu reseptör negatif yüksek metastatik özellikteki MDA-MB 231 hücre hattı ve fare deri fibroblast hücreleri L929, %10 fetal sığır serumu, %1 L- Glutamin ve %1 penisilin-streptomisin içeren DMEM içinde %5 karbon dioksit içeren 37 °C deki etüvde üretildi. HER2/neu reseptör pozitif meme kanseri hücreleri SKBR3 (ATCC-HTB-30) ise aynı şartlarda McCoy's 5a besi yeri kullanılarak çoğaltıldı. Hücreler tripsin-EDTA çözeltisi ile toplanıp PBS içerisine alındı. Hücreler QCM çipi üzerinden PBS içinde geçirildi.

QCM çipinin işlevselleştirilmesi

Çipe ligantların bağlanması ile gerçekleştirildi. Bunun için, transferrin çözeltisi 10 mg/100 ml, noç 4 ve her2/neu antikorları ise 0, 01 μg/100 ml olarak hazırlandı. Karbodiimid (5mg/200 mL) ile karıştırıldı ve +4°C'de 24 saat 37°C de inkübe edildi. Daha sonra 0, 1 M NaCl uygulanan çip son olarak PBS (pH 7,4) ile yıkanıp kullanıma hazırlandı.

Çipin özelliklerinin belirlenmesi

Öncelikle nanopartiküllerin boyut ve hidrofilisite analizleri yapıldı. Bunun için Nano Zetasizer (NanoS, Malvern Instruments, London, UK)'da 1mL nanopartikül oda sıcaklığında 90°C ışık saçılımında incelendi. Ayrıca, atomik güç (AFM) mikroskobu (Orta Doğu Üniversitesi Merkezi Laboratuvarı, Ankara) ile nanopartiküller görüntülendi. İşlevselleştirilmiş çip yüzeyinin kalınlığı ellipsometride (Nanofilm EP3-Nulling) 532 nm dalga boyunda ve 62° yansıma açısında ölçüldü. Yüzey hidrofobisini belirlemek için temas açısı ölçümleri KRUSS DSA100 (Hamburg, Germany) cihazında yapıldı. Çip yüzeyinin kimyasal analizinde FTIR (Bruker IFS 66/S, FRA 106/S, Hyperion 1000, Ramanscope II FTIR) kullanıldı.

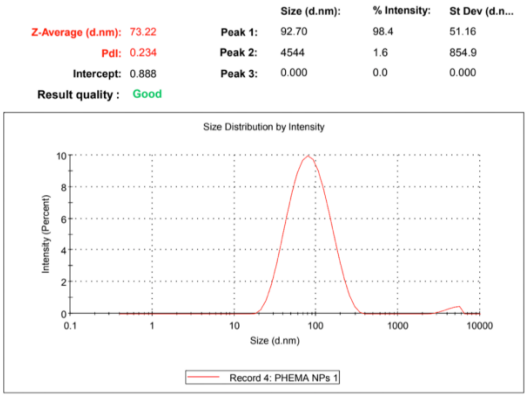
Hücrelerin QCM'de incelenmesi

Research Quartz Crystal Microbalance Monitor MAXTEK RQCM Inficon QCM aygıtında öncelikle çip 0,1 M NaCl ile 0,5 mL /min hızda Watson Marlow SCI Q 400 Peristaltic Pump peristaltik pompa ile yıkanarak bağlanmayan proteinler uzaklaştırıldı. Sonra, PBS (pH:7,4) ile yıkanan çip, hücre bağlama çalışmalarında kullanıldı. PBS içinde süspanse olmuş farklı sayıda hücre, çip yüzeyinden 0, 5 ml/min hızla geçirildi ve rezonans frekansındaki değişimler kaydedildi. Sonuçlar RQCM (Maxtek) software kullanarak hesaplandı. İşlevselleştirilmiş QCM kararlılık ve tekrar kullanılabilirlik açısından incelendi.

BULGULAR

Nanopartiküller ve çip yüzeyinin kaplanması:

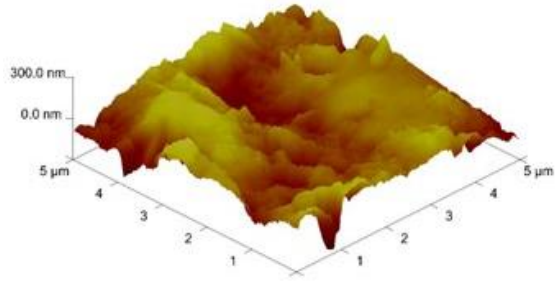
Zeta (Zζ) boyut analizi ile hesaplanan Z potansiyeli süspansiyon içerisindeki bir parçacık ile tanımlanan fiziksel bir özelliktir. Bu kavram özellikle emülsiyonların optimizasyonu için kullanılabilmektedir. Ölçüm için yeterli yoğunluğa sahip nanopartikül çözeltisi nanoboyut analizörüne ölçüm yapıldı. Nanopartikül boyutları ile ilgili ölçüm sonuçları Şekil-1'de gösterilmektedir. Zeta boyut analiziyle partiküllerin 73,22 nm ortalama çapta oldukları ve polidispersitenin 0,229 olduğu saptandı (Şekil-1).



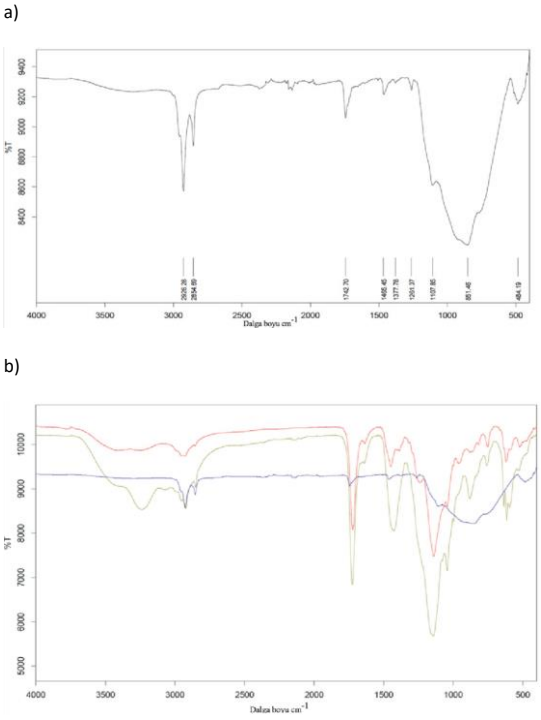
Şekil-1. Nanopartiküllerin boyutları.

Nanopartikül ile kaplanmış QCM çipindeki yüzey tabakasının kalınlığı elipsometrede incelendi. Kalınlık ölçümü 532 nanometre(nm) dalga boyunda ve 62°'lik geliş açısında gerçekleştirildi. Ölçümler; yüzey kaplanmamış ve polimerle kaplanmış QCM yüzeyi için yapıldı. Sonuçlar *Zeta-sizer* ölçümleri ile karşılaştırılarak her iki sonucun uyumlu olduğu belirlendi.

Nanopartiküllerin ölçümlerinden hesaplanan boyut 73,22 nm olduğu için 20 nm'lik boş çip üzerinde ise 87 nm'lik nanopartikül katmanı olabileceği düşünüldü. Sonuç olarak, nanopartiküllerin çip yüzeyinde homojen bir tabaka oluşturdukları saptandı. QCM çipinin kalınlık ölçümleri Tablo-1'de verilimiş ve yüzeyin atomik güç fotoğrafı ise Şekil-2'de sunulmaktadı. Nanopartiküllerin etkisiyle yüzeyde oluşan hidrofilisite temas açısı ölçümleriyle desteklendi (Tablo-1). Çip yüzeyinin kimyasal yapısı FTIR ile incelendi. Şekil-2'de görülen spectrum poliHEMA nanopartiküllerin 1722 cm^{-1} (C=O), 3417 cm^{-1} (-OH), 2925 cm^{-1} (CH_2-CH_3), ve 1139 cm^{-1} (C=C) de gözlenen tipik zirvelerini içermektedir (Şekil-3) (11).



Şekil-2. Nanopartikül kaplı QCM çip yüzeyinin atomik güç (AFM) mikroskop görüntüsü



Şekil-3. QCM çip yüzeyinin nanopartikül kaplama öncesi (a) ve ve sonrası (b) FTIR spektrumları.

İşlevselleştirilmiş QCM çipinde hücre absorpsiyonu:

Transferrin ve noç4 ile her2/neu antikoru bağlanmış ve QCM çipinin ilk aşamada 0, 1 M pH 7,4 PBS yüzeyinden peristaltik pompa yardımıyla geçirilerek dengelenmiştir. Hücreler 1 ml PBS içinde belirli sayıda süspanse edilerek çip yüzeyine uygulanmak üzere hazırlandı.

Tablo-1. Nanopartikül kaplı QCM çip yüzeyinin kalınlık değerleri ve temas açısı değerleri

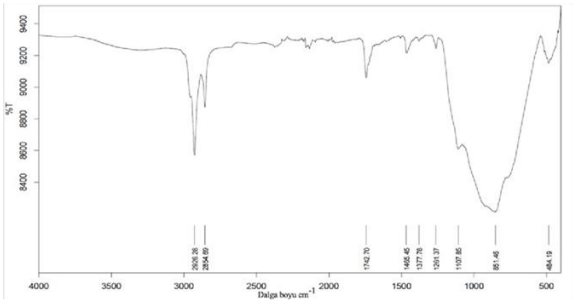
	Elipsometre (nm)	Temas Açısı (Boş QCM çip yüzeyi)
Boş QCM çip yüzeyi ölçümleri	20	**Su damlası** 85± 1.72
Nanopartikülle fonksiyonelleştirilmiş QCM çip yüzeyi ölçümleri	87	**Nanopartikül çözeltisi** 66.82± 5.71

Tablo-2. HER2, Notch4 ve transferin bağlı QCM çip için izoterm değerleri. QCM çipten geçirilen hücre sayısı süzpansiyondaki hücre/ml PBS olarak belirtilmiştir.

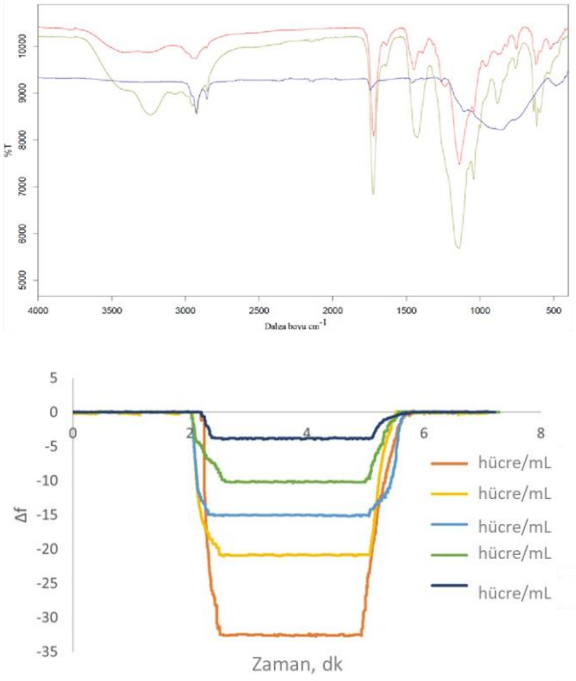
Langmuir		Freundlich		Langmuir-Freundlich	
Δ_{max}	0.63	Δ_{max}	4.806	Δ_{max}	0.84
K_A, hücre/ml	0.0076	1/n	0.22	1/n	0.05
K_D, ml/hücre	130.15	R^2	0.97	K_A, hücre/ml	0.012
R^2	0.99			K_D, ml/hücre	80.37
				R^2	0.98

Frekans dengelendikten sonra, QCM sisteminde SKBR3, MDA-MB 231 ve fibroblast hücrelerinin her biri sırayla 10-500 hücre/ml içeren süspansiyonlar halinde uygulandı. QCM çipe bağlanan SKBR3 hücrelerinin artan sayısına bağlı olarak rezonans frekansında oluşan değişimler belirlendi (Şekil-4).

a)



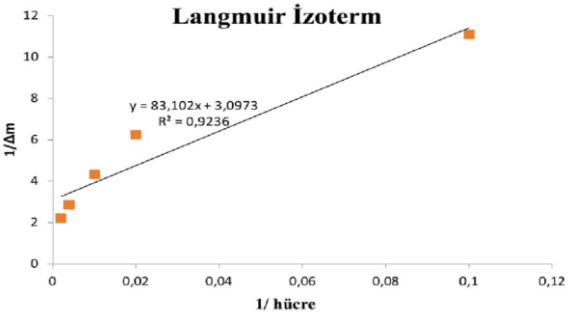
b)



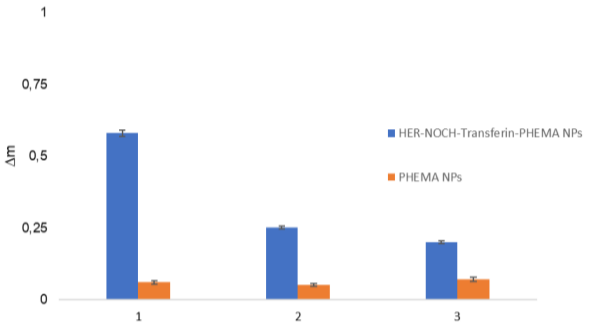
Şekil-4. Çipe bağlanan hücre sayısına bağlı frekans değişimleri

Hücreler sisteme verilirken çip yüzeyinde maksimum bağlanmanın olduğu, yani sistemin tepkisinin doğrusal forma yaklaştığı plato değerine 8 dakika geçtikten sonra ulaşıldı. Hücrelerin uzaklaştırılması için sisteme 1M Sodyum Klorür (NaCl) verildi ve tüm bağlama, çıkarma ve rejenerasyon aşamalarını içeren döngü 15 dakikada tamamlandı. Hücre sayısındaki artışa göre uygulamalardan elde edilen veriler minimum saptama değerinin (LOD) hesaplanmasında kullanıldı.

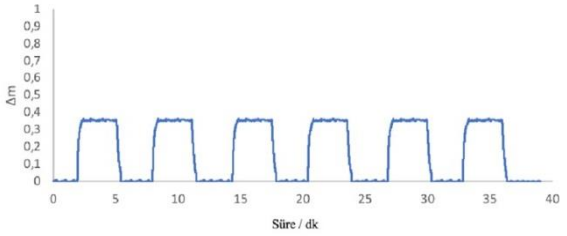
LOD değeri olarak belirtilen sensörün tespit etme limiti açıklanan çalışma koşullarında saptanan ama miktarının ölçülmesi mümkün olmayan en düşük analit derişimidir (12). LOD'nin yanı sıra sensörün tespit miktar sınırını belirten LOQ değeri ise açıklanan çalışma koşulları altında kabul edilebilir hassasiyet ve bu hassasiyetle belirlenen analitin en düşük derişimidir (13). Noç 4 ve her2/neu ile transferrin bağlı sistemin etkinliği Şekil-4'te gösterildiği gibi 4 hücre/ml LOQ değeri ise 10 hücre/ml olarak belirlenmiştir. Adsorpsiyona dayalı olan bu tip analizlerde denge reaksiyonundaki gibi belirli miktarda adsorplayıcı ile bir madde etkileştiğinde, absorbe edilen madde konsantrasyonu adsorban yüzeyde dengeye gelene kadar azalmaktadır. Adsorpsiyon Adsoppsiyon dendelendikten sonra çözeltideki adsorplanan madde miktarı sabitleşir. Adsorpsiyonda sıcaklık etken olduğu için sabit sıcaklıkta derişim başlıca etken olmaktadır. Sabit sıcaklıkta denge halinde olan çözeltiden kalan çözünen derişimine karşı birim adsoplayıcının miktarı grafik oluşturularak izotermler elde edildi (Tablo-2). Freundlich, Langmuir ve Langmuir-Freundlich izoterm modelleri olan bu kavramlar hücrelerin bağlanma şekillerini incelemekte kullanıldı. Şekil-5'te verilen doğruya göre her2/neu, noç4 ve transferrin ile bağlı olan QCM çip yüzeyi için regresyon katsayısının (R^2) en uyumlu olduğu model olarak Langmuir izoterm modeli olarak belirlendi. Bu model, adsorbanın, çözüneni bağlayabilen ve farklı eşdeğer kısımlardan oluşan ideale yakın bir katı yüzey olduğunu göstermektedir.



Şekil-5. QCM biyosensörde hücre bağlanma Langmuir izotermi



Şekil-6. Sadece nanopartikil kaplı ve işlevselleştirilmiş çiplerde hücre absorpsiyonu: 1:SKBR3, 2: MDA-MB 231, 3: Fibroblast hücreleri.



Şekil-7. QCM biyosensörün tekrarlı kullanımı.

Karşılaştırmalı analiz için gerçekleştirilen her2/neu, noç4 ve transferrin molekülünün bağlı olduğu QCM sensörde frekans değişimi (Δm) SKBR3 hücreleri için 0,58 bulundu ve yalnızca Polihidroksietilmetakrilat nanopartiküllerinin kaplı çip yüzeyi için ise bu değer 0,061 olarak bulundu. MDA-MB-231 hücreleri için ise bu değerler sırasıyla 0,251 ve 0,051, fibroblast hücreleri için de 0,201 ve 0,071'dir (Şekil-6). Seçicilik QCM sensör için önemli bir parametredir. Seçicilik özelliğini belirlemek için, QCM sensöre L929 hattı olan fare fibroblast hücreleri ve MDA-MB 231 insan meme kanseri hücreleri 10-500 hücre/ml yoğunlukta yarışmalı olarak uygulandı. Transferrinin hücre metabolizmasında rolü olmasından dolayı kanser ve kanser olmayan hücrelerin membranlarında transferrin reseptörü yer aldığı için, MDA-MB 231 hücreleri de sistemde tanınmıştır. QCM çipi; MDA-MB 231 hücrelerinde SKBR3 hücrelerine göre 1, 93 kat daha az seçicilik bulmuştur ancak yine de QCM çip tarafından MDA-MB 231 meme kanseri hücreleri tanınmıştır. Bunun nedeni, Notch4'ün MDA-MB 231 hücrelerinde aşırı eksprese edilmesi ve çip üzerinde bulunan Notch4 antikoru tarafından tanınıp yakalanmasıdır.

Tekrar kullanılabilirlik

Dengeleme bağlayıcı rejenerasyon döngüleri 10-500 tekrar edildi. HER2/neu2, Noç 4 ve transferrin bağlı QCM çipinin bağlama veriminde 5 döngü boyunca azalma saptanmadı. Sistemin tekrar kullanılabilirliği 500 hücre/ml olarak bulundu (Şekil-7).

TARTIŞMA

Kanser günümüzde önemli bir sağlık sorunu olmaya devam etmekte ve kanser mortalitesinde geç tanının etkisi bilinmekte ve erken tanının önemi vurgulanmaktadır. Bu nedenle, kullanışlı, hızlı, duyarlı ve invaziv olmayan tanı araçları geliştirilmesi ile ilgili araştırmalar devam etmektedir. Kanser biyobelirteçleri ile ilgili bilgiler bu alanda yarar sağlamaktadır. Tanı aracı olarak biyosensörlerin geliştirilmesi kanser hücrelerinin membranındaki belirteçleri hedefleyecek şekilde planlanabilmektedir. Sensörün tanımlayıcı biyoreseptör bölümünü oluşturan moleküllerin membran reseptörlerine olan afinitesi biyosensörün işleyişinde önemli rol oynamaktadır (14). Kanser hücrelerini tanıyabilecek çok çeşitli moleküller bu amaçla kullanılmaktadır. Bu moleküller arasında en etkin ve yaygın olarak seçilen ise yüksek afiniteli bağlanma kapasitesine sahip olan antikorlardır (15). Ayrıca, transferrin gibi hücre membranında reseptörü bulunan ligantlar da biyosensör tasarımına uygun moleküllerdir. Kanser hücreleri artan metabolizmalarına gereken demiri membranlarındaki transferrin reseptörlerini çoğaltarak sağlarlar. Kanser hücrelerinde Transferrin reseptörünün ekspresyonu artmış olduğu için poliferasyon, migrasyon, invazyon, apoptoz ve metastaz gibi özellikler demir ile ilişkili yolaklardan etkilenmektedir (16, 17). Transferrin reseptörlerinin normal hücrelere göre kanser hücrelerinde tümör evresine paralel olarak artış gösterdiği ve bu artışın kötü prognozla ilişkili olduğu ve kanser için bir biyobelirteç olabileceği belirtilmektedir (18, 19). Noç4 meme dokusunun normal gelişim sürecinde proliferasyon, farklılaşma ve apoptoz gibi çeşitli işlevlerde rol alması yanında meme hücrelerinde kanserleşme sürecinde etkin rol aldığı belirlenmiş bir reseptördür (20, 21). Meme kanseri hücrelerinde bulunan bu belirtecin radyasyona direnç ile de ilişkili olduğu gösterilmiştir (22). Meme kanseri hücrelerinin tiplendirilmesinde kullanılan her2/neu ise tirozin kinaz ailesinden olup epidermal büyüme faktörü reseptörünün mutant formudur.

Meme kanseri hücreleri dışında bazı kanser hücrelerinde de metastatik potansiyel ve anjiogenezle ilişkilendirilmiştir (23). Dolayısıyla, her2/neu reseptör ekspresyonu kötü prognozla da ilişkili bulunmaktadır (24).

Son yirmi yılda QCM sistemine dayalı biyosensör tasarım çalışmaları büyük ivme kazanmış ve sıvı ya da gaz ortamlardaki analitler yanında hücrelerin de tanınabileceği biyosensörler geliştirilmiştir. Bu sistemin etiketlemeye gerek duymadan çalışması, düşük maliyeti, hızı gibi sahip olduğu özellikler tanı için de kolaylıklar sağlamaktadır. Etiketlemeye gerek duyulmayan QCM gibi sistemler maliyeti ve gereken zamanı azalttıkları için oluşan tepkime veya bağlanmanın eş zamanlı ve dolaysız yürütülmesine olanak sağladıkları için etkin tanı araçları oluşturmaktadırlar (25, 26).

Bu çalışmada literatürde yer alan daha önce geliştirdiğimiz QCM-noç, QCM-transferrin ve her2/neu-QCM sistemlerinin (11, 27, 28) çoklu ligant şeklinde uygulanması ve böylece meme kanseri hücrelerini daha etkin tanımlanması amaçlandı. Bu özelliğiyle geliştirilen QCM sistemi 3 farklı reseptör-ligant etkileşiminin kullanıldığı ilk sistemdir. QCM çip yüzeyinin PHEMA ile modifikasyonunun ardından HER2, Notch4 ve transferrin bağlı çip yüzeylerinden eş zamanlı olarak hücreler geçirildi. Eş zamanlı hücre analizlerinde QCM çipin tespit limiti (LOD) değeri 4 hücre/ml olarak bulundu. LOD değeri 12 hücre olarak bulunan önceki noç 4 antikor temelli biyosensöre göre üçlü ligant bağlı QCM sensörün daha hassas olduğu belirlendi (9).

Literatürde QCM biyosensör ile ilgili kanser hücresi tanımlama çalışmaları bulunmaktadır. Örneğin, Poturnayová, A. ve arkadaşları her2/neu reseptörüne özgül bir aptasensör geliştirmişlerdir. Bu çalışmada hücre saptama limiti 550 hücre/ml olarak bulunmuştur. Çalışmada, akustik sensör yüzeyine biyotinlenmiş DNA aptamerleri bağlanmış olup yeterli hassasiyete ulaşılamamıştır (29. Poturnayová, A. ve arkadaşlarının akustik sensörde çip yüzeyinin kalın olması ve hücre katman kalınlığının kayma dalgası penetrasyon derinliğine kıyasla yüksek olması gibi sorunların düşük hassasitle ilişkili olabileceği belirtilmiştir. Diğer bir çalışmada da, Zhu, Y. ve arkadaşları SKBR3 hücrelerini de HER2 reseptörü aracılığıyla saptamışlardır. Bunun için, her2/neu antikorunu altın nanopartiküllere bağlamışlar ve cam yüzeyde karbon elektrot yüzeyinde sandviç yapısı oluşturmuşlardır . Bu sistemde LOD değeri 26 hücre/ml olarak bulunmuştur (5). Bu çalışmada geliştirilen çoklu tanıma elemanı içeren QCM biyosensör önceki çalışmalara göre yüksek afinite sağlamış olup tekrar kullanılabilir olması verimini arttırmaktadır.

SONUÇ

Literatürde yer alan çalışmalarda birçok meme kanserine ilişkin biyobelirteçler aracılığıyla sensörler veya yöntemler geliştirilse de QCM sisteminde üçlü ligant temelli biyosensör çalışması daha önce yapılmamıştır. Geliştirilen bu sistem QCM'nin hassasiyetini membran reseptörlerine özgü çoklu ligant yaklaşımıyla birleştirmiş ve iyi bir tanı aracı alternatifi oluşturmuştur.

Çıkar çatışması: Çıkar çatışması yoktur.

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Research Article / *Araştırma Makalesi*

Ege Journal of Medicine / *Ege Tıp Dergisi* 2024; 63 (3): 404-409

The prevalence of malignancy in nodular goiter in endemic and non-endemic regions

Endemik ve endemik olmayan bölgelerde nodüler guatrda malignite prevelansı

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ABSTRACT

Aim: The aim of the study was to investigate the incidence of thyroid cancer and the impact of uncontrolled iodine prophylaxis on the development of thyroid cancer in patients with single-nodular and multinodular goiter in endemic and non-endemic regions of the Republic of Azerbaijan.

Material and Methods: The study comprised 352 patients who underwent thyroid surgery for nodular goiter between 2015 and 2022. Patients were categorized into two groups and two subgroups based on endemic (n=126) and non-endemic (n=226) regions. Also according to the number of thyroid nodules 2 subgroups were defined as solitary nodular goiter (169) and multinodular goiter (183).

Results: Although there was a general decrease in the incidence of goiter disease in endemic regions due to iodine prophylaxis, an increase in the incidence of MNG was recorded (56.4%). Thyroid malignancy was detected in 20 patients (15.9%) in the endemic nodular goiter group. There were 11 (8.7%) patients with solitary nodular goiter (SNG) and 9 (7.1%) with multinodular goiter (MNG) (p≥0,05). Histopathologic evaluation revealed classical variant of papillary cancer in 19 of them (15.1%) and the follicular variant of papillary cancer in 1 (0.8%) patient. In non-endemic regions, thyroid cancer was detected in 47 cases (20.8%) with 23 (10.2%) MNG and 24 (10.6%) SNG patients. Follicular cancer was detected in 2 patients (0.7%), classical type papillary cancer in 36 patients (15.9%), microcarcinoma in 7 patients (3.1%) and medullary cancer in 2 patients (0.9%) (p≥0,05).

Conclusions: Based on the data collected from endemic regions, the uncontrolled use of iodine prophylaxis has led to a decrease in the prevalence of follicular thyroid cancer and an increase in the incidence of papillary thyroid carcinoma. Avoiding the use of iodized table salt under in regions by endemically monitored sanitary-epidemiological conditions can lead to a reduced likelihood of developing follicular cancer.

Keywords: Lodine deficiency, endemic goiter, multinodular goiter, singular nodular goiter, papillary thyroid carcinoma, follicular thyroid carcinoma.

ÖZ

Amaç: *Bu çalışmada, Azerbaycan'ın endemik ve endemik olmayan bölgelerinde nodüler guatrı olan hastalarda tiroid kanseri prevalansını ve kontrolsüz iyot profilaksisinin tiroid kanseri gelişimi üzerindeki etkisini incelemek amaçlanmıştır.*

Gereç ve Yöntem: *Çalışmaya 2015-2022 yılları arasında nodüler guatr nedeniyle opere edilen 352 hasta dahil edildi. Hastalar endemik (n=126) ve endemik olmayan (n=226) bölgelere göre iki gruba ve iki alt gruba ayrıldı. Ayrıca tiroitde olan nodül sayısına göre: soliter nodüler guatr (169) ve multinodüler guatr (183) sınıflandırıldı.*

Bulgular: *İyot profilaksisi nedeniyle endemik bölgelerde genel olarak guatr görülme sıklığında azalma olmasına rağmen, multinodüler guatr (MNG) görülme sıklığında artış (%56,4) kaydedildi. Endemik nodüler guatr hastalarının %15,9'unda tiroid malignitesi saptandı.*

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Application date: 14.11.2023 Accepted: 08.04.2024

Soliter nodüler guatr (SNG) hastalarının 11'inde (%8,7), MNG hastalarının dokuzunda (%7,1) malignite tanısı mevcuttu (p≥0,05). Histopatolojik sonuçları bu grubun 19'unda (%15,1) papiller kanserin klasik varyantı ve birinde (%0,8) foliküler varyantı şeklindeydi. Endemik olmayan bölgelerde tiroit kanseri saptanan 47 olgunun (%20,8), 23'ünde (%10,2) MNG, 24'ünde (%10,6) SNG alt grubu izlendi. Endemik olmayan bölgelerde iki hastada (%0,7) foliküler kanser, 36 hastada (%15,9) klasik tip papiller kanser, yedi hastada (%3,1) mikrokarsinom ve iki hastada (%0,9) medüller kanser saptandı (p≥0,05).

Sonuç: *Endemik bölgelerde elde ettiğimiz verilere göre kontrolsüz iyot profilaksisi kullanılması nedeniyle foliküler tiroit karsinom prevalansında azalma gözlemlenirken, papiller tiroit karsinom sıklığında artış olmuştur. Epidemiyolojik takip koşullarında iyotlu sofra tuzunun bölgelerin endemik özelliklerine göre kontrol altında tutulması foliküler kanser riskinin azalmasına yol açabilir.*

Anahtar Sözcükler: *İyot eksikliği, endemik guatr, multinodüler guatr, soliter nodüler guatr, papiller tiroit karsinom, foliküler tiroit karsinom.*

INTRODUCTION

Multinodular goiter (MNG) occurs in approximately 5-15% of the population, but in endemic regions with the increased use of neck ultrasound, this prevalence exceeds 50%. Some environmental factors, such as age, iodine intake, gender, lifestyle, and head and neck irradiation may affect the risk of thyroid nodules (1). Some studies have also shown that excessive iodine intake might contribute to nodule development, though this association remains unclear (2).

In the pathogenesis of endemic goiter, as a result of iodine deficiency, the increase in TSH level leads to follicular cell hyperplasia and eventually diffuse and nodular growth of the thyroid gland. Autonomous nodules also may develop later. Iodine deficiency, which affects approximately two billion people worldwide, is particularly problematic in some parts of the world with a natural lack of iodine and its unavailability, such as Africa, Southeast Asia, and the Western Pacific region. Azerbaijan is also iodine deficient, as indicated by a survey conducted in 1996 (in 35 districts and cities of the Azerbaijan Republic by WHO and with the participation of local specialists) revealed a 98-100% prevalence of endemic goiter among 8-14 years old school children, in endemic zones and 77-86% in non-endemic regions based on ultrasound examination. As an iodine deficient country, table salt is iodized in Azerbaijan. However, it has been determined that uncontrolled iodine prophylaxis in regions without iodine deficiency can lead to the development of goiter. This process, influenced by exogenously iodine intake, induces the pathogenesis of goiter, causing functional and morphological changes (3, 5). Several authors have suggested that thyroid tumors caused by iodine deficiency are due to chronic TSH overstimulation, possibly interacting with epidermal growth factor and insulin-like growth factor I (4).

Thyroid cancer incidence exhibits geographic variations depending on the environment and genetic factors (6). Prolonged state of TSH elevation due to thyroid dysfunction increases the risk of thyroid cancer. Recent studies indicate a higher malignancy rate in patients with multinodular goiter when compared to single nodular goiter. (7, 17, 22). The occurrence of malignancy in MNG cases ranges from 7-17% of cases. (17, 22). According to the 2009 State Statistics Committee, 16,986 patients with thyroid diseases were registered in the Republic of Azerbaijan, 53 of them were with thyroid neoplasms. Of those registered cases there were 3251 diffuse goiter, 2730 nodular goiter, 5443 thyrotoxicosis, 3363 hypothyroidism and 1060 thyroiditis (8). Preoperative diagnosis of thyroid cancer is mainly provided by minimally invasive fine needle aspiration biopsy (FNAB) examination (7, 9). FNAB, while provides crucial information for the biologic nature of the thyroid, has also been inaccurate in determining malignancy at around 25% in extensive series (10). The challenge arises when dealing with numerous nodules, as taking biopsies from each one is practically impossible in MNG cases, limiting the ability to assess the entire gland’s morphological structure (11). Additionally, even with negative cytological results from FNAB, the possibility of malignancy cannot be completely ruled out with complete accuracy (3, 6, 21). Furthermore, the relationship between MNG, malignancy, and iodine deficiency, has not been fully investigated (7). Papillary cancer malignancy is extensively documented in the relevant literature (7, 9, 15).

The aim of the current study was to investigate the incidence of thyroid cancer and the impact of uncontrolled iodine prophylaxis on thyroid cancer development of in patients with single nodular and multinodular goiter in both endemic and non-endemic regions of the Republic of Azerbaijan.

MATERIALS and METHODS

The study population included 352 patients (age 9-76) who underwent thyroid surgery for nodular goiter during 2015-2022 at the Educational Surgical Clinic of Azerbaijan Medical University and "Real hospital" (Baku city). There were 309 women (87.8%). Patients were divided into 2 groups based on the endemic and non-endemic regions, as well as into 2 subgroups according to the number of thyroid nodules as single-nodular and multinodular goiter. Thyroid functional status was assessed by routine physical examinations, thyroid hormones levels (TSH, fT3, fT4) and ultrasound examination of the gland, FNAB was performed when indicated. In patients with a suspicion of malignancy on FNAB, total thyroidectomy was performed by the same surgical team. Clinical details were reviewed considering age, gender, surgical method and histopathologic results. Patients with Graves' disease was excluded. All data were statistically analyzed (SPSS, version 20.0) using Pearson X2 (xi) and Student's t-test, respectively ($p \leq 0.05$).

RESULTS

Patients were divided into 2 groups: endemic (35.8%, n=126) and non-endemic (64.2%, n=226). Within the endemic group, subgroups were formed: SNG accounted for 43.6% (n=55) and MNG constituted 56.4% (n=71). While there were 17 men (13.5%), 86.5% were women (n=109). Clinically, 19.1% of cases (n=24) were hyperthyroid, 9.5% (n=12) were hypothyroid and 71.4% (n=90) were euthyroid. The FNAB was performed on all radiologically "suspicious" nodules and the results were classified by the Bethesda system (Table-1).

In non-endemic regions 26 patients (11.5%) were men, 200 (88.5%) were women. MNG constituted 49.5% (n=112), while SNG constituted 50.5% (n=114). Before surgery, 81% of patients (n=183) were euthyroid, 11.1% (n=25) were hyperthyroid, and 7.9% (n=18) were hypothyroid.

Results summarizing the analysis to determine the benign and malignant nature of 126 patients operated on in the endemic region are presented in Table-2.

According to the data presented in Table-2, malignant pathohistological result was 42.1% in nodes with a size of 20-29.9 mm, and 34.2% in benign ones. There was no statistical difference between the number of patients with benign and malignant forms according to the size of the nodules ($\chi 2emp=4.09$, $p \geq 0.05$).

In non-endemic regions, malignancy was most common in nodules with a 10-19.9 mm. The details of this group are presented in Table-3. Statistical analysis showed significant difference between the number of patients with benign and malignant nodules according to the size ($\chi 2emp=14.8$, $p \leq 0.05$).

In group I, thyroid cancer was detected in 20 patients (15.9%) during postoperative pathohistological examination. Eleven of them (8.7%) were on SNG patients, while 9 (7.1%) were in MNG disease. Of those cases 19 (15.1%) were classic papillary cancer, and only 1 (0.8%) patient had follicular variant of papillary cancer. There was no significant statistical difference between the groups (p=0.220) (Table-4).

Table-1. The result of FNAB in endemic and non-endemic regions.

Bethesda	Region					
	Non-endemic		Endemic		Total	
	n	%	n	%	n	%
1	0	0%	2	4%	2	1.6%
2	37	38.5%	15	30%	42	33.3%
3	4	4.2%	6	12%	10	7.9%
4	27	28.1%	13	26%	30	23.8%
5	21	21.9%	11	22%	32	25.4%
6	7	7.3%	3	6%	10	7.9%

In group II, pathohistological examinations revealed thyroid cancer in 47 patients (20.8%). Malignancy rate was similar in both SNG and MNG disease as 10.6% and 10.2% respectively in SNG and MNG cases. There were 2 cases with follicular and 36 cases with papillary cancers. (p=0,220) (Table-4).

Table-2. The size of the nodules and their pathohistological results in endemic regions.

Size of nodule	Patohistology					
	Benign		Malign		Total	
	n	%	n	%	n	%
< 10 mm	2	2.7%	2	10.5%	4	4.3%
10-19.9 mm	21	28.8%	6	31.6%	27	29.3%
20-29.9 mm	25	34.2%	8	42.1%	33	35.9%
≥ 30 mm	25	34.2%	3	15.8%	28	30.4%

Table-3. The size of the nodules and their pathohistological results in non-endemic regions.

Size of nodule	Pathohistology					
	Benign		Malign		Total	
	n	%	n	%	n	%
< 10 mm	3	1.7%	2	4.3%	5	2.2%
10-19.9 mm	39	21.8%	20	42.6%	59	26.1%
20-29.9 mm	57	31.8%	17	36.2%	74	32.7%
≥ 30 mm	80	44.7%	8	17%	88	38.9%

Table-4. The details of pathohistological diagnosis and incidence of thyroid cancer by regions.

Pathohistological diagnosis	Non- endemic	Endemic
Benign	179 (79.2%)	106 (84.1%)
Malign	47 (20.8%)	20 (15.9%)
Macro-microfollicular nodular, colloid-adenomatous goiter	146 (64.6%)	83 (65.9%)
Autoimmune thyroiditis, macro-microfollicular nodular, colloid-adenomatous goiter	5 (2.2%)	8 (6.3%)
Macro-microfollicular nodular, colloidal adenomatous tumor on Hashimoto's thyroiditis	16 (7.1 %)	9 (7.1%)
Hurtle cell (oncocytic) adenoma	3 (1.3 %)	0
Follicular adenoma	9 (4%)	6 (4.8%)
Follicular variant of papillary cancer	1 (0.4 %)	1 (0.8%)
Follicular cancer	2 (0.9%)	0
Microcarcinoma	7 (3.1%)	0
A classic variant of papillary cancer	35 (1.5%)	19 (15.1%)
Medullar cancer	2 (0.9%)	0

P>0.05, no significant statistical difference between the groups

DISCUSSION

The epidemiology of thyroid disease is profoundly influenced by various environmental factors, where even minor disparities in population iodine intake can significantly impact the occurrence and progression of thyroid abnormalities and diseases. Monitoring and regulating iodine intake, particularly in endemic regions, represent crucial elements of preventive medicine (8). Endemic

iodine deficiency is identified in various geographical regions worldwide, primarily affecting mountainous and foothill areas (10, 21). Iodine deficiency plays a pivotal role in goitrogenesis and ensuring adequate iodine intake through table salt or drinking water in endemic regions has proven to prevent deficiency and positively impact glandular diseases. However, implementing iodine prevention programs faces substantial technical and socio-economic challenges. Lack of information regarding certain causes of endemic goiter impedes the development of effective measures for disease eradication, even in regions with prolonged iodine addition to food sources (8).

In iodine-deficient regions like ours, iodized table salt is employed to combat iodine deficiency, eliminating it in the new generation but leading to notable morphological changes in already formed diffuse and nodular goiter in the older generation (8). Studies in iodine-deficient regions, such as one in Germany, revealed thyroid nodules or diffuse goiter in 33% of men and 32% of women during ultrasound examinations (12). Childhood head or neck radiation elevates malignancy risk and iodine deficiency in endemic regions has been associated with increased malignancy risk (13). Our study aligns with these findings, detecting thyroid cancer in 15.9% of patients in endemic regions, with a prevalence of papillary carcinoma (13, 14).

Global literature presents varying thyroid cancer prevalence among goiter surgery patients in endemic regions, ranging from 6.2% to 20.3% (15, 16). Our findings indicate thyroid cancer in 15.9% of patients from endemic regions, with a male/female ratio of 1/18. Non-endemic regions showed thyroid cancer in 20.8%, revealing diverse malignancy patterns compared to endemic areas, suggesting the interplay of genetic and environmental factors in malignancy development.

Follicular cancer frequency is reportedly elevated in thyroid cancer subtypes in iodine-deficient regions (17). Our study also supports this, detecting follicular cancer in 0.7% of cases in endemic areas. Long-term studies, like Harach et al.'s 40-year investigation in Argentina, highlight iodine intake's role in altering thyroid cancer subtypes (18). Recent literature emphasizes the importance of optimizing population iodine intake for preventive healthcare, linking iodine deficiency correction to a shift towards less malignant forms of thyroid cancer (19).

Additionally, complex relationships between iodine intake and thyroid tumors, including factors like delayed effects, dose thresholds, and interactions with ionizing radiation, have been reported (20.). Our findings align with these complexities, demonstrating thyroid carcinoma in 15.9% of endemic cases. Despite limitations, such as incomplete patient registration, our study provides valuable insights. The increased use of ultrasound facilitates early thyroid nodule detection. Although technology development and increased technological tool usage are common factors in the rise of papillary cancer, our study finds no statistical difference between groups, attributing this to the absence of specific radiation exposure.

In summary, our study reveals a decrease in single nodular goiter incidence and an increase in multinodular goiter due to iodine prophylaxis in endemic regions. Uncontrolled iodine prophylaxis, particularly in non-endemic regions, is associated with an increased goiter prevalence. While there's a decline in cancer incidence linked to iodine intake, there's a concurrent rise in papillary cancer. Endemic regions exhibit different malignancy patterns, with malignancy primarily found in single nodular goiter in non-endemic areas and multinodular goiter in endemic regions.

CONCLUSION

In conclusion, data from endemic regions demonstrate that uncontrolled iodine prophylaxis reduces follicular cancer prevalence but increases papillary carcinoma incidence. Restricting iodized table salt use, under controlled sanitary-epidemiological conditions in endemic regions, may decrease follicular cancer risk.

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

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Research Article / *Araştırma Makalesi*

Ege Journal of Medicine / *Ege Tıp Dergisi* 2024; 63 (3): 410-421

Aurora B kinase inhibition intensifies cisplatin cytotoxicity in MCF7 breast cancer cells

Aurora B kinaz inhibisyonu MCF7 meme kanseri hücrelerinde sisplatin sitotoksisitesini yoğunlaştırır

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ABSTRACT

Aim: Cancer, a complex and multifaceted group of diseases, poses a formidable challenge to global health. Characterised by uncontrolled cell growth and proliferation, it manifests in diverse forms, each with unique biological traits. Comprehending the intricate landscape of cancer biology stands as a fundamental cornerstone in the pursuit of tailored therapeutic interventions. This research aimed to explore the impact of inhibiting Aurora B kinase with BI-831266 on the anticancer efficacy of cisplatin in MCF7 cells, contributing to our understanding of potential treatment strategies.

Materials and Methods: Good Cell Culture Practices were conducted in this research, where MCF7 human breast cancer cells were used in order to assess the therapeutic potential of the BI-831266 and cisplatin combination. Regarding functional experiments, we employed in vitro cell proliferation assay, 2D clonogenic survival assay, 3D colony formation assay and wound-healing assay. To elucidate the molecular mechanism underlying the observed functional outcomes, SDS-PAGE and Western blotting experiments were additionally conducted.

Results: Our findings uncovered a synergistic interaction between inhibiting Aurora B kinase and treating MCF7 cancer cells with cisplatin. The combined treatment significantly increased cisplatin's cytotoxicity, hindered cancer cell migration, and influenced apoptotic pathways, as it is evident from changes in key protein expressions.

Conclusion: Our study underscores the importance of directing focus towards Aurora B kinase to amplify therapeutic outcomes of cisplatin in MCF7 breast cancer cells. This research offers valuable insights into potential combination therapies, paving the way for a more efficacious and precisely targeted approach to breast cancer treatment.

Keywords: Aurora B kinase inhibition, cisplatin, breast cancer.

ÖZ

Amaç: *Karmaşık ve çok yönlü bir hastalık grubu olan kanser, küresel sağlık açısından zorlu bir sorun teşkil etmektedir. Kontrolsüz hücre büyümesi ve çoğalması ile karakterize edilen bu hastalık, her biri benzersiz biyolojik özelliklere sahip olan çeşitli formlarda kendini göstermektedir. Kanser biyolojisinin karmaşıklığının anlaşılması, hedefe yönelik terapötik müdahalelerin geliştirilmesi için önemlidir. Bu araştırma, Aurora B kinazın BI-831266 ile inhibe edilmesinin, MCF7 hücrelerinde sisplatinin anti-tümör etkinliği üzerindeki etkisini araştırmayı ve potansiyel tedavi stratejilerini anlamamıza katkıda bulunmayı amaçlamaktadır.*

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Application date: 10.01.2024 Accepted: 29.04.2024

Gereç ve Yöntem: *BI-831266 ve sisplatin kombinasyonunun terapötik potansiyelini değerlendirmek amacıyla MCF7 insan meme kanseri hücrelerinin kullanıldığı bu araştırmada İyi Hücre Kültürü Uygulamaları gerçekleştirilmiştir. Fonksiyonel deneylerle ilgili olarak, in vitro hücre proliferasyon analizi, 2D klonojenik sağkalım analizi, 3D koloni formasyon analizi ve yara iyileşme analizi uygulandı. Gözlemlenen fonksiyonel sonuçların altında yatan moleküler mekanizmayı açıklamak için ayrıca SDS-PAGE ve Western blot deneyleri gerçekleştirildi.*

Bulgular: *Bulgularımız, MCF7 kanser hücrelerinde Aurora B kinazın inhibe edilmesi ile sisplatin tedavisi arasında sinerjistik bir etkileşimi ortaya çıkardı. Kombinasyon tedavisinin, önemli protein ifadelerindeki değişikliklerden de anlaşılacağı üzere sisplatinin sitotoksisitesini önemli ölçüde arttırdığı, kanser hücresi göçünü engellediği ve apoptotik yolakları etkinleştirdiği belirlendi.*

Sonuç: *Araştırmamız, MCF7 meme kanseri hücrelerinde sisplatinin terapötik yanıtını arttırmak için Aurora B kinaz aktivitesini hedeflemenin önemini vurgulamaktadır. Çalışmamız, meme kanseri tedavisinde daha etkili ve hedefe yönelik bir yaklaşım sunarak potansiyel kombinasyon tedavilerine değerli katkılar sağlamaktadır.*

Anahtar Sözcükler: *Aurora B kinaz inhibisyonu, sisplatin, meme kanseri.*

INTRODUCTION

Breast cancer ranks as the most frequently diagnosed cancer among women and stands as the leading cause of cancer-related mortality for women on a global scale. Its multifaceted nature necessitates a comprehensive understanding of genetic, environmental, and lifestyle factors influencing its onset and progression (1–3).

Cisplatin is a widely used platinum-based chemotherapy drug in the treatment of various cancers, including testicular, ovarian, bladder, and lung cancers. Its effectiveness stems from its ability to interfere with the DNA replication process, ultimately inducing cell death (4, 5). While it is a well-established and effective treatment for various solid tumours, such as ovarian and testicular cancers, information about its role in breast cancer has been more limited. However, the use of cisplatin in breast cancer remains an area of ongoing investigation, and its inclusion in treatment regimens is not yet considered as a standard application (6, 7).

Human Aurora kinases (Aurora A, B and C) are a family of serine/threonine protein kinases which have crucial roles in the regulation of cell cycle and the maintenance of genomic stability (8–10). Of those, Aurora kinase B is a key factor of the chromosomal passenger complex (CPC), playing a pivotal role in cytokinesis and chromosome condensation (11, 12). Dysregulation of Aurora kinases has been implicated in various cancers, making them attractive targets for anticancer therapy. Inhibition of Aurora kinases disrupts the cell cycle and induces mitotic defects, ultimately leading to cell death (13–15). Several Aurora kinase-targeted small molecule inhibitors are currently under investigation in preclinical and clinical studies (16), holding promise for the development of novel anti-neoplastic therapies with a focus on disrupting aberrant cell division in cancer cells.

In our study, we identified a synergistic interaction between Aurora B kinase inhibition and cisplatin treatment in MCF7 cancer cells. The inhibition of Aurora B kinase markedly enhanced the cytotoxic effect of cisplatin.. We further revealed that the combined inhibition impaired the migratory capacity of MCF7 cells, suggesting a dual effect of enhancing cisplatin cytotoxicity and impeding cancer cell migration. Additionally, the assessment of apoptotic markers showed significant changes in the expression levels of fundamental proteins, such as cleaved-PARP (cPARP), indicating potential modulation of apoptotic pathways by the combination treatment. These outcomes underscore the significance of targeting Aurora B kinase alongside cisplatin in terms of augmenting therapeutic responses in breast cancer.

MATERIALS AND METHODS

Chemicals

BI-831266 was kindly provided by Boehringer Ingelheim via its open innovation platform opnMe, available at https://opnme.com. Crystal violet and dimethyl sulfoxide (DMSO) were bought from Sigma-Aldrich (#c6158 and #D2438, respectively). Cisplatin was obtained from Santa Cruz (#sc-200896). Primary antibodies used in this study were acquired from various suppliers: anti-cPARP was obtained from Cell Signaling Technology (#9541S), anti-p53 was obtained from Santa Cruz (#sc-126); anti-BCL2 and anti-

proCas3 were obtained from St John's Laboratory (#STJ96943 and #STJ97448, respectively). The HRP(horseradish peroxidase)-linked anti-mouse and anti-rabbit secondary antibodies were sourced from ThermoFisher (#31460 and #32430, respectively). UltraPure™ Low Melting Point Agarose was purchased from ThermoFisher (Invitrogen - 16520050).

Mammalian cell culture techniques

DMEM, FBS, Trypsin-EDTA (0.25%) and dPBS were obtained from ThermoFisher. The antibiotic solution containing penicillin, streptomycin, and amphotericin B was bought from Capricorn (#AAS-B) Dr Alexander Hergovich (Evotec, France) kindly provided human breast cancer cell line MCF7. Unless otherwise stated, MCF7 cells were maintained in DMEM supplemented with 10% FBS. and grown in humidity-saturated incubators at 37°C supplied with 5% CO2. DMSO was used to prepare BI-831266 stock solutions stored at -80°C.

Crystal violet cell proliferation assay

Cells were seeded into 60-mm Petri dishes, treated, and grown for 24 hours before further treatment. At 10 days after seeding, cells were fixed in methanol-acidic acid solution (3:1) for 5 minutes and stained with crystal violet solution (0.5% w/v) for 15 minutes. The plates were gently washed with distilled water to eliminate surplus crystal violet and left to air-dry thoroughly. The percentage of cell proliferation was calculated according to densities of the stained cells acquired by the ImageJ program (NIH) and the data was analysed by comparing experimental groups with control conditions.

Clonogenic survival assays

Clonogenic survival experiment was carried out as indicated in (17). In brief, 1500 MCF7 cells in the exponential phase were seeded into 60-mm Petri dishes and incubated for 24 hours before being subjected to BI831266 treatment with or without cisplatin combination for an additional 24 hours. After 7-12 days incubation, colonies were initially subjected to the methanol and acidic acid (3:1) fixation, then stained with crystal violet solution (0.5% w/v). Colonies were visualised and counted by using an inverted microscope, and a cluster of at least 50 cells was defined as a colony. Plating efficiencies and survival fractions were calculated as described in (18). Three independent experiments were conducted for all clonogenic survival experiments.

Anchorage-independent colony formation assays

The anchorage-independent colony formation assay (aka soft agar) was conducted to investigate the colony-forming ability of MCF7 cells in a three-dimensional environment as described in (19) with some modifications. A 1.2% agarose solution was prepared in complete cell culture medium and 2 mL of the agarose solution was transferred into each well of the culture dish. After allowing the agarose to solidify at 4°C for 30 minutes, 2.5×10^3 cells were mixed with 0.6% agarose solution in 1:1 dilution in order to achieve a final concentration of 0.3% agarose, which was then added on top of the solidified base agar layer. Cells were incubated for at least 3 weeks, colony formation was monitored regularly and fresh medium was added every 3-4 days. Ultimately, colonies underwent staining using a 0.5% w/v crystal violet solution and were subsequently scanned.

Wound-healing assays

The wound-healing assay, a well-established two-dimensional technique, was employed to study collective migration of MCF7 cells, following the procedure outlined in reference (20). Logarithmically growing cells (4×10^5) were seeded in 6-well culture plates. After 24 hours, a straight-line scratch was created in the cell monolayer, and subsequent treatments were applied for 24 hours. Images of the wound area were captured at time points (0th, 6th, 12th, and 24th hours) using an inverted microscope (Motic-AE200). Image J software (NIH) analysed the images, and wound closure was evaluated by comparing 0 and 24-hour images, normalised to the control set as 1. Three independent experiments were conducted for all wound-healing assays.

Western blotting experiments

Western blot analysis, following the method described in reference (17), was conducted as follows: cell pellets were suspended in standard lysis buffer (SLB), incubated on ice for at least 60 minutes, and then centrifuged. The resulting soluble protein fractions were mixed with Laemmli SDS sample buffer and subjected to five-minute-heat at 95°C. 12% SDS-PAGE was used to separate proteins, which were then transferred to a PVDF membrane, and blocked with 5% skim milk in TBS-T. The membranes were then incubated with specific antibodies overnight, followed by probing with HRP-conjugated secondary antibodies and exposure to ECL substrates for chemiluminescent detection. Densitometry analysis was performed using the ImageJ program (NIH), and three

technical replicates were conducted for all Western blotting experiments. SLB: 20 mM Tris-HCl, 150 mM NaCl, 10% glycerol, 1% NP-40, 0.5 mM EGTA, 20 mM beta-glycerophosphate, 5 mM EDTA, 1 mM Na3VO4, 50 mM NaF, 0.5 mM PMSF, 1 mM DTT, 1 mM benzamidine, 1 mM leupeptin, pH 8.0; TBT-T: 50 mM Tris, 150 mM NaCl, 0.5% Tween-20, pH 7.5.

Statistical analysis

GraphPad Prism software (GraphPad, CA, USA) was used to generate graphics and study statistical analyses and results were plotted using the means ± SEM. Statistical significance of mean differences was assessed through a one-tailed unpaired Student's t-test. Differences were considered statistically significant when p-values were below 0.05 (*), 0.01 (**), 0.001 (***) or 0.0001 (****) for all experiments.

RESULTS

BI-831266 inhibits the proliferation of MCF7 cancer cells

In our study, we initially assessed the impact of BI-831266 on the proliferation of MCF7 breast cancer cells, widely employed in breast cancer research (21). Notably, BI-831266 suppressed cell proliferation, causing nearly 50% eradication at a concentration of 10 nM (Figure-1A, B). Subsequently, we investigated the IC50 values for BI-831266 and cisplatin in terms of clonogenic survival in MCF7 cells. Both compounds significantly inhibited clonogenic survival, with IC50 values of 7.67 nM for BI-831266 and 2.89 µg/mL for cisplatin (Figure-2A, B and Figure-3A, B). Overall, these findings demonstrate the anticancer effect of BI-831266 in MCF7 cells.

cancer cell survival, primarily through apoptosis.

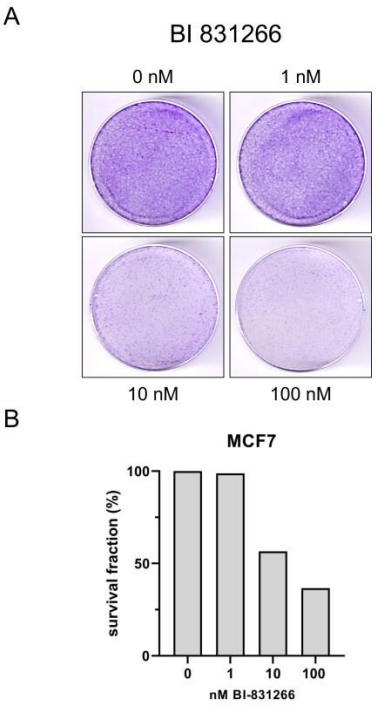

Figure-1. Treatment with BI-831266 restricts the proliferation of MCF7 cancer cells. (A) MCF7 cells were seeded into 60-mm petri dishes and treated with BI-831266 (0 - 1 - 10 - 100 nM) for 72 h. After 10 days of seeding, cells were fixed using a methanol-acidic acid solution and then subjected to staining with a 0.5% w/v crystal violet solution. (B) The ImageJ program (NIH) was utilised to determine the percentage of cell proliferation based on the densities of the stained cells, and the data were analysed by comparing experimental groups with control conditions.

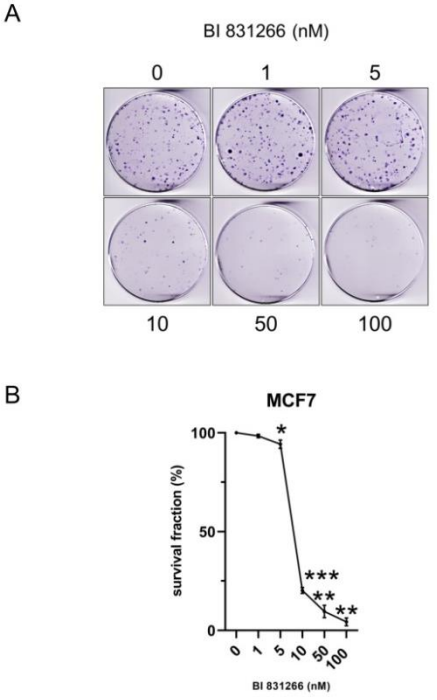

Figure-2. BI-831266 treatment impedes the clonogenic survival of MCF7 cancer cells. (A) A consistent number of MCF7 cells were seeded in 60-mm petri dishes and allowed to adhere for 24 hours before undergoing treatment with 0 - 1 - 5 - 10 - 50 - 100 nM BI831266 for 24 hours. Following an incubation period of 7-12 days, colonies were fixed with a methanol and acidic acid solution and subsequently stained with 0.5% w/v crystal violet. (B) Colony visualization and counting were performed using an inverted microscope, defining a cluster of at least 50 cells as a colony. Plating efficiencies and survival fractions were calculated according to the method outlined in reference (18) (n=3).

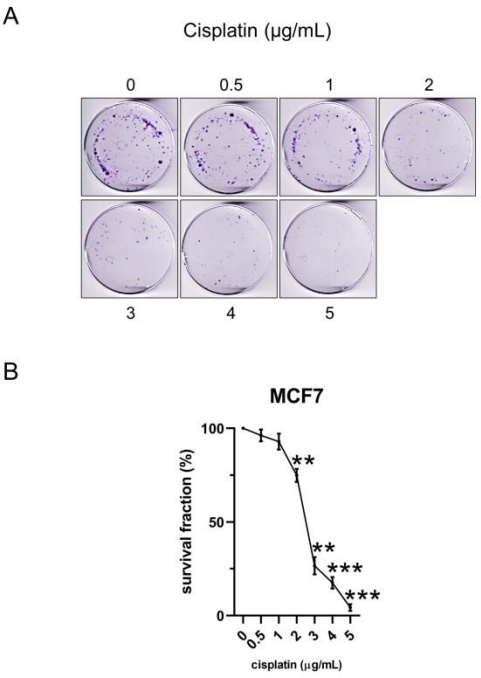

Figure-3. Cisplatin treatment inhibits the clonogenic survival of MCF7 cancer cells. (A) A consistent number of MCF7 cells were seeded in 60-mm petri dishes and allowed to adhere for 24 hours before undergoing treatment with 0 - 0.5 - 1 - 2 - 3 - 4 - 5 µg/mL cisplatin for 1 hour. Following an incubation period of 7-12 days, colonies were fixed with a methanol and acidic acid solution and subsequently stained with 0.5% w/v crystal violet.

(B) Colony visualization and counting were performed using an inverted microscope, defining a cluster of at least 50 cells as a colony. Plating efficiencies and survival fractions were calculated according to the method outlined in reference (18) (n=3).

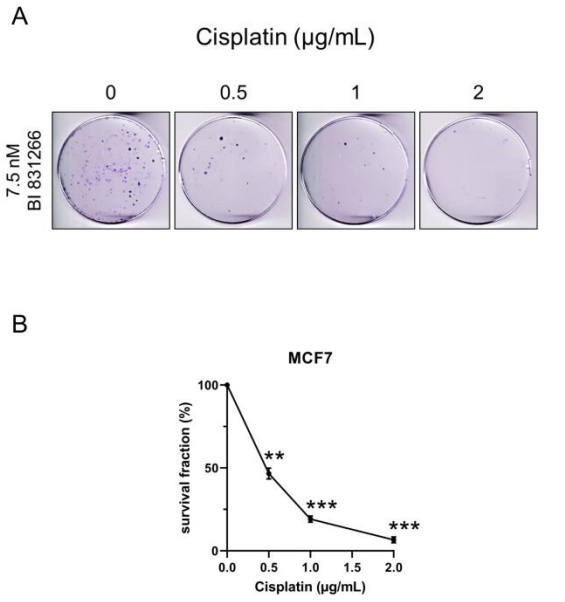

Figure-4. BI-831266 treatment potentiates the cytotoxicity of cisplatin in MCF7 cancer cells. (A) A consistent number of MCF7 cells were seeded in 60-mm petri dishes and allowed to adhere for 24 hours before undergoing treatment with 7.5 nM BI-831266 for 23 hours then combined with 0 - 0.5 - 1 - 1.5 - 2 µg/mL cisplatin for 1 hour. Following an incubation period of 7-12 days, colonies were fixed with a methanol and acidic acid solution and subsequently stained with 0.5% w/v crystal violet.

(B) Colony visualization and counting were performed using an inverted microscope, defining a cluster of at least 50 cells as a colony. Plating efficiencies and survival fractions were calculated according to the method outlined in reference (18) (n=3).

BI-831266 enhances the cytotoxic effects of cisplatin in MCF7 cancer cells

In our combination experiments, we systematically administered fixed or escalating doses of BI-831266 followed by fixed or escalating doses of cisplatin to MCF7 cells. Employing this systematic approach (22) allowed for a comprehensive exploration of potential synergistic or antagonistic interactions between the drugs. Initially, cells were treated with 7.5 nM BI 831266, a concentration corresponding to its IC50 value, for 23 hours, combined with escalating doses of cisplatin (0, 0.5, 1, and 2 µg/mL) for an additional one-hour period. The combined administration of BI-831266 with cisplatin significantly impeded cancer cell survival (Figure-4A), with 7.5 nM BI-831266 in combination with 1 and 2 µg/mL cisplatin eradicating over 75% and 90% of cells, respectively (Figure-4B).

Employing an alternate method, cells were exposed to escalating doses of BI-831266 (0, 1, 5, and 10 nM) for 23 hours, alongside a one-hour treatment with 1.5 µg/mL cisplatin. While results were not as pronounced as prior, BI-831266 significantly influenced cisplatin response in cancer cells (Figure-5A, B). Notably, treatment sequence, concentrations, and durations likely impacted outcomes, suggesting the initial approach induced further cell sensitization.

To confirm our findings, we conducted an anchorage-independent growth assay, simulating in vivo conditions. Cells in agarose were treated with 7.5 nM BI-831266 and/or 0.5 µg/mL cisplatin for at least 3 weeks. Figure-6 shows a significant reduction in colony quantity and size with combination treatment, affirming enhanced anti-neoplastic effects of cisplatin through Aurora B kinase inhibition.

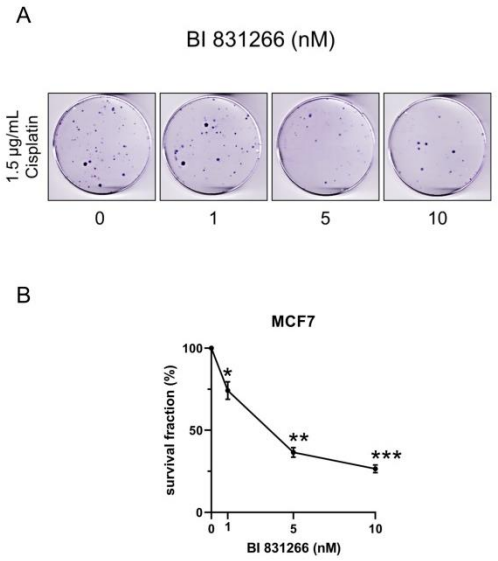

Figure-5. BI-831266 treatment increases the anticancer potential of cisplatin in MCF7 cancer cells.
(A) A consistent number of MCF7 cells were seeded in 60-mm petri dishes and allowed to adhere for 24 hours before undergoing treatment with 0 - 1 - 5 - 10 nM BI-831266 for 23 hours then combined with 1.5 µg/mL cisplatin for 1 hour. Following an incubation period of 7-12 days, colonies were fixed with a methanol and acidic acid solution and subsequently stained with 0.5% w/v crystal violet.
(B) Colony visualization and counting were performed using an inverted microscope, defining a cluster of at least 50 cells as a colony. Plating efficiencies and survival fractions were calculated according to the method outlined in reference (18) (n=3).

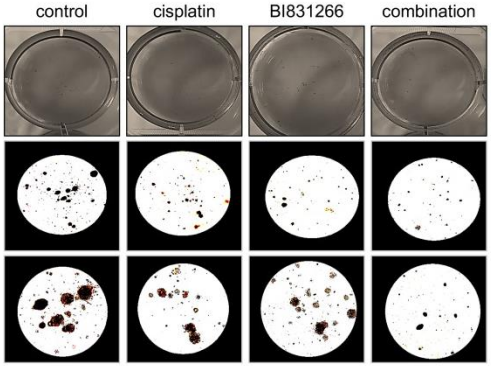

Figure-6. The BI-831266 and cisplatin combination reduces MCF7 tumour formation. A fixed percentage of agarose solution in complete cell culture medium was added to each well of a culture dish. After solidification at 4°C for 30 minutes, a pre-determined number of cells were mixed with the agarose solution and added on top of the solidified base agar layer. Cells were incubated for a minimum of 3 weeks with regular monitoring and fresh medium with the indicated drugs added every 3-4 days. Colonies were then stained with a 0.5% w/v crystal violet solution and scanned.

The combination of BI-831266 and cisplatin reduces the migratory capability of MCF7 cells

To assess the impact of the BI-831266 and cisplatin combination on MCF7 cell migration, we utilized in vitro wound-healing assays, a widely employed tool in cell biology and cancer research (23). Cells were treated with or without 7.5 nM BI-831266 and/or 0.5 µg/mL cisplatin 24 hours post-seeding, with microscopic images captured at the 6th, 12th, and 24th hours. The combination treatment demonstrated a noteworthy effect on cell migration, as evidenced by the fraction of wound area closure, surpassing the effects of individual treatments (Figure-7A, B). Collectively, these findings highlight the significant influence of the combination treatment on MCF7 cell migration.

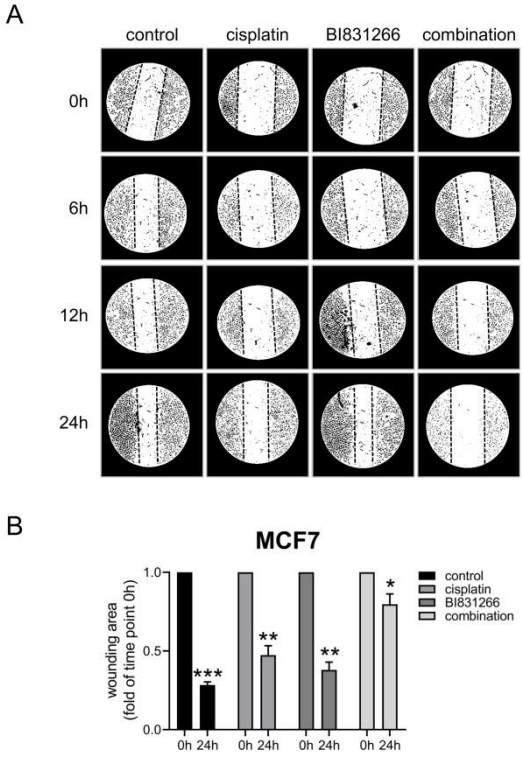

Figure-7. The BI-831266 treatment combined with cisplatin lowers the migration capacity of MCF7 cancer cells. Logarithmically growing cells were seeded in 6-well culture plates. After 24 hours, a straight-line scratch was introduced in the cell monolayer, and subsequent treatments were administered for 24 hours. Images of the wound area were captured at various time points (0, 6, 12, and 24 hours) using an inverted microscope.
(B) Image J software (NIH) analysed the images, and wound closure was assessed by comparing 0 and 24-hour images, normalised to the control set as 1 (n=3).

The BI-831266 and cisplatin combination promotes an apoptotic response of MCF7 cancer cells

To explore the mechanisms inhibiting neoplastic growth in MCF7 cells with BI-831266 and cisplatin, we conducted experiments focusing on apoptotic cell death. Western blotting included control (DMSO-treated), cisplatin alone (20 µg/mL), BI-831266 alone (10 nM and 50 nM), and two combination groups (20 µg/mL cisplatin + 10 nM or 50 nM BI-831266). Cells underwent treatment either with BI-831266 for 24 hours, cisplatin for one hour, or a combination treatment with BI-831266 for 23 hours followed by cisplatin for an additional one-hour period. Consistent with previous findings (24, 25), a one-hour acute treatment with cisplatin led to an increase in cPARP activation compared to the control (Figure-8A, B). Intriguingly, a 24-hour BI-831266 treatment significantly increased cPARP activation in a dose-dependent manner, doubling its levels (Figure-8A, B), suggesting its impact on mitosis and cellular stress contributes to apoptotic pathway activation through PARP cleavage. In comparison to both the control and individual treatments, the co-administration of cisplatin with a higher BI-831266 concentration resulted in a more pronounced induction of cPARP activation, nearly tripling in magnitude (Figure-8A, B), offering a potential explanation for the enhanced effectiveness of the combination treatment in inhibiting the cancerogenic growth of MCF7 cells.

In our investigation, we focused on the role of p53, a pivotal regulator of apoptosis, in response to cellular stress or DNA damage. Treatment with BI-831266 alone or in combination with cisplatin led to a significant increase in p53 levels (Figure-9). Surprisingly, the combination treatment did not induce a higher level of p53 compared to BI-831266 alone (Figure-9). Despite p53's known role in apoptosis and the activation of cPARP being indicative of apoptosis (26), our findings suggest that alternative pathways, independent of p53, may contribute to PARP cleavage (27, 28).

BCL-2 inhibits apoptosis by preserving mitochondrial membrane integrity and suppressing the release of pro-apoptotic factors (29–31). In line with the observations regarding cPARP, our experiments demonstrated a significant reduction in BCL-2 protein levels following treatment with BI-831266 alone or in combination with cisplatin; nevertheless, there was no significant distinction between the effects of BI-831266 alone and the combined treatments (Figure-10A, B).

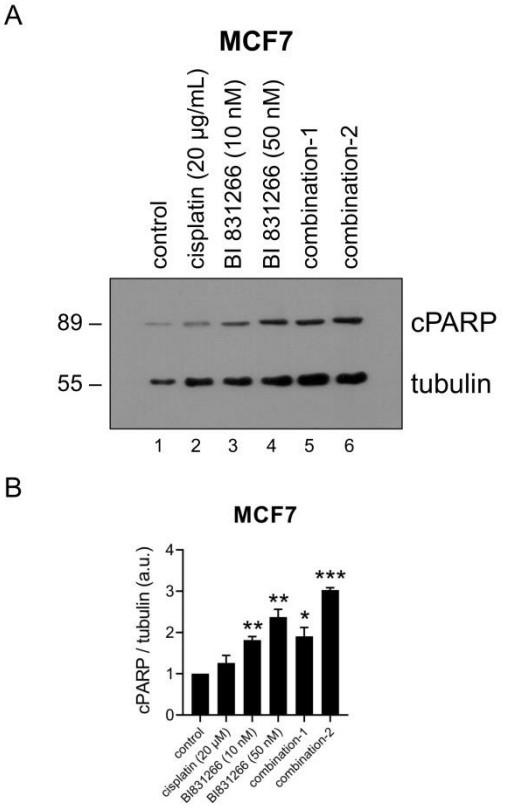

Figure-8. The BI-831266 treatment alone or in combination with cisplatin induces apoptotic cell death through PARP cleavage.

(A) Western blotting with indicated antibodies of MCF7 cell lysates from cells treated with or without the indicated drugs. The experiments were performed with distinct treatment groups, including DMSO control, cisplatin alone (20 µg/mL), BI-831266 alone (10 nM), BI-831266 alone (50 nM), combination-1 (20 µg/mL cisplatin + 10 nM BI 831266) and combination-2 (20 µg/mL cisplatin + 50 nM BI 831266). Cells underwent treatment either with BI-831266 for 24 hours, cisplatin for one hour, or a combination treatment with BI-831266 for 23 hours followed by cisplatin for an additional one-hour period. After 24 hour-treatment cells were collected and subjected to Western blotting analysis.

(B) Histograms showing the expression profile of cPARP protein, obtained by densitometric quantification of Western blots represented in A. Arbitrary units were normalised to the expression of the corresponding total protein (n=3).

Pro-Caspase 3, an inactive precursor of the apoptosis-executing enzyme Caspase 3, undergoes cleavage activation crucial for initiating programmed cell death (32). Our study

revealed that BI-831266 treatment induced apoptotic activation comparable to cisplatin, evident through pro-Caspase 3 cleavage (Figure-11A, B). Interestingly, no significant difference in pro-Caspase 3 protein levels was observed between single-agent and combination treatments (Figure-11A,B). In summary, our findings highlight that Aurora B kinase inhibition enhances the anticancer activity of cisplatin by diminishing

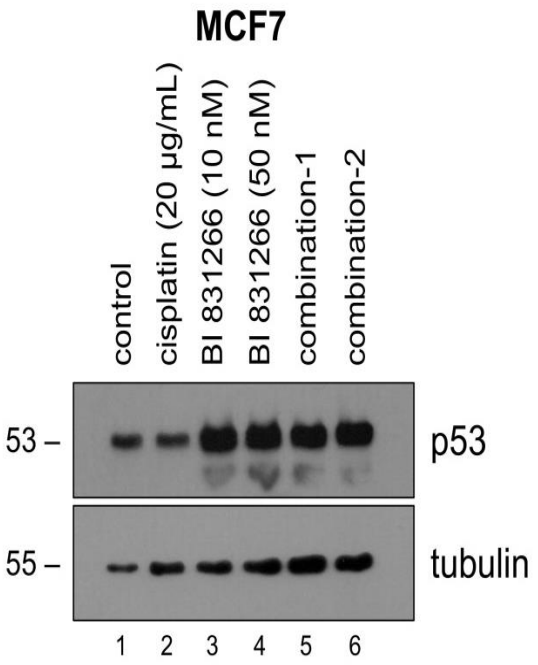

Figure-9. The BI-831266 treatment increases p53 protein levels in MCF7 cancer cells.

(A) Western blotting with indicated antibodies of MCF7 cell lysates from cells treated with or without the indicated drugs. The experiments were performed with distinct treatment groups, including DMSO control, cisplatin alone (20 µg/mL), BI-831266 alone (10 nM), BI-831266 alone (50 nM), combination-1 (20 µg/mL cisplatin + 10 nM BI 831266) and combination-2 (20 µg/mL cisplatin + 50 nM BI 831266). Cells underwent treatment either with BI-831266 for 24 hours, cisplatin for one hour, or a combination treatment with BI-831266 for 23 hours followed by cisplatin for an additional one-hour period. After 24 hour-treatment cells were collected and subjected to Western blotting analysis (n=3). Since there was no clear difference between single and combination treatments, we did not perform the densitometric analysis of the bands.

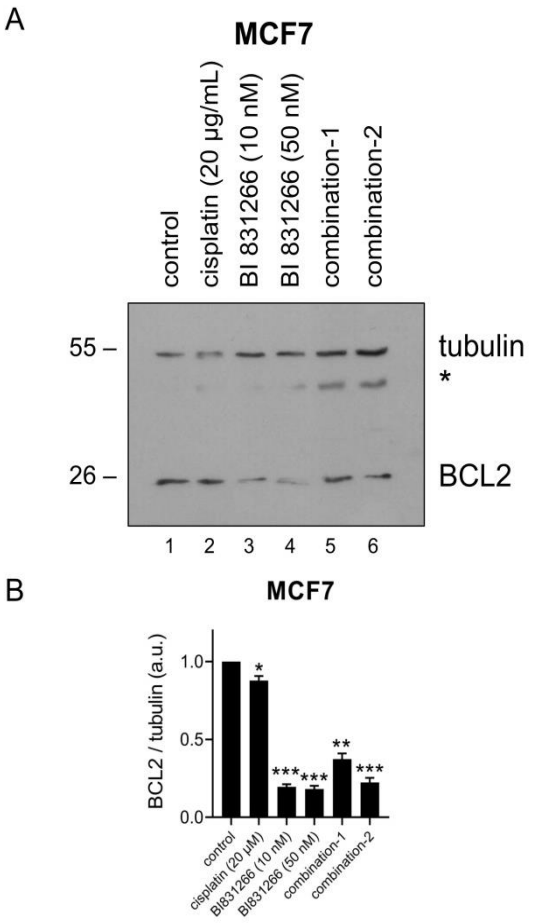

Figure-10. The BI-831266 and cisplatin treatment diminishes the level of anti-apoptotic protein BCL2.

(A) Western blotting with indicated antibodies of MCF7 cell lysates from cells treated with or without the indicated drugs. The experiments were performed with distinct treatment groups, including DMSO control, cisplatin alone (20 µg/mL), BI-831266 alone (10 nM), BI-831266 alone (50 nM), combination-1 (20 µg/mL cisplatin + 10 nM BI 831266) and combination-2 (20 µg/mL cisplatin + 50 nM BI 831266). Cells underwent treatment either with BI-831266 for 24 hours, cisplatin for one hour, or a combination treatment with BI-831266 for 23 hours followed by cisplatin for an additional one-hour period. After 24 hour-treatment cells were collected and subjected to Western blotting analysis.

(B) Histograms showing the expression profile of BCL2 protein, obtained by densitometric quantification of Western blots represented in A. Arbitrary units were normalised to the expression of the corresponding total protein (n=3). *unspecific band.

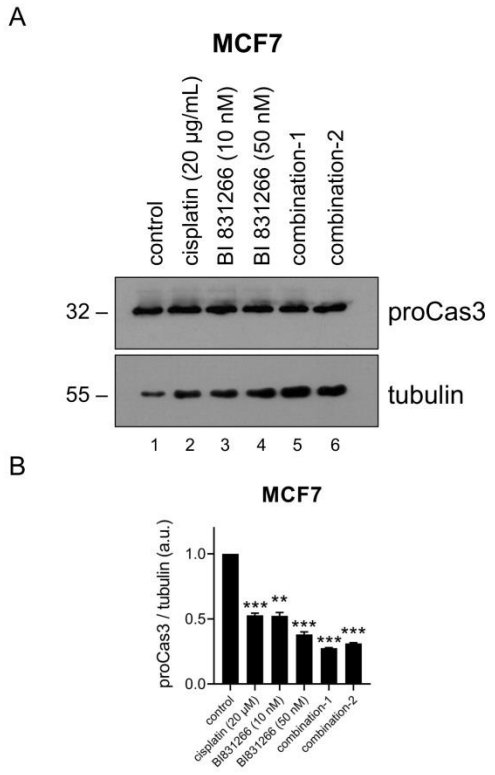

Figure-11. The combination of BI-831266 and cisplatin decreases the level of pro-Caspase 3. (A) Western blotting with indicated antibodies of MCF7 cell lysates from cells treated with or without the indicated drugs. The experiments were performed with distinct treatment groups, including DMSO control, cisplatin alone (20 µg/mL), BI-831266 alone (10 nM), BI-831266 alone (50 nM), combination-1 (20 µg/mL cisplatin + 10 nM BI 831266) and combination-2 (20 µg/mL cisplatin + 50 nM BI 831266). Cells underwent treatment either with BI-831266 for 24 hours, cisplatin for one hour, or a combination treatment with BI-831266 for 23 hours followed by cisplatin for an additional one-hour period. After 24 hour-treatment cells were collected and subjected to Western blotting analysis.
(B) Histograms showing the expression profile of BCL2 protein, obtained by densitometric quantification of Western blots represented in A. Arbitrary units were normalised to the expression of the corresponding total protein (n=3).

DISCUSSION

Breast cancer, a pervasive malignancy, epitomizes the intricate interplay of molecular factors shaping its pathogenesis. Globally, breast cancer ranks as the most common cancer among women, with an alarming incidence (3). Analysis of publicly accessible databases additionally indicated a substantial correlation between heightened Aurora kinase B expression and diminished survival rates among individuals diagnosed with breast cancer (33). Furthermore, the expression of Aurora kinase B has been recognized as a prognostic indicator in glioblastoma (34), gastric cancer (35), and oral cancer (36). Elevated expression of Aurora kinase B was also observed in prostate cancer tissues in comparison to healthy controls (37).

The fundamental roles played by Aurora kinases, coupled with their abnormal expression observed in various tumour types, have led to the exploration of several small molecule inhibitors as potential cancer treatments. Among others, BI-831266 is a powerful and specifically targeted low-molecular-weight inhibitor designed to act on Aurora kinase B. Preclinical investigations indicate that BI-831266 impedes the growth of cell lines associated with prostate cancer, human non-small cell lung cancer, and pancreatic cancer. Moreover, in murine xenograft tumour (HCT116) models, BxPC3 pancreatic adenocarcinoma, and NCI-H460 NSCLC, a constant 24-hour infusion of BI-831266 demonstrated tumour regression and inhibited growth, as reported in internal data from Boehringer Ingelheim (38). To the best of our knowledge, no preclinical study has been undertaken to explore the anti-neoplastic role of BI 831266 in breast cancer cell lines. In our research, we discovered a synergistic effect between Aurora B kinase inhibition and cisplatin treatment in MCF7 cancer cells. Specifically, we revealed that the inhibition of Aurora B kinase significantly enhanced the cytotoxicity of cisplatin. Furthermore, we found that the combination of Aurora B kinase inhibition and cisplatin impaired the migratory capacity of MCF7 cells. This finding indicates a potential dual effect, not only enhancing the cytotoxicity of cisplatin but also hampering the migratory ability of cancer cells. Finally, our investigation included an assessment of the protein expression levels of key apoptotic markers in MCF7 cells subjected to cisplatin treatment, both in the presence and absence of Aurora B kinase inhibition. Our findings revealed significant alterations in the expression levels of crucial apoptotic proteins including cPARP, suggesting that the combination of Aurora B kinase inhibition and cisplatin treatment may modulate apoptotic pathways. These findings highlight the significance of targeting Aurora kinase B in conjunction with cisplatin to enhance the therapeutic response in MCF7 breast cancer cells.

Cancer cells often develop resistance to single-agent therapies over time. Therefore, the combination of small molecule inhibitors with chemotherapy agents represents a multifaceted and effective approach to combat the complexity of cancer, addressing both the heterogeneity of tumours and the challenges associated with drug resistance. Larsen et al. found barasertib to selectively impede the growth of fulvestrant-resistant T47D breast cancer cell lines (39). Barasertib prompted the degradation of Aurora kinase B, resulting in mitotic errors, and initiated apoptotic cell death. This was substantiated by the accumulation of SubG1 cells and PARP cleavage observed in the fulvestrant-resistant cells (39). Our experiments also revealed that treatment with BI-831266 as a single agent robustly restrained the growth of MCF7 cancer cells and induced apoptosis, as indicated by the activity of cleaved-PARP. Further studies will reveal which apoptotic pathways are activated upon the BI 831266-dependent Aurora kinase B inhibition. As mentioned earlier, we found that BI-831266 treatment significantly increases the anticancer potential of cisplatin in MCF7 cells. Subsequent investigations will elucidate whether BI-831266 exhibits efficacy in targeting cisplatin-resistant MCF7 breast cancer cells. Understanding its effectiveness in resistant cells could pave the way for the development of more robust and adaptable therapeutic strategies for breast cancer patients, especially those with limited responsiveness to conventional treatments. Testing the effect of a compound in just one cell line may provide valuable initial insights into its biological activity and potential therapeutic impact within a specific context. However, relying solely on results from a single cell line has limitations. Different cell lines can exhibit diverse genetic backgrounds, phenotypic characteristics, and responses to treatments due to their unique origins and genetic alterations. Therefore, while our initial testing in a single cell line can offer valuable insights, comprehensive and reliable conclusions are further required to evaluate BI 831266's effects across a range of relevant cell lines to ensure broader applicability and relevance to diverse biological contexts.

In conclusion, by targeting Aurora B kinase alongside cisplatin, our study aligns with the ongoing efforts to address key hallmarks such as sustained proliferative signalling and evading growth suppressors, offering promising insights into more effective and targeted strategies for breast cancer treatment.

Acknowledgments

This study was financially supported by The Scientific Research Projects Coordination Unit of Bingol University (Project no: BAP-SHMYO.2023.001).

Declaration of interest statement

We received BI831266 compound gratis from Boehringer Ingelheim, aiding our research. Notably, the company had no role in experiment design, execution, analysis, or interpretation. The authors declare no other conflicts of interest.

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Araştırma Makalesi / *Research Article*

Ege Tıp Dergisi / *Ege Journal of Medicine* 2024; 63 (3): 422-427

Distal pankreatektomide pankreas güdüğünü kapatma yöntemlerinin postoperatif pankreatik fistül üzerine etkisi

Effect of pancreas stump closing methods in distal pancreatectomy on postoperative pancreatic fistula

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ÖZ

Amaç: Çalışmamızda, distal pankreatektomi (DP)'de pankreas güdüğünü kapatma yöntemleri ile postoperatif pankreatik fistül (POPF) arasındaki ilişkinin araştırılması amaçlanmıştır.

Gereç ve Yöntem: Hastanemizde 2017-2023 yıllarında DP uygulanan hastalar, retrospektif olarak tarandı. Hastalar, ISGPF 2016 tanımlamasına göre POPF gelişen grup ve gelişmeyen grup olmak üzere iki gruba ayrıldı. Güdük kapatma yöntemleri; sütürasyon, stapler kullanımı, stapler kullanımını takiben sütürasyon olmak üzere 3 grupta incelendi. Uygulanan her yöntemin sonucunda POPF görülen ve görülmeyen gruplar karşılaştırılarak yöntemlerin her birinin POPF ile ilişkisi istatistiksel açıdan değerlendirildi.

Bulgular: Çalışmaya 27 hasta dahil edildi. Hastalardan 10'una (%37,1) pankreas tümörü, 11'ine (%40,7) pankreasa invazyon gösteren başka organ tümörü, 5'ine (%18,5) travma, 1'ine (%3,7) kist hidatik nedeniyle DP uygulanmıştı. Hastaların 16'sı (%59,3) erkek, 11'i (%40,7) kadındı. Ortanca yaş 63 (44-70) idi. POPF görülmeyen hasta sayısı 20 (%74) iken POPF görülen hasta sayısı 7 (%26) idi. POPF görülmeyen grupta pankreas güdüğünü kapatma yöntemi sütürasyon olan hasta sayısı 11 (%55), stapler kullanımı olan 8 (%40), stapler kullanımını takiben sütürasyon olan 1 (%5) idi. POPF görülen grupta pankreas güdüğünü kapatma yöntemi sütürasyon olan hasta sayısı 3 (%42,8), stapler kullanımı olan 2 (%28,6), stapler kullanımını takiben sütürasyon olan hasta sayısı 2 (%28,6) idi. Gruplar arasında istatistiksel olarak anlamlı fark saptanmadı (p:0,232).

Sonuç: Çalışmamızda pankreas güdünü kapatma yöntemleri ile POPF arasında anlamlı ilişki saptanmadı. Ancak yöntemlerin birbirinden üstün olduğunu gösteren farklı çalışmalar da literatürde mevcuttur. İlerleyen dönemde konuyla ilgili daha fazla çalışma yapılması konunun aydınlatılmasında oldukça faydalı olacaktır.

Anahtar Sözcükler: Distal pankreatektomi, postoperatif pankreatik fistül, güdük kapatma.

ABSTRACT

Aim: *Our study aimed to investigate the relationship between the pancreatic stump closure methods and postoperative pancreatic fistula (POPF) in distal pancreatectomy (DP).*

Materials and Methods: *Patients who underwent DP in our hospital between 2017 and 2023 were retrospectively scanned. The patients were divided into two groups, the group that developed POPF and the group that did not develop, according to the ISGPF 2016 definition.*

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The stump closure methods used in the operation are; They were examined in 3 groups: suturing, stapler use, and stapler use followed by suturing. By comparing the groups with and without POPF as a result of each method applied, the relationship of each method with POPF was evaluated statistically.

Results: *In the study, 27 patients were enrolled. Among the patients who underwent DP, 10 had a pancreatic tumor, 11 had a tumor of another organ that invaded the pancreas, 5 had a trauma, and 1 had a hydatid cyst. 16 (59.3%) of the patients were male and 11 (40.7%) were female. The median age was 63 (44-70). While the number of patients without POPF was 20 (74%), the number of patients with POPF was 7 (26%). In the group without POPF, the number of patients whose method of closing the pancreatic stump was suture was 11 (55%), 8 (40%) had the use of stapler, and 1 (5%) had the use of stapler followed by suture. In the group with POPF, the number of patients whose method of closing the pancreatic stump was suture was 3 (42.8%), 2 (28.6%) had the use of stapler, and 2 (28.6%) had suture following the use of stapler. There was no statistically significant difference between the groups (p:0.232).*

Conclusion: *No significant relationship was found between the pancreatic stump closure methods and POPF in our study. However, there are also different studies in the literature showing that the methods are superior to each other. Further studies on the subject in the future will be quite beneficial in shedding light on the issue.*

Keywords: *Distal pancreatectomy, postoperative pancreatic fistula, stump closure.*

GİRİŞ

Distal pankreatektomi (DP); pankreas benign ve malign neoplazmları, kronik pankreatit ve pankreas hasarı ile sonuçlanan travmalar gibi çeşitli endikasyonlarla uygulanan önemli bir abdominal cerrahi prosedürdür. DP sonrasında en sık karşılaşılan ve aynı zamanda en önemli görülen komplikasyon ise postoperatif pankreatik fistüldür (POPF) (1). Literatürde DP sonrası POPF insidansı yaklaşık %20-35 olarak bildirilmiştir. Kanama, sepsis, organ yetmezliği, uzun süre hastanede kalış ve hatta ölüm riskinde artış ile ilişkili bulunmuştur (2, 3, 4). Bu nedenle POPF insidansının azaltılması, kısa dönemde morbidite ve mortalite ile uzun dönemde hasta sonuçları açısından önemli bir hedeftir. Bu hedefi gerçekleştirmek ise risk faktörlerini saptayarak POPF'u önlemeye yönelik stratejilerin geliştirilmesi ile mümkündür (5). Literatürde risk faktörlerini araştıran pek çok çalışmanın arasında DP'de pankreas güdüğünü kapatma yöntemindeki farklı tercihlerin POPF ile ilişkisini araştıran çalışmalar da mevcuttur. Ancak kapatma yöntemi tercihlerini karşılaştıran çalışmalarda farklı sonuçlara ulaşılmış olup, genel anlamda yöntemlerin birbirlerine üstünlüğü net olarak ortaya konamamıştır (Tablo-1).

Tablo-1. Pankreas güdüğünü kapatma yönteminin POPF ile ilişkisini araştıran çalışmalar ve sonuçları.

Yıl	Yazar	Araştırma türü	Hasta sayısı (stapler: sütür)	POPF tipi	POPF sıklığı (stapler : sütür)	Sonuç
2008	Okano ve arkadaşları (6)	Tek merkezli	24 : 11	POPF	3/24 : 3/11	Anlamlı fark yok
2011	Diener ve arkadaşları (21)	Çok merkezli	177 : 175	POPF	36/177 : 36/175	Anlamlı fark yok
2012	Ban ve arkadaşları (13)	Çok merkezli	224 : 164	CR-POPF	47/224 : 83/164	Stapler grubunda anlamlı olarak daha az CR-POPF görülmüş
2014	Zhang ve arkadaşları (20)	Meta-analiz	576 : 1242	CR-POPF	87/576 : 178/1242	Anlamlı fark yok
2017	Ecker ve arkadaşları (10)	Çok merkezli	1344 : 508	CR-POPF	171/1344 : 97/508	Stapler grubunda anlamlı olarak daha az CR-POPF görülmüş
2019	Shen ve arkadaşları (5)	Tek merkezli	137 : 74	CR-POPF	18/137 : 15/74	Anlamlı fark yok
2019	Maggino ve arkadaşları (4)	Tek merkezli	130 : 141	CR-POPF	24/130 : 30/141	Anlamlı fark yok
2019	Rozich ve arkadaşları (11)	Tek merkezli	86 : 170	CR-POPF	6/86 : 33/170	Anlamlı fark yok
2020	Hiyoshi ve arkadaşları (22)	Tek merkezli	23 : 14	CR-POPF	8/23 : 8/14	Anlamlı fark yok

Kısaltmalar: POPF-Postoperatif Pankreatik Fistül. CR-POPF-Klinik İlişkili Postoperatif Pankreatik Fistül.

Tablo-2. Parametreler ile POPF arasındaki ilişki.

	Tüm hastalar n=27	**POPF yok n=20**	**POPF var n=7**	**p değeri**
Yaş (yıl), ortanca (Q1-Q3)	63 (44-70)	65 (51-70)	44 (21-67)	0,104
Cinsiyet, n (%)				0,391*
Erkek	16 (59,3)	13 (65)	3 (42,9)	
Kadın	11 (40,7)	7 (35)	4 (57,1)	
VKİ (kg/m^2), ortanca (Q1-Q3)	22,5 (22,1-24,5)	22,9 (22,1-24,4)	22,5 (22,1-24,5)	0,646
PNİ, ortalama±SS	34,3±9,3	33,7±8,3	36,1±12,3	0,565
CKİ, ortanca (Q1-Q3)	3 (0-4)	3,5 (1,3-4)	2 (0-3)	0,116
ASA skoru, n (%)				0,608
1	4 (14,8)	3 (15)	1 (14,3)	
2	15 (55,6)	10 (50)	5 (71,4)	
3	4 (14,8)	4 (20)	0	
4	4 (14,8)	3 (15)	1 (14,3)	
Sigara (+), n (%)	7 (25,9)	4 (20)	3 (42,9)	0,328*
Neoadjuvan (+), n (%)	3 (11,1)	2(10)	1 (14,3)	1,000*
Pankreas konturu, n (%)				1,000*
Düz	10 (37)	7 (35)	3 (42,9)	
Dalgalı	17 (63)	13 (65)	4 (57,1)	
Preoperatif hemoglobin (g/dL), ortalama±SS	11,8±2,3	11,6±2,2	12,4±2,7	0,442
Postoperatif Hemoglobin (g/dL), ortalama±SS	10,6±1,7	10,4±1,8	11,4±1,4	0,194
Hemoglobin düşüşü (+), n (%)	19 (70,4)	15 (75)	4 (57,1)	0,633*
Multivisseral rezeksiyon (+), n (%)	18 (66,7)	15 (75)	3 (42,9)	0,175*
Splenektomi (+), n (%)	23 (85,2)	16 (80)	7 (100)	0,545*
Patoloji, n (%)				0,172
Pankreatik tümör	10 (37,1)	7 (35)	3 (42,9)	
Non-pankreatik tümör	11 (40,7)	10 (50)	1 (14,3)	
Tümör dışı	6 (22,2)	3 (15)	3 (42,9)	
Laparoskopik, n (%)	3 (11,1)	2 (10)	1 (14,3)	1,000*
Pankreas kapatma yöntemi, n (%)				0,232
Sütur	14 (51,9)	11 (55)	3 (42,8)	
Stapler	10 (37)	8 (40)	2 (28,6)	
Hem sütur hem stapler	3 (11,1)	1 (5)	2 (28,6)	
Operasyon süresi (dk), ortalama±SS	241±95	244±96	233±101	0,808
Clavien Dindo skoru, n (%)				0,388
1	13 (48,1)	10 (50)	3 (42,9)	
2	4 (14,8)	3 (15)	1 (14,3)	
3	6 (22,2)	4 (20)	2 (28,6)	
4	1 (3,7)	0	1 (14,3)	
5	3 (11,1)	3 (15)	0	

*Fisher's Exact test kullanıldı.

Kısaltmalar: ASA-Amerikan Anestezistler Birliği. CKİ-Charlson Komorbidite İndeksi. PNİ-Prognostik Nutrisyonel İndeks. SS-Standart Sapma. VKİ-Vücut Kitle İndeksi.

GEREÇ ve YÖNTEM

Veri toplama yöntemi ve araçlar

T.C. Sağlık Bilimleri Üniversitesi İzmir Tepecik Eğitim ve Araştırma Hastanesi 10/01/2024 tarihli Girişimsel Olmayan Etik Kurulu'nda 2023/12-05 numaralı karar ile onay alındıktan sonra çalışmaya başlandı. Çalışmada T.C. Sağlık Bilimleri Üniversitesi İzmir Tepecik Eğitim ve Araştırma Hastanesi'nde 2017-2023 yıllarında DP uygulanan hastalar, hastane veritabanı (Probel HBYS, v1, İzmir, Türkiye) üzerinden retrospektif olarak tarandı. Hastalar, International Study Group for Pancreatic Fistula (ISGPF) 2016 tanımlamasına göre POPF gelişen grup (Grade B, C) ve gelişmeyen grup (biyokimyasal kaçak dahil) olmak üzere iki gruba ayrıldı. Operasyonda uygulanan güdük kapatma yöntemleri ise;

sütürasyon, stapler, stapler kullanımını takiben sütürasyon olmak üzere 3 grupta incelendi. Uygulanan her yöntemin sonucunda POPF görülen ve görülmeyen gruplar karşılaştırılarak yöntemlerin her birinin POPF ile ilişkisi istatistiksel açıdan değerlendirildi.

Pankreas kapatma yöntemi

Sütürasyon yönteminde 3/0 atravmatik ipek ile devamlı dikiş ve takiben U sütürler ile takviye uygulandı. Stapler yönteminde ise lineer stapler (GIA Autosuture, 4.8 mm, DST Serisi, Covidien Medtronic, Minneapolis, ABD) kullanıldı.

İstatistiksel Yöntem

İstatistiksel analizler SPSS versiyon 25.0 yazılımı kullanılarak yapıldı. Değişkenlerin normal dağılıma uygunluğu analitik yöntemler (Kolmogorov-Smirnov/ShapiroWilk testleri) kullanılarak incelendi. Tanımlayıcı analizler normal dağılan değişkenler için ortalama±standart sapma, normal dağılım göstermeyenlerde ortanca (çeyrek değer aralığı) kullanılarak verildi. Demografik özelliklerin frekans ve yüzde değerleri verilerek tanımlayıcı istatistikleri yapıldı. Sürekli verilerde normal dağılım gösteren bağımsız gruplarda t-testi, normal dağılmayanlarda Mann-Whitney U testi kullanıldı. Kategorik verilerin analizinde Pearson's Ki-Kare veya Fisher's Exact Ki-Kare testi kullanıldı. Potansiyel risk faktörlerini bulmak için tek değişkenli analiz ve daha sonra bağımsız faktörleri tanımlamak için çok değişkenli analiz yapıldı. $p<0.05$ değeri istatistiksel olarak anlamlı kabul edildi.

BULGULAR

Çalışmaya 27 hasta dahil edildi. Hastalardan 10'una (%37,1) pankreas tümörü, 11'ine (%40,7) pankreasa invazyon gösteren başka organ tümörü, 5'ine (%18,5) travma, 1'ine (%3,7) kist hidatik nedeniyle DP uygulanmıştı. Hastaların 16'sı (%59,3) erkek, 11'i (%40,7) kadındı. Ortanca yaş 63 (44-70) idi. POPF görülmeyen hasta sayısı 20 (%74) iken POPF görülen hasta sayısı 7 (%26) idi. Pankreas güdüğünü kapatma yöntemi sütürasyon olan hasta sayısı 14 (%51,9), stapler kullanımı olan hasta sayısı 10 (%37), stapler kullanımını takiben sütürasyon olan hasta sayısı 3 (%11,1) idi. POPF görülmeyen grupta pankreas güdüğünü kapatma yöntemi sütürasyon olan hasta sayısı 14 (%51,9), stapler kullanımı olan hasta sayısı 10 (%37), stapler kullanımını takiben sütürasyon olan hasta sayısı 3 (%11,1) idi. POPF görülen grupta pankreas güdüğünü kapatma yöntemi sütürasyon olan hasta sayısı 3 (%42,8), stapler kullanımı olan hasta sayısı 2 (%28,6), stapler kullanımını takiben sütürasyon olan hasta sayısı 2 (%28,6) idi. Gruplar arasında istatistiksel olarak anlamlı fark saptanmadı (p:0,232) (Tablo-2). Tek değişkenli ve çok değişkenli analizlerin sonucunda da kapatma yöntemi ve diğer parametreler ile POPF arasında istatistiksel açıdan anlamlı ilişki saptanmadı.

TARTIŞMA

DP sonrası POPF insidansını azaltmaya yönelik çalışmalar literatürde yıllardır üzerinde durulmuş bir konudur. Son yıllarda pankreas transeksiyonunun stapler ile yapılması popülerize olmuş olsa da pankreas güdüğünün kapatılmasında hangi yöntemin uygulanması durumunda POPF insidansının azalacağına dair net bir sonuca ulaşılamamıştır (4, 6, 7). Bu anlamda konu güncelliğini korumaktadır.

Çalışmamızda DP operasyonlarında uygulanan kapatma yöntemlerinin POPF üzerine etkisi yanı sıra literatürde bahsedilen risk faktörlerinin etkinliği de araştırıldı. Sözgelimi; DP sonrası POPF için risk faktörlerinin araştırıldığı, Chong ve arkadaşlarının 43 makale üzerinden yaptığı bir meta-analizde POPF ile ilişkili bulunan başlıca risk faktörleri olarak genç yaş, düşük serum albümin değeri, uzun operasyon süresi, yüksek BMI, non-pankreatik kanser, yumuşak pankreas, kalın pankreas, açık cerrahi yöntem, splenektomi, multivisseral rezeksiyon ve vasküler rezeksiyon gösterilmiştir (8). Literatürde daha önceki çalışmalarda ileri yaş POPF için risk faktörü olarak görülmekteyken, Chong ve arkadaşlarının yaptığı meta-analizde önceki bilgilerden farklı olarak genç yaşın bir risk faktörü olduğu sonucu ortaya konmuştur (9-12). Çalışmamızda ise genel literatürle uyumlu olarak genç hastalarda DP sonrası POPF'un anlamlı ölçüde daha çok görüldüğünü saptadık.

Kapatma yöntemlerinin POPF insidansını etkilemesi konusunda literatürde farklı çalışmalar mevcuttur. Ban ve arkadaşlarının yapmış olduğu, 388 hastayı içeren multisentrik çalışmada DP yapılmış olan hastalarda stapler ile kapatmanın sütürasyon ile kapatmaya üstünlüğü ortaya konmuştur (15). Sa Cunha ve arkadaşlarının yaptığı randomize FIABLE çalışmasında stapler ile kapatmanın sütürasyon ile kapatmaya üstün olduğu sonucuna ulaşılmıştır (14). Buna karşın Futagawa ve arkadaşlarının yaptığı çalışmadaki gibi literatürde yer alan benzer geniş serili çalışmalarda sütürasyon ile kapatmanın stapler ile kapatmaya üstün olduğu vurgulanmıştır (15-17). Zhang ve arkadaşlarının yaptığı çalışma ile aynı sonuca ulaşmış olan benzer çalışmalarda

ise iki tekniğin sonuçları arasında anlamlı fark ortaya konamamıştır (18-20). Çok merkezli randomize kontrollü DISPACT çalışması ile Chong ve arkadaşlarının 2021'de yaptığı meta-analiz sonucunda da kapatma yöntemlerinin birbirine üstünlüğü olmadığı gösterilmiştir (8, 21). Bu sonuçla uyumlu olarak çalışmamızda da pankreas güdüğünü kapatma yöntemlerinin birbirine üstünlüğü saptanmadı.

Çalışmamızdaki veriler tek değişkenli ve çok değişkenli analiz ile istatistiksel açıdan değerlendirilmiştir. Fakat çalışmamızdaki hasta sayısının az olması çok sağlıklı bir alt grup analizi yapılmasını mümkün kılmamıştır. Bu durum, çalışmanın istatistiksel gücünü düşüren önemli bir faktör olarak kısıtlayıcı rol oynamıştır. Ancak bu kısıtlayıcı duruma rağmen çalışmamızdaki verileri uygun istatistiksel yöntemlerle değerlendirdik. Böylece pankreas cerrahisinde hala tartışılagelen önemli bir konuyu merkezimizdeki hastaların sonuçları ile yorumlayarak literatüre ve bilime katkıda bulunduğumuz kanaatindeyiz.

SONUÇ

DP sonrası en önemli komplikasyon olan POPF, hastanede yatış süresinin uzatması yanı sıra önemli bir morbidite sebebi olmaya devam etmektedir. Ancak literatürde uzun süredir araştırılan ve tartışılan bir konu olmasına rağmen ortak bir konsensus sağlayacak net bilgilere hala ulaşılamamıştır. Farklı sonuçların ve görüşlerin olduğu bu konuda konsensus sağlanabilmesi amacıyla ilerleyen dönemde konuyla ilgili daha fazla çalışma yapılması literatüre oldukça değerli katkılar sağlayacaktır. Özellikle de yüksek hacimli merkezler ile çok merkezli çalışmaların yapılmasına ihtiyaç vardır.

Çıkar çatışması: Çalışmamızda çıkar çatışması yoktur.

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Research Article / *Araştırma Makalesi*

Ege Journal of Medicine / *Ege Tıp Dergisi* 2024; 63 (3): 428-434

Evaluation of tetanus cases presenting to the emergency department at a tertiary hospital in Somalia

Somali'de üçüncü basamak bir hastanenin acil servisine başvuran tetanos olgularının değerlendirilmesi

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ABSTRACT

Aim: Tetanus is an infection characterized by muscle spasms and trismus caused by toxins produced by *Clostridium tetani*. Although tetanus is a vaccine-preventable disease, it continues to be a public health problem in developing countries with high mortality rates.

This study was conducted to evaluate the clinical profile and outcome of tetanus patients and determine the factors affecting the hospitalization unit, the length of hospital stay, and mortality.

Materials and Methods: This is a retrospective cross-sectional study conducted with patients diagnosed with tetanus in a tertiary care emergency department (ED) in Somalia. The relationship of demographic data, symptoms, laboratory findings, applied treatment methods with the hospitalization unit (regular inpatient floor/intensive care unit), length of hospital stay, and mortality were analyzed.

Results: Sixty-seven patients diagnosed with tetanus during a 4-year study period were included. The median patient age was 10.0 (5.0-13.0) years and 73,1% were males. Generalized muscle spasm (85,7%) was the most common symptom, benzodiazepines (95,5%.) were the most commonly used medications, and the mean length of hospital stay was 10.73±8.15 days. The rate of patients hospitalized in the ICU was 20,9%. The overall mortality rate was calculated as 19,5%. The presence of opisthotonus, neck stiffness, risus sardonicus, generalized muscle spasm, and dyspnea significantly correlated with mortality ($p<0.05$).

Conclusion: Tetanus remains a significant public health problem with high mortality in Somalia. Late-stage clinical findings at ED presentation are strongly associated with admission to the intensive care unit and mortality.

Keywords: Tetanus, treatment, mortality

ÖZ

Amaç: *Tetanos, Clostridium tetani adlı bakterinin ürettiği toksinlerin neden olduğu kas spazmları ve trismusla karakterize bir enfeksiyondur. Tetanoz aşıyla önlenebilir bir hastalık olmasına rağmen gelişmekte olan ülkelerde ölüm oranlarının yüksek olduğu bir halk sağlığı sorunu olmaya devam etmektedir.*

Bu çalışma, tetanos hastalarının klinik profillerini ve sonuçlarını değerlendirmek, hospitalizasyon ünitesini, hastanede kalış süresini ve mortaliteyi etkileyen faktörleri belirlemek amacıyla yapıldı.

Gereç ve Yöntem: *Bu çalışma; Somali'deki üçüncü basamak bir acil serviste tetanos tanısı alan hastalarla yürütülen retrospektif, kesitsel bir çalışmadır.*

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Application date: 06.12.2023 Accepted: 24.05.2024

Demografik veriler, semptomlar, laboratuvar bulguları ve uygulanan tedavi yöntemleri ile hospitalizasyon ünitesi (servis/yoğun bakım ünitesi), hastanede kalış süresi ve mortalite ilişkisi analiz edildi.

Bulgular: *Dört yıllık çalışma süresinde tetanos tanısı alan 67 hasta çalışmaya dahil edildi. Hastaların ortalama yaşı 10.0 (5.0-13.0) yıl olup %73,1'i erkekti. En sık görülen semptom jeneralize kas spazmı (%85,7), en sık kullanılan ilaç ise benzodiazepinler (%95,5) idi ve ortalama hastanede kalış süresi 10,73±8,15 gündü. Yoğun bakımda yatan hasta oranı ise %20,9 olarak saptandı. Toplam mortalite oranı ise %19,5 olarak hesaplandı. Opistotonus, boyun sertliği, risus sardonicus, jeneralize kas spazmı ve dispne varlığı mortalite ile anlamlı korelasyon göstermiştir (p<0.05).*

Sonuç: *Tetanos Somali'de mortalitesi yüksek, önemli bir halk sağlığı sorunu olmaya devam etmektedir. Acil servise başvuru sırasında geç dönem klinik bulgular olması, hastanın yoğun bakım ünitesine kabulü ve mortalite ile güçlü bir şekilde ilişkilidir.*

Anahtar Sözcükler: *Tetanos, tedavi, mortalite.*

INTRODUCTION

Tetanus; is a potentially life-threatening infectious disease caused by the *gram-positive Clostridium tetani bacillus*, associated with high mortality rates (1, 2). Tetanospasmin and tetanolysin toxins secreted by *Clostridium tetani* cause local inflammation by acting on the gangliosides in local nerve terminals (3). While trismus is mostly the first symptom, wrinkled facial expression (i.e., risus sardonicus), widespread muscle spasms with severe pain, drooling, urinary incontinence, stool incontinence, and spasm of the back muscles (i.e., opisthotonus) causing respiratory distress are the main clinical features (4).

Diagnosis is made clinically. Physical examination findings and immunization history aid in diagnostic management, but there are no definitive laboratory tests that can be helpful in diagnosis (5). On the other hand, treatment is based on the destruction of the organism with antibiotics such as penicillin G or metronidazole, neutralization of the toxin with tetanus immunoglobulin, and supportive measures such as mechanical ventilation, sedation, and muscle paralysis (6).

Tetanus is usually encountered in unvaccinated or under-vaccinated populations (4). It continues to be a public health problem in developing countries with high morbidity and mortality rates (7). Therefore, it is vital to know the relationship between clinical data and outcomes, including mortality due to its potentially fatal course.

This study retrospectively analyzed the clinical data and the factors affecting the length of hospital stay, the hospitalization unit, and mortality in patients diagnosed with tetanus in an emergency department.

MATERIALS and METHODS

Patients who were clinically diagnosed with tetanus between January 2018 and December 2021 in the Emergency Department of Somalia Mogadishu Türkiye Training and Research Hospital and subsequently admitted were retrospectively analyzed. The diagnosis of tetanus was entirely clinical and based on the presence of one or more of the following: (i) rigidity of the neck and/or abdomen/neck stiffness, (ii) lockjaw, (iii) risus sardonicus (sustained spasm of the facial muscles) or generalized muscle contractions. Patients with acute hypertonia of other causes (e.g. meningitis, encephalitis) were excluded. Patients of all age groups were included in this study. However, it did not include the patients discharged from the ED, patients who voluntarily left the hospital against medical advice, or those who had a diagnosis other than tetanus after being hospitalized. In addition, patients with incomplete data were excluded. The study was approved by the Ethical Review Committee of the same institution (2022/9184). Data including the demographic characteristics of the patients, date of presentation, clinical findings on presentation such as opisthotonus, neck stiffness, lockjaw, risus sardonicus, generalized muscle spasm, dysphagia, dyspnea, and fever were retrieved from electronic patient folders and recorded to a computer database. This database also included data regarding hospitalization units (regular inpatient floor/intensive care unit), applied treatment methods, length of hospital stay, and mortality status. Since detailed information about the area and duration of the contact could not be obtained from hospital records and patient files, these data could not be included in the study. Since tetanus vaccination data of patients were not available, vaccination history of patients and information about the duration since the last vaccination were not included in the study. Since the patients' antibody information was not available in the records, they could not be included in the study.

The patients were divided into two groups based on the hospitalization unit as regular inpatient floor (RIF) and the intensive care unit (ICU). Firstly, two groups were compared concerning demographic data and clinical findings. Subsequently, the patients were divided into two groups according to mortality status. Finally, to determine the factors affecting the length of hospital stay and mortality, the demographic and clinical patient data, including admission year and applied treatment methods, were analyzed.

Statistical Analysis

The conformity of the data to the normal distribution was evaluated with histogram, Q-Q plots, and Shapiro-Wilk test. The homogeneity of variance was tested with Levene's test. Mann-Whitney U test and independent two-sample t-test were used to compare quantitative variables between two groups. Pearson $\chi 2$ analysis and Fisher exact $\chi 2$ test were used for comparing categorical data. The data analysis was performed by the software R 4.0.0 (www.r-project.org). The significance level was accepted as $p<0.05$.

RESULTS

Overall, 67 patients were included in the study. Among these patients, 73,1% were male, and the median patient age was 10.0 (5.0-13.0) years. In our retrospective review, it was seen that the highest number of patients (n:21, 31.3%) were hospitalized in 2018. Analysis concerning symptoms and signs elucidated that generalized muscle spasm was the most common clinical finding with a rate of 55.2%.

Table-1. Demographic and clinical data of patients.

	n (%): 67
Gender	
Female	18 (26.9)
Male	49 (73.1)
Application year	
2018	21 (31.3)
2019	20 (29.9)
2020	10 (14.9)
2021	16 (23.9)
Clinical findings	
Opistotonus	9 (13.4)
Neck stiffness	26 (38.8)
Lock jaw	27 (40.3)
Risus sardonicus	22 (32.8)
Generalized muscle spasms	37 (55.2)
Dysphagia	12 (17.9)
Dyspnea	20 (29.9)
Fever	11 (16.4)
Treatment options	
Tetanus toxoid	44 (65.7)
Tetanus immunoglobulin	26 (38.8)
Benzodiazepine	64 (95.5)
Baclofen	47 (70.1)
Penicillin G	47 (70.1)
Metronidazole	60 (89.6)
Magnesium sulfate	11 (16.4)
Hospitalization unit	
Pediatric service	46 (68.7)
Neurology service	3 (4.5)
Infectious Diseases service	3 (4.5)
Neonatal ICU	8 (11.9)
Pediatrics ICU	4 (6)
Adult ICU	3 (4.5)

***** Data are expressed as n (%).

** ICU: Intensive Care Unit

Table-2. Comparison of demographic and clinical of patients with hospitalization units.

	RIF n:53 (79.1%)	ICU n:14 (20.9%)	p
Age	10 (6-13)	0 (0-15.5)	**0.041**
Gender			
Female	12 (22.6)	6 (42.9)	0.129
Male	41 (77.4)	8 (57.1)	
Clinical findings			
Opistotonus	4 (7.5)	5 (35.7)	**0.015**
Neck stiffness	20 (37.7)	6 (42.9)	0.967
Lock jaw	20 (37.7)	7 (50)	0.599
Risus sardonicus	21 (39.6)	1 (7.1)	**0.025**
Generalized muscle spasms	26 (49.1)	11 (78.6)	0.07
Dysphagia	8 (15.1)	4 (28.6)	0.257
Dyspnea	11 (20.8)	9 (64.3)	**0.003**
Fever	9 (17)	2 (14.3)	0.999

***** Data are expressed as n (%) and median (1st quartile-3rd quartile).

Table-3. Factors affecting mortality.

	Alive (n:54)	Dead (n:13)	p
Age	10 (5-13)	8 (2.5-12)	0.357
Gender			
Female	14 (25.9)	4 (30.8)	0.724
Male	40 (74.1)	9 (69.2)	
Clinical findings			
Opistotonus	3 (5.6)	6 (46.2)	**<0.001**
Neck stiffness	17 (31.5)	9 (69.2)	**0.012**
Lock jaw	22 (40.7)	5 (38.5)	0.88
Risus sardonicus	22 (40.7)	0 (0)	**0.005**
Generalized muscle spasms	25 (46.3)	12 (92.3)	**0.003**
Dysphagia	10 (18.5)	2 (15.4)	0.791
Dyspnea	11 (20.4)	9 (69.2)	**0.001**
Fever	8 (14.8)	3 (23.1)	0.47
Treatment options			
Tetanus toxoid	37 (68.5)	7 (53.8)	0.317
Tetanus immunoglobulin	21 (38.9)	5 (38.5)	0.977
Benzodiazepine	51 (94.4)	13 (100)	0.612
Baclofen	40 (74.1)	7 (53.8)	0.152
Penicillin G	36 (66.7)	11 (84.6)	0.204
Metronidazole	49 (90.7)	11 (84.6)	0.517
Magnesium sulfate	10 (18.5)	1 (7.7)	0.344

***** Data are expressed as n (%) and median (1st quartile-3rd quartile).

The comparison of treatment modalities, including vaccination, administration of immunoglobulins, drugs used for sedation and muscle spasms (i.e., benzodiazepine, baclofen, magnesium sulfate), and antibiotics (i.e., penicillin G, metronidazole), showed that benzodiazepine (i.e., diazepam or midazolam) treatment was the most frequently performed therapeutic method (95.5%). While 79.1% of the patients were admitted to the RIF, 20.9% were referred to the ICU. It was also determined that most of the RIF admissions were to the pediatrics department (68.7%), and the highest rate of ICU admissions was to the neonatal ICU (11.9%). The demographic data, clinical findings, applied treatment methods, and outcomes of the patients

are displayed in Table-1. Our analysis also included comparing the data between patients admitted to RIF and ICU. The results of this comparison are displayed in Table-2.

Our comparative analysis revealed that RIF and ICU patient groups were significantly different regarding age, presence of opisthotonus, risus sardonicus, and dyspnea ($p<0.05$). The mean length of hospitalization was calculated as 10.73±8.15 days. Analyzes were made regarding the factors affecting the hospitalization duration; applied treatment methods such as benzodiazepine, baclofen, metronidazole, and magnesium sulfate were related to the length of hospital stay ($p<0.05$).

A review concerning post-hospitalization survival status elucidated that 80.5% of the patients were discharged from the hospital, while the in-hospital mortality rate was 19.5%. Furthermore, the survival and mortality rates did not differ according to admission year ($p>0.05$) (Figure-1).

Our analysis also included a comparison based on mortality status. The results of this comparison are displayed in Table-3. Analysis concerning factors affecting mortality elucidated that opisthotonus, neck stiffness, generalized muscle spasm, and dyspnea at the time of initial presentation to ED were associated with high mortality ($p<0.05$).

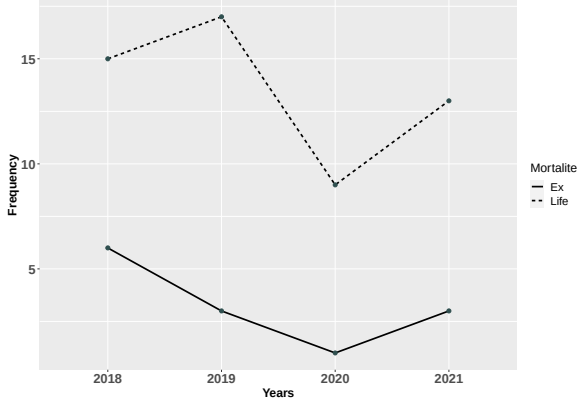

Figure-1. Mortality graph for years.

DISCUSSION

Tetanus: is a vaccine-preventable disease that remains a common cause of acute critical illness in low- and middle-income countries due to a lack of immunization (2, 8). It has a high morbidity and mortality rate due to complications such as acute kidney injury, gastrointestinal bleeding, sepsis, extensive intravascular coagulation, nosocomial pneumonia, subglottic stenosis due to long-term hospitalization, and mechanical ventilation use (6).

The Diphtheria Tetanus Toxoid and Pertussis (DTP) containing vaccine 3rd dose rate for Somalia is 42% which is based on the latest WHO/UNICEF Estimates of National Immunization Coverage (WUENIC) in 2023 (9). The World Health Assembly recommends that at least 80% of pregnant women should be vaccinated with at least two doses of tetanus toxoid-containing vaccines and that at least 80% of women of reproductive age in high-risk areas should be vaccinated with at least three rounds of tetanus toxoid-containing vaccine; but in Somalia, this rate remained at 58% as of 2019 (10). In Somalia, overall immunization rates are low due to a weak health system, inadequate immunization services, and vaccine refusal. As a result, vaccine-preventable diseases continue to occur in this region.

The mean age and gender distribution of the patients included in our study aligned with the literature (11, 12). The most common clinical finding in the presentation to the ED was generalized muscle spasm, probably due to delayed admissions to the hospital in this region. In line with this, Wang et al. found in their retrospective multicenter study that the most common symptom in tetanus patients was generalized muscle spasm (11). On the other hand, it has been reported that trismus was the most common initial symptom in these cases (4). Since tetanus is characterized by the generalization of the spasms if left untreated, we believe that Wang et al. worked on late-stage tetanus cases as we did in our study.

Treatment in tetanus cases aims to monitor the patient's condition, provide respiratory support, eliminate the source of the toxin, neutralize the unbound toxin, and prevent muscle spasms (1). In addition, tetanus immunoglobulin (TIG) neutralizes the circulating toxins, and tetanus toxoid (TT) provides active immunization (5).

Our study determined that 38% of the patients were treated with TIG and more than half with TT. Since detailed immunization data of the patients could not be reached, we do not know how the selection between TIG or TT treatments was made. In the study of Dafallah et al., diazepam was used in all patients, and baclofen was used in half of the patients for tetanus-related muscle spasms (13). Similarly, in our study, diazepam was used in almost all patients,

and baclofen was used in more than half. Diazepam was preferred by approximately 90% of patients in our study; it is thought to be widely used, primarily due to its easy accessibility and cost-effectiveness. Magnesium sulfate infusion is an adjunct to benzodiazepines for muscle spasms, especially in autonomic dysfunction (6). The low rate of magnesium sulfate in our study; may be related to a lower incidence of autonomic dysfunction symptoms such as fever, dyspnea, and the effectiveness of benzodiazepine monotherapy in the treatment of muscle spasms. It was reported in the literature that the use of metronidazole slowed disease progression and reduced mortality (4). We used metronidazole in most patients; however, we found that the selected treatment method did not affect mortality.

In a study performed in Tanzania by Chalya et al., 82.4% of the patients were referred to the ICU (14). The ICU admission rate was 20.9% in our study. Our cohort's low ICU hospitalization rate may be related to the lower mean patient age and less need for mechanical ventilator support.

In the study by Dafallah et al., the length of hospitalization was shorter than in our study (13). The inclusion of patients with late-stage clinical symptoms probably led to the prolongation of the treatment period. Also, we suggest that the relatively higher mortality rate in our study can be ascribed to the same reason (15, 16). Hasnain et al. found that the mortality rate was 28.6% in patients with tetanus, and these authors reported a significant positive correlation between age and mortality (17). Nevertheless, our analysis revealed that age, gender, and selected treatment method did not affect patient mortality.

Limitations

The most important limitation of the study is its retrospective and single-center design. Moreover, a more detailed analysis, including the vaccination status and the mechanisms of disease transmission, could not be made due to incomplete data.

CONCLUSION

Although tetanus is a vaccine-preventable disease, it is still prevalent in low- and middle-income countries with high mortality rates. Therefore, early diagnosis and treatment are essential for reducing ICU hospitalization and mortality rates.

Funding

The author(s) received no financial support for the research, authorship and/or publication of this article.

Acknowledgments

Authors thank Assoc. Prof. Dr. Sertac Cimen, Director of Education Department in Somalia Turkey Training and Research Hospital, for the support given throughout this project.

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

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Research Article / *Araştırma Makalesi*

Ege Journal of Medicine / *Ege Tıp Dergisi* 2024; 63 (3): 435-440

Anxiety and depression levels in women with hyperglycemia in pregnancy: a comparative study with normoglycemic women

Gebelikte hiperglisemisi olan kadınlarda anksiyete ve depresyon düzeyleri: normoglisemik kadınlarla karşılaştırmalı bir çalışma

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ABSTRACT

Aim: The aim of this study is to investigate the anxiety and depression levels of women with hyperglycemia in pregnancy and to compare them with normoglycemic women at similar gestational weeks.

Materials and Methods: Diabetic pregnant women (pregestational diabetes mellitus, gestational diabetes mellitus) at 32-34 weeks and normoglycemic pregnant women at similar gestational weeks were evaluated with the Hospital Anxiety and Depression Scale to determine anxiety and depression levels.

Results: In our study patients with fetal complications associated with poorly controlled diabetes, such as large for gestational age (p: 0.04) and polyhydramnios (p: 0.03), exhibited significantly lower levels of anxiety symptoms. No significant difference was found between the diabetes group and the control group in terms of anxiety and depression symptoms

Conclusion: The fact that anxiety symptoms were significantly less in polyhydramnios and large for gestational age patients, which are the result of poorly controlled diabetes, suggests that hyperglycemia may have an impact on anxiety levels.

Keywords: Anxiety, depression, diabetes mellitus, hospital anxiety and depression scale.

ÖZ

Amaç: *Bu çalışmanın amacı gebelikte hiperglisemisi olan kadınların anksiyete ve depresyon düzeylerini incelemek ve benzer gebelik haftalarındaki normoglisemik kadınlarla karşılaştırmaktır.*

Gereç ve Yöntem: *32-34 haftalarındaki Diabetik gebeler (Pregestasyonel diabetes mellitus, gestasyonel diabetes mellitus) ile benzer gebelik haftalarındaki normoglisemik gebeler anksiyete ve depresyon düzeylerinin belirlenmesi için Hastane Anksiyete ve Depresyon ölçeği ile değerlendirildiler.*

Bulgular: *Çalışmamızda diyabetin fetal komplikasyonları olan gebelik haftasına göre büyük fetüs (p: 0.04) ve polihidroamniyos (p:0.03) gelişen olgularda anksiyete semptomları anlamlı olarak daha az saptandı. Diyabet grubu ile kontrol grubu arasında anksiyete ve depresyon semptomları açısından anlamlı fark saptanmadı.*

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Application date: 18.03.2024 Accepted: 24.05.2024

Sonuç: *Kötü kontrollü diyabetin sonucu olan polihidramniyos ve gebelik haftasına göre büyük fetüs olgularda anksiyete semptomlarının anlamlı olarak az bulunması hipergliseminin anksiyete üzerinde etkisinin olabileceğini düşündürmektedir.*

Anahtar Sözcükler: *Anksiyete, depresyon, diabetes mellitus, hastane anksiyete ve depresyon ölçeği.*

INTRODUCTION

Pregnancy is an important period in a woman's life where physiological, psychological, and social changes that begin with fertilization are experienced and adaptation to these changes is necessary. Pregnant women may experience fluctuating levels of stress throughout pregnancy, and mood disorders, including depression, are commonly reported (1).

Hyperglycemia stands as a prevalent medical conditions encountered by women throughout the course of pregnancy. Statistics from the International Diabetes Federation suggest that roughly one out of every six live births, amounting to 16.8%, is delivered by women grappling with varying degrees of hyperglycemia during gestation. Hyperglycemia can cause serious complications for both mother and baby including increased risk for cesarean delivery, birth trauma, hypertensive disorders of pregnancy, macrosomia or large babies which may require an operative delivery resulting in shoulder dystocia and other birth injuries; respiratory distress syndrome; hypoglycemia; polycythemia; jaundice/hyperbilirubinemia as well as long-term risks including childhood obesity later on life (2).

Recent studies has shown an association between diabetes and depression, emphasizing that diabetes may increase the risk of depression in non-pregnant patients (3) and that depression may be a risk factor for type 2 diabetes (4). There exists limited literature concerning the psychological stress and depression levels experienced by women diagnosed with pregestational diabetes mellitus (PGDM) and gestational diabetes mellitus (GDM) (1).

In this study, we aimed to evaluate women with PGDM and GDM in terms of depression and anxiety symptoms.

MATERIALS and METHODS

This study was conducted prospectively in the obstetrics and gynecology department of a tertiary care hospital between May 2021 and August 2021.

Pregnant women with GDM or PGDM without chronic disease at 32-34 weeks of gestation, with a singleton live fetus, at a social level to read and respond to the tests and who agreed to participate in the study (Diabetes group) and a similar number of non-diabetic pregnant women (control group) were included in the study. Those with a history of psychiatric illness were excluded. Ethics committee approval for this study was obtained from the local ethical Committee (22.04.2021/1377). Participants were informed the purpose of the research.

Type 1 or type 2 diabetes diagnosed before conception was considered pregestational diabetes mellitus. GDM was defined as abnormal glucose tolerance first diagnosed during pregnancy. A fetus weighing >90th percentile for gestational age was considered large for gestational age. Polyhydramnios were diagnosed when the single deepest pocket (SDP) ≥8 cm or the amniotic fluid index (AFI) ≥24 cm.

A 75-g oral glucose tolerance test (75 g OGTT) was performed at 24-28 weeks of gestation in all cases except pregestational diabetes mellitus cases. For 75 g OGTT, the International Association of Diabetes and Pregnancy Study Groups (IADPSG) reference values (fasting blood glucose: 92 mg/dl, 1st-hour postprandial blood glucose: 180 mg/dl, 2nd-hour postprandial blood glucose: 153 mg/dl) were taken as the basis. A single high value was considered diagnostic for GDM (5).

All patients included in the study were evaluated with the Hospital Anxiety and Depression Scale (HADS) between 32 and 34 weeks of gestation at the visit after the 75 g OGTT result. As a self-reported instrument, the Hospital Anxiety and Depression Scale is composed of 14 items, seven of which provide an assessment of depression and seven of which provide an assessment of anxiety. Responses are rated on a four-point Likert scale and scored between 0-3. Developed in 1982 by Zigmond and Snaith, the Hospital Anxiety and Depression Scale has a cut-off point of 7 for anxiety and depression. According to the scale, 0-7 points are considered normal, 8-10 points are considered suspicious, and 11 points and above are considered unhealthy (6). The validity and reliability study of

this scale was conducted by Aydemir et al. in 1996 and was adapted to Turkish society. In the validity study adapted to our country by Aydemir, the cut-off point of the scale was determined as >10 for anxiety and >7 for depression (7). The objective of the scale is not to provide a diagnosis, but rather to swiftly identify the at-risk group through screening for anxiety and depression.

Statistical analysis was conducted using IBM SPSS Statistics for Windows, Version 23.0 (IBM Corporation, Armonk, NY, USA). Descriptive statistics were expressed as the mean ±standard deviation. Categorical variables are presented as numbers and percentages. Student t-test and Mann-Whitney-U test were used in comparing the continuous variables, while the Chi-Square test was used to compare categorical variables. $p < 0.05$ was considered statistically significant.

RESULTS

During the study, 96 patients were included. While 46 of these cases–were complicated with diabetes during pregnancy (Diabetes group), glucose intolerance was not detected in 50 cases (the Control group). While 27 patients complicated with diabetes were diagnosed with GDM, 19 cases were diagnosed with PGDM. A combination of diet and exercise (diet + exercise) was sufficient to maintain normoglycemia in 13 of the patients in the diabetes group, but insulin was needed in 33 of these patients. The demographic characteristics of both groups are presented in Table-1.

Large for gestational age was found in 23/96 (23.9%) pregnant women and polyhydramnios was found in 13/96 (13.5%).

The anxiety and depression status of pregnant women was determined by applying the Hospital Anxiety and Depression Scale. Accordingly, 37 (38.5%) of the 96 pregnant women who participated in the study had symptoms of depression, while 33 (34.4%) had symptoms of anxiety. In 21 (21.8%) patients, both depression and anxiety symptoms were found together. The mean anxiety score of the pregnant women who participated in the study was 9.06±4.495, while the mean depression score was 6.41±3.588. The diabetes group and the control group were compared in terms of anxiety and depression symptoms, and no significant difference was found (Table-2). Likewise, no significant difference was found between women with gestational diabetes mellitus and PGDM in terms of anxiety and depression symptoms (p:0.27).

The relationship between depression and anxiety scores in patients with large for gestational age and polyhydramnios is shown in Table-3. Anxiety symptoms were significantly lower in pregnant women with large for gestational age and polyhydramnios.

Patients in the diabetes group were compared in terms of anxiety and depression symptoms according to diagnosis and treatment regimen, and no significant difference was found (Table-4).

Table-1. Comparison of demographic characteristics of diabetes and control groups.

Parameter	Diabetes group n:46		Control group n: 50		P value
	Mean	SD	Mean	SD	
Maternal age	30.8	6.4	27.5	4.6	0.005
Gravidity	2.87	1.7	2.52	1.4	0.28
Parity	1.33	0.9	1.18	1.1	0.49
Miscarriage	0.54	1.4	0.34	0.5	0.35
Body mass index	34.4	5.5	27.8	3.4	<0.001

Table-2. Comparison of DM and control groups in terms of depression and anxiety symptoms.

	Diabetes goup n:46		Control group n: 50		P value
	N	%	N	%	
Depression score >7	17	(36.9%)	20	(40%)	0.76
Anxiety score >10	13	(28.2%)	20	(40%)	0.22

Table-3. Comparison of depression and anxiety scores in terms of Large for gestational age and polyhydramnios

	Depression score (n: 96)		P value	Anxiety score (n: 96)		P value
	>7 (n: 37)	≤7 (n: 59)		>10 (n: 33)	≤10 (n: 63)	
Large for gestational age n:23	8	15	0.67	4	19	0.04
Polyhydramnios n:13	4	9	0.53	1	12	0.03

Table-4. Comparison of depression and anxiety scores in terms of Diabetes type (GDM vs. PGDM) and Diabetes treatment (Diet + exercise and insulin)

	Depression **score**		**P value**	Anxiety score		**P value**
	>7	≤7		>10	≤10	
Diabetes type (n:46)			0.54			0.27
GDM	9	18		6	21	
PGDM	8	11		7	12	
Diabetes treatment (n: 46)			0.31			0.07
Diet + exercise	3	10		1	12	
Insulin	14	19		12	21	

DISCUSSION

Our study did not reveal any significant difference in depression and anxiety symptoms between pregnant women with and without diabetes. However, it was found that those with large–for gestational age or polyhydramnios associated with poor glycemic control had significantly fewer anxiety symptoms than those without.

There are conflicting results in the literature on depression symptoms between patients with and without a diagnosis of diabetes during pregnancy. While some studies have shown no significant difference in depression symptoms between patients with GDM and those without GDM (8-11), a systematic meta-analysis conducted in 2020 by Lee et al. found a notable increase in the risk of antepartum depression development in pregnant women diagnosed with gestational diabetes mellitus (PGDM+GDM) compared to pregnant women without a gestational diabetes mellitus diagnosis during pregnancy (RR = 1.431, 95% CI: 1.205-1.699). However, they did not find an association between PGDM (RR = 1.300, 95% CI: 0.736-2.297) and the risk of developing antepartum depression (12). In our study, no statistically significant difference was observed in depression scores between the gestational diabetes group and the control group, nor within the diabetes group (between PGDM and GDM). The discrepancies in these findings could potentially be attributed to variations in survey scales and divergent cut-off values utilized across studies.

When studies comparing pregnant women with hyperglycemia with non-diabetic pregnant women in terms of anxiety symptoms are examined, some studies reported similar levels of anxiety symptoms between diabetic pregnant women and pregnant women with normal glucose levels (13-15). A recent systematic review concluded that the diagnosis of GDM elevates the occurrence of anxiety and depression among pregnant women (16). In our study, we observed no statistically significant disparity in anxiety scores between the gestational diabetes mellitus group and the control group, nor within the diabetes mellitus groups (between PGDM and GDM).

In pregestational diabetes mellitus and GDM, a higher amount of blood glucose crosses the placenta and enters the fetal circulation and causes fetal hyperglycemia. Excess glucose in the fetus is stored as body fat –and causes macrosomia, also called "large for gestational age" (17). Data from the Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) study

show a strong linear correlation between maternal glucose concentration and large-for-gestational-age (LGA) fetuses (18). In cases of maternal hyperglycemia, the increase in fetal urine excretion is thought to be related to the increase in osmotic diuresis (19). Consequently, maternal hyperglycemia increases the risk of macrosomia and polyhydramnios. In our study, we found significantly fewer anxiety symptoms in pregnant women with large for gestational age or polyhydramnios compared with those without. Since there is a linear relationship between polyhydramnios/large for gestational age and poorly controlled maternal hyperglycemia, the question arises of whether maternal hyperglycemia has a role in the development of anxiety. To our knowledge, there is currently no published study in the literature that examines the association between glycemic control and anxiety symptoms in pregnant individuals. However, in a meta-analysis evaluating non-pregnant diabetic subjects, no strong association was found between anxiety and glycemic control (20).

In a recent study investigating the association between polyhydramnios and anxiety, polyhydramnios was found to be a risk factor for anxiety (21). Contrary to expectations, our study revealed significantly lower anxiety levels among patients diagnosed with polyhydramnios. This may be attributed to the different etiologies of polyhydramnios. The main cause of polyhydramnios in our cases was hyperglycemia, whereas, in the other study, the cause of polyhydramnios was not specified.

Our study is subject to limitations, notably the small sample size and the restricted number of cases with polyhydramnios and those categorized as large for gestational age within the control group.

The presence of both PGDM and GDM cases in the diabetes group and the comparison of diabetes cases according to treatment modalities are considered to be the strengths of our study. The limitations of our study include the small number of cases and the small number of polyhydramnios and the large for gestational age cases in the control group.

CONCLUSION

The significant decrease in anxiety symptoms in polyhydramnios and large for gestational age patients suggests that hyperglycemia or metabolic changes caused by hyperglycemia may have an impact on anxiety. Additional research is warranted to clarify the association between hyperglycemia and anxiety

Conflict of interest: The authors in the study admit that there is no conflict of interest.

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Research Article / *Araştırma Makalesi*

Ege Journal of Medicine / *Ege Tıp Dergisi* 2024; 63 (3): 441-448

Cytotoxicity assay of Türkiye's rare endemic *Helianthemum germanicopolitanum* Bornm. plant extract on HT-29 cell line

Türkiye'nin nadir endemiği Helianthemum germanicopolitanum Bornm. bitki ekstraktının HT-29 hücre hattı üzerindeki sitotoksisite analizi

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ABSTRACT

Aim: In this study, the cytotoxic potential of the endemic *Helianthemum germanicopolitanum* Bornm. plant against colon cancer is investigated.

Materials and Methods: This study pioneers the investigation of medicinal applications of the *H. germanicopolitanum* plant, specifically targeting the HT-29 human colon cancer cell line. The phytochemical profile of the aerial parts of the plant, especially the flavonoid content, was analyzed using High Performance Liquid Chromatography (HPLC). Cytotoxic effects were then evaluated by WST-1 assays on the HT-29 cell line; this revealed time- and dose-dependent inhibition of cancer cell growth.

Results: These results also highlight the need for comprehensive research into *H. germanicopolitanum*'s unique flavonoid composition and its broader implications in cancer treatment.

Conclusion: These results also highlight the need for comprehensive research into *H. germaicopolitanum*'s unique flavonoid composition and its broader effects in cancer treatment.

Keywords: *Helianthemum germanicopolitanum*, colon cancer, HT-29 cell line, flavonoids, cytotoxicity.

ÖZ

Amaç: *Bu çalışmada endemik Helianthemum germanicopolitanum Bornm. bitkisinin kolon kanserine karşı sitotoksik potansiyeli araştırılmıştır.*

Gereç ve Yöntem: *Bu çalışma, özellikle HT-29 insan kolon kanseri hücre hattını hedef alarak H. germanicopolitanum bitkisinin tıbbi uygulamalarının araştırılmasına öncülük etmektedir. Bitkinin topraküstü kısımlarının fitokimyasal profili, özellikle de flavonoid içeriği Yüksek Performanslı Sıvı Kromatografisi (HPLC) kullanılarak analiz edilmiştir. Sitotoksik etkiler daha sonra HT-29 hücre hattı üzerinde WST-1 deneyleri ile değerlendirilmiştir; bu, kanser hücresi büyümesinin zamana ve doza bağlı inhibisyonunu ortaya çıkarmıştır.*

Bulgular: *Flavonoid profillerinin ve sitotoksisitenin karşılaştırmalı analizleri, benzer türler üzerindeki mevcut literatüre göre yapılmıştır. Bu çalışmanın bulguları, H. germanicopolitanum'un kolon kanseri tedavisinde terapötik uygulamaları olan biyoaktif bileşiklerin kaynağı olarak tıbbi açıdan potansiyelinin belirlenmesine ışık tutmaktadır.*

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Application date: 26.02.2024 Accepted: 30.05.2024

***Sonuç**: Bu sonuçlar ayrıca, H. germanicopolitanum bitkisinin eşsiz flavonoid bileşimi ve kanser tedavisindeki daha geniş etkileri hakkında kapsamlı araştırmalara duyulan ihtiyacı vurgulamaktadır.*

***Anahtar Sözcükler:** Helianthemum germanicopolitanum, kolon kanseri, HT-29 hücre hattı, flavonoidler, sitotoksisite.*

INTRODUCTION

Cancer is the cause of one in six deaths worldwide in 2018 (1). The last parts of the large intestine other than the rectum is called the colon, and its cancer is called colon cancer, and all cancers of the large intestine are called colorectal cancer (2). Colorectal cancer is the third most common type of cancer worldwide and the second leading cause of cancer-related deaths worldwide, accounting for approximately 10% of all cancer cases (3). Incidence and mortality rates in colon cancer show large geographical differences, with the highest incidence rates in Europe, Australia, and New Zealand, while the highest mortality rates were observed in Eastern Europe (3).

The relationship between humans and plants dates to 1.2 million years ago (4). Considering fossil records, primitive man's use of plants as medicine for treating diseases dates back at least 60,000 years ago (5). Medicinal plants are used in the treatment of various diseases around the world (6).

Plants are very important natural treasures in traditional medicine thanks to the phytochemicals in their composition (7). Medicinal plants, which have important roles in the fight against cancer, contain secondary metabolites such as alkaloids, tannins, flavonoids, pigments, and terpenoids, which do not have an active role in their growth (1). These compounds show anticancer properties by causing DNA repair, suppressing cancer-inducing enzymes, increasing immunity, producing anticancer enzymes, and inducing antioxidant activity (8).

Türkiye is a very rich country in terms of endemic plant species, and one-third of approximately 9,000 medicinal and aromatic plants are endemic (9). It is known that some species of *Helianthemum* Adans., which are widespread in our country, have anti-constipation and astringent effects, and in some countries, species of this genus are also used as anti-inflammatory, antiulcerogenic, wound healing, antimicrobial, cytotoxic and antidiabetic agents (10, 11). In addition, some *Helianthemum* species contain phytochemicals of high medical importance and rich antioxidants, confirming their use in the treatment of various human diseases (12, 13).

Türkiye is home to 4 genera (*Cistus*, *Fumana*, *Tuberaria*, *Helianthemum*) and 37 taxa of Cistaceae, of which about ∼19% are endemic. *Helianthemum* is represented by 19 taxa in Turkey, 4 of which are endemic (14), giving an endemism rate of 21, which ranks first in Turkish Cistaceae species. *Helianthemum germanicopolitanum* Bornm., whose vernacular name is 'özgegüngülü' (14), is a rare endemic plant native to Çankırı province and grows in gypsum/marly areas (15). There are studies in the literature emphasizing the medical importance of *H. germanicopolitanum*, which is endemic in Türkiye, in diseases such as diuretics, constipation, and hemorrhoids (16, 17, 18).

Helianthemum germanicopolitanum, which is less popular because its growing area is limited to Çankırı province (Turkey), is an endangered plant. The fact that studies revealing the effects of *H. germanicopolitanum*, whose medical importance has been emphasized in several studies, on any cancer are not included in the literature causes our research to gain momentum. Therefore, this study aimed to investigate the cytotoxic effect of the plant by determining the active compounds of the aerial parts of endemic *H. germanicopolitanum*, which may be useful in the treatment of colon cancer. Local medical sources use the plant material as an exploration to cure various diseases and situations (etc. scar regeneration and replenishment), transforming such plants from nature to modern medicine needs dosing with toxicity tests then *in vitro* and *in vivo* respectively. In this study, we investigated the first step to understand the potential of the plant for medical use.

Local sources, observations, and limited literature reported the anorectal usage of this plant material. To support this claim, we started our study with the human colon cancer cell line of HT-29. This cell line may mimic neovascularization, hemorrhoids, and anal fissures. Such disorders can be classified as gastrointestinal tract invasions. Characteristics of

HT-29 may be implemented from the cecum to the anus as one. According to our current knowledge, this study is the first to investigate colorectal medical aspects of specimen usage.

MATERIALS and METHODS

Plant Material

Helianthemum germanicopolitanum is a suffruticose, perennial herb, with erect flowering stems up to 30 cm tall (Figure-1a). The leaves are elliptic or oblong, and stellate-tomentose. The inflorescence is branched or simple, and laxly 3-6 flowered. Corolla is yellow. Flowering time is in June (19). The habitat of the species is gypsum-rich soils (Figure-1b).

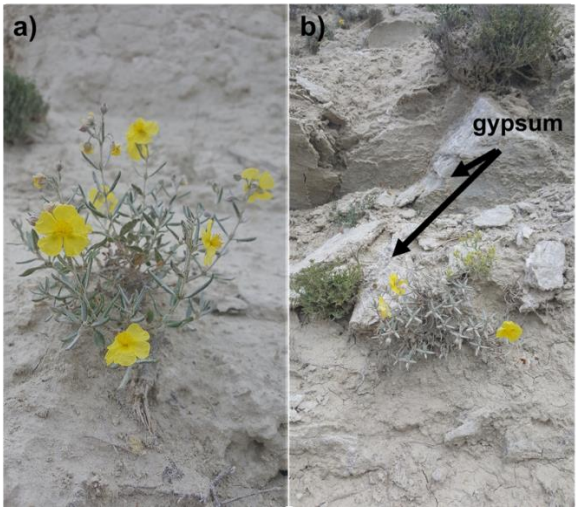


Figure-1. (a) *Helianthemum germanicopolitanum* habit (from Çankırı province), (b) Gypsum habitat where the plants grow (Photos by A. Kayabaş Avşar)

H. germanicopolitanum was collected from gypsum habitats on Çankırı-Korgun road (Coordinates: 40°38’55.1"N, 33°36’29.7"E, Çankırı, Turkey) in May-June 2021. Samples were identified taxonomically according to Flora of Turkey and the East Aegean Islands (19). The identification of plant species was made by the author (A. Kayabaş Avşar).

The whole plant samples were taken individually from their natural habitat, cleaned of soil and dirt using a fine paintbrush or by gently blowing on the sample followed by a wash with distilled water.

Plant Extraction

The aerial parts of the *H. germanicopolitanum* were used in the study. The roots of this perennial plant were not included in the analysis because they contain large amounts of cellulose due to their woody structure. Since the plant is an endemic and rare species, all aerial parts were analyzed as a single piece, considering the protection of the flora in nature. To increase the surface area before extraction and thus increase the extraction efficiency, the plant material was dried and made as homogeneous and small-sized particles as possible.

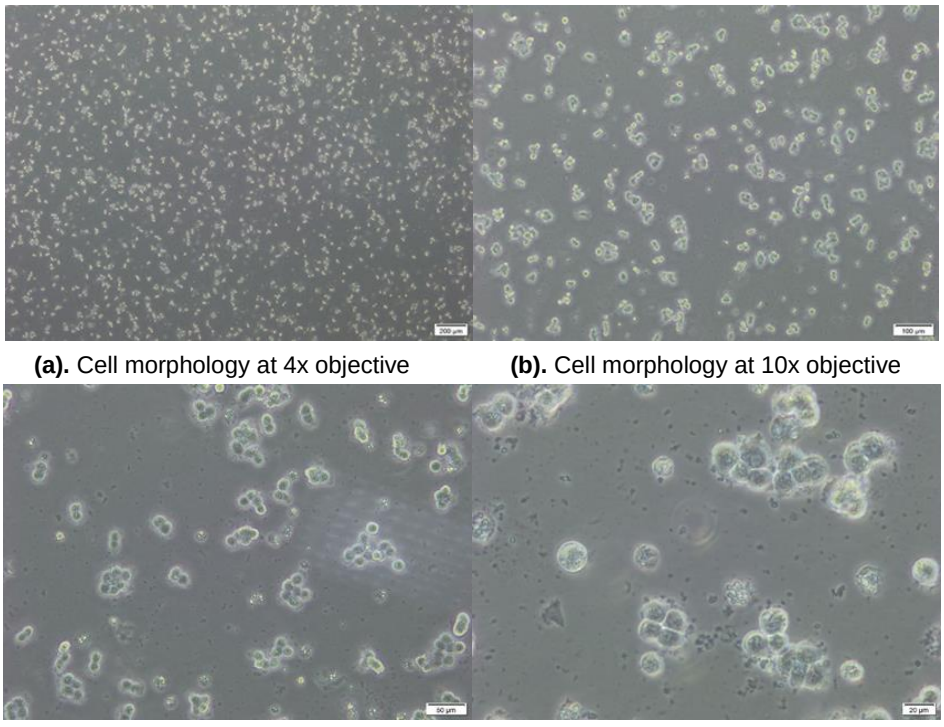
The extraction efficiency depends on how much and how long the plant material is in contact with the solvent and the choice of the appropriate solvent for extraction. Extraction time and temperature are important parameters during the extraction of plant particles with increased surface area. As extraction time, 20 g of plant material and 400 ml of methanol were extracted at 50^{o}C using the soxhlet device the first siphon was completed in the 20th minute and the next 4 siphons were completed at 15-minute intervals and the extraction process was carried out at the most appropriate time and temperature. After the extraction process was finished, the solvent was removed from the rotary evaporator system. The extract obtained was powdered with the help of a lyophilizer device and made ready for the next experimental procedures (20).

High Purity Liquid Chromatography (HPLC) Analysis

HPLC-DAD analysis was performed with a Thermo Ultimate 3000 HPLC (Thermo Scientific, Dionex, Bremen, Germany). The system components consisted of a pump, an autosampler, a column oven, and a diode array detector. An ODS RP C18 column (250 x 4.6 mm, 5 µm, Thermo Scientific, Bremen, Germany) was used for all separations. Liquid chromatography separations were performed using the following solvents: A-Ultrapure water: H_3PO_4 (0.2%) and B-acetonitrile. Before the last injection, the column was equilibrated for 3 min at initial conditions. The flow rate was 0.8 mL/min, and the column temperature was set at 30 °C. Detection was performed at 205 nm and the UV spectra of all samples were scanned between 190-400 nm (21).

Cell Culture and Treatment

HT-29 cells, a colon cancer cell line, were obtained from Ege University Faculty of Medicine, Department of Histology and Embryology. Dulbecco’s Modified Eagle’s Medium (DMEM) and Fetal Bovine Serum (FBS) (Gibco, USA) were purchased. Culture conditions are validated morphologically as seen in the Figure-2.



(a). Cell morphology at 4x objective **(b).** Cell morphology at 10x objective

(c). Cell morphology at 20x objective **(d).** Cell morphology at 40x objective

Figure-2. Cell morphology of HT-29 on culture conditions on 4x (a), 10x (b), 20x (c) and 40x (d) respectively.

HT-29 cholangiocarcinoma cells frozen at P/17 were thawed and plated with Dulbecco's Modified Eagle's medium prepared by adding 10% Fetal Bovine Serum, 1% Penicillin-Streptomycin and 1% L-Glutamine and grown at 37°C in an incubator with 5% CO_2. Cells were seeded in 25 cm^2 cell culture flasks for 24 hours. Afterward, the cells were passaged and seeded into 75 cm^2 flasks.

Colon cancer cells were divided into control groups and dose-treated groups. HT-29 cells in all groups were grown in a complete medium. HT-29 cells were treated with *H. germanicopolitanum* doses (0, 5, 10, 50, 100 and 1000 µg/ml) for 24, 48 and 72 hours respectively and stored in the incubator. Cell cytotoxicity was evaluated by WST-1 assay in 96-well plates.

Cell Proliferation Assay

Cell proliferation experiments were performed. HT-29 colon cancer cells removed after passaging were seeded in 96 well plates at $8x10^3$ cells per well. In this study, cell cytotoxicity was measured with the Water-Soluble Tetrazolium-1 WST-1 Assay Kit (Cell Proliferation) (ab65475, Abcam, USA) kit according to the protocol determined by the manufacturer. The experiment was started by incubating the cells for 24 hours and allowing them to adhere to 96 well plates. When the cells reached 90% density, 10 µl WST-1 reagent was added to the cells in each 96 well according to the WST-1 kit (Assay Kit (Cell Proliferation) (ab65475, Abcam, USA) procedure, and the cells were incubated for 1 hour, the *H. germanicopolitanum* concentrations we prepared were added to the wells and left for 24, 48 and 72 hours of incubation. At 490 nm wavelength, readings were taken in 3 replicates (22).

As explained above, approximately $8x10^3$ HT-29 cells/mL were seeded in triplicate and 10 µl of WST-1 reagent was added. The cells were then incubated in the incubator for 1 hour and the absorbance at 490-520 nm was measured every 30 minutes.

RESULTS

To obtain cytotoxicity results we first investigated the HPLC profile of the plant and compared it with the literature, after that toxicity assay was investigated.

HPLC

The High-Performance Liquid Chromatography (HPLC) analysis of the *H. germanicopolitanum* extract has provided insightful findings,

demonstrating the existence of a diverse array of flavonoids (Figure-3). This conclusion is drawn from the distinctive peaks discerned in the analysis results. However, to gain a comprehensive understanding and ascertain the precise composition, a meticulous and in-depth investigation becomes imperative. Identifying the specific types of flavonoids within the extract requires a detailed examination, ensuring accuracy in characterizing the phytochemical profile of this plant.

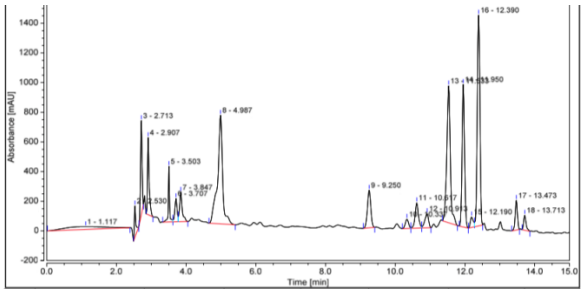


Figure-3. HPLC profile of *Helianthemum germanicopolitanum* extract.

Cytotoxicity

In the study, WST-1 analysis was performed to show the cytotoxic effects of the plant on the HT29 cell line, and when the results obtained were interpreted, it was found that the substance showed a time/dose-dependent effect. Increasing the concentration or prolonging the exposure time shows a more severe inhibition of cell growth. As shown in Figure-4 and Figure-5, the IC50 values of the substance were determined as 26.45, 37.81, and 36.21 µg/ml after 24, 48 and 72 hours of exposure.

Cell viability was determined by WST-1 analysis on the HT-29 cell line. At the end of the exposure period, it was observed that the plant showed an anti-proliferative effect on the cell line depending on time and dose. As a result of the analyses performed in previous studies Kılıç et al. evaluated the extracts of *H. ledifolium* varieties growing naturally in Turkey in terms of *in vitro* anti-trichomoniasis activity against *Trichomonas vaginalis* and reported that these extracts inhibited the proliferation of *T. vaginalis* dose-dependently from the 4th hour (23). In this study, the IC50 value of *H. glomeratum* was reported as 62.92 µg/mL (23), in their study with *H. oelandicum*, Ağca et al. investigated the inhibition activities of ethanol and aqueous solutions of plant extracts against α glucosidase for *in vitro* hypoglycemic activity determination and found IC50 values of 2.52±0.01 and 3.21±0.01 µg ml^{-1}, respectively (10). When these values were compared with the standard compound acarbose with IC50 value of 0.90±0.01 µg ml^{-1}, strong inhibition activities were found. As a result of all these evaluations, it was stated that the antioxidant, anti-inflammatory and hypoglycemic activity of the ethanol extract was higher than the aqueous extract (10), in other study investigating the wound healing ability of *H. canum*, Küpeli Akkol et al. reported that *H. canum* extract significantly reduced cell viability at doses higher than 156 µg/mL and had no toxic effect at low doses, but according to SRB assay results, it was toxic at high doses. In the RTCA test results, the IC50 value of *H. canum* extract at 24 hours was determined as 2.7 mg/mL (11). The results obtained from plants in this study are like previous studies / not like previous studies.

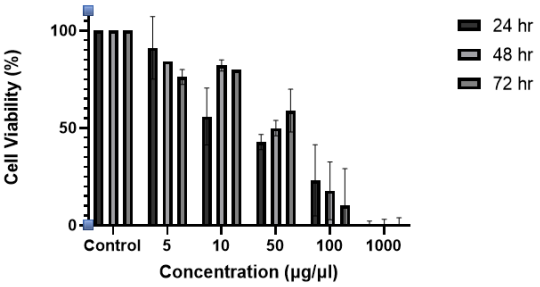


Figure-4. Cell proliferation measurements by WST-1 colorimetric method. Viability of HT-29 cells after 24, 48 and 72 hours of exposure to different concentrations.

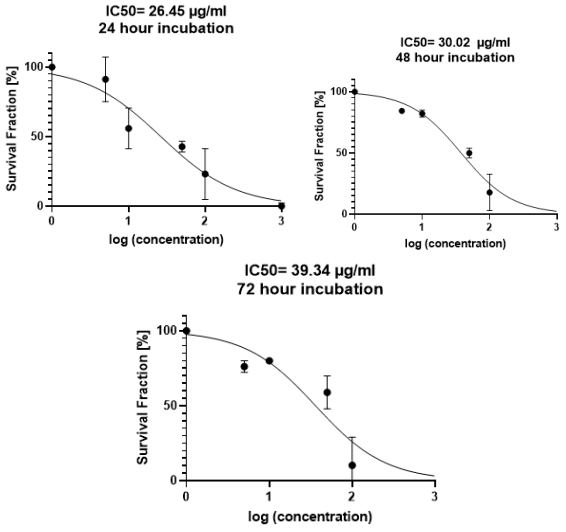


Figure-5. IC50 values were plotted in the GraphPad Prism program based on the data obtained from WST-1 analysis. HT-29 cell lines were exposed to 5-1000 µm/ml concentrations for 24, 48 and 72 hours and analyzed by WST-1 assay. The data obtained were normalized by comparison with the control group.

DISCUSSION

Members of the genus *Helianthemum* are known to be particularly native to the Mediterranean region and usually grow on sandy, stony, and calcareous soils. There are many different subspecies within this plant genus, each containing its specific flavonoid compounds (24, 25). *H. germanicopolitanum*, which we focused on in our study, is an endemic plant species specific to Çankırı and its surroundings. Local people use this plant in the form of ointment for the treatment of hemorrhoids and superficial injuries. However, comprehensive scientific studies on this plant are limited. Therefore, in our study, we investigated the cytotoxic effects of *H. germanicopolitanum* in detail. Our findings contribute to our understanding of the potential therapeutic effects of this special plant.

The root, stem, and leaves of the plant should be extracted separately to determine the flavonoids they contain. Flavonoids are components consisting of colored pigments with antioxidant properties found in plants. The main task of these components is to support the biological functions of the plant (26, 27).

The properties of flavonoids found in extracts obtained separately from the roots, stems, and leaves of plants of the genus *Helianthemum* have been studied in detail in the research (24, 28). Extracts obtained from these plants and fruits in various types include flavones such as apigenin, flavanones such as eriodictyol, hesperetin and naringenin, flavonols such as quercetin, kaempferol, myricetin and isorhamnetin, isoflavanoids such as genistein and daidzein, anthocyanins such as cyanidin, delphinidin, malvidin, pelargonidin, petunidin, peonidin, and flavonols such as epicatechin and proanthocyanidin (25).

In this first study on *H. germanicopolitanum*, the plant was extracted without separating the root, stem, and leaves. The graph we obtained after HPLC analysis (Figure 2) clearly shows that *H. germanicopolitanum* contains various flavonoids. However, specific analysis methods, such as NMR (Nuclear Magnetic Resonance Spectroscopy), need to be used to determine which flavonoids it contains (25, 29).

Flavonoids combat oxidative stress at the cellular level by reducing free radicals formed in the body. This contributes to maintaining a healthy cellular environment by protecting cells thanks to their antioxidant properties. Furthermore, flavonoids are known to reduce inflammation and exert anti-inflammatory effects (30, 31, 32).

Some types of flavonoids can regulate the cell cycle. They can inhibit the growth of cancerous tissue by stimulating apoptosis (programmed cell death) and inhibition of angiogenesis (formation of blood vessels). These properties emphasize the potential anti-cancer effects of flavonoids (33).

It is also known that flavonoids can regulate the gut microbiota. Some research suggests that flavonoids may have positive effects on stomach ulcers and inflammation. In addition, it is reported to have therapeutic and helpful properties for the digestive system by regulating intestinal motility. These diverse biological effects of flavonoids help us understand the potential positive health effects of these compounds from plant foods (34, 35, 36).

Terfassi et al. isolated a new type of flavonoid, 5,7,2′,4′,5′-pentahydroxyflavone 3-O-β-D-galactopyranoside and its derivatives from *H. getulum* Pomel. The structures of the extracts were determined using mass spectrometry and NMR techniques. Within the scope of this study, it was determined that 5 of the 13 flavonoids obtained from *H. getulum* Pomel were discovered for the first time in endemic plants of the genus Helianthemum. According to the results of the research, flavonoids are isolated by Siham Terfassia et al. were reported to have antidiabetic and antioxidant properties (37).

Plescia et al. carried out phytochemical analyses on *Helianthemum lippii*, an endemic plant native to Italy, and investigated the biological activities of the plant. According to the results of the studies, the extracts obtained were reported to have cytotoxic and antimicrobial properties (38).

Küpeli Akkol et al. investigated in detail the wound-healing mechanism of *H. canum* (L.) Baumg, which is known for its wound-healing properties. LC/MS-MS was used for phytochemical analysis of the extract obtained from the plant. Anti-inflammatory effects were evaluated through Interleukin 1, Interferon ɣ, and Interleukin 6 levels in fibroblast cells. In addition, histopathological analyses, collagenase, hyaluronidase, elastase enzyme inhibitors, and hydroxyproline estimation analyses were also performed. The results obtained show that the quinic and myricetin content of *H. canum* promotes wound healing by supporting

hydroxyproline production and wound contraction (11).

HT-29 cell line is a type of cancer derived from colon or colorectal adenocarcinoma cells lining the lining of the rectum, from which colorectal cancer originates. It is a model system frequently used in scientific research to understand the molecular mechanisms, biological properties, and potential treatment methods of colorectal cancer (39).

CONCLUSION

Our research reveals in detail that the *H. germanicopolitanum* plant species contains various flavonoids. Cytotoxicity studies of flavonoids in the extracts obtained because of the extraction procedures on colorectal cancer cell lines show that flavonoids offer anticarcinogenic effects by activating cell death pathways at certain concentrations. Our results are in line with the results of previous cytotoxic studies with flavonoids. These important findings emphasize the need for a more in-depth study of flavonoids in *H. germanicopolitanum* and the need for further research on these important compounds. The results obtained offer new insights into potential therapeutic applications and health effects, which may inspire future research.

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

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Research Article / *Araştırma Makalesi*

Ege Journal of Medicine / *Ege Tıp Dergisi* 2024; 63 (3): 449-456

The role of neutrophil/lymphocyte ratio, platelet/lymphocyte ratio and mean platelet volume in diagnosis of hydrosalpinx

Hidrosalpinks tanısında nötrofil/lenfosit oranı, trombosit/lenfosit oranı ve ortalama trombosit hacminin rolü

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ABSTRACT

Aim: Our study aimed to investigate the role of Neutrophil/Lymphocyte Ratio (NLR), Platelet/Lymphocyte Ratio (PLR), and Mean Platelet Volume (MPV) values in the diagnosis of patients who had Hydrosalpinx.

Materials and Methods: Between March 2018 and June 2023, 349 patients who underwent hysterosalpingography (HSG) due to the diagnosis of infertility in our hospital were included. Demographic and clinical data of 89 patients with hydrosalpinx detected on imaging were compared with 260 patients with normal Hsg findings.

Results: Neutrophil and Platelet levels were found to be significantly higher in the Hydrosalpinx-group than the control group (p<0.001). The presence of Hydrosalpinx was found to be significantly higher in patients who were diagnosed with secondary infertility (p<0.001). Neutrophil and Platelet levels were significantly higher in the bilateral-Hydrosalpinx-group than the unilateral-Hydrosalpinx-group (p:0.036, p:0.012, respectively). The NLR and PLR were found to be significantly higher in the bilateral-Hydrosalpinx-group than the unilateral–Hydrosalpinx-group (p:0.038, p:0.009, respectively). MPV were found to be significantly lower in the bilateral-Hydrosalpinx-group than the unilateral-Hydrosalpinx-group (p:0.011).

Conclusion: The findings of our study support the literature data on the relationship between NLR,PLR,MPV, and chronic inflammatory processes. These markers deserve to be evaluated again and again in prospective and controlled studies, in which they will be considered together with clinical findings, to investigate their ability to predict the diagnosis of Hydrosalpinx, its severity, and clinical outcomes in infertile patients.

Keywords: Hydrosalpinx, infertility, platelet/lymphocyte ratio, neutrophil/lymphocyte ratio, mean platelet volume.

ÖZ

Amaç: *Bu çalışmanın amacı hidrosalpinksli hastaların tanısında Nötrofil/lenfosit oranı, Platelet/lenfosit oranı ve Mean Platelet volume değerinin rollerini ortaya koymaktır.*

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Application date: 29.03.2024 Accepted: 02.06.2024

Gereç ve Yöntem: *Mart 2018 – Haziran 2023 tarihleri arasında hastanemizde infetilite tanısı nedeniyle histerosalpingografi (HSG) işlemi uygulanan 349 hasta dahil edilmiştir. Görüntülemede hidrosalpinks saptanan 89 hastanın demografik ve klinik verileri, normal Hsg bulguları olan 260 hasta ile karşılaştırıldı.*

Bulgular: *Hidrosalpinks grubunda nötrofil düzeyi ve platelet düzeyi, kontrol grubuna kıyasla anlamlı yüksek tespit edilmiştir (p<0.001). Sekonder infertilite tanılı hastalarda hidrosalpinks varlığı anlamlı yüksek saptanmıştır (p<0.001). Bilateral hidrosalpinks grubunda nötrofil düzeyi ve platelet düzeyi unilateral hidrosalpinks grubu ile kıyasla anlamlı yüksek saptanmıştır (p:0.036, p:0.012 sırasıyla). Bilateral hidrosalpinks grubunda platelet/lenfosit oranı ve nötrofil/lenfosit oranı, unilateral hidrosalpinks grubu ile kıyasla anlamlı yüksek saptanmıştır (p:0.038, p:0.009 sırasıyla). Bilateral hidrosalpinks grubunda mean platelet volüme düzeyi, unilateral hidrosalpinks grubuna göre anlamlı düşük saptanmıştır (p:0.011).*

Sonuç: *Çalışmamızdaki bulgular Platelet/lenfosit oranı, Nötrofil/lenfosit oranı ve Mean Platelet volume ile kronik inflamatuar süreçler arasındaki ilişkiye dair önceki literatürü desteklemektedir. Bu belirteçlerin, infertil hastalarda, hidrosalpinks tanısını, hastalığın şiddetini ve klinik sonuçlarını tahmin etme yeteneklerini araştırmak için, klinik bulgularla birlikte ele alındıkları prospektif, kontrollü çalışmalarda yeniden değerlendirilmeyi hak etmektedir.*

Anahtar Sözcükler: *Hidrosalpinks, infertilite, platelet/lenfosit oranı, nötrofil/lenfosit oranı, mean platelet volume.*

INTRODUCTION

It is considered that many different mechanisms are effective in the relationship between Hydrosalpinx and infertility. Hydrosalpinx, as its name suggests, occurs when the fallopian tubes become obstructed and filled with clear fluid (1). It can affect one or both fallopian tubes simultaneously, with infertility becoming inevitable if it occurs in both (1). Pregnancy can theoretically be achieved when Hydrosalpinx is unilateral, but its detrimental effects on fertility remain significant (2). Although the precise mechanism reducing pregnancy rates in the presence of Hydrosalpinx has not been fully elucidated, there is a possibility that the fluid within the tube may exert toxic effects on the embryo, and its spread to the endometrial tissue during ovulation may have a toxic impact on the embryo as well (3). Hysterosalpingography (HSG) is used as the gold standard diagnostic criterion for the investigation of tubal pathologies, particularly in infertility etiology (4). HSG is a diagnostic radiologic procedure that is widely used as a first-line investigation for assessing fallopian tube patency (5). Hydrosalpinx typically develops due to adhesions resulting from previous infections, endometriosis, and fallopian tube surgeries (5), which can cause narrowing or blockage of the fallopian tubes at their ends (6). The presence of infections and endometriosis sets the stage for chronic inflammatory processes (6). In recent years, there has been increasing recognition of the role of inflammatory markers in various diagnoses (7). Blood tests monitoring the neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) are employed to assess the body's inflammatory responses and infections (8). The average NLR is typically less than 2.5, while a high ratio might be a sign of an inflammatory illness. The typical PLR is less than 150, and a high ratio might point to an infection or inflammatory reaction within the body (9). PLR and NLR values are commonly used to identify illnesses and inflammatory reactions in the body. However, elevated PLR and NLR values might potentially be a sign of infections or other inflammatory diseases including cancer (9). The Mean Platelet Volume (MPV) is a statistic showing the average platelet volume that is assessed during blood tests (10). Blood clotting involves thrombocytes. MPV is utilized to assess the platelets' functioning and activation levels (10). An increased proportion of larger platelets is associated with the progression of an inflammatory state, often following procoagulation, intracellular production of pro-inflammatory cytokines, granule degranulation, and the release of splenic platelets into circulation (11). These cells rapidly migrate to the site of inflammation upon activation (12), which explains the decreased MPV values observed in patients with ongoing inflammation (13). A comprehensive analysis of hematological parameters can provide physicians with important knowledge in the diagnosis of Hydrosalpinx. It is

very important to uncover the relationship between Hydrosalpinx, which occurs as a result of chronic inflammatory processes, and inflammatory markers. The present study aimed to investigate the roles of NLR, PLR, and MPV inithe diagnosis of individuals with Hydrosalpinx.

MATERIALS and METHODS

This study was designed as a retrospective observational case-control study and was designed in line with the Helsinki Declaration Principles. Informed consent forms were received from the individuals in this current research. This study was started after receiving Ethics Committee approval number 2024/229 from our hospital. The present study comprised 349 individuals who had sought treatment at our infertility clinic between March 2018 and June 2023. These individuals were diagnosed with either primary or secondary infertility and underwent Hysterosalpingography (HSG) to explore the causes of their infertility. HSG scans for all patients were conducted within the first 5 days following the conclusion of menstruation. Laboratory data and HSG results of the participants were retrospectively collected from patient records and hospital databases. Participants were divided into two groups based on whether Hydrosalpinx was detected in their HSG results. Among the patients included in the study, 89 participants with Hydrosalpinx and 260 patients without Hydrosalpinx were divided into two separate groups. Demographic data such as age, gravida, parity, Body Mass Index (BMI), etc., were retrospectively compared between the groups with and without Hydrosalpinx. The groups with and without Hydrosalpinx were compared retrospectively with regard to preoperative complete blood count (Platelet, Lymphocyte, Neutrophil, Leukocyte, PLR, NLR, and MPV). Conditions that might cause changes in blood parameters, such as the presence of endometriosis, presence of adenomyosis, hematological diseases, chronic systemic diseases, anticoagulant use, and oral contraceptive use, were considered as exclusion criteria. Neutrophil, Lymphocyte, and the Beckman Coulter Gen-S System instrument (Beckman-Coulter Inc.) were utilized in our laboratory to conduct a complete blood count, during which Mean Platelet Volume (MPV) characteristics were assessed. The PLR parameter was calculated by dividing the platelet count by the absolute lymphocyte count, while the NLR parameter was obtained by dividing the absolute neutrophil count by the absolute lymphocyte count.

Statistical Analysis

For the analyses of the data, the SPSS 26.0 (IBM Inc., Chicago, IL, USA) was utilized. The Kolmogorov-Smirnov Test was employed to determine the normalcy of the analysis. In addition to the statistical methods (mean±standard deviation) for identification, the Independent t-test was employed to compare the pair groups in the study data evaluation, and the qualitative data were compared using the Chi-Square Test. There was a 95% Confidence Interval (CI) used in the analysis of the result values. For the p-value, less than 0.05 indicated statistical significance.

RESULTS

In the present research, the average age was 29.55 ± 4.01 in the group with Hydrosalpinx and 29.36 ± 4.53 in the control group, with no significant difference found between the groups (p: 0.704). Hemoglobin levels were found to be 9.94 ± 2.88 g/dL in the Hydrosalpinx group and 10.29 ± 2.79 g/dL in the control group, with no significant difference observed between the groups (p: 0.327). However, the neutrophil level was determined to be 6.60 ± 1.45 in the Hydrosalpinx group and 5.64 ± 1.11 in the control group, showing a significantly higher level in the Hydrosalpinx group (p<0.001). The platelet level was 291.60±64.91 in the hydrosalpinx group and 256.82±56.940 in the control group, with a significant difference observed between the two groups (p<0.001). The NLR was 2.68±3.63 in the hydrosalpinx group and 2.10±0.44 in the control group, with no significant difference observed between the groups in this regard (p:0.134). The PLR was 123.22±168.85 in the hydrosalpinx group and 94.21±24.57 in the control group, with no significant difference observed between the groups (p:0.108). The MPV was 8.02±1.24 in the hydrosalpinx group and 8.28±1.71 in the control group, with no significant difference observed among the groups (p:0.124) (Table-1).

There were 264 patients in the primary infertility group, with 53 (20.1%) patients diagnosed with hydrosalpinx, and 211 (79.9%) patients without hydrosalpinx. In the secondary infertility group, there were 85 patients, with 36 (42.4%) patients diagnosed with hydrosalpinx, and 49 (57.6%)

patients without hydrosalpinx. When comparing patients in terms of primary and secondary infertility and the presence of hydrosalpinx, hydrosalpinx was found to be significantly more prevalent in secondary infertile participants (p<0.001) (Table-2).

Table-1. Comparison of clinical and demographic data based on the existence of Hydrosalpinx.

	Hydrosalpinx (+) n:89	**Hydrosalpinx (-) n:260**	**p**
Age(years)	29.55±4.01	29.36±4.53	*0.704
Body Mass Index (kg/m^2)	22.74±1.71	22.49±1.45	*0.208
Hemoglobin (g/dL)	9.94±2.88	10.29±2.79	*0.327
Neutrophil (n/mL)	6.60±1.45	5.64±1.11	*<0.001
Platelet (n/mL)	291.60±64.91	256.82±56.940	*<0.001
Lymphocyte (n/mL)	3.16±0.49	3.00±0.80	*0.860
NLR	2.68±3.63	2.10±0.44	*0.134
PLR	123.22±168.85	94.21±24.57	*0.108
MPV (fl)	8.02±1.24	8.28±1.71	*0.124

*Independent sample t-test, BMI: Body mass index, NLR: Neutrophil/lymphocyte ratio, PLR: Platelet/lymphocyte ratio, MPV: Mean platelet volume

Table-2. The Relationship between infertility type and Hydrosalpinx.

	Primary Infertility 264-(%)	**Secondary Infertility** 85-(%)	**Total** 349-(%)	**p**
Hydrosalpinx (-)	211-(79.9%)	49-(%57.6)	260-(74.5%)	*<0.001
Hydrosalpinx (+)	53-(%20.1)	36-(%42.4)	89-(%25.5)	

* Chi Square test

Table-3. Comparison of clinical and demographic data according to the Hydrosalpinx type.

	Bilateral Hydrosalpinx n:53	**Unilateral Hydrosalpinx** n:36	**p**
	Mean ±SD		
Age (years)	29.66±5.1	28.92±3.76	*0.451
Body Mass Index (kg/m^2)	22.44±1.37	22.56±1.57	*0.697
Hemoglobin (g/dL)	9.97±2.98	9.91±2.78	*0.936
Neutrophil (n/mL)	6.64±1.35	6.14±1.46	*0.036
Platelet (n/mL)	305.66±57.1	270.89±70.91	*0.012
Lymphocyte (n/mL)	3.12±0.48	3.22±0.51	*0.361
NLR	2.52±0.28	2.07±0.62	*0.038
PLR	99.76±23.40	86.03±24.25	*0.009
MPV (fl)	7.72±2.51	8.66±0.62	*0.011

*Independent sample t-test, BMI: Body mass index, NLR: Neutrophil/lymphocyte ratio, PLR: Platelet/lymphocyte ratio, MPV: Mean platelet volume

In our study, the average age was 29.66±5.1 in the group with bilateral hydrosalpinx and 28.92±3.76 in the unilateral hydrosalpinx group, and no significant difference between the groups (p:0.451). The neutrophil level was 6.64±1.35 in the bilateral hydrosalpinx group, 6.14±1.46 in the unilateral hydrosalpinx group, and was significantly higher in the bilateral hydrosalpinx group (p:0.036). The platelet level was 305.66±57.1 in the bilateral hydrosalpinx group,

270.89±70.91 in the unilateral hydrosalpinx group, and was significantly higher in the bilateral hydrosalpinx group (p:0.012). NLR was 2.52±0.28 in the bilateral hydrosalpinx group, 2.07±0.62 in the unilateral hydrosalpinx group, and was significantly higher in the bilateral hydrosalpinx group (p:0.038). PLR was 99.76±23.40 in the bilateral hydrosalpinx group, 86.03±24.25 in the unilateral hydrosalpinx group, and was found to be significantly higher in the bilateral hydrosalpinx group (p:0.009). MPV was 7.72±2.51 in the bilateral hydrosalpinx group, 8.66±0.62 in the unilateral hydrosalpinx group, and was significantly lower in the bilateral hydrosalpinx group (p:0.011). There are 20 patients in the right unilateral hydrosalpinx group and 16 patients in the left unilateral hydrosalpinx group (Table-3).

DISCUSSION

As one of the causes of primary and secondary infertility, Hydrosalpinx can be seen bilaterally or unilaterally as the cause of a chronic inflammatory process or a previous surgery. Although the Hysterosalpingography Method is used commonly for the diagnosis of Hydrosalpinx, the widespread use of inflammatory markers in gynecological diseases in recent years has brought to mind its possible relationship with Hydrosalpinx. Many recent investigations have proven that NLR, PLR, and MPV might have the potential as markers of gynecological inflammatory disorders and obstetric problems and malignancies (e.g., endometriosis, adenomyosis, adnexal torsion, endometrial hyperplasia, and preeclampsia) (14-17). In our study, we found that there was no significant difference in NLR, PLR and MPV levels between the hydrosalpinx group and the non-hydrosalpinx group.

It is already known that Systemic inflammation brought on by infections and adhesions results in a reduction in lymphocytes and an increase in neutrophils. NLR is a straightforward indicator of the inflammatory response that shows how well the cellular immune system is able to counteract the degree of systemic inflammation brought on by infection and adhesions (18, 19). The first cells to reach the infection site are Neutrophils. The increase in the Neutrophils causes a decreased lymphocytes, causing increased NLR, which is the primary parameter investigated in this present research (18, 19).

In the research conducted by Duan et al., a significant relationship was found with the NLR level, which is considered an inflammation indicator in infertility patients (20). In another research in the literature, unexplained infertile patients were analyzed by employing a comparison with the control group, and no differences were reported in terms of NLR and Neutrophil parameters (21). It is considered that the contradiction between studies in the literature occurred depending on the cause of infertility. In this current research, no significant differences were detected between the Hydrosalpinx group and non-Hydrosalpinx group based on NLR. However, Neutrophil levels were observed to be significantly greater in the Hydrosalpinx group.

In the literature, in an investigation in which infertile patients were evaluated, a negative relationship was detected between PLR and implantation (21). In the study conducted by Duan et al., a significant relation was detected with the PLR level, which is considered an indicator of inflammation in infertility patients (20). It was proved in an investigation that was conducted by Yang et al. to be highly associated with inflammation in infertile patients who were diagnosed with endometriosis. A positive correlation was found with PLR value in infertile women (22). In the literature, a correlation was found with PLR value in unexplained infertile patients and in patients who had infertility detected as a cause of endometriosis. In this present research, contrary to the literature data, no significant differences were detected between the group with and without Hydrosalpinx based on PLR and Platelet counts. However, the relationship with Hydrosalpinx has not been analyzed directly in other studies in the literature.

A study conducted by Avcıoğlu et al. reported that in order to differentiate between the early and severe stages of endometriosis, which results in infertility, MPV and other Platelet indicators may be useful biomarkers (23). In an infertility-based research by Li et al., MPV was found to be significantly elevated in women with PCOS when made a comparison to women without PCOS (24). In our study, contrary to the literature data, no significant differences were reported between the group with and without Hydrosalpinx based on MPV. The relationship with Hydrosalpinx has not been directly evaluated in other studies in the literature.

Patients who had primary and secondary infertility were evaluated in a previous study conducted by Al Subhi T et al., and the Hydrosalpinx rate was found to be 19% in primary infertile patients and 29% in secondary infertile patients, and a significant difference was uncovered (25). In the study of Benksim et al., no significant differences were detected based on tubal factor prevalence in the etiology of primary and secondary infertility (26). The rate of Hydrosalpinx was observed to be significantly greater in individuals who were diagnosed with secondary infertility in our study. The reason for the contradiction in studies in the literature might be that tubal factor and the presence of Hydrosalpinx are considered as two separate criteria.

Bilateral tubal obstruction was shown to be the most typical reason of infertility in women, and the most typical reason of obstruction of the fallopian tubes was pelvic inflammatory disease in a study that was conducted by Abebe et al. (27). In the investigation conducted by Ambildhuke et al., tubal obstruction was shown to be the most typical reason of infertility in females (28). In the resarch of Elsharif et al., the relation between previous infections and pelvic inflammatory disease was reported to be the most typical reason of tubal obstruction in women (29). In the research conducted by Seçkin et al., the NLR was found to be significantly higher in individuals who were diagnosed with pelvic inflammatory disease (30). In this present research, the NLR was found to be significantly greater in individuals who had bilateral Hydrosalpinx than in patients who had unilateral Hydrosalpinx. Considering the relationship between previous infections and pelvic inflammatory disease in the etiology of bilateral Hydrosalpinx, our data were found to be compatible with the literature data. In the research conducted by Guo et al., the PLR was found to be significantly greater in patients who had endometriosis and pelvic adhesion (31). The PLR in patients who had bilateral Hydrosalpinx was found to be significantly greater in our study than in individuals who had unilateral Hydrosalpinx. Considering the relationship between chronic inflammatory processes such as pelvic adhesions and endometriosis in the etiology of bilateral Hydrosalpinx, our data were found to be compatible with the literature data. In the study that was conducted by Hocaoğlu et al., MPV was found to be significantly lower in individuals who were diagnosed with pelvic inflammatory disease (32). MPV in patients who had bilateral Hydrosalpinx was observed to be significantly lower than in individuals who had unilateral Hydrosalpinx in our study. Considering the relationship between previous infections and pelvic inflammatory disease in the etiology of bilateral Hydrosalpinx, our data were found to be compatible with the literature data.

CONCLUSION

The current findings corroborate information from earlier studies about the connection between chronic inflammatory processes, NLR, PLR, and MPV. As far as the authors are aware, this is the first research to compare and contrast MPV, PLR, and NLR as inflammatory markers in patients who have Hydrosalpinx. NLR, PLR, and MPV seem to be useful markers that can be used for the diagnosis of bilateral Hydrosalpinx and merit a second assessment in prospective, controlled trials where they are taken into account with clinical results to look at their potential to predict the diagnosis of hydrosalpinx, the severity of the condition, and clinical outcomes in individuals who are sterile.

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

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Research Article / Araştırma Makalesi

Ege Journal of Medicine / *Ege Tıp Dergisi* 2024; 63 (3): 457-465

Content analysis of YouTube™ videos related to anesthesia practices in circumcision surgery in children

Çocuklarda sünnet ameliyatında anestezi uygulamaları ile ilgili YouTube™ videolarının içerik analizi

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ABSTRACT

Aim: The aim of our study was to evaluate the accuracy and reliability of information provided to patients by videos on the application of anesthesia in pediatric circumcision surgery on the video platform YouTube™, an Internet information source.

Materials and Methods: The keywords "anesthesia in circumcision surgery" and "anesthesia in circumcision surgery in children" were typed into the search bar on YouTube™. A total of 220 videos were viewed. The date of upload, number of views, duration, number of likes and dislikes, source of upload, and number of comments were recorded. The reliability and quality of the video were assessed using the Global Quality Scale (GQS) and the modified DISCERN scale.

Results: After exclusion criteria, a total of 38 videos were evaluated. The videos were divided into four groups according to the anesthesia method mentioned/recommended in the content. When comparing between the groups in terms of GQS score, modified DISCERN score and video content, it was observed that the mean scores of the videos in the local anesthesia group were statistically significantly lower than those in both the general and local anesthesia groups, separated by age ($p<0.001$) When the videos were evaluated according to the GQS score, 15 videos (39.4%) were of low quality, 15 videos (39.4%) were of medium quality, and 8 videos (21%) were of high quality. The duration, number of interactions, modified DISCERN score, and video content score of high-quality videos were significantly higher than those of medium and low-quality videos ($p<0.05$).

Conclusion*:* The YouTube™ video platform has a narrow range of information about anesthesia in pediatric circumcision surgery, and the content of videos on this topic is mostly inadequate.

Keywords: Circumcision, anesthesia, children, YouTube, internet.

ÖZ

Amaç: *Çalışmamızın amacı bir internet bilgi kaynağı olan YouTube™ video platformunda yer alan çocuklarda sünnet cerrahilerinde uygulanan anestezi uygulamaları ile ilgili videoların hastalar için sağladığı bilginin doğruluğu ve güvenilirliğinin değerlendirilmesidir.*

Gereç ve Yöntem: *Araştırma için YouTube™ sayfasında arama çubuğuna 'sünnet cerrahisinde anestezi' ve 'çocuklarda sünnet cerrahisinde anestezi' anahtar kelimeleri yazıldı. Toplamda 220 video izlendi. Videoların yüklenme tarihi, süresi, görüntülenme sayısı, beğenme ve beğenmeme sayısı, videoyu yükleyen kaynak, yorum sayısı kaydedildi. Videonun güvenilirliği ve kalitesi modifiye DISCERN ölçeği ve Global Quality Scale (GQS) ölçeği kullanılarak değerlendirildi.*

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Bulgular: *Dışlama kriterlerinden sonra toplamda 38 video değerlendirildi. Videolar içeriğinde bahsedilen/önerilen anestezi yöntemine göre dört gruba ayrıldı. GQS skoru, modifiye DISCERN skoru ve video içeriği açısından gruplar aralarında kıyaslandığında, lokal anestezi grubundaki videoların puan ortalamalarının yaşa göre ayırarak hem genel hem lokal anestezi grubundaki videolardan istatistiksel olarak anlamlı düşük olduğu gözlendi (p<0,001). Videolar GQS skoruna göre değerlendirildiğinde 15 videonun (%39,4) düşük kalitede, 15 videonun (%39,4) orta kalitede, 8 videonun (%21) yüksek kalitede olduğu görüldü. Yüksek kaliteli videoların süreleri, etkileşim sayıları, modifiye DİSCERN skoru ve video içeri puanlaması orta ve düşük kaliteli videolara göre anlamlı yüksek bulundu (p<0,05).*

Sonuç: *YouTube™ video platformunda çocuklarda sünnet cerrahisinde anestezi hakkında dar bir bilgi yelpazesi mevcuttur ve bu konu ile ilgili videolarının içeriği çoğunlukla yetersizdir.*

Anahtar Sözcükler: *Sünnet, anestezi, çocuklar, YouTube, internet.*

INTRODUCTION

Circumcision is the surgical cutting of the foreskin (prepuce) covering the glans to expose the tip of the penis. It is one of human history's oldest and most performed operations. In our country, almost all boys are circumcised, while it has been reported that 30% of men aged 15 years and older are circumcised worldwide, the majority of whom are Muslim men (1).

It is very important to provide appropriate anesthesia and analgesia for circumcision. Circumcisions can be performed under local anesthesia, sedation, or general anesthesia. While pediatric surgeons generally prefer to perform circumcision under general anesthesia, some surgeons use local anesthesia. The child's age or personal experience may be a factor in these preferences. Each method of anesthesia has advantages and disadvantages depending on the child and the experience and time management of the person performing the circumcision. Circumcision is a relatively common and significant source of stress for children, although they do not feel pain when it is performed under local anesthesia. All pediatric surgical procedures can cause emotional distress and trauma to children and their families because of the fear and excitement experienced by psychologically unprepared children (2). General anesthesia also carries a risk of mortality due to various life-threatening complications such as respiratory, circulatory and allergic complications, and there is a significant increase in the incidence of nausea and vomiting in children after general anesthesia (3). In addition, general anesthesia is considered a disadvantage by many surgeons or families because it requires operating room conditions, an experienced team, and is more expensive (4).

The Internet, which is accessible to a large part of the world's population, has become one of the most widely used sources of information today due to its wide variety of information sources (5). People view the Internet as a valuable source of health information and use it to research their health conditions before seeking professional help (6). YouTube™ is the second most used website and video- sharing platform in the world, easily accessible through smartphones, computers, and televisions (7). There is no control mechanism before sharing videos on YouTube™, making it a subjective site that can be useful for users but can also lead to misleading information (8, 9). Today, the YouTube™ video platform has become very popular for medical searches. Studies evaluating the content of videos on the YouTube™ video platform about various diseases and their treatments have raised concerns about the accuracy and reliability of the video content, and it has been reported that the information provided by these videos is not homogeneous (10-12).

There is no study in the literature analyzing the videos on the YouTube™ video platform about the use of anesthesia in pediatric circumcision, which is highly questioned by parents and accurate information is needed. In this study, we aimed to evaluate the quality and accuracy of the information content of videos on the YouTube™ video platform about anesthesia applications in pediatric circumcision.

MATERIALS and METHODS

Study design and participants

In our study, the Turkish videos related to the application of anesthesia in circumcision surgery in children on the YouTube™ video platform, which is an online video-sharing resource, were

reviewed on March 31, 2024. Publicly available videos on YouTube™ were evaluated, and as no human participants/animals were involved, no ethics committee approval was required in this study, as in similar studies (13, 14).

First, the search history was cleared, and the videos were searched by entering the keywords "anesthesia in circumcision surgery" and "anesthesia in circumcision surgery in children" into the search engine. Previous studies of Internet search engines have found that more than 90% of users evaluate the first 3 pages of search results (15). In our study, the videos on the first 3 pages for each keyword were evaluated, and a total of 220 videos were viewed. All videos were carefully analyzed by both researchers to determine which videos to include/exclude in the study.

All videos were carefully analyzed by the researchers to determine which videos to include/exclude in the study.

Exclusion criteria for the study (Figure-1);

1. The video language is not Turkish
2. Irrelevant to the topic
3. Music in the video
4. Lack of audio in the video
5. Repetition of the same video

For each video included in the study, the URL address, video duration (seconds), number of views, number of likes, number of dislikes, number of comments, time elapsed since upload (days), anesthesia method mentioned/recommended in the video (local, general, local + general, both local and general by separating the methods according to the age of the child), person narrating the videos (physician (pediatric surgeon-urologist-pediatrician-anesthesiologist), patient, other), target audience (patient, healthcare professional, unknown). The parameters view rate [number of views/time since upload x 100%] and interaction index [(number of likes - number of dislikes) / number of views x 100%] were calculated (10).

Assessment of reliability

The reliability of the video was assessed using the modified DISCERN (m DISCERN) scale in terms of the reliability and completeness of the information contained in the content. The DISCERN scale was designed to assess the quality of written information about treatment options for any health problem in individuals using health services. The m DISCERN has been adapted from the original version and includes five yes-no questions (16):

1. Is the video clear, concise, and understandable?
2. Does it use reliable sources of information?
3. Is the information presented balanced and unbiased?
4. Are additional sources of information provided for the patient?
5. Are areas of uncertainty/controversy addressed?

Each "yes" answer is scored as 1 point and each "no" answer is scored as 0 points, and the reliability of the information in the video is scored between 1 and 5.

Assessment of quality

The Global Quality Scale (GQS) used to assess the quality of videos has a scoring system ranging from 1 to 5. Video flow, usability, and quality can be assessed using the GQS; 1-2 points indicate low quality, 3 points indicate medium quality, and 4-5 points indicate high video quality (16).

The following scoring system was used in this study:

1 Low quality, poor site flow, most information missing, not useful at all for patients.

2 Overall low quality and poor site flow. Some information is available, but many important topics missing, very limited use for patients.

3 Medium quality, suboptimal flow, some important information adequately discussed but others insufficient, partially useful to patients.

4 High quality, generally good flow. Includes most relevant information, but some topics are missing, useful for patients.

5 High quality and good flow, very useful for patients. Provides complete and clear information.

Evaluation of video content

A list of 10 questions was prepared by the researchers about the topics we expected to be included in the content to create an informative video about anesthesia practices in circumcision surgery. For each answer in the video, 1 point was determined, and the total score was recorded.

Topics that we expect to be in every video content,

1. General information about circumcision surgery (its performance, technique, etc.)

2. General information about anesthesia (types; general anesthesia, local anesthesia)

3. Detailed information about the proposed type of anesthesia

4. Advantages and disadvantages of one type of anesthesia over another

5. Age range for which anesthesia is recommended or not recommended

6. Information about what to do before surgery

7. Information on what to do in the postoperative period

8. Information about complications

9. Knowledge of anesthesia consent requirements

10. Information about the appropriate areas where the procedure should be performed and by whom it should be performed.

Statistical analysis

Statistical evaluations in our study were performed with the program SPSS for Windows 20.0 (IBM SPSS, Chicago). In summarizing the data, nominal data were presented as numbers and percentages (%), and measured data were presented as mean (±standard deviation) and median (minimum-maximum). Normal distribution variables were assessed by the Kolmogorov-Smirnov test. Mann-Whitney U test was used for non-parametric variables and chi-squared test for categorical data. $p<0.05$ was considered statistically significant.

RESULTS

A total of 220 videos were viewed. A total of 182 videos were excluded from the study because 155 of them were "not related to the topic", 3 were "foreign language", 19 were "duplicate", 4 were "no sound", and 1 video was only music. A total of 38 videos were included in the study, and the evaluation of the general characteristics of the videos is shown in Table-1. The videos included in the study were divided into 4 groups according to the anesthesia methods mentioned/recommended in the content. In 7 videos it was mentioned that circumcision should be performed only under general anesthesia, in 14 videos only under local anesthesia, in 3 videos under general plus local anesthesia, and in 14 videos it was mentioned that it should be performed either under local or general anesthesia, depending on the age of the child. It was observed that 14 of the videos were prepared by 'a urology specialist', 12 by 'a pediatric surgery specialist', 4 by 'a pediatric urology specialist', 4 by 'a general practitioner', 1 by 'a anesthesia and resuscitation specialist', and 3 by ' a non-physician'. The mean GQS score of the videos in the study was 2.71±0.95 and the mean m DISCERN score was 1.92±1.19.

The mean number of views of the videos was 11576.02±27707.93 and the mean number of likes was 100.13±359.58. In addition, it was observed that there was no dislike in any of the videos in the study. When the content evaluation criteria of the videos in the study were evaluated, it was observed that the mean scores were 2.44±2.32 (Table-1). When video duration, number of views, number of likes, number of comments, interaction index, and viewing rate were compared between groups, the rates were higher in the group that mentioned both local and general anesthesia according to age but the results were not statistically significant (Table-2). When the videos in the study were compared between groups in terms of GQS score, m DISCERN score, and video content, it was observed that the mean scores of the videos in which local anesthesia was mentioned/suggested were statistically significantly lower than the videos in which both general and local anesthesia were mentioned/suggested, separated by age anesthesia methods ($p<0.001$) (Table-2).

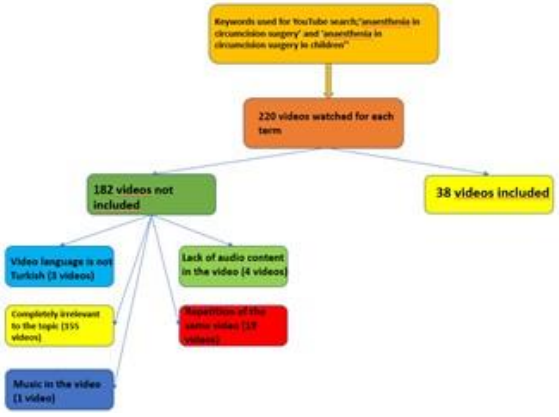

Figure 1. Flowchart of video selection according to exclusion criteria.

All videos in the study were evaluated with a GQS score and grouped into low, medium, and high quality according to the quality of information provided. 15 videos (39.4%) were low quality, 15 videos (39.4%) were medium quality, and only 8 videos (21%) were high quality. The duration of high-quality videos was significantly longer than that of low-quality videos (p=0.001). The number of interactions of medium quality videos was significantly lower than that of high-quality videos (p=0.024). The m DISCERN scores of high-quality videos were significantly higher than those of low and medium quality videos (p=0.000 and p=0.045, respectively). In addition, the m DISCERN scores of medium quality videos were significantly higher than those of low-quality videos (p=0.024). When comparing according to video content scores, it was observed that the mean video content scores of low-quality videos were significantly lower than medium and high-quality videos (p=0.005 and p=0.000) (Table-3).

The distribution of parameters included in all videos in the study is shown in Figure-2. Among the identified criteria for evaluating video content, most of the videos contained information about "general information about anesthesia (types; local, general)" (44.7%) and "advantages and disadvantages of the proposed anesthesia method compared to another" (42.1%). None of the videos in the study provided information about 'the need for consent for anesthesia'. The video content was evaluated in the groups classified according to the anesthesia methods mentioned/recommended, as shown in Table-4. When the video content was evaluated according to groups, it was observed that the required parameters were more in the group where both local and general anesthesia were mentioned/recommended according to age.

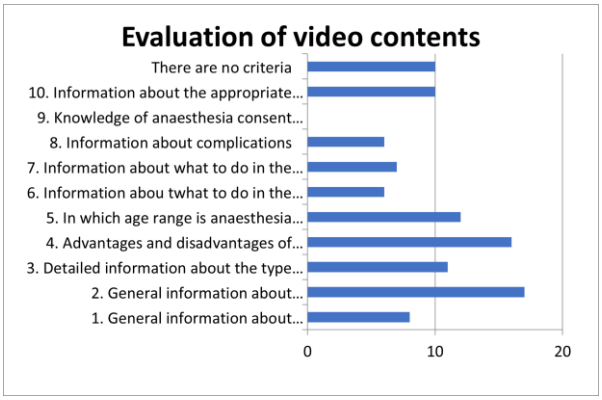

Figure 2. Evaluation of videos according to the information they contain

There was a significant positive correlation between the duration of the videos in the study and the GQS score, the m DISCERN score, and the video content scores (Table-5).

Table-1. Evaluation of the general characteristics of the videos (n=38).

	Mean ± SD	**Mean (min – max)**
Video duration	120.02 ± 126.15	73 (20 - 480)
Number of views	11576.02 ± 27707.93	2170 (37 - 161000)
Number of likes	100.13 ± 359.58	7 (0 - 2100)
Number of dislikes	0 ± 0	0 (0 - 0)
Number of comments	17.52 ± 66.43	0 (0-330)
Number of interactions	0.68 ± 0.67	0.46 (0 – 2,91)
Number of days published	1341.84 ± 1064.64	1059.50 (210 – 4015)
Viewing rate	964.77 ± 2104.78	220.05 (3.03 – 11027.00)
GQS score	2.71 ± 0,95	3 (0 - 4)
m DİSCERN score	1.92 ± 1.19	2 (0 – 5)
Video content score	2.44 ± 2.32	2 (0 – 8)

GQS; Global Quality Scale, m DİSCERN; Modified DİSCERN

Table-2. Comparison of the content of the videos according to the anesthesia method mentioned/recommended.

	General (n=7)		Local (n=14)		General+local (n=3)		Both general and local, separated by age (n=14)		
	Mean ± SD	**Median (min - max)**	**Mean ± SD**	**Median (min - max)**	**Mean ± SD**	**Median (min - max)**	**Mean ± SD**	**Median (min - max)**	**p**
Video duration (sec)	111 ± 137	63 (40 -420)	73 ± 92	40 (20 - 375)	100 ± 25	97 (76 - 126)	176 ± 149	128 (27 - 480)	0.055
Number of views	5618 ± 7351	1300 (127 - 19000)	7432 ± 10444	2923 (37 - 33127)	10169 ± 17175	403 (103 - 30000)	19001 ± 43683	3049 (268 - 161000)	0.906
Number of Likes	8 ± 7	5 (1 -21)	41 ± 51	14 (0 -146)	18 ± 26	3 (2 -48)	224 ± 582	10 (1 -2100)	0.804
Number of comments	1 ± 2	0 (0 -5)	1 ± 4	0 (0 -13)	9 ± 14	2 (0 -26)	44 ± 107	1 (0 -330)	0.252
Interaction index	0.35 ± 0.25	0.36 (0.05 - 0.78)	0.7 ± 0.67	0.57 (0 - 2.7)	1.19 ± 1.5	0.49 (0.16 -2.91)	0.74 ± 0.6	0.61 (0.05 - 1.86)	0.509
Number of days on air	1716 ± 1679	720 (360 - 4015)	1145 ± 780	945 (210 - 2555)	1470 ± 1283	1370 (240 -2800)	1325 ± 970	1060 (240 - 3600)	0.993
Viewing rate	241.2 ± 151.2	210,3 (34,7 - 473,2)	958.9 ± 1528.3	321.1 (3 - 5641)	381.1 ± 597.5	42,9 (29,4 -1071)	1457.5 ± 3096.1	211,3 (36.3 - 11027)	0.792
Video content	3 ± 3	3 (0 -8)	1 ± 1	0 (0 -3)	1 ± 1	2 (0 -2)	4 ± 2	4 (1 -7)	<0.001
GQS score	3 ± 1	3 (2 -4)	2 ± 1	2 (0 -3)	3 ± 1	3 (2 -3)	3 ± 1	4 (2 -4)	0.001
m DİSCERN score	2 ± 1	2 (1 -3)	1 ± 1	1 (0 -2)	2 ± 1	2 (1 -2)	3 ± 1	3 (1 -5)	<0.001

p<0.05 is statistically significant

GQS; Global Quality Scale, m DİSCERN; Modified DİSCERN

Table-3. Evaluation of video features according to the quality of videos determined by GQS score.

	Low quality (n=15)		Medium Quality (n=15)		High Quality (n=8)		p value
	mean±sd	**Median (min-max)**	**mean±sd**	**Median (min-max)**	**mean±sd**	**Median (min-max)**	
Video duration	68.2±87.6	40 (20-375)	80.2±36.4	76 (34-139)	291.7±152.4	328 (30-480)	0.001
Number of views	9390.0±10243.5	5120 (37-33127)	4310.7±7674.7	1300 (103-30000)	29297.1±56991.9	1782 (268-161000)	0.581
Number of Likes	35.2±47.8	12 (0-146)	13.1±23.3	4 (0-85)	385.0±748.1	11 (2-2100)	0.140
Number of comments	2.2±3.6	0 (0-13)	3.0±6.8	0 (0-26)	73.5±136.6	0,5 (0-330)	0.890
Number of interactions	0.5±0.6	0,4 (0-2.7)	0.5±07	0.3 (0-2.9)	1.1±0.5	1,1 (0,3-1,8)	0.021
Number of days published	1588.8±1149.7	1370 (210-4015)	1418.2±1145.6	1024 (240-3600)	735.5±398.7	710 (330-1460)	0.189
Viewing rate	864.0±1459.2	363 (3-5641)	342.3±475.6	162 (13-1670)	23206±3968.8	253 (74-11027)	0.402
m DİSCERN score	1.0±0.6	1 (0-2)	2.0±0.7	2 (1-3)	3.5±0.9	3 (2-5)	0.000
Video content score	0.6±1.1	0 (0-4)	2.6±1.2	3 (1-5)	5.6±1.9	6 (2-8)	0.000

p<0.05 is statistically significant

GQS; Global Quality Scale, m DİSCERN; Modified DİSCERN

Table 4. Evaluation of video contents according to the mentioned/recommended anesthesia methods.

	Total (n/%) (n=38)	General (n=7)	Local (n=14)	General+local (n=3)	Both general and local, separated by age (n=14)
1. General information about circumcision (technique etc.)	8 (21.0%)	2	0	0	6
2. General information about anesthesia (types; general, local?)	17 (44.7%)	2	1	2	12
3. Detailed information on the type of anesthesia recommended	11 (28.9%)	1	2	2	6
4. Advantages and disadvantages of one type of anesthesia over another	16 (42.1%)	6	3	0	7
5. At what age is anesthesia recommended or not recommended?	12 (31.5%)	1	2	0	9
6. Information about what to do in the preoperative period	6 (%15.7)	3	0	0	3
7. Information about what to do in the postoperative period	7 (18.4%)	2	2	0	3
8. Information about complications	6 (15.7%)	1	0	0	5
9. Knowledge of the need for anesthesia consent	0 (0.0%)	0	0	0	0
10. Information about the appropriate areas where the procedure should be performed and by whom.	10 (26.3%)	4	0	0	6
There are no criteria	10 (26.3%)	1	8	1	0

Table-5. Evaluation of the relationship between the duration of the videos and GQS score, m DISCERN score and video content scores.

	GQS score	m DISCERN score	Video content score
Video duration (p değeri/ r)	0.000 (r=0.574)	0.000 (r=0.652)	0.000 (r=0.700)

p<0.05 is statistically significant

GQS; Global Quality Scale, m DİSCERN; Modified DİSCERN

DISCUSSION

The aim of this study was to evaluate the quality and reliability of Internet information on anesthesia for circumcision. Circumcision has been performed for thousands of years for cultural, religious, aesthetic, and public health reasons and remains one of the most common surgical procedures performed worldwide. This surgery, usually performed in childhood, can be one of the most traumatic experiences of childhood due to the pain experienced. Therefore, it is very important to provide appropriate anesthesia and analgesia for circumcision (17). Circumcision is performed under two types of anesthesia, local anesthesia and general anesthesia. These methods of anesthesia depend on many factors, such as the physician, the parents' wishes, the age of the child, and the environment in which they are used.

YouTube™, the second most visited website in the world, has become a popular resource for patients seeking information about medical conditions and general health information (18, 19). It has been reported that video-based resources will grow rapidly in the next few years and that videos will become people's primary source of information (20, 21). At the same time, recent studies have identified YouTube™ as a useful tool for physicians to promote their services and disseminate general health information (22, 23). However, the unregulated nature of this open-access media platform allows for the simultaneous presentation of videos that provide quality/useful information as well as videos that provide misleading/false information.

In a study by Koller U et al (24) analyzing a total of 133 arthritis-related YouTube™ videos, it was reported that 84-86% of the videos were of poor quality, with only 2-4% having excellent information content. In a study evaluating 114 YouTube™ videos about implants, the information content of the videos was generally low (25). Menziletoglu D et al (26) analyzed 107 YouTube™ videos on impacted wisdom tooth surgery and reported that 30.85% had low-quality information and only 16.82% had high- quality

content. They stated that the majority of these high-quality videos contained accurate and useful information because they were uploaded by healthcare professionals. When the quality of the 38 videos about anesthesia methods in circumcision surgery was evaluated according to the GQS score after the exclusion criteria in our study, 39.4% were of low quality, 39.4% were of medium quality and only 21% were of high quality. The source of 92% of the videos was physicians, but only 1 of them was an anesthesiology and reanimation physician. Therefore, we think that the information content on anesthesia methods is insufficient.

When we examined the content of the videos, we found that the most common topic was general information about anesthesia (types of anesthesia; general and local anesthesia) and information about the advantages and disadvantages of the proposed anesthesia method compared to the other. However, there was very little information about the age at which anesthesia methods used in circumcision surgery are appropriate, what should be done in the preoperative period, what complications can occur intraoperatively, and what the patient can expect in the postoperative period. Again, we believe that the reason for this is that most video sources belong to physicians other than anesthesiologists and resuscitators. When patients meet face-to-face with anesthesia and resuscitation physicians, the physicians discuss all the topics examined in this study, and if they have any questions, they have the opportunity to resolve them immediately. It may be more accurate for the patient to receive information from the physician face-to-face. Considering the fact that patients use the Internet so much to get information, we believe that anesthesiologists and resuscitators should prepare YouTube™ videos for more detailed and accurate information about anesthesia for circumcision surgery.

In a survey study conducted in Ankara province of our country, it was reported that 13.3% of the circumcision's of 1235 children were performed by traditional circumcisers and the remaining part was performed by physicians, and these were pediatric surgeons or urologists (27). In our study, the majority of video sources were physicians and the majority of them were pediatric surgeons and urologists. The fact that circumcision surgery is mostly performed by these two specialists explains the fact that YouTube™ video resources on this topic are more prevalent in these specialties.

It was found that the GQS scores of the videos in our study were positively correlated with the duration, number of likes, number of comments, number of interactions, and view rate of the videos. The results of the study are similar to the results of the study by Öztürk & Gümüş, who evaluated videos on the YouTube™ video platform about dental treatment under general anesthesia in children (28).

The content of YouTube™ video platform has a variable structure due to the video results that change daily according to subjective search criteria (keyword selection, video viewing time, interest, etc.) or uploaded-deleted video results. This is the first limitation of the study. As in other studies, the fact that the data collection method is instantaneous also affects the results of the current study. As new videos are uploaded or deleted from the YouTube™ video platform, the results of the study will also vary. In addition, only Turkish language videos were analyzed in this study. The inclusion of other languages in the analysis may also affect the results of the study. This is the second limitation of the study.

CONCLUSION

On the YouTube™ video platform, there is a narrow range of information about anesthesia for pediatric circumcision, and the content of the videos on this topic is mostly inadequate. Most of the videos deal mainly with the surgical side of circumcision surgeries, and general information about anesthesia methods used in these surgeries, general information about complications that should or may occur in the preoperative, intraoperative, and postoperative periods is almost not included. Therefore, parents whose children will be circumcised may find it difficult to access accurate information about the anesthesia used during circumcision surgery from the videos on the YouTube™ video platform.

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

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Research Article / Araştırma Makalesi

Ege Journal of Medicine / *Ege Tıp Dergisi* 2024; 63 (3): 466-473

Dietary interventions for reducing atherosclerosis and heart attack risk: a cross-sectional study of coronary artery disease patients

Ateroskleroz ve kalp krizi riskini azaltmaya yönelik diyet müdahaleleri: koroner arter hastalığı hastalarının kesitsel bir çalışması

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ABSTRACT

Aim: Cardiovascular diseases, particularly atherosclerosis and heart attacks, pose significant health threats worldwide. We aimed to examine the complex relationship between dietary habits and cardiovascular health, in the context of the role of various dietary components.

Materials and Methods: This descriptive cross-sectional study consists of coronary artery patients who applied to the cardiology outpatient clinic of the hospital between June 14 and August 30, 2022. The sample of the study consists of 116 participants diagnosed with coronary artery disease (CAD). The food consumption frequency data were calculated using the BeBiS (Nutrition Information System) computer program. The nutrient values calculated by BeBis were evaluated according to the "Dietary Reference Intake" (DRI). A Mediterranean Diet Index score of ≤7 indicates a low level of adherence to the Mediterranean diet, while scores of 8-9 or ≥10 indicate higher adherence levels.

Results: The average age of the participants in the study is 57.84±13.38 years (range 31-80 years). 46.5% of the participants are in the 51-70 age group, 25.7% are over 70 years old, and 27.7% are in the 31-50 age group. The participants were divided into two groups based on whether they have a diagnosis of type 2 diabetes mellitus. In terms of the Mediterranean Diet Scale classification, 56% of the patients scored ≤7 points, 33% scored 8-9 points, and 11% scored ≥10 points.

Conclusion: Reducing saturated fats, trans fats, and excess sodium intake plays an important role in maintaining optimal heart health. We think it is important to include omega-3 fatty acids, antioxidants and fiber-rich foods in one's diet. By promoting a better understanding of the Mediterranean diet and its potential health benefits, health professionals can contribute to improving dietary behaviors and overall health outcomes, especially in societies with a high prevalence of overweight and obesity.

Keywords: Atherosclerosis, heart attack, cardiovascular health, dietary strategies, nutrition.

ÖZ

Amaç: *Kardiyovasküler hastalıklar, özellikle ateroskleroz ve kalp krizleri, dünya çapında önemli sağlık tehditleri oluşturmaktadır. Bu çalışmanın amacı, bu hastalıklarla ilişkili risk faktörlerini azaltmak için beslenme stratejilerini araştırmaktır.*

Gereç ve Yöntem: *Bu tanımlayıcı kesitsel çalışma, 14 Haziran- 30 Ağustos 2022 tarihleri arasında hastanenin kardiyoloji polikliniğine başvuran koroner arter hastalardan oluşturmaktadır. Çalışmanın örneklemini koroner arter hastalığı (KAH) tanısı almış 116 katılımcı oluşturmaktadır. Besin tüketim sıklığı BeBiS (Beslenme Bilgi Sistemi) bilgisayar programı kullanılarak değerlendirilmiştir. BeBis tarafından hesaplanan besin değerleri "Diyet Referans Alımı"na (DRI) göre derecelendirilmiştir. Akdeniz Diyeti İndeksi puanı ≤7, Akdeniz diyetine düşük düzeyde uyumu gösterirken, 8-9 veya ≥10 puanları daha yüksek düzeyde uyumu göstermektedir.*

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Application date: 15.08.2024 Accepted: 21.08.2024

Bulgular: *Çalışmaya katılanların yaş ortalaması 57,84±13,38 yıldır (aralığı 31-80 yıl). Katılımcıların %46,5'i 51-70 yaş grubunda, %25,7'si 70 yaş üstü ve %27,7'si 31-50 yaş grubundadır. Katılımcılar tip 2 diabetes mellitus tanısı olup olmadıklarına göre iki gruba ayrıldı. Akdeniz Diyeti Ölçeği sınıflamasına göre hastaların %56'sı ≤7 puan, %33'ü 8-9 puan, %11'i ise ≥10 puan aldı.*

Sonuç: *Doymuş yağları, trans yağları ve aşırı sodyum alımını azaltmak, optimum kalp sağlığını korumada önemli bir rol oynar. Kişinin diyetine omega-3 yağ asitleri, antioksidanlar ve lif açısından zengin yiyecekleri dahil etmenin önemli olduğunu düşünüyoruz. Akdeniz diyeti ve potansiyel sağlık yararları hakkında daha iyi bir anlayış teşvik ederek, sağlık profesyonelleri özellikle aşırı kilo ve obezitenin yaygın olduğu toplumlarda diyet davranışlarını ve genel sağlık sonuçlarını iyileştirmeye katkıda bulunabilirler.*

Anahtar Sözcükler: *Ateroskleroz, kalp krizi, kardiyovasküler sağlık, diyet stratejileri, beslenme*

INTRODUCTION

Cardiovascular diseases, foremost among them atherosclerosis and heart attacks, represent enduring challenges to global public health, exerting a substantial toll on individuals and healthcare systems. Atherosclerosis, the progressive buildup of plaque within arterial walls, serves as a pivotal precursor to various cardiovascular complications, with heart attacks emerging as critical and potentially life-threatening events. Against this backdrop, the intricate interplay between dietary patterns and cardiovascular health has gained increasing recognition. This article embarks on a comprehensive exploration of the multifaceted relationship between nutrition and the prevention of atherosclerosis and heart attacks (1).

The escalating prevalence of cardiovascular diseases underscores the urgency of developing effective preventive strategies, and dietary interventions have emerged as a promising avenue. Scientific literature consistently underscores the profound impact of dietary choices on cardiovascular outcomes, emphasizing the need for informed nutritional strategies to curb risk factors associated with atherosclerosis and heart attacks (2).

In our study, we aimed to examine the complex relationship between dietary habits and cardiovascular health, in the context of the role of various dietary components, such as fruits, vegetables, whole grains, healthy fats, adequate water intake, low glycemic index foods, and sodium, in atherosclerosis and heart attack risk. We also evaluated the potential protective effects of certain nutrients, such as omega-3 fatty acids and antioxidants, and the benefits of a fiber-rich diet.

MATERIALS AND METHODS

Study design

This descriptive cross-sectional study consists of coronary artery patients who applied to the cardiology outpatient clinic of the hospital between June 14 and August 30, 2022.

The sample was determined through simple random sampling. All selected samples from the universe were reached. The sample of the study consists of 116 participants diagnosed with coronary artery disease (CAD). The study included individuals who volunteered to participate and were communicative. Individuals with communication difficulties, pregnant women, and those diagnosed with cancer were excluded from the study.

Data Collection

Information regarding the socio-demographic characteristics of the participants was obtained through the Introduction Information Form, while data determining their dietary habits were assessed using the Mediterranean Diet Scale based on the frequency of food consumption. The data for the study were collected through face-to-face interviews. The Introduction Information Form includes general characteristics of individuals, such as age, gender, body weight, height, Body Mass Index (BMI), waist circumference, marital status, education level, presence of chronic diseases, and information on smoking and alcohol consumption.

Data related to the participants' height, weight, and waist circumference were collected by a single researcher. Height was measured using a height gauge with 1 mm intervals, and weights were measured with a precise electronic scale with 0.1 kg accuracy. Body Mass Index (BMI) was calculated using the formula "BMI (kg/m2) = Body Weight (kg) / Height2 (m)" based on the participants' height and weight.

Participants' BMIs were classified according to the World Health Organization (WHO) BMI classification. BMI values of 18.5-24.9 are classified as normal, 25.00-29.99 as pre-obese, and ≥30.00 as obese.

Biochemical data were obtained from participants' medical records and recorded in the general survey form. The biochemical parameters included in the study are fasting blood glucose, Hemoglobin A1C (HbA1C), total cholesterol (T-Chol), triglycerides (TG), high-density lipoprotein cholesterol (HDL-Chol), low-density lipoprotein cholesterol (LDL-Chol), aspartate aminotransferase (AST), alanine aminotransferase (ALT), red blood cell (RBC), Hemoglobin (HGB), and Hematocrit (Hct) serum levels.

To determine individuals' food consumption, the food frequency questionnaire form includes categories such as dairy and products, meat and products, grains, fruits and vegetables, fats, and other foods. Food consumption frequency questionnaire included the consumption frequencies and quantities of foods within the basic food groups recorded over the past month, and daily food consumption amounts were calculated. The data on food consumption frequency were obtained through face-to-face interviews. The food consumption frequency data were calculated using the BeBiS (Nutrition Information System) computer program, which indicates the macro and micronutrient quantities of foods (3). The nutrient values calculated by BeBis were evaluated according to the "Dietary Reference Intake" (DRI). Age groups were categorized into four categories according to the DRI: 19-30, 31-50, 51-70, and >70 years. To assess adherence to the Mediterranean diet, the 14-item Mediterranean Diet Scale used by Martinez-Gonzales et al. in the PREDIMED study was employed (4). The scale consists of 14 questions aimed at evaluating diet quality and, particularly, adherence to the Mediterranean diet. The questions are answered with "yes" or "no," with "yes" being scored as one point and "no" as zero points. The total score obtained from the scale is examined in three categories. A Mediterranean Diet Index score of ≤7 indicates a low level of adherence to the Mediterranean diet, while scores of 8-9 or ≥10 indicate higher adherence levels.

Statistical Analysis

The data of the study were analyzed using IBM SPSS Statistics 20.0. Descriptive statistics, including mean, standard deviation, maximum, minimum, and percentage values, were utilized to assess the distribution of the data. The Kolmogorov-Smirnov test was employed to check the normality of the data, and since the significance values were greater than 0.05, parametric tests were used for advanced analyses. The Chi-square test was applied to evaluate the relationship between two categorical variables. For detecting relationships among three or more variables, the One-Way Analysis of Variance (ANOVA) test was used, and the homogeneity of variances was assessed using the Levene test. In post-hoc analysis, the Tukey Honestly Significant Difference (HSD) test was utilized. The statistical significance level in the study was considered as $p<0.05$.

The study was approved by the Ethics Committee of Gaziantep City Hospital (2024/65, 15/05/2024). The Declaration of Helsinki protocol was followed in the research protocol Written informed consent was obtained from each patient prior to their inclusion in the study.

RESULTS

Table-1 provides the mean and standard deviations along with the lower and upper values of age, BMI, and waist circumference measurements according to individuals' genders. The average age of the participants in the study is 57.84±13.38 years (range 31-80 years). 46.5% of the participants are in the 51-70 age group, 25.7% are over 70 years old, and 27.7% are in the 31-50 age group. According to the World Health Organization's BMI classification, 47.5% of the participants are overweight, 34.7% are obese, and 17.8% have normal body weight. According to the World Health Organization's waist circumference cutoff points, 68.3% of the participants have a high waist circumference. Regarding education levels, 65.3% of the participants completed primary education, 18.8% completed high school, and 15.8% graduated from university/postgraduate studies. The rate of non-smokers is 76.2%, and the rate of non-alcohol consumers is 93.1% (Table-1).

All participants in the study have been diagnosed with coronary artery disease (CAD). The participants were divided into two groups based on whether they have a diagnosis of type 2 diabetes mellitus, and the mean, standard deviation, and lower-upper values of their biochemical findings are shown in Table 2. It was determined that 26.7% of the participants have type 2 diabetes mellitus. Fasting blood glucose and HbA1C levels are higher in individuals with type 2 diabetes mellitus ($p<0.05$) (Table-2).

In Table 3, participants were categorized based on gender, age, BMI, the presence or absence of Type 2 Diabetes Mellitus (DM), and whether they had high or normal waist circumference. In terms of the Mediterranean Diet Scale classification, there was no statistically significant differences were observed. The participants were further subgrouped based on the medical diagnosis of Type 2 Diabetes Mellitus and Coronary Artery Disease (CAD), and their Mediterranean Diet Scale classifications were compared. No statistically significant differences were found in these comparisons either. In terms of the Mediterranean Diet Scale classification, 56% of the patients scored ≤7 points, 33% scored 8-9 points, and 11% scored ≥10 points (Table-3).

In Table 4, there was no statistically significant difference between the Mediterranean Diet Scale score classification and biochemical findings ($p>0.05$) (Table-4).

Table-1. Mean values of age, body mass index, and waist circumference measurements of individuals.

	Female (n=47)	Male (n=54)	P value	Total (n=101)
Age, years	56.47±12.44	61.72±13.77	0.448	57.84±13.38
BMI, kg/m2	26.73±5.11	27.55±5.13	0.696	26.48±4.35
Waist circumference, cm	96.88±13.71	99.49±12.56	0.611	97.33±12.86

Table-2. Comparison of biochemical findings

	CAD (n=74)	CAD+DM (n=27)	P-value	Total (n=101)
Glucose, mg/dL	105.2±17.86	144.67±62.78	0.021*	114.48±38.23
HbA1C	5.869±0.61	7.098±1.66	0.017*	6.216±1.16
Total Cholesterol, mg/dL	198.47±45.76	182.94±42.41	0.377	193.02±44.62
LDL-C, mg/dL	137.94±39.18	118.75±38.64	0.363	131.68±40.36
HDL-C, mg/dL	45.69±12.86	45.78±10.02	0.731	45.71±12.09
Triglycerides, mg/dL	155.17±85.69	147.63±70.23	0.575	152.47±80.49
ALT, U/L	21.48±11.41	22.18±17.42	0.278	21.68±13.87
AST, U/L	20.04±6.76	21.58±21.58	0.146	20.36±9.76
RBC	4.67±0.51	4.50±0.61	0.331	4.62±0.55
HGB	13.89±1.39	13.64±1.81	0.219	13.79±1.53
HCT	41.92±9.75	39.33±5.38	0.461	41.24±8.95

Chi-square/Independent t-tests, *$p<0.05$, CAD: Coronary Artery Disease, DM: Diabetes Mellitus, HbA1C: Hemoglobin A1C, LDL-C: Low-Density Lipoprotein Cholesterol, HDL-C: High-Density Lipoprotein Cholesterol, ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, RBC: Red Blood Cell, HGB: Hemoglobin, HCT: Hematocrit

Table-3. Comparison of Mediterranean Diet Scale Classification by Participants' Demographic Characteristics and Anthropometric Measurements

Mediterranean Diet Scale Classification	<=7		8-9		>=10			
Characteristics								
	Num	Per	Num	Perc	Num	Perc	P.val	Mean±SD P.va
Gender								
Female (n=47)	31	56.0	18	33.5	7	9.7	0.897	7.20 ±1.812 (4-10) 0.474*
Male (n=54)	35	55.9	21	34.7	8	11.0		7.41 ±1.699 (3-11)
Age, years								
31-50 (n=26)	15	60.0	8	33.3	6	8.3	0.948	7.30±1.941(3-11) 0.972**
51-70 (n=57)	32	54.2	21	36.3	6	8.5		7.24±1.791(3-11)
>70 (n=25)	13	49.6	11	38.4	5	12.0		7.29±1.971 (3-11)
BMI, kg/m2								
18.5-24.9 (n=21)	11	44.3	11	30.7	5	11.4	0.412	7.19 ±2.061 (3-11) 0.593**
25-29.9 (n=51)	28	52.8	17	32.5	8	13.6		7.36±1.594 (3-11)
>=30 (n=39)	25	68.4	10	26.8	3	5.6		7.05±1.821 (3-11)
Waist circumference								
Normal (n=37)	16	49.2	17	35.2	5	12.0	0.762	7.30±1.945 (3-11) 0.993*
High (n=70)	42	71.1	20	28.9	8	11.0		7.28±1.874 (3-11)
Diagnosis								
CAD (n=78)	46	51.7	31	38.5	10	13.0	0.041	7.33±1.942 (3-11) 0.048**
CAD+DM (n=22)	19	57.4	10	42.6	6	11.1		7.18±1.902 (4-10)
Education Level								
Primary School (n=58)	48	62.4	24	30.9	7	6.7	0.527	7.28±1.887 (3-11) 0.695**
High School (n=34)	11	50.0	9	45.0	6	14.3		7.39±1.958 (3-11)
University/Postgraduate (n=16)	9	45.0	7	35.3	4	10.3		7.82±1.819 (3-11)

*Chi-square test, **ANOVA, BMI: Body Mass Index

Table-4. Comparison of individuals' biochemical findings based on the Mediterranean Diet Scale classification.

Biochemical Parameters	Mediterranean Diet Scale Classification	<8 (n=45)	8-9 (n=28)	>9 (n=30)	P-value
Glucose, mg/dL	115.20±10.112	101.45±24.081	119.76±44.292	118.46±0.284	0.349
HbA1C	6.32±1.0823	6.174±0.9172	6.482±1.1311	6.77±.426	0.387
Total Cholesterol, mg/dL	198.37±53.486	185.42±41.958	190.79±38.375	193.53±0.528	0.298
LDL-C, mg/dL	141.98±45.827	132.40±37.552	130.91±38.365	137.43±0.737	0.361
HDL-C, mg/dL	44.61±13.702	46.48±14.991	43.14±11.896	45.53±0.614	0.346
Triglycerides, mg/dL	147.76±85.239	128.12±58.677	139.59±54.904	136.55±0.416	0.271
ALT, U/L	22.03±13.260	19.67±8.243	24.94±21.802	23.56±0.247	0.319
AST, U/L	19.12±7.406	20.01±6.847	27.92±23.122	27.44±0.042*	0.389
RBC	4.66±0.602	4.54±0.624	4.59±0.541	4.48±0.715	0.259
HGB	13.79±1.682	13.37±1.207	13.94±1.468	13.69±0.592	0.318
HCT	40.82±10.036	38.51±4.418	39.61±4.765	41.53±0.626	0.334

ANOVA, *p<0.05, HbA1C: Hemoglobin A1C, Total Cholesterol (T-Kol), LDL-C: Low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol, TG: Triglycerides, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, RBC: Red blood cell, HGB: Hemoglobin, HCT: Hematocrit

DISCUSSION

The findings of this study underscore a significant concern regarding the dietary habits of patients diagnosed with coronary artery disease (CAD). The notably low adherence to the Mediterranean diet, observed in the majority of participants, highlights a critical gap in dietary practices that are essential for managing and potentially reducing the risk of further cardiovascular events. With 56% of the patients classified as having low adherence to the Mediterranean diet and only 11% demonstrating high adherence, these results reflect a pressing need for more effective dietary interventions and education. Higher adherence to the Mediterranean diet has been associated with lower mortality, cardiovascular disease, metabolic disease, and cancer risks. Mechanisms underlying the beneficial effects of the Mediterranean diet include reductions in blood lipid levels, inflammatory and oxidative stress markers, improvement in insulin sensitivity, endothelial function, and anti-thrombotic effects, possibly attributed to bioactive components such as polyphenols, monounsaturated, and polyunsaturated fatty acids, or dietary fiber (5-8).

Adherence to the Mediterranean diet may have positive effects on disease-related issues and mortality in CAD patients (9, 10). A meta-analysis has shown a 29% reduction in major cardiovascular events (myocardial infarction, stroke, or cardiovascular death) associated with adherence to the Mediterranean diet (11). Another meta-analysis suggests an average 40% decreased risk associated with the incidence of CAD and mortality. The protective effects of the diet are particularly associated with olive oil, fruits, vegetables, and legumes. Similarly, a meta-analysis examining three randomized clinical controlled trials reported that adherence to the Mediterranean diet has a beneficial impact on the overall incidence of CAD and total myocardial infarction (12). Another meta-analysis, examining six randomized clinical controlled trials, indicated that the Mediterranean diet provides protection against major vascular events, coronary events, stroke, and heart failure, but it does not affect all-cause mortality or cardiovascular mortality (13). Similarly, another meta-analysis of 14 randomized clinical controlled trials reported beneficial effects of the Mediterranean diet on endothelial function (14).

It has been determined that approximately 80% of the participants in this study are overweight or obese. When participants are grouped according to their BMI and compared based on the classification of the Mediterranean Diet, there was no statistically significant difference among the groups (15). In a study examining the relationship between obesity and CAD through meta-analyses, it was observed that mortality in individuals with diabetes, hypertension, or coronary artery disease exhibited a U-shaped relationship with BMI. While a slight excess weight in elderly patients may initially show a protective effect against mortality due to sarcopenia, ongoing weight gain increases the risk of mortality. Therefore, it is crucial to prevent obesity in these patients. Meta-analyses have demonstrated that adherence to the Mediterranean diet has positive effects on reducing body weight and BMI or preventing weight gain. This effect is expected to increase further with energy restriction, increased physical activity, and adherence to the Mediterranean diet for more than six months (16).

Among the participants in this study, 25.7% have been diagnosed with Type 2 diabetes mellitus. When comparing groups with and without a diagnosis of Type 2 diabetes mellitus based on the classification of the Mediterranean Diet, there was no statistically significant difference. However, it was observed that the group with Type 2 diabetes mellitus had higher levels of fasting blood glucose and HbA1c. Therefore, increasing adherence to the Mediterranean diet is particularly important in patients with both diabetes and CAD. Although diabetes poses a risk for CAD, studies have shown no significant difference in the severity of CAD between individuals with and without diabetes. Additionally, it has been reported that an increase of 1 mmol/L in serum glucose independently increases the risk of CAD by 43%. A meta-analysis of eight randomized controlled trials demonstrated that the Mediterranean diet significantly reduces HbA1c but is not effective in reducing glucose parameters (17, 18). Another study evaluating nine randomized controlled trials indicated that adherence to the Mediterranean diet reduces HbA1c, fasting plasma glucose, and fasting insulin. In a different meta-analysis, adherence to the Mediterranean diet for more than six months was reported to have more favorable effects on glycemic control compared to low-fat diets (19).

In this study, no statistically significant differences were found in the mean values of Total Cholesterol (T-Col), LDL-Cholesterol (LDL-Col), HDL-Cholesterol (HDL-Col), and Triglycerides (TG) among the participants classified according to the Mediterranean Diet Scale. The Mediterranean diet's positive effects on endothelial dysfunction are attributed to its low cholesterol content. A meta-analysis of eleven randomized controlled trials provided strong evidence that the Mediterranean diet has a positive effect on TG, T-Col, and HDL-Col (20).

Approximately one-fourth of the participants in our study smoke. Smoking is an independent risk factor for CAD, particularly contributing to the formation and spreading of coronary artery plaques. Studies have shown that smokers develop 4% more plaques than non-smokers. Additionally, smoking increases oxidative stress, endothelial dysfunction, and atherosclerosis, thereby raising the risk of CAD and adversely affecting the prognosis in CAD patients (21). Adherence to the Mediterranean diet can counteract the damaging effects of smoking and potentially prevent its harms (22). In a study examining various education methods related to the Mediterranean diet, including individual counseling, computer-based personalized counseling, group education, internet-based training, cooking classes, and printed materials, participants who received education demonstrated statistically significant increases in the intake of vegetables, legumes, nuts, fruits, whole grains, seeds, olive oil, polyunsaturated fatty acids, and monounsaturated fatty acids. Moreover, improvements were observed in Total Cholesterol, LDL-Cholesterol, Total Cholesterol/HDL-Cholesterol ratio, insulin resistance, BMI, body weight, and waist circumference measurements. Another study reported that including cooking applications in educational programs tripled adherence to the Mediterranean diet (23).

This study has certain limitations. The data is limited to a single center, and the cross-sectional design prevents making causal inferences.

CONCLUSION

Our study reveals a concerningly low adherence to the Mediterranean diet among participants, which aligns with the high prevalence of overweight and obesity in the study population. These findings emphasize the critical need for targeted, continuous, and effective nutritional education programs. By focusing on improving dietary habits and promoting the Mediterranean diet, healthcare professionals can play a pivotal role in addressing these health issues.

The implementation of personalized counseling, group education sessions, and interactive approaches like cooking classes can significantly enhance participants' understanding and adoption of healthier eating patterns. Such initiatives are not only essential for raising awareness but also for empowering individuals to make sustainable dietary changes that improve long-term health outcomes.

In conclusion, enhancing adherence to the Mediterranean diet through well-structured and consistent nutritional education efforts is vital for improving overall health, particularly in populations at risk due to overweight and obesity. Continued efforts in this direction will be crucial for fostering healthier communities and reducing the burden of diet-related chronic diseases.

Conflict of interest: The authors declare no competing interests.

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Research Article / Araştırma Makalesi

Ege Journal of Medicine / *Ege Tıp Dergisi* 2024; 63 (3): 474-483

Prevalence of metabolic syndrome patients with systemic sclerosis

Sistemik skleroz hastalarında metabolik sendrom sıklığı

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ABSTRACT

Aim: To determine the prevalence of metabolic syndrome (MetS) in Turkish systemic sclerosis (SSc) patients.

Materials and Methods: In this cross-sectional, single-centre study, 76 SSc patients admitted to the outpatient clinic of our tertiary care hospital between July and September 2021 were included. The National Cholesterol Education Programme's Adult Treatment Panel (NCEP-ATP III) criteria were used to define metabolic syndrome (MetS). The relationship between MetS and SSc organ involvement and disease characteristics was investigated.

Results: According to the ATP III criteria, 37 cases (48.7%) were identified as having MetS. The prevalance of MetS increased with advancing age (40-45 years: 25%, 46-60 years: 48.4%, >60 years: 62.9%). The cases were divided into two groups according to the presence or absence of MetS. Patients with MetS had higher mean age (58.2±9.4 vs. 51.6±13.5, p=0.015) and lower modified Rodnan skin scores (14 vs. 22, p=0.019). The groups were comparable regarding disease subtype, duration and activity, organs/systems involved and disease-related damage.

Conclusion: Although the prevalence of MetS in SSc patients in our study was higher than that observed in the general population of our country, the prevalence of MetS did not increase when compared to the similar age group. MetS was thought to be related to age and gender predominance rather than the disease itself. Although mRSS was found to be significantly lower in patients with MetS, its sensitivity to predict MetS was found to be low. Nevertheless, our data suggest that the risk of MetS should be considered in SSc patients.

Keywords: Systemic sclerosis, metabolic syndrome, prevalence, insulin resistance.

ÖZ

Amaç: *Türk sistemik skleroz (SSc) hastalarında metabolik sendrom (MetS) prevalansının saptanması amaçlamıştır.*

Gereç ve Yöntem: *Kesitsel ve tek merkezli çalışmaya, Temmuz-Eylül 2021 tarihleri arasında üçüncü basamak hastanemizin polikliniğinde başvuran 76 SSc olgusu alındı. MetS, National Cholesterol Education Program's Adult Treatment Panel (NCEP-ATP III) kriterlerine göre tanımlandı. MetS'in SSc organ tutulumu ve hastalık özellikleri ile ilişkisi incelendi.*

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Bulgular: *ATP III kriterlerine göre MetS, 37 olguda (%48.7) saptandı. MetS sıklığının ilerleyen yaşla birlikte arttığı (40-45 yaşta: %25, 46-60 yaşta: %48.4, >60 yaşta: %62.9) görüldü. Olgular MetS olup olmamasına göre 2 gruba ayrılarak karşılaştırıldı. MetS olan olguların yaşlarının daha yüksek (58.2±9.4'e karşı 51.6±13.5, p=0.015) ve modifiye Rodnan deri skorlarının daha düşük olduğu görüldü. (14'e karşı 22, p=0,019). Hastalık tipi, süresi, aktivitesi, tutulan organlar/sistemler ve hastalık ilişkili hasar bakımından da grupların benzer nitelikte oldukları görüldü.*

Sonuç: *Çalışmamızda SSc hastalarında saptanan Mets sıklığı ülkemiz genel popülasyonuna göre daha yüksek olsa da benzer yaş grubuna göre değerlendirildiğinde MetS sıklığının artmamış olduğu görülmektedir. MetS'in hastalığın kendisinden ziyade yaş ve cinsiyet baskınlığına bağlı olduğu düşünülmüştür. MetS'li hastalarda mRSS anlamlı olarak düşük saptanmasına rağmen, MetS'i öngörme duyarlılığı düşük bulunmuştur. Yine de verilerimiz SSc hastalarında MetS riskinin dikkate alınması gerektiğini ve daha geniş hasta gruplarında ileri çalışmalara ihtiyaç olduğunu ortaya koymaktadır.*

Anahtar Sözcükler: *Sistemik skleroz, metabolik sendrom, prevalans, insulin direnci.*

INTRODUCTION

Systemic sclerosis (SSc), also known as scleroderma, is a chronic autoimmune disease that affects both the skin and internal organs (1). The disease is characterised by vascular dysfunction and progressive fibrosis (1). The aetiology of this disease remains unclear, and it is associated with high mortality and morbidity rates (2, 3).

Metabolic syndrome (MetS) is a condition characterised by a cluster of metabolic disorders, including insulin resistance, obesity, dyslipidaemia and hypertension. The National Cholesterol Education Programme Adult Treatment Panel III (NCEP ATP III) criteria are used to define MetS. The aforementioned criteria encompass abdominal obesity, elevated triglyceride levels, diminished HDL cholesterol levels, elevated blood pressure, and elevated fasting plasma glucose levels (4). It is established that MetS is a significant risk factor for the development of cardiovascular disease and diabetes.

An increased prevalence of MetS has been reported in numerous rheumatological disorders, including gout, systemic lupus erythematosus (SLE), ankylosing spondylitis, rheumatoid arthritis, and antiphospholipid antibody syndrome (5). The prevalence of MetS in patients with SSc and the relationship between these two conditions remain a topic of debate in the scientific community (6-8). Systemic sclerosis (SSc) is characterised by microvascular changes and fibrosis, which are associated with systemic inflammation and autoimmune reactions. It can therefore be postulated that the inflammatory and vascular damage observed in SSc patients may predispose them to the development of MetS. Conversely, both diseases have some common pathological pathways, including oxidative stress associated with increased leptin and reactive oxygen radicals (ROS) and decreased adipokine levels (9-15). Furthermore, there are reports indicating that SSc fibrosis interacts with metabolic pathways via connective tissue growth factor (CTGF) and peroxisome proliferator-activated receptor gamma (PPARG) (16-19). It has been demonstrated that the enhanced sensitivity of SSc fibroblasts to CTGF-mediated collagen synthesis is mediated by insulin (16). Furthermore, it is established that reduced PPARG expression, which is closely associated with insulin resistance, is also a contributing factor in SSc fibrosis (17). While decreased PPARG expression is linked to fibrosis, PPARG activation with rosiglitazone has been observed to mitigate SSc fibrosis in mouse models (18, 19).

The existing literature on the prevalence of MetS in patients with SSc is limited, and there is a paucity of data on the relationship between these two conditions. Additionally, there is evidence indicating that the prevalence of MetS varies across different racial groups (20). The objective of this study was to investigate the prevalence of MetS according to the Adult Treatment Panel III (ATPIII) criteria in patients with SSc in the Turkish population. To this end, the prevalence of MetS in patients with SSc was calculated according to the ATPIII criteria, and the relationship between the presence of MetS and disease characteristics was analysed.

MATERIALS and METHODS

Patient Selection

A single-centre, cross-sectional study was conducted on consecutive patients who had applied to the internal medicine-rheumatology outpatient clinic of Ege University Faculty of Medicine Hospital between July 2021 and September 2021. Patients who met the diagnosis of SSc according to the 2013 European League Against Rheumatism (EULAR) / American College of Rheumatology (ACR) classification criteria (21) and who were aged 18 years or over were included in the study, provided that they had given written consent to participate. Patients with other concomitant rheumatological diseases, active treatment for any malignancy, and pregnancy were excluded from the study. The study was approved by the Clinical Research Ethics Committee of Ege University Faculty of Medicine (29.07.2021, Decision no:21-7.1T/19) and conducted in accordance with the principles of the Declaration of Helsinki. No support was received from any institution/organisation in the conduct of the study.

Demographic and Anthropometric Characteristics

The follow-up files were consulted in order to obtain information pertaining to the age, number of pregnancies, menstrual status and smoking status of the subjects. The weight and height of all patients were measured by the same researcher (UO) using a scale with a calibrated height measuring stand. Body mass index (BMI) was calculated as weight in kilograms divided by height in square metres. The waist circumference of all patients was measured by the same researcher (UO) using a non-flexible measuring tape. The midpoint between the anterior superior iliac crest and the lowest rib was identified as the measurement point. The measurement was conducted at the end of normal expiration with the patient in an upright position. Blood pressure readings for all patients were recorded by the same individual using a calibrated manual sphygmomanometer in the sitting position after a 30-minute rest period.

Laboratory Parameters

The total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), C-reactive protein (CRP) and fasting blood glucose (FBS) results were retrospectively obtained from the Patient Information Management System (PIMS) of Ege University Hospital. Given that the lipid parameters of the patients had already been screened on an annual basis in accordance with the recommendations set forth by the Dyslipidaemia Guideline of the Turkish Society of Endocrinology and Metabolism (annual dyslipidaemia follow-up in chronic inflammatory diseases), no further tests were deemed necessary. The results of the samples obtained between 08:00 and 10:00 a.m., following at least eight hours of fasting, during a patient visit within the past year were taken into consideration.

Characteristics of the Disease

The patients were divided into two groups, namely limited and diffuse cutaneous, on the basis of the extent of skin involvement and other clinical features (22). The current modified Rodnan skin score (mRSS) (range 0-51) was determined by physical examination by the same experienced rheumatologist (23). The disease activity was calculated with the Revised European Scleroderma Therapy (EUSTAR) Activity Index (RAI) score (24), while the disease burden/damage level was determined with the Scleroderma Clinical Trials Consortium Damage Index (SCTC-DI) score (25), both of which were calculated by the same rheumatologist (FYZ). A RAI score of 2.5 or greater was considered indicative of active disease. The maximum SCTC-DI score was 55, which was interpreted as indicating a low damage score (less than 5), a medium damage score (between 6 and 12), and a high damage score (equal to or greater than 13). The dates of onset of Raynaud's phenomenon and disease diagnosis, the presence of digital ulcers, the organs and systems involved, the presence of comorbidities and the current treatment information (including the duration of corticosteroid use and the cumulative corticosteroid dose) were obtained from the patient follow-up files.

In the evaluation of the involved organs and systems, a diagnosis of interstitial lung disease (ILD) was made based on the presence of characteristic pulmonary function tests and high-

resolution computed tomography (HRCT) findings. A diagnosis of pulmonary arterial hypertension (PAH) was made on the basis of right heart catheterisation, with a mean pulmonary arterial pressure (mPAP) of greater than 25 mmHg and a pulmonary capillary wedge pressure (PCWP) of less than 15 mmHg. Left ventricular diastolic dysfunction, myocarditis, pericarditis, pleural effusion exceeding 1 cm on echocardiogram, and arrhythmia requiring treatment were deemed to represent cardiac involvement. Gastroesophageal reflux disease necessitating the use of proton pump inhibitors and dysphagia with manometry evidence were deemed to represent oesophageal involvement, whereas constipation not attributable to any other cause for a period exceeding six months and diarrhoea attacks necessitating the administration of antibiotics were considered to indicate intestinal involvement. Renal failure accompanied by malignant hypertension was defined as scleroderma renal crisis. Tendon rubbing sound, arthritis and myositis were defined as locomotor system involvement. The data related to the involved organs and systems were obtained from the patient follow-up files.

Statistical Analysis

The statistical analyses of the data were conducted using the SPSS (IBM Statistics for Windows, Version 25.0, Armonk, NY: IBM Corp.) programme. The conformity of the data to a normal distribution was evaluated using the Shapiro-Wilk test. The Mann-Whitney U test was employed for the comparison of data that do not present a normal distribution, while the Chi-square test was used for the comparison of categorical variables. The correlations of continuous variables were evaluated by Spearman correlation analysis. The cut-off value for the presence of MetS was determined by ROC analysis using the Youden J index, and the data were presented as mean ± standard deviation (SD) and n (%). A p-value of less than 0.05 was considered statistically significant.

RESULTS

The study cohort comprised 76 individuals with SSc, 70 of whom were females (92.1%) and 6 males. The mean age of the participants was 54.8 ± 12.0 years, with a median disease duration of 10 (5-13.5) years. The median Rodnan skin score was 19 (10-26), with 57.9% of patients presenting with limited and 42.1% with diffuse cutaneous systemic sclerosis. According to the RAI score, 49.3% of the patients exhibited evidence of active disease. According to HRCT, 54 patients (71.1%) exhibited interstitial lung disease, 7 patients (92%) demonstrated pulmonary hypertension, as confirmed by right heart catheterisation, and 32 patients (42.1%) exhibited involvement of the locomotor system. All patients exhibited oesophageal involvement, while intestinal involvement was observed in 25 patients (32.9%).

According to the ATP III criteria, MetS was identified in 37 patients, representing a prevalence of 48.7%. When analysed according to age groups, no patients below the age of 40 years were found to have MetS. However, the frequency of MetS increased with advancing age, with 25% of patients aged 40-45 years, 48.4% of patients aged 46-60 years, and 62.9% of patients aged over 60 years having MetS.

The patients were divided into two groups according to the presence or absence of the MetS and compared in terms of patient and disease characteristics. The age of patients in the MetS group was found to be significantly higher than that of patients without MetS (58.2 ± 9.4 vs. 51.6 ± 13.5, p = 0.015). In contrast, no significant differences were observed between the groups with regard to patient characteristics, including gender, smoking status, number of pregnancies, and postmenopausal status. Additionally, the groups exhibited comparable disease types, durations, activities, and disease-related damage. The distribution of demographic and disease characteristics according to the presence of MetS is summarized in Table-1.

Table-2 illustrates the distribution of subjects according to their metabolic profiles. As expected, the subjects with MetS exhibited higher blood pressure, waist circumference, body mass index (BMI), and lipid levels, as well as lower high-density lipoprotein (HDL) levels, compared to those without MetS. The prevalence of diabetes mellitus (DM) and hypertension (HT) was higher in subjects with MetS, whereas the

prevalence of other diseases, including coronary artery disease (CAD), hyperlipidaemia and hypothyroidism, was similar.

A comparison was also conducted between patients with and without MetS in order to determine whether there were any differences in the organs and systems involved. When the involvement of the skin was categorised as limited or diffuse, no significant difference was observed between the groups. However, when mRSS, a quantitative assessment of skin involvement, was used, it was noted that the median mRSS was lower in those with MetS. The median mRSS was 14 (6-24) in those with MetS and 22 (14-28) in those without MetS (p=0.019). Nevertheless, no significant difference was observed between the two groups with respect to organ involvement. Table-3 presents a comparison of organ involvement in patients with SSc according to the presence of MetS.

Table-1. Descriptive characteristics of the patients and the disease.

Feature	MetS (+) (n=37)	MetS (-) (n=39)	All group (n=76)	*p* value
Age*	58.21±9.36	51.58±13.46	54.81±12.04	**0.015**
Gender (F:M)	35:2	35:4	70:6	0.675
Current smokers n(%)	10 (27.0)	12 (30.8)	22 (28.9)	0.719
Gestation†	3 (2-4)	2 (1.5-3)	2 (2-4)	0.182
Menopause n(%)	31 (83.8)	25 (64.1)	56 (73.7)	0.073
Disease subtype n(%)				
Limited cutaneous	24 (64.9)	20 (51.3)	44 (57.9)	0.231
Diffuse cutaneous	13 (35.1)	19 (48.7)	32 (42.1)	
Disease duration†	10 (5-13)	10 (5-14)	10 (5-13.5)	0.815
Active disease (RAI ≥2.5) n(%)	16 (43.2)	20 (51.3)	36 (47.4)	0.412
SCTC-DI score†	8 (5-12)	8 (5-12)	8 (5-12)	0.613
Autoantibodies				
Anti-centromere n (%)	8 (21.6)	6 (15.4)	14 (18.4)	0.160
Anti-Scl70 n (%)	21 (56.8)	23 (59.0)	44 (57.9)	
Anti-nucleolar n(%)	3 (8.1)	3 (7.7)	6 (7.9)	
Anti-Ro n (%)	5 (13.5)	2 (5.1)	7 (9.2)	
Anti-RNP n (%)	0	5 (12.8)	5 (6.6)	
Steroid exposure (months) †	24 (0-60)	8 (0-60)	12 (0-60)	0.543
Cumulative steroid dose (mg) †	2880 (300-10710)	1440 (0-9360)	2070 (0-10035)	0.514

MetS: Metabolic syndrome**; F**: female; **M**: male; **RAI**: Revised activity score; **SCTC-DI**: Scleroderma clinical trials consortium damage index; **Anti-Scl70**: Anti-topoisomerase antiboides; **Anti-RNP**: anti-ribonucleoprotein antibodies; * mean±SD, †median (d interquartile range)

Table-2. Comparison of metabolic features in systemic sclerosis patients with and without metabolic syndrome.

Feature	MetS (+) (n=37)	MetS (-) (n=39)	All group (n=76)	p value
Systolic BP (mmHg) *	124.02±17.17	111.64±15.24	117.67±17.26	**0.001**
Diastolic BP (mmHg) *	75.27±10.36	70.56±9.25	72.85±10.02	**0.040**
Waist circumference (cm)*	97.50 (92-103)	92 (82-100)	93 (88-102)	**0.015**
Weight (kg) †	68.80 (58.60-74.40)	61.90 (54.20;72.40)	65.30 (55.85-74.30)	0.262
Height (cm)*	155.16±8.51	159.25±7.13	157.26±8.05	**0.026**
BMI (kg/m^2) †	28.20 (24.70-30.30)	24.30 (21-29.30)	26.80 (22.90-30.10)	**0.026**
TC (mg/dL)*	204.54±47.15	187.87±35.16	195.98±42	0.084
LDL (mg/dL)*	123.67±38.65	107.53±28.29	115.39±34.48	**0.041**
HDL (mg/dL) †	47 (39-54)	57 (53-64)	54 (44.50-61)	**<0.001**
TG (mg/dL) †	155 (109-218)	92 (68-122)	114.5 (80-158.5)	**<0.001**
Serum glucose (mg/dL) †	92 (86-105)	87 (82-91)	88.50 (83.50-94.50)	**0.003**
Comorbid diseases n(%)				
DM	10 (27.0)	0	10 (13.2)	**0.001**
Hyperlipidaemia	2 (5.4)	1 (2.6)	3 (3.9)	-
CHD	3 (8.1)	1 (2.6)	4 (5.3)	0.294
Hypothyroidism	3 (8.1)	5 (12.8)	8 (10.5)	1.000
Malignancy	6 (16.2)	2 (5.1)	8 (10.5)	0.115
Hypertension	19 (51.4)	11 (28.2)	30 (39.4)	**0.048**

MetS: metabolic syndrome, **BP:** blood pressure, **BMI:** body mass index**, TC:** total cholesterol; **LDL:** low density lipoprotein, **HDL:** high density lipoprotein, **TG:** Triglyceride; **DM**: diabetes mellitus, **CHD:** coronary heart disease, ***** mean ±SD, **†**median (interquartile range).

Tablo-3. Distribution of organ involvement in patients with systemic sclerosis according to the presence of metabolic syndrome.

Feature	MetS (+) (n=37)	MetS (-) (n=39)	All group (n=76)	p value
mRSS†	14(6-24)	22 (14-28)	19 (10-26)	**0.019**
ILD n(%)	27 (73.0)	27 (69.2)	57 (75)	0.719
% FVC*	84.47±21.56	90.92±21.90	87.8±21.8	0.206
DCLO*, mLCO/min/mm	58.55±18.57	64.47±17.05	61.5±17.9	0.164
PAH	4 (10.8)	3 (7.7)	7 (9.2)	0.708
Cardiac involvement n (%)	11 (29.7)	6 (15.4)	17 (22.4)	0.134
Renal involvement n (%)	2 (5.4)	1 (2.6)	3 (3.9)	0.610
Locomotor involvement n(%)	16 (43.2)	16 (41.0)	32 (42.1)	0.845
Digital ulcers n(%)	21 (56.8)	27 (69.2)	48 (63.2)	0.260
Active digital ulcers n(%)	2 (5.4)	3 (7.7)	5 (6.6)	0.190
GIT involvement				
Oesophageal n(%)	37 (100)	39 (100)	76(100)	0.567
Intestinal n(%)	11 (29.7)	14 (35.9)	25 (32.9)	

MetS: metabolic syndrome; **mRSS**: modified Rodnan skin score; **ILD:** interstitial lung disease; **FVC**: forced vital capacity; **DLCO**: diffusing capacity of the lung for carbon monoxide; **PAH**: pulmonary arterial hypertension; **GIT**: gastrointestinal tract.

Given that the median mRSS was observed to be lower in patients with MetS, a ROC analysis was conducted to determine the mRSS cut-off point predictive of the presence of MetS. The specificity and sensitivity of the mRSS≤11 cut-off value in predicting MetS were calculated to be 84.62% and 45.95%, respectively (AUC: 0.656, 95% CI 29.5-63.1, p=0.014), as illustrated in Figure-1. However, the relationship was weak, and no statistically significant difference was found when the subjects were divided into two groups according to the mRSS predictive value and compared in terms of demographic factors, comorbid diseases and MetS risk factors (age, smoking, number of pregnancies, presence of menopause, cumulative steroid dose, blood pressure, waist circumference, height and weight, BMI, TC, HDL, LDL, TG, ACS and DM, HT, CAD).

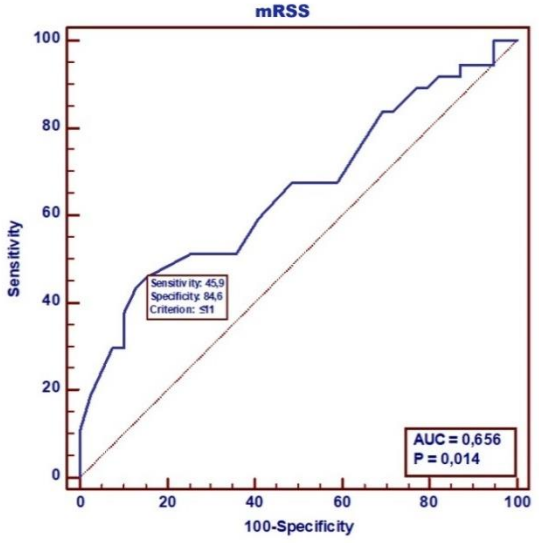

Figure-1. ROC analysis for the relationship between mRSS and MetS.

AUC: Area under curve; mRSS: Modified Rodnan Skin Score; MetS: Metabolik sendrom; ROC: Receiver Operating Characteristic

DISCUSSION

The aim of this study was to investigate the prevalence of metabolic syndrome in Turkish patients with systemic sclerosis. The prevalence of MetS in patients with SSc was found to be 48.7% in the present study. In accordance with the ATP III definition, the prevalence of MetS in the general population of Turkey is estimated to be between 32.9 and 33.9 percent (26-27). In this regard, the 48.7% prevalence of MetS observed in SSc patients suggests a higher prevalence compared to the general population. On the other hand, it is widely acknowledged that gender is a significant determinant of the prevalence of metabolic syndrome. Kozan et al. demonstrated that the prevalence of MetS was significantly lower in men (28%) than in women (39.6%) (27). In the TEKHARF2012 study, it was reported that the prevalence of MetS was 45.1% in men and 54.5% in women (28). Similarly, Abacı A. et al. (26) revealed that the prevalence of MetS was 26.8% in men and 38.3% in women in their meta-analysis. It was therefore considered that the high prevalence of MetS in SSc may be attributed to the dominant female gender, given that the female population ranged between 50-55% in all MetS prevalence studies in Turkey, whereas the female population in our study was 92.1%.

The prevalence of MetS is also influenced by age. A number of studies have demonstrated that the prevalence of MetS increases with age in both sexes (26-28). In the study by Kozan O. et al., the prevalence of MetS was reported as 10.7% in men and 9.6% in women in the 20-29 age group, while it was 49% in men and 68.6% in women over the age of 70 (27). In the TEKHARF 2012 study, the prevalence of MetS was reported as 36.5% in men and 36.7% in women in the <50 age group, 48% in men and 52% in women in the 50-59 age group, and 54.3% in men and 68.1% in women in the 60-69 age group (28). Upon evaluating the results according to age groups, we observed that MetS was not present in any patients below the age of 40 years. However, with advancing age, there was a notable increase in the frequency of MetS (40-45 years: 25%, 46-60 years: 48.4%, >60 years: 62.9%). The high prevalence of MetS, reaching 62.9%, particularly among patients over 60 years of age, serves to reinforce the pivotal role of age as a risk factor. In conclusion, given that the majority of our patients (92.1% of whom were women) were over 40 years of age (and under 70 years of age), it can be concluded that the prevalence of MetS in SSc is consistent with the data for our country and similar to that observed in the general population.

Given the absence of a comparable study conducted in our country examining the prevalence of MetS in patients with SSc, it is not feasible to conduct a disease-specific analysis of our findings. Nevertheless, two studies conducted in patients with rheumatoid arthritis, another inflammatory rheumatological disease, have reported a prevalence of MetS between

17.3 and 27%, which is comparable to that observed in healthy controls (29–30). Furthermore, the international literature on this topic is also limited (6-8, 31). In studies conducted in Italy, the prevalence of MetS in patients with SSc was reported to be between 10.6% and 19.3% (6, 7). Similarly, a prevalence of 20% was reported in a Korean study (31). Compared to the prevalence we found; these rates are quite low. On the other hand, when the healthy Turkish population was considered, it was reported that the prevalence of MetS was higher compared to Americans, Koreans, Chinese, Japanese and Mongolians, and comparable to those of Mexican, South Asian and Iranian origin (27). Similarly, a study conducted in 2015 in 55 SSc cases from a single centre in Mexico showed that the prevalence of MetS in SSc was 36.4% (8). Therefore, considering that the prevalence of MetS has gradually increased over the years in our country and probably in the whole world, it can be assumed that the results obtained in our study are compatible with the literature. Nevertheless, it is obvious that further studies should be carried out with a larger number of patients in regions with different demographic and geographical characteristics, both in our country and in the world.

In our study, we did not find an association between disease subtype (limited or diffuse SSc) and MetS. Many studies in the literature have not shown an association between MetS and disease subtypes (6-7, 31). On the contrary, Peralto-Amaro et al. suggested that limited SSc was associated with insulin resistance and MetS (8). This may be due to the fact that the criteria they used to diagnose MetS were different from those used in other studies. In general, data from our study and the literature support that the development of MetS may occur independently of scleroderma subtypes. Apart from disease subtypes, we did not find any differences between the groups in terms of disease duration, activity and disease-related damage. Similar to our study, previous studies have not shown a significant association between disease characteristics and the frequency of MetS (6-7, 31). On the other hand, when we analysed mRSS, one of the disease characteristics, we found that patients with MetS had lower mRSS (p:0.019), contrary to other reports (7,31). When ROC analysis was performed, we found that the mRSS cut-off point predicting the presence of MetS was ≤11 (specificity: 84%, sensitivity: 45.95%, AUC: 0.656, p:0.014). Considering that mRSS is an important marker associated with visceral organ involvement and mortality in diffuse SSc, we believe that our data should be confirmed in more patients and in different ethnic groups (32).

As expected, we found that blood pressure, waist circumference, BMI, lipid levels and blood glucose levels were higher, and HDL levels were lower in patients with MetS. In addition, the higher prevalence of DM and HT in patients with MetS suggests that these diseases may be associated with MetS. However, there was no difference between the groups in the prevalence of other diseases such as coronary heart disease, hyperlipidaemia and hypothyroidism, suggesting that MetS is more specifically associated with some metabolic factors.

Our study has some limitations. First, the number of patients with SSc was small. Therefore, multivariate analyses to investigate risk factors for the presence of MetS could not be performed. Therefore, the causality between clinical characteristics and MetS in SSc patients could not be fully assessed. Secondly, only cases attending a single tertiary centre were included in this study, which may lead to selection bias. The majority of cases reside in the coastal regions of the Aegean Sea, and different lifestyles may influence the prevalence of MetS. Therefore, the study population in our study may differ from the general population and SSc patients in other centres. Thirdly, our study did not include a control group of healthy individuals, and the interpretations regarding the prevalence of MetS were based on the results obtained from the country in previous years. Finally, our study did not include the physical activity of the subjects, which may influence the presence of MetS.

CONCLUSION

The prevalence of MetS in patients with SSc is higher than in the general population. However, when analysed according to age and gender, the results are comparable. It has been demonstrated that age is an important risk factor in the development of MetS, and the degree of skin involvement may also be effective. These findings emphasise that MetS should not be ignored in scleroderma patients, and cardiovascular risk management of these patients should be considered. Further studies in larger patient populations are needed, as MetS is

affected by factors such as age, gender, ethnicity, nutrition and exercise habits.

ACKNOWLEDGEMENTS

The initial draft is authored by the researchers themselves. Subsequently, the English manuscript undergoes refinement and polishing by DeepL Write, adhering to native language standards. After using the tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Conflict of interest: There are no conflicts of interest. The authors did not receive support from any organization for the submitted work. The authors have no financial or non-financial competing interests to declare that are relevant to the content of this article.

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Case Report / *Olgu Sunumu*

Ege Journal of Medicine / *Ege Tıp Dergisi* 2024; 63 (3): 484-486

An unusual complication in a patient with Hodgkin lymphoma: bilateral auricular hematoma

Hodgkin lenfomalı hastada sıra dışı komplikasyon: bilateral auriküler hematom

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ABSTRACT

Hodgkin lymphoma is lymphoid neoplasm in which the malignant cells admixed with a heterogeneous population of non-neoplastic inflammatory cells. A combination of Bleomycin, Doxorubicin, Dacarbazine and Vinblastine (ABVD) is the frequently used chemotherapy protocol for Hodgkin lymphoma. We present a patient with Hodgkin lymphoma who developed bilateral auricular hematoma after a first-time administration of ABVD treatment in this case report. A 27-year-old female patient was applied to the hematology outpatient clinic with a swelling in the neck for two weeks. An excisional biopsy performed from the left axillary region. The pathology report demonstrated classical Hodgkin lymphoma was noticed to be consistent with mixed cellular type. First dose ABVD protocol was administered to the patient. One week after chemotherapy, swelling and discoloration were observed in the patient's bilateral auricles without a history of trauma. The patient was consulted to the Department of Otorhinolaryngology-Head and Neck Surgery. Bilateral drainage of auricular hematoma was performed immediately. Intravenous ciprofloxacin was also administered. The auricular hematoma did not reoccur after the subsequent chemotherapy treatments.

Keywords: Hodgkin lymphoma, auricular hematoma, adverse reaction, chemotherapy treatment, side effect.

ÖZ

Hodgkin lenfoma; malign hücrelerin, neoplastik olmayan inflamatuar hücrelerle heterojen bir şekilde karıştığı lenfoid neoplazmdır. Doksorubisin, Bleomisin, Vinblastin ve Dakarbazin (ABVD) kombinasyonu, Hodgkin lenfoma için en sık kullanılan kemoterapi rejimidir. Bu olgu sunumunda, ilk kez ABVD tedavisi uygulandıktan sonra bilateral auriküler hematom gelişen Hodgkin lenfomalı bir hastayı sunuyoruz. 27 yaşında kadın hasta, iki haftadır devam eden boyunda şişlik şikâyeti ile hematoloji polikliniğine başvurdu. Sol aksiller bölgeden yapılan eksizyonel biyopsi sonucunda patoloji raporu klasik Hodgkin lenfomanın mikst hücreli tip ile uyumlu olduğu saptandı. Hastaya ilk doz ABVD protokolü uygulandı. Kemoterapiden bir hafta sonra travma öyküsü olmayan hastanın iki taraflı kulak kepçelerinde şişlik ve renk değişikliği gözlendi. Hasta Kulak Burun Boğaz-Baş Boyun Cerrahisi bölümüne konsülte edildi. Kulak hematomunun iki taraflı drenajı hemen yapıldı. Ayrıca intravenöz siprofloksasin uygulandı. Kulak hematomu sonraki kemoterapi tedavilerinden sonra tekrar ortaya çıkmadı.

Anahtar Sözcükler: *Hodgkin lenfoma, auriküler hematom, advers reaksiyon, kemoterapi tedavisi, yan etki.*

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INTRODUCTION

Hodgkin lymphoma is lymphoid neoplasm in which the malignant cells admixed with a heterogeneous population of non-neoplastic inflammatory cells. In the United States, Europe, and other economically developed regions, HL accounts for approximately 10% of all lymphomas 0.6% of all cancers, and 0.2% of all cancer deaths (1-3). ABVD is the frequently used chemotherapy regime for Hodgkin lymphoma. The commonest acute adverse effects of this regimen, include neutropenia, nausea/vomiting, alopecia, infections, constipation, anemia, thrombocytopenia and mucositis (4). We present a patient with Hodgkin lymphoma who developed bilateral auricular hematoma after a first time administration of ABVD treatment in this case report.

Case

A 27-year-old female patient was applied to the hematology outpatient clinic with a swelling in the neck for two weeks. Physical examination revealed multiple pathological lymphadenopathies at the left cervical region and left axilla. An excisional biopsy performed from the left axillary region. The pathology report demonstrated classical Hodgkin lymphoma was noticed to be consistent with mixed cellular type. Moreover, there were cervical, supraclavicular, axillary, and mediastinal involvements in PET CT. Therefore, the patient with more than two nodal involvements above the diaphragm on PET CT was evaluated as stage IIB according to the Ann Arbor classification. The patient's hemoglobin and lactate dehydrogenase (LDH) levels were 11,2 g/dl and 321 U/L respectively. Other biochemical, hemogram and activated partial thromboplastin time (aPTT)-prothtrombin time (PT) were normal.

First dose ABVD protocol was administered to the patient. One week after chemotherapy, swelling and discoloration were observed in the patient's bilateral auricles without a history of trauma (Figure-1). The patient was consulted to the Department of Otorhinolaryngology-Head and Neck Surgery. The physical examination demonstrated, fluctuating auricular hematomas located at the cavum concha and cymba concha bilaterally. However bilateral external auditory canals and tympanic membranes were normal. In the systemic examination, there was no history of bleeding diathesis such as non-steroidal drug use, aspirin (acetylsalicylic acid), anticoagulant drug, hypothyroidism, liver-kidney disease, alcohol use, and connective tissue disease. Moreover, blood count, PT and aPTT were normal. Bilateral drainage of auricular hematoma was performed immediately (Figure-1). Intravenous ciprofloxacin was also administered. The auricular hematoma did not reoccur after the subsequent chemotherapy treatments.

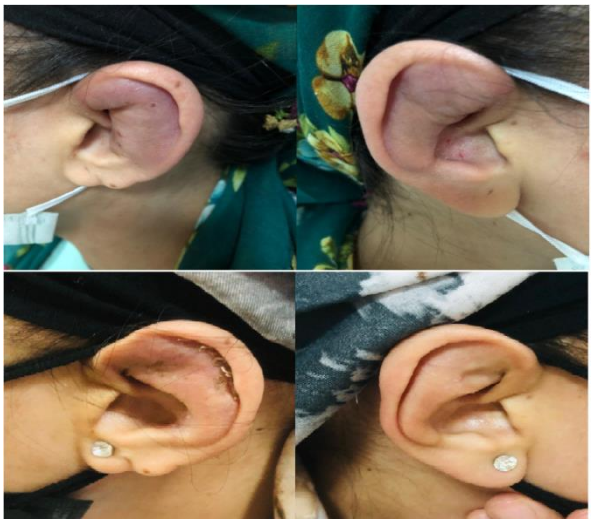

Figure-1. The upper images show bilateral ear hematoma after chemotherapy, and the lower images show drainage of the hematoma.

DISCUSSION

Auricular hematoma generally occurs after trauma including contact sports such as wrestling, boxing, and martial arts, earring placement, wrestling, or motor vehicle accidents (5). Interestingly, bilateral hematoma was detected in our patient without a sign of trauma. Of note, the auricular hematoma is an ear nose throat (ENT) emergency that might progress to chondritis and chondronecrosis. Therefore, bilateral auricular hematoma drainage and antibiotic treatment were applied immediately.

As far as we know, no case in the English literature developed auricular hematoma after chemotherapy for HL.

In literature, there are case reports with side effects in the outer ear after chemotherapy particularly related to the cytarabine treatment. Cytarabine-related side effects are usually manifested by swelling and redness in the ear (6-10). Moreover, chondritis has been reported in a patient with lung cancer to whom docetaxel and carboplatin therapy was administered (10).

CONCLUSION

Auricular hematoma that developed after ABVD treatment was observed for the first time in a patient with Hodgkin lymphoma. Although ear side effects are more common after cytarabine, it is noteworthy that unusual side effects may occur after any chemotherapy agent.

Ethics and competing interest

Consent for publication: Informed consent was obtained from the patient included in the study.

Availability of data and material: The datasets used and/or analyzed during the current study are available from the corresponding author.

Conflict of interest: The authors have no competing interest.

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Review / *Derleme*

Ege Journal of Medicine / *Ege Tıp Dergisi* 2024; 63 (3): 487-500

Peripheral nerve injuries: the recent surgical management strategies

Periferik sinir yaralanmaları: güncel cerrahi yönetim stratejileri

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ABSTRACT

Aim: Numerous individuals with peripheral nerve injuries (PNIs) have permanent disability, which is a major health concern. There are a number of potential causes of PNIs, including piercing injuries, compression, stretch, and ischemia. These injuries can present with a variety of clinical symptoms.

Materials and Methods: In order to clarify the many forms of injury, the peripheral nerve's anatomy is thoroughly explained in this review, which attempts to revisit key PNI ideas. In addition, the specific pathophysiological processes that follow a peripheral nerve damage and the related variables that might either support or undermine the body's ability to regenerate itself depending on PNIs classifications are also mentioned. Next, the recent therapeutic neurosurgical approaches that are accessible in cases of PNIs are described.

Results: Following our overview of the previous literatures on neurosurgical strategies for the management of PNIs, we can observe that surgical procedures are unfortunately very expensive and that their use has been limited due to a variety of adverse effects, such as immunosuppression, chromosomal abnormalities, and tumorigenicity.

Conclusion: In accordance with the source, location and extent of the injury, there are currently advantages to treating PNIs with both surgical and non-surgical approaches. These days, it is possible to identify innovative techniques with the aid of good information regarding incidences, existing practice, outcomes, and study types. Despite a great deal of research on this topic, full functional recovery is still a problem that has to be solved.

Keywords: Peripheral Nerve, Injuries, Surgical, Strategies

ÖZ

Amaç: *Periferik sinir yaralanmaları (PSY) yaşayan birçok birey kalıcı sakatlıklarla karşı karşıya kalmakta ve bu durum önemli bir sağlık sorunu oluşturmaktadır. PSY, delici yaralanmalar, sıkışma, gerilme ve iskemik gibi çeşitli nedenlerden kaynaklanabilir ve her biri farklı klinik semptomlarla ortaya çıkar. Bu derleme, periferik sinir anatomisinin ayrıntılı bir açıklamasını sunarak temel PSY kavramlarını yeniden gözden geçirmeyi amaçlamaktadır. Ayrıca, periferik sinir hasarını takip eden patofizyolojik süreçleri araştırmakta ve PSY sınıflandırmalarına dayalı olarak vücudun kendini yenileme kapasitesini destekleyen veya engelleyen faktörleri vurgulamaktadır. İncelemede ayrıca, PSY'lerin tedavisinde mevcut olan güncel terapötik nöroşirürji yaklaşımları da tanımlanmaktadır.*

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Gereç ve Yöntem: *Bu derleme, çeşitli yaralanma türlerini aydınlatmak amacıyla periferik sinirlerin anatomisini titizlikle tartışmaktadır. Periferik sinir yaralanmalarının tetiklediği spesifik patofizyolojik mekanizmalara dalmakta ve yenilenmeyi etkileyen ilgili faktörleri incelemektedir. Ayrıca, PSY'lerin ele alınmasında kullanılan güncel terapötik nöroşirürji stratejilerini de açıklamaktadır.*

Bulgular: *PSY'lerin yönetimi için nöroşirürji stratejilerine dair önceki literatürlerin gözden geçirilmesi, cerrahi prosedürlerin genellikle gerekli olmasına rağmen, çok pahalı olduğunu ve immün supresyon, kromozomal anormallikler ve tümör oluşumu gibi çeşitli yan etkiler nedeniyle kullanımının sınırlı kaldığını göstermektedir.*

Sonuç: *Yaralanmanın kaynağı, yeri ve kapsamı dikkate alındığında, PSY'lerin tedavisinde cerrahi ve cerrahi olmayan yaklaşımların her ikisi de avantaj sağlamaktadır. İnsidans, mevcut uygulamalar, sonuçlar ve araştırma türlerine ilişkin kapsamlı verilerle desteklenen alandaki gelişmeler, yenilikçi tedavi tekniklerine yol açmıştır. Bununla birlikte, tam fonksiyonel iyileşme sağlamak, geniş kapsamlı araştırma çabalarına rağmen hala önemli bir zorluk olarak kalmaktadır.*

Anahtar Sözcükler: *Periferik sinir, yaralanma, cerrahi, stratejiler.*

INTRODUCTION

PNI is known as damage or illness to the nerves that exit the central nervous system (CNS) to the body's remaining organs, while about 3% of trauma patients may exhibit PNIs (1,2). Throughout history, the majority of our understanding of peripheral nervous system (PNS) and PNIs has come from combat experiences (3). While caring for the wounded during World War II, Sir Herbert Seddon developed his PNI classification system in 1942 (4). However, in contemporary times, PNIs are frequently encountered in trauma situations unrelated to combat. These injuries have the ability to significantly alter a person's life and are frequently linked to high rates of morbidity, which raises the possibility of severe disabilities. These disabilities affect patients for the rest of their lives because they frequently manifest in young people who are working age (5).

Numerous factors, including trauma, compression, illness, or inflammation, can cause damage to peripheral nerves (6). According to etiological surveys, motor vehicle crashes (MVCs) account for the majority of PNIs (46%) and are followed by motorcycle crashes (9.9%) (7).On the other hand, explosions and shrapnel are the most frequent causes of PNIs during warfare (8). In general, gunshot wounds, falls, industrial accidents, stab wounds, vehicle versus pedestrian injuries, recreational motor vehicle collisions (e.g., snowmobiles), and assaults are other major causes of PNIs (7). Furthermore, 17.4% of surgically treated PNIs are iatrogenic injuries brought on by medical or surgical procedures, per one study (9).

PNIs can be blunt or sharp, transected or lacerated, depending on the form of injury; the nerves can also be shifted, stretched, contused, or even partially separated, resulting in neuromas or lesions in continuity (8,10). In the affected part of the body, these injuries may impair nerve function and result in symptoms like pain, weakness, numbness, or tingling. Depending on the location and extent of the damage, the symptoms will vary in intensity. It is important to note that because of their anatomical route, which places them superficially and in close proximity to a bone structure or joint, some nerves are more susceptible to damage and can be injured by compression or stretching. For instance, injury to the radial nerve (which runs down the humerus shaft's spiral groove) brought on by an incorrect extended sitting posture in a chair or “Saturday night palsy” (11). Clinically, this kind of damage manifests as weakness in the wrists and fingers. Other instances include injuries to the ulnar nerve and the common peroneal nerve in the lower extremities. Ulnar nerve damage following surgery is a common issue that accounted for up to 17% of cases in one cohort. It arises from a patient's malposition, which compresses or stretches the ulnar nerve at the elbow level (12). The common peroneal nerve might be compressed in the lithotomy position between the leg holder and the fibular head, especially in thin patients or during long procedures (13).

Depending on the circumstances, PNIs may require surgery, physical therapy, medication, or other treatments. The past few decades have seen improvements in peripheral nerve surgery outcomes due to the understanding of nerve

regeneration, advancements in microsurgical methods, and ongoing molecular biology research.

Anatomical Structure of Peripheral Nerve

Each peripheral nerve is made up of several longitudinal axon configurations known as "fascicles," which are encased in 3 layers of connective tissue. These layers include blood vessels that supply trophic support for the nerve fibres and sustain the fascicles (14–16). All of the fascicles that make up the peripheral nerve's outermost layer are called the epinerium, and the primary component of the epinerium is the areolar connective tissue, which permits nerve contraction and expansion (16). The inner part of the epinerium coats every fascicle and is filled with blood channels that moisten the nerve and some adipose tissue, while the exterior coating covering the entire nerve provides anatomical form and mechanical protection (15).

The perinerium, a thin, dense connective layer that encircles each fascicle separately, is the middle layer. Consequently, aids in preserving structural homeostasis and safeguarding the endoneurial environment (15,16). The endonerium, a thin layer of collagen fibres that envelops each axon inside the fascicle, is the final component of the inner layer. Although this layer is very elastic and has a narrow network of microvessels and capillaries, it provides minimal mechanical protection (15,16).

Every single myelinated axon has a close relationship with Schwann Cells (SC). Since these glial cells construct a fatty multi-layered membrane (many layers of SC membranes connected with secreted proteins) that isolates the axon, they are able to produce laminin-rich sheets of myelin. To improve the pace at which neural electrical impulses propagate along nerve fibres, myelin is crucial. The impulse travels in waves when the nerve fiber is demyelinated. However, conduction happens by a saltatory propagation in myelinated fibres. The increase in electric resistance along the cell membrane is another myelin function that contributes to a quicker impulse. SCs are the primary extrinsic mediators of peripheral nerve regeneration in addition to their function in the myelination process (16).

Pericytes, contractile cells linked to the endothelium lining of microvasculature that regulate blood flow and capillary dilatation, are another significant cell type found in the peripheral nerve environment. In the endoneurial microenvironment as well as the brain-nerve barrier, these cells support homeostasis (16,17). An outline of the anatomy of peripheral nerves is shown in Figure-**1**.

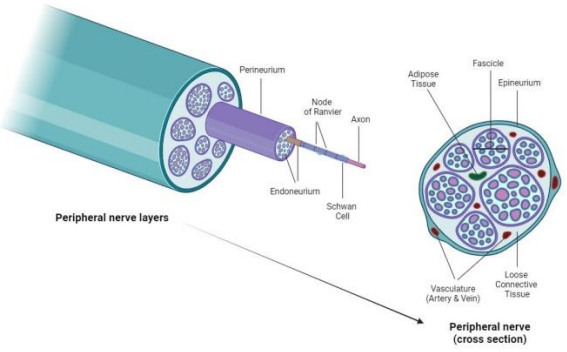

Figure-1. Diagrammatic illustration of a typical peripheral nerve (Created by using biorender.com and kleki.com).

Peripheral nerve injuries classification and implications

Experts have categorized PNIs into various classes based on their severity. This classification scheme facilitates the successful discussion of nerve pathophysiology and the choice of appropriate treatment by scientists and medical professionals (18).

1. Nerve injuries classified by Seddon

Sir Herbert Seddon developed what is known as Seddon's classification in 1943, dividing peripheral nerve injuries into three primary grades based on the degree of damage to the nerve's axons and connective tissue as well as the presence of demyelination. These consist of axonotmesis, neurotmesis, and neuropraxia (8). Table-**1** provides a succinct explanation of these injuries along with their effects.

2. Sunderland's nerve injury classification

In 1951, Sir Sydney Sunderland defined PNIs by further subdividing them into five grades based on the discontinuity of multiple layers of connective tissues. Table-2 summarizes the Seddon's and Sunderland's classifications.

Table-1. The classification of nerve damage by Seddon.

Neuropraxia	Axonotmesis	Neurotmesis
- This type of damage results in the partial or total loss of the nerve's ability to spread action potentials, while the vital axonal continuation is fully retained. Segmental demyelination of the nerve fibres is related to this condition. Although paralysis occurs, there is no peripheral deterioration (19). - The most vulnerable neurons are the motor neuronal fibres, which initially lose their ability to function and eventually regain it (20). - "Saturday night palsy" is an illustration of neuropraxia, where pressure builds up on a nerve while a person is asleep. On its own, this illness usually gets better in 12 weeks (21).	- Axonotmesis is the second grade of injury, characterised by extensive destruction to the nerve fibres that results in intact peripheral degeneration (22,23). In this kind of injury, the layers of connective tissue and the structures that are tightly connected to nerve fibres preserve the interior structures to an appropriate degree (24). - In this instance, neuropraxia is more worthy of the retrieval since it is good and spontaneous, yet Wallerian degeneration and axonal regrowth occur throughout (25). - Usually, no surgical intervention is required in this case (25).	- Neurotmesis, the third grade of nerve injury, damages the components of neural connective tissue and affects the perineurium, epineurium, and/or endoneurium. The nerve fibre is completely split into two ends, which results in total paralysis (26). - One unique characteristic of this damage is the Wallerian degeneration and axonal regrowth. This involves loss of the blood-brain barrier, axonal misdirection, and intraneural damage, all of which limit the healing process (25,27). - Surgery is now required for healing due to the injuries that resulted in damage to the perineurium and epineurium (25,27).

Table-2. The Seddon's and Sunderland's classifications of PNIs.

Seddon's Classification	**Neuropraxia**	**Axonotmesis**	**Axonotmesis**	**Axonotmesis**	**Neurotmesis**	
Sunderland's classification	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Grade 6 (According to MacKinnon)
Causes	Compression, traction, mild crush, and local ischemia	Nerve crush	Nerve crush	Nerve crush	Nerve transection and laceration	Closed traction damage, gunshot or stabbing wounds (28)
Pathophysiology	Nerve conduction block, continuity of connective tissues & axons	Axon division, but the connective tissue's layers are all still intact (29)	Endoneurial layer and myelin sheath are separated (30)	Axon with a detached perineurium, endoneurium, and myelin sheath (30)	Axon with detached endoneurium, perineurium, epineurium, and myelin sheath (31)	All grades engaged, mixed injuries (28)
Surgery	Not usually	Not usually (18)	Not usually	Usually necessary; protocol is dependent on the findings	Necessary; prompt nerve regeneration or repair (27)	Surgical exploration and intraoperative electrodiagnostic methods; nerve transplant or reconstruction (28)
Recuperation	Complete (Hours up to a few weeks)	Complete (Weeks to months) (18)	Incomplete and variable (Months)	Incomplete and variable, depending on injury and treatment (Months to Years)	Incomplete (Months to years)	Incomplete (Months to years)

Advances in treatment options and tactics for PNIs

Early nerve exploration and nerve restoration are two extrinsic factors that affect ho w long it takes for an injured nerve to heal. On the other hand, the rate of axonal regeneration can be as low as 1-2 mm/day, and there is no known medication that might quicken this process (32). After the muscle is denervated, the irreversible motor unit degeneration begins 12 to 18 months later and can last up to 26 months (33). Figure-2 illustrates potential surgical and non-surgical strategies for treating PNIs.

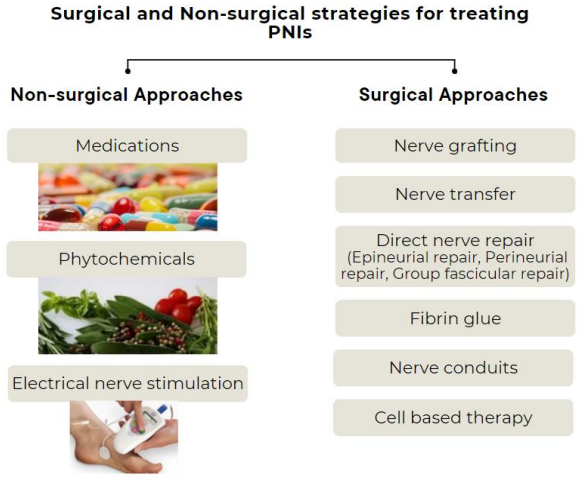

Figure-2. Potential surgical and non-surgical strategies for treating PNIs. (Created by using MS powerpoint).

Surgical treatment methods to restore peripheral nerve function

Following a PNI, there are six main neurosurgical categories of therapy treatments utilized to help facilitate the recovery of motor and sensory function.

1. Nerve grafting

Nerve grafting is a process whereby nerves from the same species are transplanted to fill up nerve gaps larger than 2 cm. By using this procedure, the fascicles' connective tissue should be severed rather than just one fascicle, and the gap be cut longer than the lesion. In respect to the lesion within normal tissue, the fascicles should be dissected at both the proximal and distal ends (34). The diameter of the host and donor nerves, the length of the nerve grafts, the number of fascicles, the pattern of fascicles, the cross-sectional area and form, and the patient's preferences are some of the criteria that should be taken into account when choosing a nerve donor (35). Nerve autografts, also known as autologous nerve grafts, and nerve allografts are the two types of nerve grafting that will be discussed in Table-3.

2. Nerve transfer

Applications: This surgical technique is employed to treat nerve injuries that result from total loss of motor and sensory function (45). If there is a significant injury to the proximal nerves, this may be the only reconstructive option available. For middle- and high-level injuries, the reconstruction is best done using extended nerve grafts to transplant the distal motor nerve. This technique shortens the time and distance needed for regeneration and permits segmentation in the planes of uninjured and unscarred tissues (46). In addition, it involves surgically reestablishing and reorganizing auxiliary motor units in order to restore the functional loss and sensibility (34).

Advantages: Because the surgical location for the nerve transfer is away from the damage site and uses identifiable, healthy tissues rather than the crushed or scarred tissues present at the injury site, it can be regarded as superior to nerve grafting. It permits the nerve's reinnervation into the intended muscle while maintaining the anatomical and biomechanical integrity of the nerve (47).

Disadvantages: Following nerve transfer, clinical outcomes take several months to manifest and require specialized technical knowledge. The validity of this approach is limited by the availability of donors and is extremely costly (48). Transfer of nerves cannot be viewed as a conventional therapeutic approach given the drawbacks.

3. Direct nerve repair

The most effective treatment for axonotmesis and neurotmesis is direct nerve restoration using microsurgical techniques to provide endurance or continuity between the distal and proximal part of the nerves (49). Direct nerve repair with microsurgical procedures to give endurance or continuity between the distal and proximal part of the nerves is the most successful treatment for axonotmesis and neurotmesis (35). Three categories of nerve repair treatments are included in Table-4; group fascicular repair, perineurial repair, and epineural repair (50).

4. Fibrin glue

Applications: Fibrin glue works by employing fibrin sealants, a sticky substance, to facilitate

primary sutureless healing. It is regarded as a successful method of preventing suturing for nerve cooptation (55,59).

Benefits: Fibrin glue repair guarantees reduced fibrosis, fewer inflammatory reactions, and a quicker recovery period (60). The primary benefit of fibrin glue is in its rapid and effortless application for nerve repair in emergency situations when a skilled surgeon is not available (61). However, it is not suitable for serious damage. A perfect sealant should possess particular mechanical, structural, and biological characteristics and shouldn't impede the process of regeneration (55).

Drawbacks: The usage of human blood in commercially available sealants is their greatest drawback, since it can lead to the spread of infection, fibrosis, toxicity, and necrosis (62). This has led to the discovery of a novel heterologous fibrin sealant (HFS) produced from snake venom. It can stop fluid loss, shorten the duration of operation, and lessen bleeding (63).

5. Nerve conduits

Applications: Nerve conduits act as a link between the damaged nerve's proximal and distal stumps. They can be employed in place of nerve autografts and offer a scaffold for axonal regrowth. Recently, researchers have concentrated on creating conduits as a different kind of treatment, particularly for complicated abnormalities (52). By inserting distal and proximal stumps into both of the nerve conduit's ends, this approach enables axonal regeneration from the proximal stump through the conduit and selectively grows into the typical pathways in the distal nerve stump. Based on the materials they are made of, conduits are divided into two categories: synthetic conduits (more classified into non-degradable and degradable conduits) and biological conduits (64–66).

Table-3. Nerve grafts types; A) Nerve autografts, and B) Nerve allografts.

	A) Nerve autografts (Autologous grafts)	**B) Nerve allografts**
Applications	- The gold standard for peripheral nerve repair is autografts (36). - Autologous grafts improve recovery for more proximal injuries, severe nerve injuries, and lengthy nerve deficits (>3 cm) (37). - Donor nerve grafts are typically taken from expandable sensory nerves, such as the lateral and medial antebrachial nerves, the superficial sensory branch of the radial nerve, the dorsal cutaneous branch of the ulnar nerve, and the lateral femoral cutaneous nerve (38). - Different nerve autografts, such as cable, single, vascularized, interfascicular and single nerve autografts, have been employed, depending on the degree of the lesion (37).	- One of the best substitutes for nerve autografts is nerve allograft. For the purpose of nerve transplantation, allograft nerves are extracted from cadavers or donors (39). Allografts of cadaveric nerves are widely available and contain donor SCs and endoneural architecture, which promote regeneration (40). - Systemic immunosuppression is necessary in this procedure to prevent graft rejection, and the donor's stem cells serve as facultative antigens in addition to helping in remyelination. Furthermore, the systemic immunosuppression is transient and can be reversed if the host SCs have migrated sufficiently (around 24 months) (40,41).
Advantages	- Autologous grafts offer the best outcomes since it doesn't trigger an immune response and involves elements that promote neuron regeneration, such as Schwann cells, basal lamina, neurotrophic factors, and adhesion molecules (42).	- It avoids the morbidity of the donor site, is easily accessible, and comes in an endless supply (20).
Disadvantages	- Although autologous nerve grafts have been shown to produce positive outcomes, there are certain drawbacks to this procedure, such as restricted tissue availability, graft-related complications, donor-site morbidity, nerve function loss, scarring, the need for a second incision, formation of neuromas, limited supply, and possible differences in tissue size (42).	- The recovery results are good even though the treatment is too expensive and requires expertise to perform (43,44). - Tumor development and opportunistic infections are two of the several negative outcomes of immunosuppression (20).

Table-4. The three categories of direct nerve repair treatments.

	A) Epineural repair	B) Perineurial repair	C) Group fascicular repair
Applications	This method of repairing damaged nerves includes merely sewing the outer sheath of the injured nerve and can be applied to primary as well as secondary neural repairs (51).	- Hashimoto and Langley originally published a description of this method in 1917 (52). - Because it is a simpler and speedier approach that also entails just a little disruption of the nerve's internal structure, it is a superior option for suturing the epineurium and for large acute nerve lacerations (53).	When a nerve is lacerated and the branches of transected nerves are clearly arranged and identifiable inside the main trunk, this procedure is simple (34).
Advantages	- Its benefits include low magnification, speedy execution, avoidance of intra-neural contents, and ease of use (54). - The most crucial technique for achieving a tension-free natural connection with no loss of nerve tissue and exact alignment of the nerve fascicles is to perform nerve repair after nerve alignment (55).	After proper localization of fibers at nerve terminals, this nerve repair procedure has proven to be more beneficial in terms of calming and neural pathways, while also covering the neurophysiological and morphological elements of it (53).	Both the proper coordination of the motor and sensory fascicles and the avoidance of cross-innervation of motor sensory nerves are possible (34).
Disadvantages	None	Greater fibrosis at the nerve suture site, a lengthier surgical period, and discontinuities in fasciculi one-to-one are some of the disadvantages of this approach (56,57).	There are currently several drawbacks, such as a lengthy surgical procedure, which make it impractical (58).

Advantages: The conduits stop surrounding tissues from leaking into a gap between the stumps. Furthermore, axon regeneration after a nerve injury is facilitated by the abundance of neurotrophic substances in these conduits (53). The capacity of a conduit to create the perfect environment for neuronal repair is by far its greatest benefit. For this reason, the perfect nerve conduit should have the following characteristics: it should be thin, porous, biocompatible, permeable, flexible, biodegradable, compliant, and have the right surface for neuro-inductivity and neuro-conductivity (67, 68).

6. Cell-based treatment

The main drawbacks of current previous therapies are their inability to adequately fill in big gaps and their slow rate of nerve regeneration. In order to get around these restrictions, cell-based therapy was created to provide nourishing cells to the location of the lesion in an effort to hasten neuron regeneration, which could eventually take the place of all existing surgical treatments (69). Because stem cells may differentiate into specialized cell types and self-renew, they are used in cell-based therapy (70). SCs are the most researched therapeutic models, although other types of stem cells (Box-1) have also produced amazing results.

Box-1. Types of stem cells used in cell-based therapy for PNIs:

Schwann cells-mediated therapy

SCs are the most significant and preferred seed cells since they are the primary peripheral nervous system functional cells that promote myelination and regeneration (71). They are essential for nerve regeneration because they promote the manufacture of neurotrophic factors such as neuropeptide Y, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), platelet-derived growth factor, and neurotrophic factor (BDNF) (72). Moreover, SCs have the ability to migrate, remyelinate, modulate the immune system, and multiply themselves. The improvement of injured nerve regeneration and repair can be attributed to all of these variables. Neural crest cells are the primary source of SCs in cell-based therapy. The axonal regeneration process is accelerated by the transplantation of SCs seeds into nerve conduits. Regretfully, they are difficult to come by and have a sluggish proliferation to huge numbers (71).

Embryonic stem cells (ESCs)

The benefits of using ESCs are numerous and include the ability to produce an endless supply of cells, strong differentiation potential, and long-term proliferation capacity. However, the main issue with using these cells for transplantation is ethical issues (73).

Neural stem cells (NSCs)

NSCs have the ability to differentiate into neurons and glial cells; however, due to the difficulty in harvesting these cells and the possibility of neuroblastoma formation, their usage is restricted (74).

Bone marrow-derived stem cells (BMSCs)

The ability to develop into SC-like cells exists in BMSCs (BMSC-SCs). Studies have revealed that BMSCs' capacity for differentiation is weaker than that of NSCs (71).

Fetal stem cells (FSCs)

Fetal stem cells can be extracted from amniotic fluid, amniotic membrane, umbilical cord, and Wharton's jelly. Both umbilical cord-derived mesenchymal stem cells (UC-MSCs) and amniotic tissue-derived stem cells (ATDSCs) possess the ability to differentiate and proliferate. Two key advantages of fetal-derived stem cells are their low immunoreactivity and ease of acquisition. Regretfully, ethical issues are also fetal-derived stem cells' main disadvantage (75).

Adipose stem cells (ADSCs)

ADSCs have a high capacity for angiogenesis and increase the perfusion of injured neurons (76).

Dermal skin-derived precursor stem cells (SKP-SCs)

SKP-SCs can differentiate into any type of cell, including glial and neuronal cells, and are located in the dermis. They are said to quicken nerve regeneration (77).

Hair follicle stem cells (HFSCs)

One special quality of HFSCs is their ability to differentiate into SCs without the need for genetic modification. Studies on animals have shown that employing HFSCs can promote nerve healing (78).

Induced pluripotent stem cells (iPSCs)

The employment of substitute cells, such as iPSCs, has become necessary due to a number of disadvantages related to stem cells. Although they exhibit improved neural regeneration, their application has been constrained by their tumorigenicity, immunosuppressive need, and chromosomal abnormalities (79).

Conclusion and Future Directions

PNI is a well-known health problem that, depending on the degree of nerve damage, presents a wide range of signs and symptoms. There is a wealth of information on the pathogenic mechanisms of PNI and its regeneration, but there is still a dearth of trustworthy treatments that guarantee full and precise functional recovery. The process of recovery is extremely sluggish, and even with the use of numerous treatment techniques, full functional restoration remains unattainable. We have attempted to highlight the benefits and drawbacks of the current PNI neurosurgical treatments in this review. Currently, there are benefits to treating PNIs using both surgical and non-surgical methods. Regrettably, surgical techniques are highly costly, and their application has been restricted because of a number of side

effects, including immunosuppression, chromosomal abnormalities, and tumorigenicity.

Acellular human nerve allografts: Scientists are working on acellular human nerve allografts (ANAs) at the moment in an effort to get rid of immunosuppressants (41,80). The extracellular matrix (collagen, laminin, and growth factors) and internal neuronal structure are retained in ANAs that are extracted from SCs and myelin (81,82). The migrating SCs of the host are involved in the regeneration process with ANAs. Therefore, even if ANAs perform well in studies, they remain ineffective for lengthy nerve repairs (20,80). In the future, ANAs enhanced with growth factors and seed cells might enhance the results of surgical repair for a sizable gap in PNIs (20,83). Therefore, despite the wide range of applications and advancements in grafts, more advancements with improved prognoses are still required. Resolving the immunosuppression issue would be a significant advancement in this discipline.

Nerve sealants: It will be a significant advancement in this sector if more genuine and reasonably priced nerve sealants are found in the future to overcome the disadvantages of fibrin glue. Additionally, this endeavour might lessen the population that is afflicted with PNI.

Competing interest: The authors have no competing interest.

Abbreviations

PNIs	Peripheral nerve injuries
CNS	Central nervous system
PNS	Peripheral nervous system
SCs	Schwann cells
MVCs	Motor vehicle crashes
HFS	Heterologous fibrin sealant
NGF	Nerve growth factor
BDNF	Brain-derived neurotrophic factor
CNTF	Ciliary neurotrophic factor
FSCs	Foetal stem cells
ESCs	Embryonic stem cells
NSCs	Neural stem cells
BMSCs	Bone marrow-derived stem cells
BMSC-SCs	Bone marrow-derived stem cells Schwann cells-like
ATDSCs	Amniotic tissue-derived stem cells
UC-MSCs	Umbilical cord-derived mesenchymal stem cells
ADSCs	Adipose stem cells
SKP-SCs	Dermal skin-derived precursor stem cells
HFSCs	Hair follicle stem cells
iPSCs	Induced pluripotent stem cells
ANAs	Acellular nerve allografts

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Çalışma koşulları doktorların dikkat performansını etkiliyor olabilir mi?

Could working conditions affect doctors' attention performance?

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Sayın Editör,

Kadıoğlu ve Yılmazer 'in (2023) Ege Tıp Dergisi'nde yayınlanan "Sigara içen doktorlarda yetişkin tip dikkat eksikliği ve hiperaktivite bozukluğunun değerlendirilmesi" başlıklı makalesini okuduk (1). Çalışmanın örneklemini oluşturan doktorlarda dikkat eksikliği hiperaktivite bozukluğu (DEHB) ile ilgili belirtilerin yüksek oranda saptanmasının, üzerinde durulması gereken önemli bir konu olduğunu düşündük. Bu sebeple bazı katkılarda bulunmak istedik.

Dikkat eksikliği hiperaktivite bozukluğu, dikkatsizlik, hiperaktivite ve dürtüsellik ile karakterize yaygın görülen nörogelişimsel bir bozukluktur (2). Çocukluk çağında başlar ve hastaların bir kısmını yetişkinliğe kadar etkiler. DSM tanı sistemleri, çocukluk döneminde ve yetişkinlik döneminde saptanan DEHB tanısının aynı hastalık olduğunu varsaymaktadır. Ancak erişkin dönemde konulan DEHB tanısının, çocukluk döneminde konulan DEHB tanısı ile örtüşme oranının düşük olduğunu, bu hastalıkların farklı hasta gruplarını temsil ediyor olabileceğini bildiren çalışmalar bulunmaktadır (2). Erişkin yaşta saptanan DEHB'nin, çocukluk DEHB'siyle aynı çok genli yatkınlık faktörlerini paylaşmadığı, nöropsikolojik testlerde belirgin bozuklukla ilişkili olmadığı ve çeşitli ruhsal bozukluklarla önemli ölçüde ilişkili olduğu gösterilmiştir (2). Ayrıca erişkin yaşta DEHB tanısı konulurken kişilerin çocukluk belirtilerini yanlış hatırlama veya olduğundan farklı beyan etme eğilimleri tanı konmasını güçleştirebilir (3). Bu sebeplerle erişkin DEHB tanı güvenilirliğinin düşük olduğu ile ilişkili güncel tartışmalar devam etmektedir.

İlgili araştırmaya 128 doktorun katıldığı, katılımcılara Erişkin Dikkat Eksikliği ve Hiperaktivite ölçeği uygulandığı, ölçek sonuçlarına göre katılımcılardan 34'ünde "yüksek ihtimalle DEHB", 92'sinde "çok yüksek ihtimalle DEHB" ve 2'sinde "düşük ihtimalle DEHB" saptandığı belirtilmiştir. Bu durum katılımcıların %98,4'ünün yüksek veya çok yüksek ihtimalle DEHB olduğu anlamına gelmektedir. Oysa genel toplumda erişkin DEHB yaygınlığının yaklaşık olarak % 4.4 olduğu düşünülmektedir (3). Bu çalışmanın örneklemini oluşturan doktorların, üniversite sınavından iyi bir derece aldıkları, uzun süreli bir eğitim sürecini devam ettirebildikleri ve tıp fakültesi gibi ağır akademik çalışma gerektiren bir üniversiteden başarı ile mezun olabildikleri göz önüne alındığında; genel bilişsel işlevlerinin toplum ortalamasına göre daha yüksek olabileceği tahmin edilebilir. Bu durumda doktorların kendilerinde DEHB sıklığının bu kadar yüksek belirtilmesinin sebebi ne olabilir?

Doktorlar ve sağlık çalışanlarının stres seviyelerinin genel çalışan nüfusa kıyasla belirgin şekilde daha yüksektir (4). Türkiye'de çalışan doktorların ağır çalışma koşulları ve yetersiz muayene sürelerinden şikayetçi oldukları bilinmektedir (5). Çok yüksek sayıda hastayı, kısa sürede, yeterince mola vermeden, hızla, hasta yoğunluğu baskısı altında muayene etme şeklindeki uygulama günümüz sağlık sistemi işleyişinde son derece sıktır. Bu çalışma düzeninde dikkat süresi kısalmakta, bu kısa süreli fakat yoğun odaklanmış dikkatin mesai saati boyunca sürmesi beklenmektedir.

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Doktorların bu koşullarda normal bir insanın kapasitesinin üstünde dikkat ve bilişsel performans beklentileri kendilerinde dikkat eksikliği belirtileri deneyimlerine neden oluyor olabilir. Aynı zamanda iş ortamındaki yoğun uyaran ve iş yükünün yarattığı stres, dikkati bir konuda uzun süre sebat ettirme konusundaki kronik eksiklik doktorların hem mesai saatleri içerisinde hem de mesai saatleri dışındaki dikkat, odaklanma, odaklanmayı sürdürme becerilerini olumsuz etkiliyor olabilir. Bunlar genel toplumun maruz kaldığı fazla uyaran maruziyeti ve dikkat sürelerini gittikçe kısaltan teknoloji kullanım alışkanlıklarıyla birlikte değerlendirildiğinde doktorların %98,4'ünün yüksek/çok yüksek DEHB belirtisi belirtmelerini daha anlaşılır kılabilir.

Sonuç olarak, doktorlarda saptanan "yüksek olasılıklı DEHB" oranları, bu tanının erişkinlikte konulması ile ilişkili düşük tanı güvenilirliği ile ilişkili olabilir. Ancak doktorların kendilerinde yüksek derecede dikkat sorunları olduğunu belirtmelerinin nedenlerinin araştırılmasına ve doktorların çalışma koşullarını iyileştirmek için yapılacak düzenlemelere ihtiyaç vardır.

Anahtar Sözcükler: Erişkin Dikkat Eksikliği Hiperaktivite Bozukluğu, Dikkat, İş Stresi

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Late complication of Roux-en-Y Gastric Bypass: Marginal ulcer perforation

Roux-en-Y Gastrik Bypass'ın geç komplikasyonu: Marjinal ülser perforasyonu

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ABSTRACT

In recent years, morbid obesity has tended to increase as a human problem. Surgical options are used in cases where medical treatments are not a solution. One of the surgical options is Roux en-Y gastric bypass surgery (RYGB). RYGB is more effective than other surgical options and is one of the most frequently preferred methods (1, 2). We present a female patient who presented to our emergency department with marginal ulcer perforation 1 year after RYGB.

Keywords: Late complication, marginal ulcer, perforation.

ÖZ

Son yıllarda morbid obezite bir insanlık sorunu olarak artma eğilimindedir. Tıbbi tedavilerin çözüm olmadığı durumlarda cerrahi seçeneklere başvurulmaktadır. Cerrahi seçeneklerden biri de Roux en-Y gastrik bypass ameliyatıdır (RYGB). RYGB diğer cerrahi seçeneklere göre daha etkilidir ve en sık tercih edilen yöntemlerden biridir. Acil servisimize RYGB'den 1 yıl sonra marjinal ülser perforasyonu ile başvuran bir kadın hastayı sunuyoruz.

Anahtar Sözcükler: *Geç komplikasyon, marjinal ülser, perforasyon.*

A 38-year-old woman was admitted to our emergency department with severe abdominal pain and nausea. The patient had no history of non-steroidal anti-inflammatory and steroid use. There was no history of alcohol consumption and diabetes mellitus. She was a cigarette smoker. After RYGB surgery, the patient had dyspeptic complaints and occasional abdominal pain. Recently, the pain tended to increase. Control endoscopy was not performed within 1 year after RYGB. Physical examination revealed diffuse abdominal tenderness and rebound. computed tomography showed free air in the abdomen. The patient was operated under emergency conditions. During the operation, a 2 cm perforation area was observed in the gastrojejunostomy anastomosis line (**Figure-1)**. The perforated area was closed with omentoplasty. The biopsy taken from the perforated area was reported to be compatible with ulcer.

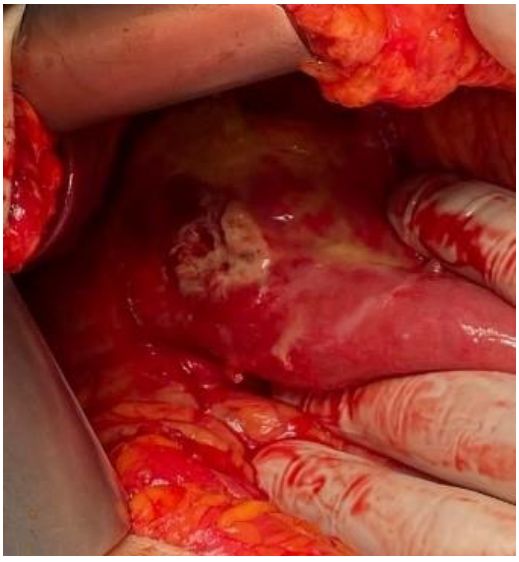

Figure-1. Perforated area after RYGB

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Marginal ulcer (MU) in gastro-jejunal anastomosis after RYGB is seen in 1-4.6%. The duration of occurrence of MU changes between 1 month and 6 years (2). Late perforation due to MU is seen with a rate of 0.6-16% in patients with RYGB (3). Mortality rates related with gastric perforations may rise up to 30% (1, 4, 5). Therefore, close follow-up and treatment of RYGB patients is important. We recommend gastroscopy for RYGB patients with dyspeptic stomach complaints.

Gastroscopy should be performed before the first 6 weeks if postoperative bleeding and stenosis are considered, and after the first 8 weeks if MU is considered (6). Biopsy should be taken for *Helicobacter pylori* and eradication should be performed if *H. pylori* is present. Smoking alone may be sufficient in the pathophysiology of MU in patients with RYGB (7, 8). Therefore, risk factors that may lead to the development of MU in patients with RYGB should be well identified. The seriousness of MU and its complications should be clearly explained to patients.

Conflict of interest: There is no conflict of interest.

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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.

Dergide yayımlanması kabul edilse de edilmese de sisteme yüklenmiş olan dosyalar arşivlenirler.

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Açık Erişim ve Makale İşleme

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Yazar(lar)dan yazılarının yayımı için herhangi bir ücret talep edilmez.

Okuyucular dergi içeriğini akademik veya eğitsel kullanım amaçlı olarak ücretsiz indirebilirler. Dergi herkese, her an ücretsizdir. Bunu sağlayabilmek için dergi Ege Üniversitesi'nin mali kaynaklarından, editörlerin ve hakemlerin süregelen gönüllü çabalarından yararlanmaktadır.

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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.

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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*)*.

Dergide yayımlanmak üzere gönderilen yazıların daha önce başka bir yerde yayımlanmamış veya yayımlanmak üzere gönderilmemiş olması gerekir. Daha önce kongrelerde sunulmuş çalışmaların Editöre gönderilen Ön Yazıda belirtilmesi gerekir. Makale, yazar(lar)ın daha önce yayımlanmış bir yazısındaki konuların bir kısmını içeriyorsa, bu durumun da Ön Yazıda belirtilmesi ve yeni başvuru dosyaları ile birlikte önceki makalenin bir kopyasının da dergi sayfasına yüklenmesi gereklidir.

Yazarlık kriterlerini karşılamayan ancak çalışmaya katkısı olan kişi, kurum veya kuruluşların isimlerine "Teşekkür" bölümünde yer verilebilir.

Çıkar çatışması: Çalışmaları ile ilgili taraf olabilecek tüm kişisel ve finansal ilişkilerin bildirilmesinden yazarlar sorumludur. Ticari bağlantı veya çalışma için maddi destek veren kurum(lar) varlığında kullanılan ticari ürün, ilaç, firma vb. ile nasıl bir ilişkinin olduğu veya herhangi bir çıkar çatışmasının olmadığı Çıkar Çatışması Formu'na doldurularak sisteme yüklenmeli ve metinde "Çıkar Çatışması" bölümünde belirtilmelidir. Çıkar çatışması formu http://icmje.org/conflicts-of-interest/ adresinden edinilmelidir.

İntihal taraması: Ege Tıp Dergisi hiçbir şekilde intihale izin vermemektedir. Bu nedenle, dergiye gönderilen tüm yazılar ön değerlendirme sürecinde intihal tarama programı (*iThenticate* ve benzerleri) ile en az bir kez taranır. Belirlenen oranın üzerinde benzeşime sahip yazılar değerlendirmeye alınmadan yazara iade edilir.

YAZI TÜRLERİ

Yazılar, elektronik ortamda egetipdergisi.com.tr veya dergipark.gov.tr/etd adreslerinden birisi ile sisteme giriş yapılarak gönderilebilir. Yazı türlerinin içermesi gereken bölümler ile ilgili bilgilere "Yazının Hazırlanması" başlığı altında yer verilmiştir.

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.

YAZININ HAZIRLANMASI

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.

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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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Main Text

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:

- Original Articles: Turkish title, abstract and keywords / English title, abstract and keywords / Introduction / Materials and Methods / Results / Discussion / Conclusion / Conflict of Interest / (if present) Acknowledgements / References / Tables (with captions and notes) / Figure captions.

- Case Reports: Turkish title, abstract and keywords / English title, abstract and keywords / Introduction / Case Report / Discussion / Conclusion / Conflict of Interest / (if present) Acknowledgements / References / Tables (with captions and notes) / Figure captions.

- Clinical Image: Turkish title / English title / Case / Conflict of Interest / References / Figure captions.

- Technical Note: Turkish title / English title / Technical note / Conflict of Interest / (if present) Acknowledgements / References / Tables (with captions and notes) / Figure captions.

Title

The title should be short, easy to understand and must define the contents of the article and should be written both in Turkish and English.

Abstracts

Abstract should be in both English and Turkish and should consist “Aim, Materials and Methods, Results and Conclusion” in original articles. The purpose of the study, the setting for the study, the subjects, the treatment or intervention, principal outcomes measured, the type of statistical analysis and the outcome of the study should be stated in this section (up to 250 words). Abstract should not include reference. In Case Reports abstracts should not be divided into sections and do not exceed 200 words. Clinical Image, Technical Note and Letter to the Editor are not required abstracts.

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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*

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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.

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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

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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.

Journal article on the Internet

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*

Website

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.

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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.*

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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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LETTER TO THE EDITOR

Volume: 63
Issue: 3
September - 2024

egetipdergisi.com.tr

ISSN 1016 - 9113