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İÇİNDEKİLER / CONTENTS

ARAŞTIRMA MAKALESİ / ORIGINAL ARTICLE

ESKİSEHİR MEDICAL JOURNAL
ESKİŞEHİR TIP DERGİSİ
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Original Article / Araştırma Makalesi

9-12 AY ARASI ÇOCUKLARDA PROFİLAKTİK DEMİR DESTEĞİNE UYUMUN VE ETKİNLİĞİNİN DEĞERLENDİRİLMESİ

EVALUATION OF COMPLIANCE AND EFFECTIVENESS OF PROPHYLACTIC IRON SUPPLEMENTATION IN CHILDREN BETWEEN 9-12 MONTHS

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ABSTRACT

Introduction: Iron deficiency anemia (IDA) is a common nutritional problem worldwide, but it is more common in underdeveloped and developing countries. Iron deficiency (ID) is the most important cause of anemia, especially in infants aged 6 months to 2 years and in adolescence. This study aimed to evaluate the rate of iron prophylaxis applied to children aged 6 to 12 months, whether it was applied in the appropriate dose, and the factors that reduce compliance with the medication.

Methods: We included children aged 9 to 12 months who applied to the well-child clinic between January 2020 and December 2020. Routine blood counts were performed on the children to investigate whether they used iron prophylaxis, their nutritional habits, and how they used iron supplements. We evaluated the data we obtained by classifying the children according to their anaemia status.

Results: It was found that iron prophylaxis was recommended in 548 (98.6%) of the cases. We found that 349 (62.8%) cases took iron prophylaxis more than three days a week, while 207 (37.2%) used it less than three times a week or not at all. Regular and appropriate dose use was in 193 (34.7%) of 556 children among all participants in the study. 363 (65.3%) children received irregular or low-dose iron supplements. While we detected anaemia in 61.6% of those who did not use iron prophylaxis regularly, we detected anaemia in 38.4% of those who used iron prophylaxis regularly.

Conclusions: Iron prophylaxis is recommended for almost all families. A significant portion of families cannot continue iron supplementation for various reasons. However, a significant portion of those who use it regularly receive insufficient doses of iron supplementation. In this case, the incidence of iron deficiency anemia increases and the applied iron prophylaxis support becomes inadequate. In order for iron supplementation to be successful, it is necessary to start iron prophylaxis and to pay attention to the appropriate dose in growing children and to keep the children under follow-up.

Keywords: Iron prophylaxis, infancy, underuse, iron deficiency anemia

ÖZET

Giriş: Demir eksikliği anemisi (DEA) tüm dünyada yaygın bir beslenme sorunu olmakla birlikte özellikle gelişmemiş ve gelişmekte olan ülkelerde daha sık görülmektedir. Demir eksikliği (DE) özellikle 6 ay–2 yaş arası süt çocuklarında ve ergenlik çağında aneminin en önemli nedenidir. Bu çalışmada, 6-12 ay arası çocuklarda uygulanan demir proflaksisinin ne oran da uyguladığını, uygun dozda uygulanıp uygulanmadığını ve ilaca uyumu azaltan etkenlerin değerlendirilmesi amaçlandı.

Yöntemler: Ocak 2020-Aralık 2020 tarihleri arasında 9 ay ile 12 ay arası sağlam çocuk polikliniğine başvuran çocuklar dâhil edildi. Çocuklardan rutin kan sayımı yapılarak demir profilaksisini kullanıp kullanmadıkları, beslenme alışkanlıkları ve demir desteğini nasıl kullandıkları araştırıldı. Elde ettiğimiz verileri çocukları anemi durumuna göre sınıflandırarak değerlendirdik.

Bulgular: Olguların 548'ine (%98,6) demir profilaksisinin önerildiği saptandı. Olguların 349'unun (%62,8) demir profilaksisini haftada üç günden fazla aldığını, 207'sinin (%37,2) ise haftada üçten az ya da hiç kullanmadığını tespit ettik. Tüm araştırmaya katılanlar içinde düzenli ve uygun dozda kullanım 556 çocuk içinde 193 çocukta (%34,7) idi. 363 (%65,3) çocuk ya düzensiz ya da düşük dozda demir desteği almaktadır. Demir proflaksisini düzenli kullanmayanlarda %61,6 oranında anemi tespit ederken, demir proflaksisini düzenli kullanan %38,4 anemi tespit ettik.

Sonuç: Demir profilaksisi hemen hemen bütün ailelere önerilmektedir. Ailelerin önemli bir kısmı demir desteğine çeşitli nedenlerden dolayı devam edememektedir. Bununla birlikte düzenli olarak kullananlarında önemli bir kısmı yetersiz dozda demir desteği almaktadır. Bu durumunda demir eksikliği anemisinin görülme sıklığını arttırmaktadır ve uygulanan demir proflaksisi desteğinin yetersiz kalmasına neden olmaktadır. Demir desteğinin başarılı olması için demir profilaksisini başlamanın yanında büyüyen çocuklarda uygun dozda kullanıldığına da dikkat etmek ve çocukları takipte tutmak gerekmektedir.

Anahtar Kelimeler: Demir profilaksisi, süt çocukluğu, Yetersiz kullanım, Demir eksikliği anemisi

GİRİŞ

Demir dünyada bol bulunan bir element olmasına rağmen, çocuklarda demir eksikliği anemisi (DEA) özellikle gelişmekte olan ülkelerde daha sık görülmekle birlikte tüm dünyada yaygın bir beslenme sorunudur (1). Demir eksikliği (DE) her yaş grubunda görülürken özellikle 6 ay–2 yaş arası çocuklarda ve ergenlik çağında çok daha sık görülmektedir. Çocukluk döneminde anemi sıklığı gelişmiş ülkeler %4-20 arasında iken, gelişmekte olan ülkelerde %70

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gibi yüksek oranlara çıkabilmektedir (2). Türkiye'de 5-59 aylık çocuklarda aneminin yaygınlığı Dünya Sağlık örgütü (DSÖ) tarafından %30 olarak bildirilirken, bölgesel farklılıklara göre %15-60 oranları arasında farklılıklar göstermektedir (3). Bu verilere ek olarak sanayileşmiş ülkelerde 0-4 yaş arasında %20,1, 5-14 yaş arasında ise %5,9 (gelişmekte olan ülkelerde %39 ve %48,1) görülür (4).

Özellikle iki yaş ve altı çocuklarda demir eksikliği anemisinin büyüme, gelişme ve bilişsel fonksiyonları önemli etkileri nedeniyle, tarama programlarıyla aneminin takip edilmesi ve tedavisinin desteklenmesi gerekmektedir. Bu nedenle aneminin erken tanı ve tedavisi çok önemlidir. DEA'yı önlemek veya tedavi etmek için uygulanan herhangi bir strateji bölgenin yerel koşullarına, etiyolojiye ve prevalansa uygun olmalıdır. Türkiye Cumhuriyeti Sağlık Bakanlığı çocuklarda nutrisyonel demir eksikliği anemisi sıklığını azaltmak amacıyla 2004 yılından itibaren "Demir Gibi Türkiye" programını başlatmıştır.

Gerek ülkemizde gerekse başka toplumlarda demir eksikliği anemisini önlemeye yönelik uygulanan programlardan istenilen sonuç alınamamıştır. Bu tür tarama ve destek programlarının başarılı bir şekilde sürdürülmesi önemlidir. Ancak birçok nedenden dolayı bu programlardan istenilen sonuçlar alınamamaktadır. Bizim bu çalışmayı yapmakta amacımız 9-12 ay arası çocuklarda demir desteği programına ailelerin ne oranda uygun hareket ettikleri ve bu programa ne kadar uyum sağlandığını ve başarısızlığa neden olan faktörleri ortaya koymaktır.

GEREÇ VE YÖNTEM

Prospektif gözlemsel olarak planladığımız bu çalışma için 1 Ocak 2020 – 31 Aralık 2020 tarihleri arasında Bursa Yüksek İhtisas Eğitim ve Araştırma Hastanesi Çocuk Sağlığı ve Hastalıkları kliniği sağlam çocuk polikliniğine 9 – 12 ay arası tam kan sayımı için başvuran çocukları dâhil ettik. Demir gibi Türkiye programı dâhilinde 4-6 ay arası çocuklara demir profilaksisinin başlanması ve en az üç ay devam edilmesi önerilmektedir. Biz de çalışmamız için en az üç aylık kullanım periyodunu değerlendirebilmek adına 9-12 ay arası çocukları araştırmamıza dâhil ettik. Araştırmamızdan akut enfeksiyon bulgusu, metabolik hastalığı ve doğumsal anomalisi olanlar, DEA nedeniyle demir tedavisi alanlar, talasemi taşıyıcıları, demir profilaksisi dışında multivitamin veya herhangi bir ilaç kullanımı olan hastaları çalışma dışı bırakıldı. Çocuğun velisinden onam aldıktan sonra çocuklardan tam kan sayımı için kan örneği alındı. Alınan kan örneği ile birlikte çocukların beslenme alışkanlıklarını değerlendirdiğimiz bilgi formu çocukların velileri ile yüz yüze görüşerek dolduruldu. Bu bilgi formunda çocukların demir proflaksisine ne zaman başlandığı, hangi dozdan ne kadar süre kullandıkları gibi soruların yansıra, ne kadar anne sütü kullandıkları, ek gıdaya ne zaman geçiş yaptıkları demirden zengin beslenme durumlarını değerlendirmek amaçlı ne kadar kırmızı et tükettikleri gibi sorular bulunmaktadır. Bunun yanı sıra özellikle demir proflaksisine neden devam etmediklerini öğrenmek amaçlı hem seçenek içeren hem de açık uçlu sorular yönelttik. Çocukların tam kan sayımı sonuçlarına göre çocuklar için yaş ve cinsiyete göre Dünya Sağlık örgütü tarafından belirlenen hemoglobin değerine göre anemi varlığını değerlendirdik. Sağlık Bakanlığı tarafından önerilen +3 değerlikle ferrik demirden en az 1 mg/kg/gün alım "uygun doz" kullanım olarak kabul ettik. Haftada en az üç gün ve üzeri kullanım ise düzenli kullanım olarak değerlendirildi. Haftada üç günden daha az ya da 1mg/kg'dan daha az demir desteği sağlanmasını da "doğru olmayan" kullanım olarak tanımlandık.

İstatistik

İstatistiksel analiz Statistical Package for the Social Sciences (SPSS 20.0) paket programı kullanılarak yapıldı. Araştırmamız için kullandığımız tanımlayıcı istatistiksel verileri olgu sayısı(N) ve yüzde (%) ile ifade ettik. Araştırmamızda elde ettiğimiz sayısal verilerin normal dağılımını değerlendirmede Kolmogorov - Simirnov testi kullandık. Normal dağılım göstermeyen verilerin tanımlayıcı istatistiklerinde ortanca ve çeyrekler arası veriler kullanılırken normal dağılım gösteren veriler için ortalama ve standart sapma kullanıldı. Kategorik verilerin ikili karşılaştırmasında Pearson ki-kare testi kullanıldı. İkili sayısal normal dağılım gösteren grupların karşılaştırmasında bağımsız Gruplar t testi kullanılır iken, normal dağılım göstermeyen sayısal verileri ile kategorik verilerin analizinde Mann-Whitney U testi kullanılmıştır. İkili bağımsız verilerin korelasyon analizinde normal dağılım göstermeyen sayısal veriler ve kategorik verilerin korelasyon incelemesinde spearman testi kullanıldı. Tüm analizler için p değeri <0,05 değeri istatistiksel olarak anlamlı kabul edildi.

BULGULAR

Çalışmaya 556 çocuk dâhil edildi. Çocukların 286'sı (%51,4) erkek, 270'i (%48,6) kız idi (erkek/kız: 1,1). Olguların yaş ortanca değeri 10 (IQR:3) ay idi. Ortanca anne sütü kullanım süresi 4 (IQR:3) ay, tamamlayıcı beslenmeye başlama yaşı ise ortalama 5,92±0,51/ay olarak tespit ettik. Olguların 216'sı (%38,8) ilk 6 ay sadece anne sütü ile beslenirken, 36'sı (%6,4) doğumundan itibaren mama ile çocuğunu beslediğini belirtti. Olguların 107'si (%19,2) inek sütü kullanımı vardı. Olguların kırmızı et tüketimini değerlendirildiğinde 46'sında (%7,8) et tüketiminin haftada bir öğünden az veya hiç olmadığını, 113'ünün (%20,3) haftada bir öğün, 309'unda (%55,6) haftada iki öğün, 73'ünde (%13,1) haftada üç öğün ve 15'inde (%2,7) haftanın her günü et tükettiğini saptadık. (Tablo 1)

Olguların 548'ine (%98,6) demir profilaksisinin önerildiği saptandı. Olguların 349'unun (%62,8) demir profilaksisini haftada üç günden fazla aldığını, 207'sinin (%37,2) ise haftada üçten az ya da hiç kullanmadığını tespit ettik. Ortanca demir profilaksisi kullanım süresi 6 (IQR:2) ay, kullanılan demir dozu ortanca değeri 6 (IQR:1)damla/gün

Tablo 1. Olguların Demografik Özellikleri

	N (%)
Cinsiyet	
Erkek	286 (%51,4)
Kadın	270 (%48,6)
Yaş*	10 (3) ay
İlk altı ay sadece anne sütü	216 (%38,8)
Ortalama anne sütü tüketimi*	4 (3) ay
Tamamlayıcı beslenmeye geçiş**	6 ±0,5
Formül mama kullanımı	
Evet	334 (%60,1)
Hayır	222 (%39,9)
Formül mama başlangıç yaşı*	6 (3) ay
İnek sütü kullanımı	
Evet	107(%19,2)
Hayır	449(%80,8)
Kırmızı et tüketimi	
Haftada birden az / yok	46 (%7,8)
Haftada bir kez	113 (%20,3)
Haftada iki kez	309 (%55,6)
Haftada üç kez	73 (%13,1)
Her gün	15 (%2,7)

* Veriler normal dağılım göstermediği için ortanca ve çeyrekler arası değerler belirtilmiştir.

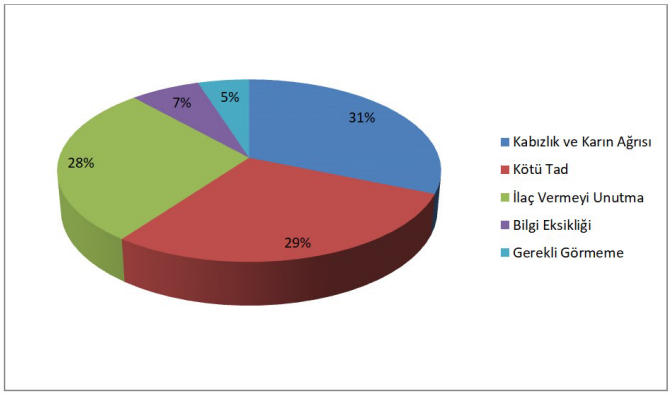
** Veriler normal dağılım gösterdiği için ortala ve standart sapma kullanılmıştır.

olarak tespit ettik. Demir profilaksisi kullanmayan ailelerin (n:207), 51'inin (%24,6) kabızlık ve karın ağrısı yaptığı için, 47'sinin (%22,7) tadı kötü olduğu için, 46'sının (%22,2) ilacı vermeyi ihmal ettikleri için demir profilaksisini vermediklerini belirttiler. Diğer nedenlere baktığımızda ise ailelerin 8'inin (%3,9) demir kullanımını gerekli görmedikleri için ve 11 (%5,3) ailenin bu konu hakkında bilgilerinin olmadığını tespit ettik (Tablo 2, Grafik 1).

Tablo 2.Ailelerin demir profilaksisi kullanım durumu

Demir proflaksisi önerildi mi?	
Evet önerildi	548 (%98,6)
Hayır önerilmedi	8 (%1,4)
Demir proflaksisi kullanımı	
Evet kullanıyorum (≥ 3 gün /hafta)	349 (%62,8)
Hayır kullanmıyorum (≤ 2 gün/hafta)	207 (%37,2)
Demir kullanım süresi (ay)*	6 (IQR:2)
Demir kullanım miktarı (damla/gün)*	6 (IQR:1)
Demir proflaksisi kullanmama nedenleri	
Karın ağrısı /kabızlık nedeniyle	51 (%24,6)
Tadı kötü	47 (%22,7)
İhmal ediyorum / Unutuyorum	46 (%22,2)
Gerekli olduğunu düşünmüyorum	8 (%3,9)
Önerilmedi / Bilgim yok	11 (%5,3)

*Veriler normal dağılım göstermediği için ortanca değer ile çeyrekler arası (interquatile range) değer belirtilmiştir.



Grafik 1. Ailelerin Demir Proflaksisini Vermeme Nedenleri

556 çocuğun 349'u (%62,8) düzenli olarak (haftada en az üç gün) demir proflaksisi verirken, 207 (%37) aile düzenli olarak ilaç vermediğini belirtti. Düzenli olarak profilaksi verdiğini belirten ailelerin çocuklarının ağırlıklarına göre uygun dozda demir desteği verip vermediklerine baktığımızda düzenli kullanan 349 çocuğun 156'sı (%44,7) <1mg/kg/gün, 123'ü (35,2) 1-2mg/kg/gün ve 70'i (%20,1) >2mg/kg/gün olacak şekilde demir proflaksisi vermektedir. (Tablo 3). Çocukların 193'ü (%34,7) düzenli (>3 gün /hafta) ve uygun dozda (>1mg/kg/gün) demir proflaksisi kullanımı mevcuttu. 363 (%65,3) çocuk ya düzensiz ya da düşük (yetersiz) dozda demir desteği alıyordu.

Tablo 3. Ailelerin demir proflaksisine uyumunun değerlendirilmesi

	n	**%**
Düzenli olarak demir desteği verenler	349	62,8
<1mg/kg demir kullananlar	156	44,7
1 – 2 mg/kg demir kullananlar	123	35,2
>2mg/kg demir kullananlar	70	20,1
Düzenli olarak demir desteği vermeyenler	207	37,2

Olguların 219'unda (%39,4) anemi tespit ettik. Anemi oranında yaş ve cinsiyete göre istatistiksel bir fark yoktu. Anemi daha fazla et tüketenlerde, uygun doz demir desteği alanlarda, uzun süre demir desteği alanlarda daha düşük orandaydı ($p<0,001$). Demir proflaksisini düzenli kullanmayan 207 çocuğun 135'inde (%65,2), demir proflaksisini düzenli kullanan 349 çocuğun 84'ünde (%24,1) anemi vardı (Tablo 4). Demir proflaksisini düzenli kullanan çocukların ortalama hemoglobin değeri 11,5 gr/dl iken, düzenli kullanmayan grubun ortalama hemoglobin değeri 10,4 gr/dl olarak geldi ($p<0,005$). Olguların demir profilaksisi kullanım süresi ve dozu ile hemoglobin değerleri arasındaki korelasyona baktığımızda hemoglobin değerinin hem doz ($r=0,397$) hem de kullanım süresiyle ($r=0,270$) pozitif yönde bir korelasyonunun olduğunu gördük ($p<0,005$).

Tablo 4. Anemisi olan ve olmayan olguların demografik özellikleri

	Anemi		
	Var	Yok	p
Yaş	10,50±1,15	10,45±1,19	0,664
Cinsiyet			0,774
Erkek	111 (%50,7)	175 (%51,9)	
Kız	108 (%49,3)	162 (%481)	
Düzenli demir kullanımı			<0,005
Evet	84 (%24,1)	265 (%75,9)	
Hayır	135 (%65,2)	72 (%34,8)	
Kırmızı et tüketimi			<0,005
Haftada bir	66 (%58)	47 (%42)	
Haftada iki	120 (%38)	189 (%62)	
Haftada üç ve daha fazla	10 (%11)	78 (%90)	

TARTIŞMA

Çocukluk çağında en sık görülen anemi demir eksikliği anemisidir ve gelişmekte olan ülkelerde başta olmak üzere tüm dünyada DEA önemli bir halk sağlığı sorunu olmaya devam etmektedir (5). Dünya genelinde DEA'yı önlemeye yönelik uygulanan tün stratejilere rağmen DEA gelişmekte olan ülkelerde 4 yaş altında anemi oranı %24 civarındadır (6). Semptom olup olmadığına bakmaksızın çocuklarda DE veya DEA derhâl tedavi edilmelidir (7). Bu yaş grubu (özellikle beş yaş altı), çocukların kognitif fonksiyonlarının geliştiği, okul performanslarının olumsuz yönde etkilenebileceği ve davranış problemlerinin ortaya çıkma ihtimalinin olduğu yaşlardır (7). Ülkemizde çocuklarda yapılan çeşitli çalışmalarda farklı yaş grupları, bölgeler ve topluluklarda fazla oranlarda bulunsa da genelde DEA sıklığı oldukça yüksek bulunmuştur. Ülkemizde çocukluk yaş grubunda yapılan çeşitli araştırmalarda DEA sıklığı %15,2 ile %62,5 arasında bildirilmiştir (8, 9, 10). Sağlık Bakanlığı DEA'yı önleme ile ilgili olarak 2004 yılında ''Demir Gibi Türkiye'' programıyla demir profilaksi projesi başlatmış ve bakanlık 2017 kayıtlarına göre demir eksikliği anemisi sıklığı çocuk yaş grubunda %30'lardan (%12-80), %6,3'e gerilemiştir (8). Çetinkaya ve ark.'ın 2005'te İstanbul'da yaptıkları çalışmada yaşları 7-24 ay arasındaki 3.117 çocukta demir eksikliği prevalansı %61,6 oranında saptamışlardır (11). Çalışmamızda anemi oranı %39,4 olarak saptandı. Hastanemizin sosyokültürel olarak düşük ve orta düzeyde ailelerin yoğun olarak bulunduğu bir yerde bulunması nedeniyle anemi prevalansını Türkiye ortalamasından daha yüksek olarak bulduğumuzu düşünmekteyiz.

Nöromotor ve beden gelişiminin en aktif olduğu süt çocukluğu döneminde özellikle gelişmekte olan ülkelerde sosyoekonomik düzey göz önünde bulundurularak DSÖ, anemi prevalansının yüksek olduğu ülkelerde oral demir profilaksisi önermektedir (4). Sağlık Bakanlığı tarafından 2004 yılında başlatılan ''Demir Gibi Türkiye'' programına göre miadında doğan bebeklere 4.aydan itibaren 1 mg/kg/gün dozunda; prematüre ve 2500 gr altında doğan bebeklere 2. aydan 2 mg/kg/gün dozunda elementer demir desteği sağlanması önerilmektedir. Rehberde demir profilaksisine en az 5 ay veya 1 yaşına kadar devam edilmesi önerilmektedir (8). Çalışmamızda ailelerin %98,6'sına herhangi bir sağlık kuruluşundan demir desteğinin önerildiği saptanırken, olguların 349'unun (%62,8) demir profilaksisini haftada üç günden fazla aldığını, 207'sinin (%37,2) ise haftada üçten az ya da hiç kullanmadığını tespit ettik. Ortanca demir profilaksisi alma süresi 6 (IQR:2) ay, kullanılan demir dozu ortanca değeri 6 (IQR:1) damla/gün olarak tespit ettik. Sonuçları değerlendirdiğimizde, ilgili sağlık ocakları tarafından ailelere demir desteği çok yüksek oranda önerildiği görülmektedir. Ancak, demir profilaksisine devam etmeme oranları %37,2 gibi yüksek saptanmıştır. Kılıç ve ark.'ının yaptığı, 540 çocuğun değerlendirildiği bir çalışmada; çocukların %85,4'üne demir profilaksisi önerildiği ancak %57'sinin profilaktik demir desteğini kullandığını görülmektedir. Bu çalışmada demir takviyesi alan hastaların yaklaşık %35'inin demir profilaksisinden vazgeçtiği gözlenmiştir (12).

Çalışmamıza katılanların %98,6'sına herhangi bir sağlık kuruluşu tarafından demir desteği önerildiğini belirtmiştik. Ancak düzenli ve uygun doz alımlarını değerlendirdiğimizde 349 (%62,8) aile düzenli olarak demir desteği verdiklerini belirtirken ailelerin 207'si (%37,2) düzenli olarak demir desteği vermediklerini belirttiler. Bununla birlikte düzenli olarak demir desteği veren 349 ailenin uygun doz verip vermediklerine baktığımızda 156'sının (%44,7) yetersiz dozda demir desteği verdiklerini tespit ettik. Bu açıdan baktığımızda araştırmaya katılan 556 çocuğun sadece 193'i (%34,7) hem düzenli hem de uygun dozda demir proflaksisi aldığını tespit ettik. Bu sonuçlar ile birlikte her ne kadar ailelere yüksek oranda demir profilaksisi önerilse de uygun dozda ve düzenli olarak kullanım çok düşük orandadır. Demir profilaksi programlarının başarısız olduğunu belirten uluslararası yayınlar bulunmakta ve ülkelerin kültürel sosyoekonomik özelliklerine bağlı olarak süt çocukluğu döneminde demir eksikliği yaygın olarak görülmeye devam etmektedir (13). İlarslan ve ark.'ının yapmış oldukları çalışmada %44,3 oranında katılımcının demir profilaksisini kullanmadıkları tespit edilmiştir (10). Bu sonuca benze şekilde Yalçın ve ark.'ının yaptıkları çalışmada profilaktik demir desteği kullanmayanların oranının %31,2, olarak belirtilmiştir (14). Bizim yaptığımız çalışmada düzenli olarak demir desteği vermeyenlerin oranları %37,2 olup bu çalışmalar ile benzer sonuçlar çıkmıştır. Ancak bu çalışmalardan farklı olarak bizim çalışmamızda ailelerin uygun dozda demir desteği verip vermediklerini değerlendirdiğimizde araştırmaya katılanların %65,3'ünün esasında yeterli dozda demir desteği vermediklerini tespit ettik.

Yapılan çalışmalarda, ailenin gereksiz bularak kullanmak istememesi, ihmal (unutkanlık), karın ağrısı ve kabızlık gibi yan etki endişesi, tat bozukluğu, sağlık çalışanı tarafından bilgilendirilmeme, gibi sebeplerden dolayı demir profilaksisine devam etmedikleri belirtilmiştir (15). Bizim çalışmamızda tespit ettiğimiz nedenlere baktığımızda, ailelerin %24,6 karın ağrısı, kabızlık yapması, %22,7 tat bozukluğu, %22,2 ihmal, %3,9 gerekli olmadığını düşünmesi, %5,3 ailenin de bu konu hakkında bilgisinin olmadığını tespit ettik.

Çalışmamızda demir profilaksisini düzenli kullanan 349 çocuğun 84'ünde (%24,1) anemi tespit ettik. Bunula birlikte düzenli demir profilaksisi kullanmayanlarda ise anemi oranı %65 gibi yüksek bir orandaydı. Yurdakök ve ark'ı 4-7. aylar arasında demir desteğinin çocuklarda demir eksikliğini önleyemediğini daha uzun süreli destek verilen çalışmalara ihtiyaç olduğunu belirtmişlerdir (16). Çalışmamızda da demir profilaksisi alanlarda anemi oranını %24 bulmamız Yurdakök ve ark.'nın belirttiği gibi profilaksi sürelerinin daha uzun süre olması gerektiği yorumu da yapılabilir. Ancak araştırmamız neticesinde vurgulamak istediğimiz bir nokta ise profilaksi süresinin uzun tutulması yanında çocukların yeterli dozda da demir desteği almadıklarından da kaynaklanabilmektedir. Çünkü araştırmamıza katılanların sadece %34,7'si hem uygun dozda hem de düzenli olarak demir desteği verdiklerini tespit ettik. Zaten araştırmamız sonuçlarında da demir süresi ve verilen demir dozu ile çocukların hemogram değerleri arasında pozitif korelasyon da vardı. Çalışmamızda düzenli proflaksi verilen çocuklarda da DEA oranının %24,1 yüksek olması, büyük olasılıkla ailelerin yetersiz dozda demir desteği verdiklerini göstermektedir.

Çalışmamıza dâhil edilen çocukların beslenme şekilleri incelendiğinde, ortanca anne sütü kullanım süresi 4 (IQR:3) ay, tamamlayıcı beslenmeye başlama yaşı ise ortalama 5,92±0,51 ay olduğunu bulduk. Olguların 216'sı (%38,8) ilk 6 ay sadece anne sütü ile beslenirken, 36'sı (%6,4) doğumundan itibaren mama ile çocuğunu beslediğini belirttiler. Olguların 107'sinde (%19,2) inek sütü kullanımı vardı. Formül mamaya başlama ay ortalaması 3±1,29 ay olduğu görüldü. DSÖ, bebeklerin ilk 6 ay sadece anne sütü ile beslenmesini, 6. aydan sonra, anne sütüne ek olarak demirden zengin içerikli ek gıdalarla tamamlayıcı beslenmeye geçilmesini önermektedir (3). Anne sütünün hem anne hem bebek açısından bilinen yararlarına rağmen, ilk 6 ay sadece anne sütü ile beslenme ve sonrasında emzirmeye devam etme süresi dünyada ve ülkemizde istenilen düzeyde değildir. ESPGAN (Avrupa Pediatrik Gastroenteroloji Hepatoloji ve Beslenme Derneği) ve DSÖ çocuklarda 6. aydan sonra tamamlayıcı beslenmeye başlanmasını ve anne sütüne 2 yaşına kadar devam edilmesini, inek sütünün 1 yaşından sonra beslenmeye eklenmesini önermektedir (4, 17).

İyi bir demir kaynağı olan kırmızı et ile ilgili çeşitli çalışmalarda, çocukların kırmızı et tüketimi ile demir durumları arasında pozitif yönlü korelasyon saptanmıştır (18). Korğalı ve ark.'nın çalışmasında kırmızı et tüketimi ile demir eksikliği arasında pozitif bir ilişki saptanmış ve haftada 3 öğünden az öğün kırmızı et tüketimi olan çocuklarda demir eksikliği anemisi olma olasılığı 4.73 kat daha yüksek bulunmuştur (19). Çalışmamızda değerlendirilen çocuklarda haftada en az bir defa kırmızı et tüketim oranı %91,7 idi. Ancak literatürde yeterli et tüketimi olarak tanımlanan haftada 3-5 gün en az bir öğün et tüketimine baktığımızda olguların sadece %15,8'nin yeterli miktarda et tükettiğiniz tespit ettik. Tüm dünya geneline bakıldığında kırmızı et tüketimi yüksek olan bölgelerde anemi prevalansı düşüktür (20). Çalışmamızda ve benzer çalışmalarda görüldüğü gibi, önemli bir demir kaynağı olan kırmızı et tüketiminin yetersiz olması ile demir eksikliği arasında yakın bir ilişki bulunmaktadır.

Çalışmamızda ilk altı ayda sadece anne sütü ile beslenen bebeklerde demir profilaksisi kullanım oranları daha yüksek çıkmıştır. Bu durum hem anne sütünün demir desteğinin daha iyi olduğunu hem de anne sütü ile beslenmeye özen gösteren ailelerin demir profilaksisine de daha iyi uyum sağladıklarını göstermektedir. Altıncı aydan sonra anne sütünün tek başına verilmesi, bebeğin artan demir gereksinimine yetmediği için bu dönemde de tek başına anne sütü ile beslenme durumunda DE/DEA kolayca ortaya çıkmaktadır. Eksiklik oluşmaması ve gereksinimin karşılanabilmesi için 6 ay sonrasında demirden zengin ek gıdalara başlanmalıdır. Bu sebeple erken çocukluk çağında anemi oranlarını düşürmenin en etkili yollarından biri de demir profilaksisi yanında tamamlayıcı beslenme döneminden başlayarak demirden zengin besinlerin ve kırmızı etin çocuğun menüsüne dâhil edilmesidir (21).

SONUÇ

Sonuç olarak, demir eksikliği dünyadaki en sık rastlanan beslenme sorunudur. Demir eksikliği anemisi; azalmış fizik ve öğrenme kapasitesi, konsantrasyon azalması, unutkanlık, enfeksiyonlara yatkınlık, zekâ ve motor gelişim geriliği, davranış bozukluğu ve psikolojik gelişim geriliği gibi çok önemli klinik sonuçlara yol açmaktadır. Demir elementinin özellikle nörokognitif gelişim üzerindeki etkileri göz önünde bulundurulduğunda başta DSÖ olmak üzere toplumlar süt çocukluğu döneminde demir desteğine önem vermektedirler. Araştırmamız neticesinde hemen hemen herkese demir profilaksisi önerilirken ilacı vermeyi unutma ve tat ve karın ağrısı gibi yan etkilerden dolayı ailelerin profilaksiye devam etmedikleri görüldü. Bununla birlikte profilaksiye devam edenlerinde önemli bir kısmını yetersiz dozda demir desteği aldıklarını tespit ettik. Araştırmamız neticesinde şunu söyleyebiliriz ki toplumumuzda demir desteğine başlamada ciddi bir aksama görülmüyor ancak ailelerin ilaca devam etmesi ve büyüyen çocuğun kilosuna

uygun demir ilacı alıp almadığını noktasında ciddi aksaklılar olduğu kanaatindeyiz. Demir destek programlarının başarıya ulaşması noktasında özellikle birinci basamak sağlık takiplerinde ve çocuk hekimliği pratiğinde ailelerin demir desteğine devam edip etmediklerinin kontrolünün yanı sıra büyüyen çocukların ağırlıklarına uygun miktarda desteği aldıklarının da takip edilmesi gerekmektedir. Koruyucu sağlık hizmetlerinin çok önemli olduğu günümüzde ''Demir Gibi Türkiye'' programının daha başarılı ilerlemesi adına uygun sağlık politikalarının uygulanması gerekmektedir.

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Yazarlık Katkısı: Fikir/Kavram: TM, MB, Tasarım/Dizayn: TM, MB, AE, Denetleme/Danışmanlık: MB, AE, Veri Toplama ve/ veya işleme: TM, Analiz ve/veya Yorum: TM, MB, Literatür Taraması: TM, Makalenin Yazımı: TM, MB, AE, Eleştirel İnceleme: AE.

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Original Article / Araştırma Makalesi

INTERNAL COMPRESSION THERAPY FOR DEEP VENOUS İNSUFFICIENCY EARLY RESULTS IN PATIENTS

DERIN VENÖZ YETMEZLIĞI OLAN HASTALARDA İNTERNAL KOMPRESYON TEDAVISININ ERKEN DÖNEM SONUÇLARI

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ABSTRACT

Introduction: In the treatment of deep venous insufficiency (DVI), in addition to surgical interventions, internal compression therapy (ICT) using a paravalvular leak device, a percutaneous method, can also be employed. This study aims to present the early outcomes of ICT application in patients diagnosed with deep venous insufficiency exhibiting reflux at the saphenofemoral junction.

Methods: This study included 28 patients with deep venous insufficiency who underwent ICT. The patients were followed for 6 months. Clinical findings and venous Doppler ultrasound (USG) results were recorded. Our study was conducted with a retrospective method.

Results: There was a significant reduction in the mean reflux duration before the procedure and at 6 months post-procedure. Significant improvement was observed in the postoperative Venous Clinical Severity Score (VCSS) and Aberdeen Varicose Vein Questionnaire (AVVQ) scores in all patients (p<0.001).

Conclusions: The aim of ICT is to narrow the vein diameter and achieve valve coaptation at the saphenofemoral junction. Consequently, we believe that ICT treatment significantly contributes to the improvement of deep venous insufficiency by preventing or reducing reflux in the venous system.

Keywords: deep venous surgery, venous reflux, deep venous insufficiency, venous insufficiency, internal compression therapy

ÖZET

Giriş: Derin venöz yetmezlik (DVY) tedavisinde cerrahi tedavilerin yanı sıra perkütan bir yöntem olan paravalvüler sızıntı cihazı ile internal kompresyon tedavisi (ICT) de kullanılabilmektedir. Bu çalışmada safenofemoral birleşkede reflü akımı olan derin venöz yetmezlik tanılı hastalarda ICT uygulamasının erken dönem sonuçlarını sunmayı amaçladık.

Yöntemler: Bu çalışmaya derin venöz yetmezliği olan ve ICT uygulanan 28 hasta dahil edildi. Hastalar 6 ay boyunca takip edildi. Klinik bulgular ve venöz Doppler USG sonuçları kaydedildi. Çalışmamız retrospektif yöntem ile yapılmıştır.

Bulgular: İşlem öncesi ve işlemden 6 ay sonra ortalama reflü süresinde anlamlı azalma görüldü. Ameliyat sonrası VCSS ve AVVQ skorlarında tüm hastalarda anlamlı iyileşme görüldü (p<0,001)

Sonuç: ICT'nin amacı,safenofemoral birleşke bölgesinde ven çapını daraltmak ve kapak koarktasyonunu sağlamaktır. Sonuç olarak, ICT tedavisinin venöz sistemdeki reflüyü önleyerek veya azaltarak derin venöz yetmezliğin iyileşmesine önemli katkı sağladığına inanıyoruz.

Anahtar Kelimeler: Derin ven cerrahisi, venöz reflü, derin venöz yetmezlik, venöz yetmezlik, internal kompresyon tedavisi

INTRODUCTION

Chronic venous insufficiency (CVI) is a common disease affecting almost half of adult population. CVI can include various pathologies, ranging from insufficiency in superficial or deep vessels to their blockage. Studies show that 90% of CVI cases is superficial venous insufficiency, 30% is deep venous insufficiency and 20% is the perforating vein insufficiency (1). In addition, a wide range of clinical manifestations can be observed in patients, ranging from superficial varicose changes to chronic skin changes with ulceration. CVI can hamper people's daily activities and cause loss of work and time (2).

The presence of reflux in the deep venous system, such as the femoral vein, popliteal vein, is defined as deep venous insufficiency. Deep venous reflux (DVR) can be caused by a primary etiology such as valve absence or valve dysfunction, or it can also be a complication of proximal obstruction. In patients with DVR, symptoms are usually severe, and at least half of the symptomatic patients have DVR (3, 4).

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Although there are various and effective treatment methods for the treatment of the superficial venous insufficiency, treatment for deep venous reflux and deep vein occlusions is limited to compression therapy in addition to medical treatment and wound care if there are ulcers (3).

Chronic venous insufficiency (CVI) has specific scoring methods to determine the clinical severity of the disease, with one of the most commonly used being VCSS. The scoring system consists of 10 parameters comprising clinical criteria determined by both the physician and the patient. Scoring is conducted by assigning scores ranging from 0 to 3 to the parameters to assess clinical severity (5).

The etiological causes of DVR are Es (post-thrombotic syndrome), Ep (primary deep valve insufficiency) and Ec (congenital valve malformation) according to the CEAP classification. In patients with post thrombotic syndrome (Es), serious damage to the valves occurs due to fibrosis and retraction, and valve repair might be unlikely. In primary deep valve insufficiency (Ep), there is an expansion of the valve ring due to elongation of the free edges of the valves and expansion in the commissures. This group is commonly found in the etiology of chronic venous insufficiency cases (6). In the treatment of these patients, methods such as valve reconstructions such as internal and external valvuloplasty, vein transplantation, and external tape have been reported in the literature. However, these invasive methods are technically difficult, wound complications are frequent, and success rates are low. Therefore, invasive approaches are not widely practiced today (2-7).

In the present study, therefore we applied a polymer consisting of a mixture of cyanoacrylate and hyaluronic acid between the deep vein and the muscle fascia in patients with CVI due to primary deep valve insufficiency. We aimed to evaluate the effectiveness and safety of this procedure.

MATERIALS AND METHODS

This study was initiated with the ethical approval numbered 26, dated 11.02.2021, obtained from the Ethics Committee of the Faculty of Medicine at Eskişehir Osmangazi University Hospital. Our study was conducted with a retrospective method.

A total of 28 patients admitted to our clinic with the diagnosis of primary femoral venous insufficiency between January of 2020 and June of 2021were treated with ICT. All the patients were diagnosed with femoral vein insufficiency through performing Doppler ultrasonography (DUS) by a radiologist. Although compression stockings were recommended to all of these patients, their compliances were low and all the patients were receiving medical treatment.

The patients with isolated deep vein insufficiency without concomitant superficial venous insufficiency were included in the present study. The patients with reflux of 2 sec and above in DUS were included. On the other hand, the patients with a diagnosis of postthrombotic syndrome and deep vein thrombosis, with congenital deep vein insufficiency, with valve anomalies, with superficial venous insufficiency or perforating vein insufficiency were excluded from the study.

The patients one day before and after the surgery; then one, three and six months after the surgery were checked to assess their clinical presentation and quality of life. The severity of the disease was assessed using the CEAP (Clinical-Etiology-Anatomy-Pathophysiology) classification and the Venous Clinical Severity Score (VCSS). The quality of life assessment of the patients was performed using the Aberdeen Varicose Vein Questionnaire (AVVQ) questionnaire. The results of DUS performed in the controls were compared. Success of the procedure was defined as no reflux in the deep vein valves in the early period and showing less than 1 second of reflux after six months.

All surgical procedures were performed under local anesthesia and under sterile conditions. The patients were placed on their backs and the position was given so that the lower limbs were slightly flexed. DUS was used to confirm the location of the deep vein valves and the severity of reflux. Vein diameter and reflux times were measured at the location of the valves in the femoral vein approximately 1 cm below the saphenofemoral junction.

Two 6F cannulas were inserted on the anterior and medial facets of the femoral vein in the space between the muscle fascia and the deep vein through the Seldinger method. A polymer (ICT) consisting of cyanoacrylate and hyaluronic acid (Invamed, Ankara, Turkey) was injected into the space till the gap between the valves filled. In this way, the vessel diameter was reduced. After it was confirmed that the reflux flow ended with DUS, the cannulas were withdrawn and the procedure was terminated.

Statistical analysis

While continuous data were expressed as Mean ± SE, categorical data were expressed as a percentage (%). Shapiro Wilk's test was used to investigate the conformity of the data to the normal distribution. The dependent sample t-test was used to compare the values at different measurement times with a normal distribution. IBM SPSS Statistics 21.0 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, N.Y.: IBM Corp.) was used. A value of $p<0.05$ was accepted as the criterion for statistical significance.

RESULTS

A total of 28 patients were included in the present study. While 15 of them were female and 13 were male. The average age of the patients was 46 ± 11 (37 - 72). The right leg was operated in 16 patients and the left leg was operated in 12 patients. The mean duration of symptoms presented was 12 ± 8.8 years. According to the CEAP classification performed before the procedure, 11 of the patients were C4, 8 were C5 and 9 were C6 (Table 1).

Table 1: Demographic and Clinical Features

	mean ± SD	minimum	maximum
Age	46 ± 11	37	72
Average time for the symptoms (year)	12 ± 8,8		
	n	**%**	
Gender			
Male	13	46.42	
Female	15	53.58	
Operated side			
Right	16	57.14	
Left	12	42.86	
CEAP classification			
Clinic	**n**		**%**
C4	11		39.28
C5	8		28.57
C6	9		32.15

*CEAP scoring is used to determine the severity of postthrombotic syndrome. C (clinical), E (etiology), A (anatomy), P (pathology) C4: Skin Changes (Pigmentation or Lipodermatosclerosis), C5: Previously present but now healed venous ulcer, C6: Currently present active venous ulcer

The procedure was performed under local anesthesia on all 28 patients. The average amount of polymer applied was 2.8 ml (2.0 – 3.6). The mean duration of the procedure was 16.8 ± 14min, and the mean discharge time was 4.2 ± 2.5 hours. The mean distance between the end points of the valves was 3.8 mm (2.1-4.4). Dec After the procedure, cooptation was achieved on all valves.

The mean femoral vein diameter before the procedure was 12.5 ± 0.88 (10.5 - 14.8) mm but it determined to be 9.44 ± 0.76 (8.8 - 11.4) 6 months after the procedure ($p < 0.001$). On the other hand, while the mean reflux time before the procedure was 3.2 (2 - 8) sec it measured to be 0.2 sec (0 – 1) 6 months after the procedure ($p < 0.001$) (Table 2).

Table 2: Results Related to the Procedures

	(Mean ±SD)	(min - max)	p
Amount of the polymers applied (ml)	2.8	(2.0 – 3.6)	
Time for the procedure (minute)	16.8 ± 14	(12 - 25)	
Diameter of femoral vein (mm)			
Pre-operation	12.5 ± 0.88	10.5 – 14.8	
Postoperative 6th month	9.44 ± 0.76	8.8 – 11.4	**p<0.001**
Preoperative distance between the tips of the valves of the veins (mm)	3.8	2.1 – 4.4	
Reflux finding in CDUS (second)			
Pre-operation	3.2	(2 - 8)	
Postoperative 6th month	0.2	(0 - 1)	**p<0.001**

*CDUS : Color Doppler Ultrasonography

There was a significant improvement in postoperative VCSS and AVVQ scores in all patients. Before and 6 months after the procedure, VCSS scores were 9 (6 – 11.5) and 5 (4-8), respectively ($p<0.001$). Likewise, the AVVQ values before and 6 months after the procedure was 30 (28-35) and 19 (15-25), respectively ($p<0.001$). Complete recovery was observed in 6 of 9 patients with ulcers with an average duration of 8 weeks (66.6%) (Table 3).

Table 3: Complications Observed with the Patients

	n	%
Number of the patients with complications	3	10.7
Severe pain and sensitivity	2	7.1
Hematoma	0	0
Ecchymosis	1	3.5
Induration	1	3.5
Thrombophlebitis / Cellulite	0	0
Deep vein thrombosis / Pulmonary emboli	0	0

No significant morbidity or mortality related to the procedure was observed. No serious complications such as deep vein thrombosis, pulmonary embolism, phlebitis developed in any of the patients. Only two patients developed pain and sensitivity while one patient developed localized ecchymosis and induration (Table 4).

Table 4: Clinical Assessment

	Pre-operation n (min-max)	Post-operation 6 th month n (min-max)	p
VCSS	9 (6 – 11,5)	5 (4 – 8)	**P<0,001**
AVVQ	30 (28 - 35)	19 (15 - 25)	**P<0,001**

*Vcss: Venous Clinical Severity Score,
*AVVQ: Aberdeen Varicose Veins Questionnaire

DISCUSSION

In the present study, the short- and medium-term results of internal compression therapy (ICT) in primary deep valve insufficiency (Ep) were analyzed. The results showed that no serious side effects occurred during the 6-month follow-up. The results obtained have confirmed that the procedure was safe and feasible in the treatment of venous insufficiency.

Corrective surgery on the DVR is not often performed. Most surgens consider it risky and ineffective. In fact, this type of operation is not aggressive and the complication rate is low. However, surgical procedures are still controversial in terms of effectiveness, and there is no suitable treatment for deep venous insufficiency (8, 9).

Furthermore, CVI significantly reduces the quality of life of patients and causes significant economic burden as well as loss of productivity of the people. For these reasons, the treatment of these patients becomes even more important. Conventional treatment includes regular exercise, leg elevation, compression therapy such as compression stockings, and wound care. Although these treatment methods are very important, they usually fail due to low compliance rate of patients (8, 9).

Surgical repair or replacement of deep vein leafs was first described by Kistner in 1968 (10). Aftermath, external and internal valvuloplasty, valve transplantation and valve transposition from the saphenous vein or axillary vein, and many other open surgical procedures such as wrapping the dilated segment around the heads have been reported. These methods have not found widespread use due to the presence of wound complications and ulcer recurrence in a significant proportion of patients (8, 9). Makhatilov et al in their study in 2009 used an external endoscopic support system for the femoral valves and presented successful results. However, the number of patients treated in their study was limited to 24 (11).

Studies on the placement of prosthetic valves mounted in metal stents or similar structures by the endovascular method are mostly experimental practices. In 2021, Thodur Vasudevan et al. used a system with four integrated components, which they called the BlueLeaf system, and offered a treatment option by creating valves into the femoral and popliteal veins using an endovascular method (12). In their study, at least one monocuspid valve was successfully formed in 13 of 14 patients and no serious complications were noted. However, the number of patients in their study were few and long-term results were not available.

Moreover, ICT is a treatment method aimed at restoring venous valve functions. A polymer consisting of cyanoacrylate and hyaluronic acid is used and administered percutaneously around the vessel. Hyaluronic acid causes an increase in connective tissue, while cyanoacrylate takes

on the role of a skeleton that supports the vein environment by creating dense tissue. In this way, it is aimed to reduce the diameter of the vein and ensure the cooptation of the valves. In recent years, there are several studies available using this system (13,15). We aimed to investigate the effectiveness of ICT in the present study. All of our patients were patients with primary valve insufficiency (Ep). We excluded the patients with post-thrombotic insufficiency and valve aplasia since there is no treatment for etiologic deficiencies from the present study.

In their study in 2020, Yavuz et al. used ICT as a paravalvular leakage system in 286 patients with primary deep venous insufficiency and presented their mid-term clinical results (14). Furthermore, using the same method in 2021 Eroğlu et al. conducted a similar study and presented the clinical results of 18 months in 30 patients (13). Gultekin et al. A study of 27 patients reported a significant decrease in patients' postoperative VCSS scores (16). In all these studies, significant improvements in patients' VCSS scores were reported after the procedure and they emphasized that the ICT procedure was safe and effective. Our present study further supported their observations. In short, valve cooptation was achieved and reflux was eliminated through achieving adequate venous diameter narrowing in all our patients after ICT application. Besides, there was a significant decrease in VCSS and AVVQ values 6 months after the procedure compared to preoperative values and overall patient satisfaction was high. Finally, none our patients developed serious complications.

The most significant limitation of the current study is that it is a retrospective, single centered study and the data presented are based on records in patient files.

CONCLUSION

The vast majority of primary deep valve insufficiencies can be safely treated with ICT. Long-term results and randomized trials are needed to support the present results.

Ethics Committee Approval: The necessary permission to conduct the research was received from Eskişehir Osmangazi University Faculty of Medicine Ethics Committee (decision number 12.01.2021-26).

Informed Consent: Informed consent for the procedure was obtained from all patients, and they were informed that their data could be used in the study later.

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

Authorship Contributions: Idea/Concept:CO,TT Design:ASK, TT Supervision:CO, Data Collection and Processing:ASK, TT, Analysis or Interpretation:ASK, TT Literature Search:ASK, Writing:TT, Critical Review:CO,ASK,TT, References and Fundings: ASK, TT, Materials: CO, ASK,TT.

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Original Article / Araştırma Makalesi

EVALUATION OF SERUM FIBROBLAST GROWTH FACTOR LEVELS IN ACUTE ISCHEMIC STROKE

AKUT İSKEMIK İNMEDE SERUM FIBROBLAST BÜYÜME FAKTÖRÜ DÜZEYLERININ DEĞERLENDIRILMESI

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ABSTRACT

Introduction: Stroke is a leading cause of death worldwide and remains very difficult to treat. Stroke results in brain damage through a cascade of events, including inflammatory responses and apoptosis. Serum basic fibroblast growth factor (bFGF) promotes the survival of nerve cells, stimulates new vessel formation, mesodermal remodeling, cell division, and cell migration, and accelerates wound healing. In this study, we evaluated serum bFGF levels in patients with ischemic stroke and examined the relationship between the clinical status of ischemic stroke patients and the control group.

Methods: We prospectively evaluated 96 patients (38 female and 58 male) admitted to our Neurology Clinic between February 2012 and October 2012, whose diagnosis of ischemic stroke was confirmed within 24 h after the onset of ischemic stroke. The control group comprised 48 age- and sex-matched healthy volunteers (32 female and 16 male) without vascular risk factors. the initial neurologic evaluation was performed using the National Institutes of Health Stroke Scale (NIHSS) to determine the severity of the stroke, and blood samples were obtained from all patients included in the study within 24 h from the onset of stroke to measure serum bFGF levels. These data were compared with data from control subjects.

Results: Serum bFGF levels of the ischemic stroke patients and the control group were 6.6±8.8 pg/ml and 4.6±4.3 pg/ml, respectively. A significant difference was noted between serum bFGF levels of both ischemic stroke patients and control subjects (p=0.005). Serum bFGF levels did not correlate significantly with NIHSS scores of patients with ischemic stroke (p>0.05).

Conclusions: We found that serum bFGF levels were significantly increased after acute ischemic stroke. The increase in serum bFGF levels in ischemic stroke patients may be a protective response to reduce brain damage.

Keywords: Basic fibroblast growth factor, Intracerebral ischemia, Angiogenesis, Acute ischemic stroke

ÖZET

Giriş: İnme, dünya çapında önde gelen bir ölüm nedenidir ve tedavisi hala çok zordur. İnme, inflamatuar yanıtlar ve apoptozis de dahil olmak üzere bir dizi olay yoluyla beyin hasarına neden olur. Serum bazik fibroblast büyüme faktörü (bFGF), sinir hücrelerinin hayatta kalmasını destekler, yeni damar oluşumunu, mezodermal yeniden şekillenmeyi, hücre bölünmesini ve hücre göçünü uyarır ve yara iyileşmesini hızlandırır. Bu çalışmada, iskemik inmeli hastalarda serum bFGF düzeylerini değerlendirdik ve iskemik inmeli hastaların klinik durumu ile kontrol grubu arasındaki ilişkiyi inceledik.

Yöntemler: Şubat 2012 ile Ekim 2012 arasında Nöroloji Kliniğimize başvuran ve iskemik inme tanısı iskemik inmenin başlangıcından sonraki 24 saat içinde doğrulanan 96 hastayı (38 kadın ve 58 erkek) prospektif olarak değerlendirdik. Kontrol grubu, vasküler risk faktörleri olmayan 48 yaş ve cinsiyete eşleştirilmiş sağlıklı gönüllüden (32 kadın ve 16 erkek) oluşuyordu. İlk nörolojik değerlendirme, felcin şiddetini belirlemek için Ulusal Sağlık Enstitüleri İnme Ölçeği (NIHSS) kullanılarak yapıldı ve çalışmaya dahil edilen tüm hastalardan, serum bFGF seviyelerini ölçmek için inmenin başlangıcından itibaren 24 saat içinde kan örnekleri alındı. Bu veriler, kontrol deneklerinden alınan verilerle karşılaştırıldı.

Bulgular: İskemik inme hastalarının ve kontrol grubunun serum bFGF seviyeleri sırasıyla 6,6±8,8 pg/ml ve 4,6±4,3 pg/ml idi. Hem iskemik inme hastalarının hem de kontrol deneklerinin serum bFGF seviyeleri arasında anlamlı bir fark kaydedildi (p=0,005). Serum bFGF seviyeleri, iskemik inme hastalarının NIHSS puanlarıyla anlamlı bir şekilde ilişkili değildi (p>0,05).

Sonuç: Akut iskemik inmeden sonra serum bFGF seviyelerinin anlamlı şekilde arttığını bulduk. İskemik inme hastalarında serum bFGF düzeylerindeki artış beyin hasarını azaltmak için koruyucu bir yanıt olabilir.

Anahtar Kelimeler: Temel fibroblast büyüme faktörü, İntraserebral iskemi, Anjiyogenez, Akut iskemik inme

INTRODUCTION

Ischemic stroke is defined as an acute cerebrovascular disease resulting from the occlusion of blood vessels that prevent blood flow to the brain (1). Stroke is a major cause of death worldwide. The incidence of ischemic stroke is increasing in developing countries and is among the leading causes of disability (2). In addition, the number of individuals

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experiencing the paralytic effects of stroke is increasing day by day (3).

Neurorestorative progression in stroke is characterized by angiogenesis, neurogenesis, and synaptic plasticity, which are beneficial for functional recovery (4). Newly formed blood vessels speed up cerebral blood flow in the ischemic area by supplying oxygen and nutrients to the ischemic area and improve neurological function (5). Therefore, promoting angiogenesis is a promising therapeutic strategy for the treatment of ischemic stroke. New-generation biochemical markers such as serum bFGF levels may be helpful in the diagnosis of ischemic stroke patients, stroke type and mortality rates.

Fibroblast growth factors (FGFs) are polypeptide growth factors involved in numerous processes, including cell growth, development, neuronal function, metabolism, proliferation, migration, apoptosis, wound repair, and angiogenesis (6). FGFs support blood vessels to help deliver nutrients to the brain and other organs (7). Thanks to their homeostatic functions, they promote tissue repair and accelerate wound healing (8). The angiogenic and neurotrophic properties of FGFs suggest that they may be effective in the treatment of ischemic stroke. FGFs also contribute to neuroprotection, neuroregeneration, vascular protection, angiogenesis, and blood-brain barrier protection after ischemic stroke. As such, FGFs may be candidate agents that act via multiple pathways to improve the outcomes of stroke patients. Thus, bFGF will provide new information on the recovery of ischemic stroke and will be helpful in treatments.

The aim of this study was to determine the serum levels of basic fibroblast growth factor (bFGF) in patients with ischemic stroke and the control group and to determine the role of bFGF in stroke diagnosis through its correlation with National Institute of Health Stroke Scale (NIHSS) scores in patients with ischemic stroke.

METHODS

The prospective study was conducted in the Department of Neurology between February 2012 and October 2012 on 96 patients (38 female and 58 male) who were hospitalized following diagnosis of ischemic stroke, which was confirmed by neuroradiological examination performed within 24 h after the event. These patients received treatment following detailed anamnesis, as well as physical and neurological examinations.

Ethics approval was obtained from the Local Ethics Committee before initiating the study (decision no: 2011/223; dated 01.12. 12).

Those with a history of systemic diseases, including chronic neurologic disease, head trauma, uremia, liver cirrhosis, cancer, and chronic lung and liver diseases, were excluded.

Our group consisted of 48 age-matched healthy volunteers (32 women and 16 men) who were relatives of patients who applied to the neurology outpatient clinic and did not have any vascular risk factors. Detailed history of vascular risk factors, medications used, neurologic examination results, NIHSS scores, and blood pressure data were recorded at the Department of Neurology.

For the initial neurologic evaluation, the level of consciousness, conscious response to questions, responsiveness to commands, extraocular movements, visual fields, facial paralysis, arm and leg motor movements, limb ataxia, sensation, aphasia, dysarthria, and degree of neglect were assessed using the NIHSS score. For the measurement of bFGF levels, 10 ml of blood was drawn from the antecubital vein of patients within 24 h after the onset of stroke and from all healthy volunteers. Blood samples collected in gel tubes used for biochemical testing were left to clot for 20-30 minutes and then centrifuged at 8000-10000 rpm for 10 minutes. Serum was separated and placed in individually labeled Eppendorf tubes and stored at -50°C until the day of analysis. On the day of analysis, frozen serum samples were allowed to thaw at room temperature. Serum bFGF levels were measured using an enzyme-linked immunosorbent assay kit (Human bFGF, Ray Biotech, GA, USA, P09038). Additionally, blood tests were performed to measure possible significant differences in hs-CRP and hemogram values between the patient and normal control groups.

Statistical analysis

SPSS (Statistical Package for Social Sciences) 11.5 PC (SPSS Inc., "SPSS 11.5 for Windows," Chicago, IL, USA, 2002.) program was used for all statistical analyses. The conformity of the data to normal distribution was assessed using the Kolmogorov-Simirnov test. For descriptive statistics, number, percentage, mean ± standard deviation (ss), median, minimum (min), and maximum (max) values were used. Since the data did not conform to normal distribution, Mann Whitney U, Kruskal Wallis tests and Spearman correlation analysis were used in comparisons between groups. The chi-square test (and/or Fisher's exact test) was used to analyze categorical variables. A p-value of <0.05 was accepted to indicate statistical significance.

RESULTS

The study included a total of 96 ischemic stroke patients (58 [78.4%] males and 38 [54.3%] females) and 48 controls (16 [21.6%] males and 32 [45.7%] females). Distribution of stroke status of the study group according to sociodemographic characteristics (Table 1). Descriptive characteristics of the patient and control groups are presented in Table 2.

The mean bFGF levels of the 15 patients who died due to stroke and the 81 patients who survived were 5.9±5.4 pg/ml

Table 1. Distribution of stroke status of the study group according to sociodemographic characteristics

Sociodemographic Characteristics	Stroke			Test Value x^2;p
	Yes (Patient) n(%)[a]	None (Normal Control) n(%)[a]	Total n(%)[b]	
Age Group				
Under 65	20(57.1)	15(42.9)	35(24.3)	1.887; 0.187
65 and over	76(69.7)	33(30.3)	109(75.7)	
Gender				
Male	58(78.4)	16(21.6)	74(51.4)	9.396; 0.002
Female	38(54.3)	32(45.7)	70(48.6)	
Total	96(66.6)	48(33.3)	144(100.0)	

a: Row percentage
b: Column percentage
Note: This table displays descriptive statistics for each data. The estimated statistics are n(%) and/or mean, minimum, maximum and standard deviation (SD)

and 6.6±8.8 pg/ml, respectively (p=0.361).The white blood cell count (WBC) differed significantly between the ischemic patients and control groups (p<0.0001). There was no significant difference between the groups in terms of platelet count (p=0.497), hematocrit (p=0.309), and hemoglobin level (p=0.12). LDL levels were significantly higher in normal controls. There was no correlation between serum bFGF level and NIHSS score in patients with ischemic stroke (p>0.05), whereas a moderately positive correlation existed between NIHSS score and Hs-CRP (High-sensitive C-reactive protein) level (r=0.413; p=0.011).

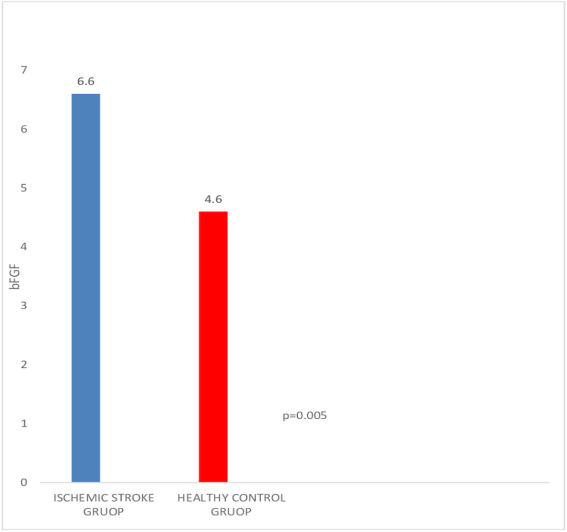

Figure 1. bFGF levels between ischemic stroke and healthy control groups

As shown in Figure 1, bFGF levels differed significantly between the ischemic stroke and healthy control groups (p=0.005).

Table 2. Distribution of stroke status of the study group according to related variables

Related Variables	Patient (Median) Q1-Q3	Control (Median) Q1-Q3	Test Value	
			u	p
WBC (1000x)	7.9(6.4-9.8)	6.4 (5.2-7.8)	1.353	<0.001*
Hct (%)	39.9 (36.3-43.0)	41.1 (38.8-43.2)	2.554	0.289
Hs-CRP (mg/dl)	16.2 (10.3-28.5)	6.9 (4.2-8.9)	6.881	<0.001*
Hb	13.2 (11.8-14.2)	13.7 (12.9-14.3)	2.748	0.060
MCV	88.2 (84.0-92.0)	89.0 (87.0-92.0)	2.576	0.248
bFGF (pg/ml)	349.2 (56.7-1029.4)	255.4 (0.0-782.3)	1.991	0.214
NIHSS scores	5.0(2.0-11.5)	0.0 (0.0-0.0)	9.966	<0.001*
LDL	106.0 (88.4-130.8)	125.8 (105.2-152.8)	2.631	<0.001*

Note: This table displays descriptive statistics for each data. The estimated statistics are mean, minimum, maximum and standard deviation (SD)
Abbreviations:WBC: White blood cell count; Hct:Hematocrit; Hs-CRP:High-sensitive C-reactive protein; MCV: Mean corpuscular volume; Hb: Hemoglobin; bFGF: Basic fibroblast growth factor;
NIHSS; National Institutes of Health Stroke Scale ; LDL, Low-density lipoprotein

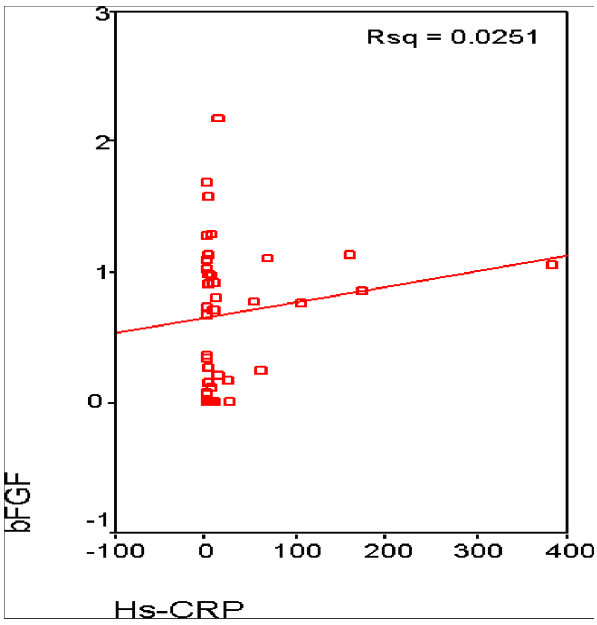

Figure 2. Correlation between Hs-Crp and bFGF values

As seen in Figure 2, any statistically significant correlation was found between Hs-Crp and bFGF values (p= 0.694).

DISCUSSION

Polypeptide growth factors are important for molecular and cellular processes involved in functional recovery and wound healing after an episode of acute stroke. Brain injury triggers the release of different growth hormones to protect the brain from excitotoxicity, hypoxia, acidosis, and oxidative stressThe main source of the growth hormone bFGF in the brain is glial cells, although it is also expressed by other types of neurons. Together with other fFGFs, bFGF plays an important role in many different processes, such as nerve regeneration, chronic inflammation, and wound healing. bFGF is a potent angiogenic factor that promotes the migration and proliferation of endothelial cells and also plays a role in other metabolic processes (9). Angiogenesis, which is associated with survival, is accelerated in the brain tissue of stroke patients. Increased bFGF levels were found mostly in the ischemic penumbra region of stroke patients to have contributed to increased angiogenesis and blood flow (10). bFGF protects neurons against toxic insults and ischemic neuronal disorders (11). After experimentally induced stroke mouse model, bFGF treatment was found to promote neurogenesis, reduce infarct volume and accelerate functional recovery. bFGF levels increase after cerebral ischemia, which may be related to increased vascularization. A study by Lanfrankomi et al. (12) supported the neuroprotective role and neurogenesis effects of bFGF. However, a phase II/III trial conducted in North America was stopped after review of data from 300 patients with acute ischemic stroke due to higher mortality rates in the bFGF-treated group compared to the control group (13). A European-Australian phase II/III randomized study in 286 acute ischemic stroke patients confirmed that 5 or 10 mg trafermin (recombinant human bFGF: rhbFGF) or placebo infused intravenously for 24 h did not provide any significant neuroprotection versus intravenous administration, but instead caused dose-dependent damage. Moreover, hypotension and increased mortality rates were noted among treated patients (14).

Guo H et al. conducted a study in China and found a significant increase in serum bFGF levels in 30 acute ischemic stroke patients compared to the control group (15). Relatively higher serum bFGF levels were also observed in our study. Angiogenesis is important to help the neuronal tissue recover from neurological deficits after cerebral ischemia and ensure neuronal survival. bFGF is a potent stimulator of angiogenesis occurring after cerebral ischemia. FGFs can be used in the treatment of stroke due to their pharmacological effects on multiple targets, including the ability to directly promote neuronal survival, increase angiogenesis, protect against blood-brain barrier disruption, regulate microglia, reduce infarct size, and improve neurological function (16). In a study examining the roles and therapeutic potential of fibroblast growth factors in the treatment of ischemic stroke demonstrated the protective effects of bFGFs in a stroke model (17).

Serum FGF levels in patients with acute ischemic stroke were found to be significantly higher than those in the control group (18). In healthy individuals, very low or even undetectable levels of bFGF were recorded, whereas increased levels of bFGF were found in the serum of patients with diabetes and coronary artery disease (19). In a previous study, bFGF levels were found to be significantly higher in the serum of 16 patients with intracerebral hemorrhage and 28 patients with ischemic stroke compared to healthy controls (20). In our study, we similarly found increased bFGF levels in the serum of patients with ischemic stroke. It is known that hs-CRP levels are high in patients with stroke (21). Similarly, in our study, Hs-CRP levels were found to be higher in the case group than in the control group. Increased serum bFGF levels may be a protective response to prevent secondary damage after acute ischemia in patients with ischemic stroke. Unlike the previous study, the relationship of bFGF levels with clinical findings was also investigated in our study. The bFGF levels was not found to be associated with NIHSS scores. These findings showed that levels of bFGF increase after ischemic stroke, and serum bFGF levels may provide valuable information for predicting infarct size and clinical prognosis after ischemic stroke.

In our study, the sample size was not large enough, with 96 patients and 48 controls. Since findings on changes in bFGF levels over time in patients with acute cerebral infarction were not reported, more evidence would support stronger results.

In this study, serum bFGF levels are high in the initial phase of acute cerebral infarction. Further studies are needed to determine the changes in bFGF levels in serum after days of cerebral infarction.

CONCLUSION

In conclusion, this study showed that serum bFGF levels significantly increase after acute ischemic stroke. The increase in bFGF levels in these patients may be a protective response to reduce brain damage or may be related to angiogenesis. Due to an urgent need for the development of new and more effective medications, future research on bFGFs should be conducted to achieve better treatment outcomes with bFGFs among ischemic stroke patients. Continued research on the utility of bFGF as a novel drug candidate in the treatment of cerebral ischemia will provide new insights into the involvement of bFGF in neuronal recovery after ischemic stroke.

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Informed Consent: An informed consent form was obtained from the patient/patient's representative to collect and publish the patient's clinical information.

Authorship Contributions: Idea/Concept:ŞŞ, MŞ, SD, HA, HD, Design: ŞŞ, MŞ, SD, HA, HD, Supervision: ŞŞ, MŞ, SD, HA, HD, Data Collection and Processing: ŞŞ, MŞ, SD, HA, HD, Analysis or Interpretation: ŞŞ, MŞ, SD, HA, HD, Literature Search:ŞŞ, MŞ, SD, Writing: ŞŞ, MŞ, SD, Critical Review: ŞŞ, MŞ, SD, References and Fundings: -, Materials: ŞŞ, MŞ, SD.

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Original Article / Araştırma Makalesi

THE EFFECT OF PLATELET RICH PLASMA IN BONE TUNNEL TENDON-BONE HEALING

KEMIK TÜNEL İÇI TENDON-KEMIK IYILEŞMESINDE TROMBOSITTEN ZENGIN PLAZMANIN ETKISI

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ABSTRACT

Introduction: In our study, we aimed to evaluate the effect of platelet-rich plasma (PRP) obtained from autologous blood on rabbit intra-tunnel tendon bone healing using biomechanical and histologic parameters.

Methods: The hind legs of 31 New Zealand albino rabbits were used in the study. After the extensor digitorum longus tendon of both hind legs was removed from the proximal attachment site, the same leg was placed in a bone tunnel opened in the tibia. In the P "platelet" group, PRP was injected into the tunneled part of the tendon, while in the K "control" group, the tunneled part of the tendon. In addition, each group was divided into two groups "six and twelve weeks old". After sacrification, the bone-tendon junction, which was removed together with the tibia and tendon, was pulled in a pulling device at a speed of 5mm/second. The machine recorded the maximal endurance force (ULF). Using these values, stiffness and energy absorption were calculated. Three specimens each from the sixth and twelfth groups were examined histologically.

Results: At 6.-12. weeks, all biomechanical parameters were higher than in the control group and the increase was significant in all three parameters at twelfth week and in two of the parameters (ULF, stiffness) at sixth week ($p<0.05$)

Conclusions: As a conclusion of the study, it was determined by biomechanical and histological examinations that PRP application positively enhanced healing in the intra-tunnel healing model created in the New Zealand albino rabbit. PRP application can be successfully used to accelerate tendon-bone healing.

Keywords: Anterior cruciate ligament, Platelet-rich plasma, Tendons

ÖZET

Giriş: Çalışmamızda otolog kandan elde edilen trombositten zengin plazmanın (TZP) tavşan tünel içi tendon kemik iyileşmesine etkisini, biyomekanik ve histolojik parametreler kullanılarak değerlendirmeyi amaçladık.

Yöntemler: Çalışmada 31 adet Yeni Zelanda tipi albino tavşanın arka bacakları kullanıldı. Her iki arka bacağın ekstensör digitorum longus tendonu proksimal yapışma yerinden alındıktan sonra, aynı bacak tibiada açılan kemik tünel içine yerleştirildi. P "trombosit" grubunda tendonun tünelde kalacak kısmına üç ayrı yerden insülin enjektörü ile TZP enjekte edildi, K "Kontrol" grubuna tendonun tünelde kalacak kısmına boş insülin enjektörüyle üç defa girildi. Ayrıca her grup kendi içerisinde "altı ve oniki haftalık olarak" ikiye ayrıldı. Sakrifikasyon işleminden sonra tibia ve tendonla birlikte çıkartılan kemik-tendon bileşkesi çekme cihazında 5mm/saniye hızında çekildi. Çekme işlemi, tendon, kemik-tendon bileşkesinden ayrılıncaya veya tendon kopuncaya kadar sürdürüldü. İşlem sırasında maksimum dayanma kuvveti (ULF) ve kopana kadar geçen süredeki uzama miktarı, makine tarafından kaydedildi. Bu değerler kullanılarak stiffness (katılık) ve enerji absorbsiyonu hesaplandı. Altıncı ve onikinci gruplardan üçer adet denek histolojik olarak incelendi.

Bulgular: Altıncı ve onikinci haftalarda biyomekanik parametrelerin hepsi kontrol grubuna göre yüksek bulundu ve onikinci haftadaki üç parametrenin tamamında, altıncı haftadaki üç parametrenin ikisinde (ULF, katılık) yükseklik anlamlıydı. ($p<0,05$)

Sonuç: Çalışma sonucunda Yeni Zelanda tipi albino tavşanda oluşturulan tünel içi iyileşme modelinde TZP uygulamasının,iyileşmeyi olumlu şekilde arttırdığı biyomekanik ve histolojik incelemelerle belirlendi. TZP uygulaması, tendon-kemik iyileşmesini hızlandırmak için başarıyla kullanılabilir.

Anahtar Kelimeler: Ön çapraz bağ, Trombositten zengin plazma, Tendon

INTRODUCTION

The injury of anterior cruciate ligament rupture (ACLR) is a well-known knee injury that can occur when the knee joint is stressed accounting for around 80% of all sports-related knee injuries (1,2). The treatment often requires reconstruction surgery in which autograft tendons are widely used. Among the alternatives, bone-patellar tendon-bone (BTB) and four-strand hamstring tendon (HT) are the most commonly preferred ones. One of the advantages of HT which makes the technique a more favorable choice, is having lower rates of donor site morbidity compared to BTB

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cases. However, the increased healing duration of the tendon to the bone inside the tunnel, which prolongs the rehabilitation period is a significant downside of the method (3,4). Aiming to speed up the healing process various materials and methods have been used including bone morphogenetic protein-2 (BMP-2), bone morphogenetic protein-2 gene transfer, bone morphogenetic protein-7 (BMP-7), bone growth factor mixture (BGF), periosteum, bone marrow stromal cells, bone marrow, and periosteum and a decrease in the duration was observed. The ease of application, cost, and availability have caused some of these products and methods to be less preferred, and recently the use of growth factors found in platelets has become more frequent (5-14). Platelet-rich plasma (PRP), which involves abundant amounts of growth factors in a concentration of platelets exceeding 1.0 × 106 platelets/µl is shown to improve bone tissue restoration and is presently used in ACLR reported in numerous studies with inconsistent results (15-16). The aim of this study was to demonstrate the effectiveness of local PRP application on tendon histology and mechanical properties in procedures where in-tunnel bone tendon healing is expected, such as ACL surgery, by injecting PRP into the surgical field.

METHODS

Ethics

The study was approved by the Ethics Committee of the Faculty of Medicine of the Erciyes University (12/11/08 No: 08/62) and the study permit was obtained from the Experimental and Clinical Research Center of Erciyes University. A total of 31 female, adult, New Zealand albino rabbits were provided from the Experimental and Clinical Research Center of Hakan Cetinsaya Experimental and Clinical Research Center. The study was conducted in accordance with the principles of the Care and Treatment Guidelines. (www.nap.edu/catalog/5140.html).

Study Design and Subjects

The number of rabbits in this animal experiment study was determined by “sample size analysis” and 31 female rabbits were selected for the study. Since the female of these rabbits is larger, female rabbits are preferred for surgery. Rabbits were kept in rooms with a temperature of 24 Cº and a humidity of 45%. They were fed with 150 g of artificial feed and 100 ml of water daily. The rabbits used in the study were randomly divided into two groups. Group A: The group sacrificed 6 weeks after the surgical procedure. Group B: The group sacrificed 12 weeks after the surgical procedure. The hind legs of the subjects were used in the study. Group A consisted of 15 and Group B consisted of 16 subjects. Three rabbits from each group were randomly separated for histological study, while the remaining were planned to undergo biomechanical tests.

PRP Preparation

The ear for blood collection was shaved and wiped with xylol. 10 cc of blood was collected in 10 cc EDTA tubes by using a 24 G intraket. As a technique for obtaining platelet-rich plasma, Yokota described was used (17). The blood taken from the rabbit ear vein was first centrifuged for 10 minutes at 1500 rpm and the plasma remaining on top was taken with the buffy coat and generally separated from the red blood cells. Then the plasma was spun at 2000 rpm for 10 minutes and the platelet-poor part on top was separated. The plasma remaining at the bottom was spun again at 2500 rpm and the lowest part of 1/3 was accepted as PRP, which corresponded to approximately 1.5 cc. While the platelet concentration rate in PRP was 3.66±1.29 times higher than the whole blood platelet concentration rate before the intervention, a 3.93±0.95-fold increase was achieved in the group planned for sacrifice at the end of the 12th week. All these procedures were performed under sterile conditions.

Surgical Technique

All rabbits were fasted for four hours before the operation. Xysilazine HCL (Rompun®-Bayer Pharmaceuticals San. Turkey) and 10 mg/kg I.M (intramuscular), 40 mg/kg I.M ketamine (Ketalar®-Eczacıbaşı İlaç San. Turkey) was performed for induction of general anesthesia. This procedure provided anesthesia for about 30 minutes. The operations were performed by two orthopedicians under sterile conditions. After general anesthesia, a tunnel was opened manually using a K wire in the tibia metaphysis and the extensor digitorum longus tendon was prepared. Into the left knee using an insulin syringe PRP was injected from three different points aiming to provide that two-thirds of the PRP was preserved inside the tunnel. Then, the tendon was passed through the tunnel and sutured to the surrounding soft tissue medially; the remaining part of the PRP was given inside the tunnel. In order to ensure that the tendon was exposed to the same trauma in the right knee, the part that would remain inside the tunnel was entered in the same way with an empty insulin syringe from the corresponding symmetrical three marks. The tendon was passed through the tunnel and sutured to the surrounding soft tissues medially. No restraint was applied to the animals after the operations and the animals were allowed to move freely in the cage. The rabbits in group A were sacrificed at the end of the 6th week and the ones in group B at the end of the 12th week. After the sacrifice procedure, the bone was removed in making sure that the tendon-bone junction was not damaged. Three pairs of specimens were separated from each group for histology. The remaining materials were placed in refrigerators at -20 Cº to be stored until the biomechanical measurement day (18). The specimens separated for biomechanical tests were labeled as P and K, with P indicating the PRP-injected tissue and K for the control sample. The labels were numbered 6 and 12

indicating the time the sample was obtained. For example, P6 belongs to the tissue of the PRP-injected subject which was sacrificed at the end of the 6th week, and K12 indicates the sample of the control subject sacrificed at the end of the 12th week.

Biomechanical measurements

In the study, the Instron® 4411 (Instron Corp.®-England) tensile machine was used. The tensile machine laboratory temperature was fixed at 20 ºC and the humidity rate was fixed at 56%. The specimens, which were previously removed from the cooler and thawed at room temperature, were placed in the tensile device. The preload of the device was set to one Newton (N). Ten cycles of stretching and relaxing up to one N were performed and "preconditioning" was accepted as achieved, the tendon-bone complex was stretched at a speed of five mm/s until the breaking point. The ultimate load failure (ULF), energy absorption, and stiffness values formed during the process were recorded in a digital environment.

Histological examinations

The blocks to be histologically examined on a rotary microtome (Microm, HM 360, Germany) 5-7 µm sections were taken. Sections stained with Hematoxylin-Eosin, Mallory Trichrome and alcian blue method; generalized bone and tendon morphology, structure of the tendon-bone junction and tendon healing process evaluated. Histologic examinations were performed under a microscope at 40x magnification.

Statistical analysis

Statistical calculations were performed using the SPSS package program. The variables used in the study were examined using the Kolmogorov-Smirnov distribution test which showed that the variables were normally distributed. The "unpaired student's t" test (a test of significance between two means) was used in the comparison between the samples obtained at the end of the 6th and the 12th week. The mean and standard deviation were used in presenting the continuous variables. The "p" value less than 0.05 was accepted as significant.

RESULTS

A total of 31 rabbits were divided into four groups. Group A included 15 subjects treated with PRP and were sacrificed at the end of the 6th week and Group B with 16 control cases sacrificed at the end of the 12th week.

Biomechanical analysis results

The analysis of the ULF, hardness, and energy absorption values of the subjects that were treated using PRP showed that although all recorded values were higher in the P12 group, a statistically significant difference occurred for hardness.

The subgroup comparison of the subjects sacrificed at the end of the 6th week (Group A) showed a similar presentation of higher recorded values for all three parameters of the rabbits treated using PRP (P6). In detail, in comparison to K6, P6 subjects were 39% more successful in ULF, 66% more successful in hardness, and 34% more successful in energy absorption. The statistical analysis findings, on the other hand, showed that except for energy absorption results, the ULF and hardness scores were significantly different (Table 1).

Table 1. Analysis results of parameters of group A (subjects sacrificed at the end of the 6th week)

	Right (Control)	Left (PRP)	t	p
ULF (N)	31,69± 7,66	44,13± 7,76	3,86	0,001
Energy Absorption	10,35 ± 6,46	13,85 ±4,92	1,48	0,151
Hardness (N/mm)	8,07± 3,84	13,29 ± 2,92	3,74	0,001

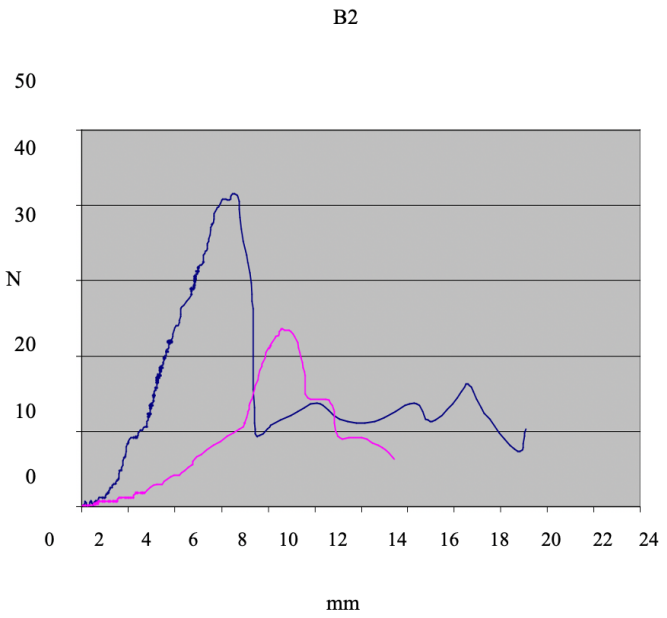
The rabbits treated with PRP of Group B presented similar but increasingly higher values. P12 subjects were 84% more successful in ULF, 149% more successful in hardness, and 101% more successful in energy absorption. The difference in the findings was statistically significant for all three parameters (Table 2). The effectiveness of PRP is seen in the force-extension curve of the second rabbit which was sacrificed at the end of the 12th week. (Graph 1).

Table 2. Analysis results of parameters of group B (subjects sacrificed at the end of the 12th week)

	Right (Control)	Left (PRP)	t	p
ULF (N)	28,89± 17,14	55,30± 0,97	3,38	0,002
Energy Absorption	6,93 ± 5,08	14,49 ± 4,80	3,89	0,001
Hardness (N/mm)	8,13± 4,67	20,17 ± 7,62	4,85	0,000

Histological Examination Results

In the histological assessment of the Group A subjects, there was a significant increase in vascularization in P6, while irregular fibrous tissues were observed in K6. The thickness of the fibrovascular connective tissue at the bone-



Graph 1. Force-extension curve of subject 2 (Group B, blue: P12, pink: K12)

tendon interface was less organized in the control cases than in the PRP. In both groups, collagen fibrils were mostly arranged parallel to the tendon-bone axis, but especially in the PRP group, the presence of perpendicular ones (similar to Sharpey fibers) was noted. In the histological assessment of the Group B rabbits, the cartilage tissue in the transition area on the right side was narrow and irregular, however, there was wide and regular cartilage tissue in the tendon-bone transition area in the PRP group. At the end of the 12th week, it was observed that the bone growth into the tendon and the restructuring process in the PRP group developed faster than in the control group, and a thinner intervening connective tissue was observed. In the PRP group, it was determined that the bone trabeculae were better integrated with the tendon. Besides, the cartilage layer connected to the bone tissue by the transition zone and the collagen fibrils perpendicular to them were clearly noticeable. (Figure 1)

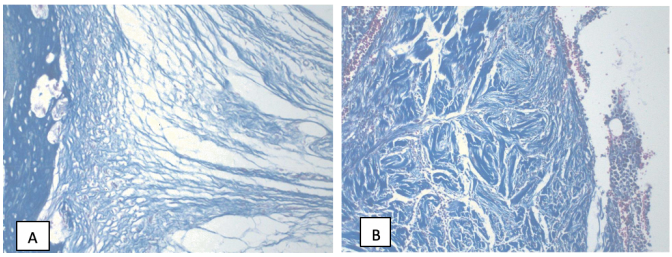


Figure 1. 12th week light microscope alcian blue staining images, A: PRP group B: Control group

DISCUSSION

This experimental study, which tested tendon-bone healing biomechanically and histologically, found significant differences in parameters in both areas.

After reconstruction surgeries using hamstring or patellar tendons, the tunnel area is the area where the graft strength is weakest until complete bone-tendon or bone-bone healing occurs within the bone tunnel. Therefore, its movement is partially restricted until healing is achieved. Rehabilitation procedures should be started as early as possible to prevent complications due to long-term immobilization such as muscle atrophy, joint contractures, and reflex sympathetic dystrophy, to regain joint range of motion and to ensure early return to daily activation. Such goals may be possible with quality and rapid bone-bone or bone-tendon healing. Tunnel healing is the osteointegration of the tendon to the bone, basically a tenodesis phenomenon. It has been shown that the use of PRP in rotator cuff repair has positive clinical results (19). Our study also helps provide a histological and biomechanical basis for such studies. Additionally, our study may not be interpreted for cases including only the anterior cruciate ligament, but also for cases where the basic principle is based on the tenodesis, including but not limited to tendon transfers or tendon repairs that are torn from the junction. Based on the results of our findings, the recovery period can be shortened and rehabilitation can be started earlier in surgeries require tendon-bone healing.

In recent years, the use of hamstring tendons as autografts in anterior cruciate ligament reconstruction surgery has increased. Many methods have been introduced using this graft to support the bone-tendon healing process, involving periosteum wrapping of the tendon-bone junction; application of purified bone marrow, BMP-2, BMP-7, bone growth factor, and BMP- 2 gene transfer (5-7,10,12). The literature focused on the positive effects of growth factors on healing have clearly demonstrated the improved effects. However, despite all these promising effects, the supply of growth factors is difficult and the economic cost is high. Thus, the use of growth factors is limited. In fact, these factors play a partial role in the wound-healing cascade and affect each other in harmony, increasing and decreasing at different times in tissue healing. In this context, instead of giving these factors one by one, using platelets that contain all these factors may mimic the natural process and increase effectiveness.

The factors contained in platelet-rich plasma have positive effects on chemotaxis, cell proliferation, angiogenesis, extracellular matrix formation, and remodeling (20). A study conducted by Rozman (21) in 2007 supports most of these effects, but considering that remodeling begins in the 26th week, it was suggested that growth factors cannot remain active for such a long time and therefore cannot play an active role in remodeling. There are also examples of research conducted at more frequent intervals (2, 4, 8, 12, and 24 weeks), such as the study by Rodeo et al. (22), but similar studies in the literature show that significant biomechanical and histological changes in the bone-tendon healing process occur between the 6th and 8th weeks

postoperatively and that there are no sharp changes in healing after this period (23).

Anderson et al. (24) applied bone growth factor to the bone-tendon junction in a rabbit model to accelerate bone-tendon healing and reported statistically significant ($p<0.001$) 85% higher average ULF values in the first eight weeks compared to the control group. In our study, the ULF values at the end of the 6th and 12th weeks were significantly higher in the PRP group

than in the control cases. The findings indicate that a 39% higher average ULF value was reached in the sixth week and 84% higher at the end of the 12th week. However, it is important to note that the parameters of stiffness and energy absorption were not assessed in the study by Anderson et al. The stiffness and energy absorption levels of a tendon are as important as its strength at the moment of rupture (ULF). Tendons exposed to a force undergo an elongation process, and the ratio of this force to the amount of elongation in the tendon is called stiffness and its unit is N/mm. Studies conducted focusing on the matter report that repaired tendon stiffness values do not reach normal tendon values (25). Therefore, the higher the study hardness value, the closer it is to normal it is expected to be. In our study, hardness was significantly higher in the PRP group than in the control group at the end of the 6th and the 12th weeks.

The energy absorption value is the amount of energy absorbed by the tendon-bone complex until rupture, in other words, the result value of the parameter will be parallelly increased to the higher amount of force the tendon can handle. In our study, energy absorption was significantly higher in the PRP group than in the control group at the end of the 6th and the 12th weeks. In the study conducted by Lyras et al. (26) in 2009 on the effect of PRP in patellar tendon ruptures, ULF, stiffness, and energy absorption were assessed and on the 14th day, a high average of 72.2% in ULF, 53.1% in stiffness, and 39.1% in energy absorption was observed. In a study conducted with quadriceps tendon graft, the need for painkillers, the time to use crutches, and the time to reach the target joint range were found to be reduced in the PRP group. Such effects may also contribute to early inflammation (27).

Histologically, an irregular, thin cartilage formation was observed at the tendon-bone transition in the control group, while a regular and thicker cartilage layer was encountered in the PRP group. Rodeo et al. (22) examined bone-tendon healing in dogs histologically and biomechanically and concluded that the endurance of the bone-tendon junction is increased in parallel to the formation of new bone tissue, and the maturation and mineralization of the recovery tissue. Similar results were observed in the study conducted by Arnoczky et al. (23). In both studies, the bone-tendon junction that was subsequently formed differed from the natural structure histologically. Histologically the natural structure is an ideal attachment form with its four-layered transition feature in addition to important biomechanical properties. In the structure, since the tension in the tendon is distributed equally to the entire attachment area through the fibrous cartilage, excessive load is prevented from being applied to a single region increasing the total durability of the entire junction. The literature lacks studies describing accurate formation of the natural structure that is formed in a short time along the entire tunnel histologically. In the study conducted by Shino et al. (28) in dogs, in addition to early findings regarding healing of the tissue, a four-layered natural structure was partially observed between 30 and 52 weeks after implantation. In our study, the regular, thick cartilage tissue formed between collagen fibers and bone at the end of the 12th week in the PRP group presented similar properties to the four-layered transition in the natural tendon-bone junction. The finding is also parallel to the results with significant differences in the biomechanical study, since a narrower, irregular cartilage layer was observed in the control group, with predominantly fibrous tissue was observed in the recovering tissue. Although the regular cartilage tissue seen in the PRP group in our study was not encountered in some studies conducted using a similar model with using periosteum, successful results have been reported (7,8).

Limitations

The structure we subjected to biomechanical testing is not a homogeneous tissue; it is a complex consisting of tendon, tendon-bone junction, and bone parts. In our study, the complex was evaluated as a whole without isolating any parts. In other words, the complex's structural tensile properties were examined and the properties of the isolated healing tissue (material properties) as a homogeneous tissue were not assessed. In order to analyze the material properties much more sophisticated laboratory devices with high resolution and speed cameras and marking systems are required. Since our study is an extra-articular study model, it may be interpreted to a wide range of surgical models based on tenodesis.

Another limitation of our study is that the surgical field was not in contact with joint fluid and histologic scoring could not be performed.

CONCLUSION

In surgical procedures based on in-tunnel tendon healing, such as anterior cruciate ligament or tendon transfer, local application of PRP can contribute to graft integration and durability both biomechanically and histologically. The application of PRP can improve surgical success by allowing early, aggressive rehabilitation.

Ethics Committee Approval: Ethics approval was obtained from Faculty of Medicine of the Erciyes University.

Informed Consent: No need.

Authorship Contributions: Idea/Concept: SK, Design: SK,MU, Supervision: SK, MU, Data Collection or Processing: MU,SK,CY, Analysis or Interpretation: MU,CY, ÖC, Literature Search: SK, MU, Writing: SK,MU, Critical Review: SK, MU, CY, ÖC, Fundings: -, Materials: SK, MU.

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Original Article / Araştırma Makalesi

RESVERATROL SUPPRESSES CELL VIABILITY AND INVASION IN PANCREATIC CANCER CELLS

RESVERATROL, PANKREAS KANSERI HÜCRELERINDE HÜCRE CANLILIĞINI VE INVAZYONUNU BASKILAR

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ABSTRACT

Introduction: Pancreatic cancer is a challenging disease to diagnose and treat due to its asymptomatic progression and high mortality rate. It is often identified at an advanced stage, where it typically metastasizes to other organs. To address these challenges, scientific studies explore various treatments, including natural and anticancer compounds like resveratrol. This study investigates the effects of resveratrol on pancreatic cancer cells.

Methods: The study used PANC-1 pancreatic cancer cells cultured *in vitro*. Cell viability in resveratrol-containing media was measured using the MTT assay. Safe, toxic, and IC50 doses for a 24-hour period were determined. Morphological changes were analyzed using hematoxylin-eosin staining. In invasion experiments, a wound-healing assay was performed by scraping cells in a 6-well plate and photographing their movement in resveratrol media.

Results: Resveratrol increased cell viability at concentrations up to 10 µM, showed similar viability to the control at 50 µM, and reduced viability below 50% at 200 µM. The IC50 dose was calculated as 189.5 µM. At concentrations above 100 µM, significant disruptions in nuclear and cytoplasmic structures were observed. Additionally, cell movement ceased at 100, 150, and 189 µM doses, showing dose-dependent inhibition of cell invasion ($p<0.05$).

Conclusions: Resveratrol, a naturally occurring compound found in many fruits and plants, demonstrated cytotoxic and anti-metastatic effects on PANC-1 cells. This study highlights its potential role in pancreatic cancer treatment, emphasizing the importance of continued research to combat this aggressive disease effectively.

Keywords: PANC-1 cells, cell viability, anti-metastatic, resveratrol, anti-proliferative

ÖZET

Giriş: Pankreas kanseri, asemptomatik ilerlemesi, teşhis ve tedavisinin zor olması nedeniyle yüksek ölüm oranlarına neden olan bir hastalıktır. Genellikle ileri bir aşamada tanımlanır ve bu nedenle başka organlara metastaz yapar. Bu hastalığın tedavisindeki zorluklar nedeniyle araştırmacılar bazı antikanser özelliği yüksek moleküllerin de pankreas kanserine etkisini araştırmışlardır. Çalışmamızda, bu moleküllerden biri olan resveratrolün pankreas kanseri hücrelerine olan potansiyel etkilerini araştırdık.

Yöntemler: Çalışmamızda, *in vitro* kültüre edilmiş PANC-1 pankreas kanseri hücreleri kullanılmıştır. Resveratrol içeren midyumlarda hücre canlılığı, MTT testi kullanılarak ölçülmüştür. Resveratrolün 24 saatlik güvenli, sitotoksik ve IC50 dozları belirlenmiştir. Morfolojik değişiklikler hematoksilin-eozin boyama kullanılarak analiz edilmiştir. İnvazyon deneylerinde, 6 kuyucuklu plakalarda yara iyileştirme testi gerçekleştirilmiştir.

Bulgular: Resveratrol 10 µM'a kadar hücre canlılığını arttırmış, 50 µM'da kontrole benzer canlılık göstermiş ve 200 µM'da hücre canlılığını %50'nin altına düşürmüştür. IC50 dozu 189,5 µM olarak hesaplanmıştır. 100 µM'nin üzerindeki konsantrasyonlarda, nükleer ve sitoplazmik yapılarda önemli bozulmalar gözlenmiştir. Ek olarak, resveratrol hücre hareketini 100, 150 ve 189 µM'larda durdurarak hücre hareketinde doza bağlı inhibisyon göstermiştir ($p<0,05$).

Sonuç: Bu çalışma ile kanser proliferasyonunu ve hareketini inhibe edici etkiye sahip bir bileşikle pankreas kanseri tedavisine katkıda bulunma amaçlanmıştır. Birçok meyve ve bitkide bulunan ve doğal olarak oluşan bir bileşik olan resveratrol, PANC-1 hücreleri üzerinde sitotoksik ve anti-metastatik etkiler göstermiştir. Çalışmamız sonuçlarına göre, bu alanda daha fazla derinlemesine çalışmalara ihtiyaç olduğu açıktır.

Anahtar Kelimeler: PANC-1 hücreleri, hücre canlılığı, anti-metastatik, resveratrol, anti-proliferatif

INTRODUCTION

Pancreatic cancer is a type of cancer characterized by its aggressive nature, late-stage diagnosis, and poor prognosis. More than 200,000 people die from pancreatic cancer each year. According to the American (US) Cancer Society, approximately 56,000 pancreatic cancer cases were diagnosed in the USA in 2019, and approximately 45,000 of them died. This rate ranks third after lung cancer and colorectal cancer. Increases in pancreatic cancer-related death rates have been reported in America and European countries, indicating that the disease is widespread worldwide. Pancreatic cancer incidence varies by gender, with the incidence being 50% higher in men than in women. This cancer is more common in older adults. The etiology of

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pancreatic cancer includes familial transmission, smoking, chronic pancreatitis, diabetes, excess weight, and heavy exposure to certain chemicals (carcinogens). Currently, surgery, chemotherapy, radiotherapy and palliative care methods are used in the treatment of pancreatic cancer (1, 2). Increasing and developing diagnostic and treatment options for this type of cancer, which is very difficult to diagnose and treat, is a very important goal for scientists. More basic and clinical research is needed on these issues. Many scientific studies have shown that eating foods high in antioxidants has significant effects on reducing cancer risk and progression (3, 4).

Resveratrol is a polyphenol compound found in many fruits, especially in grapes. It has antioxidant, anti-cancer, chemo-preventive, antiviral, cardio-protective, anti-aging, anti-nociceptive, and life-prolonging properties that have been shown in many studies. Recently, many studies have shown that Resveratrol has inhibitory effects on the proliferation and metastasis of cancer cells (5, 6). Resveratrol has also been shown to have significant results in pancreatic cancer. Resveratrol is effective on pancreatic cancer by acting on many pathways and steps in cell physiology (proliferation, metastasis, apoptosis, pancreatic cancer stem cells, chemoradiosensitization, etc.). For example, Jiang et al. found resveratrol has been shown to inhibit the proliferation and metastasis of pancreatic cancer cells via fibroblasts in cancerous tissue. (7, 8).

Our aim in this study is to investigate the efficacy of resveratrol, which has been shown to have anticancer properties in many studies, in pancreatic cancer, which is very difficult to treat, on the basis of cell viability, morphology and cell movement, which is an important feature in metastasis, on the PANC-1 cell line.

METHODS

Cell Culture

PANC-1 cells (ATCC, CRL-1469) were cultured in 75 cm2 flasks (TPP, 90076) in DMEM high glucose, with L-glutamine (Capricorn, DMEM-HA) containing 10% fetal bovine serum (Sigma, F9665) and 1% penicillin/streptomycin (Sigma, P4333) at 37°C in an incubator (PHCbi) containing 5% CO2. When the cells in the flasks reached 70% density, the cells were subcultured or taken into the experiment.

Preparation of Resveratrol Doses

For preparing experimental resveratrol (Sigma, R5010) doses, resveratrol was dissolved in dimethyl sulfoxide (Sigma, 41640) to create a stock solution. Experimental doses were prepared by diluting from this stock solution. The experimental doses were as follows; 2, 2.5, 5, 10, 40, 50, 100, 150, 200 μM. The stock solution of resveratrol was stored at -20 degrees.

Cell viability assay

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma, M5655) test was used to determine cell viability in the experiments. In MTT tests, 9 doses of resveratrol (2, 2.5, 5, 10, 40, 50, 100, 150, 200 μM) were used. When the cells in the flasks reached sufficient maturity, they were collected from the flasks, counted with a thoma slide (Marienfeld Superior) and seeded into 96-well cell culture plates at 5000 cells per well (TPP, 92096). After allowing the cells to adhere for 24 h, the media were removed by inverting the plates. And then the cells were exposed to different resveratrol concentrations for 24 h. After 24 hours, the resveratrol-containing media were removed and media containing 0.5 mg/ml MTT were added to the wells and incubated for 2 hours. After the MTT-containing media were removed, 0.1 ml DMSO was added to the wells. The optical density of the wells was measured at 570 nm using an ELISA reader (BioTek 800TS). The cell viability rate of the control group that did not receive resveratrol was accepted as 100%. MTT analyses were repeated three times to increase the reliability of the results.

Morphological examination of PANC-1 cells

Hematoxylin-eosin (Moslab) staining was performed to examine the morphological changes of PANC-1 cells. First, sterile coverslips were placed on the bottoms of 6-cell culture flasks (TPP, 92006). Then, the cells collected from the flasks were counted and 3x105 cells seeded into these 6-well plates. After the cells adhered to the coverslips, different doses of resveratrol (40, 50, 100, 150 and 189 μM) were added to the cells' media and waited for 24 hours. At the end of the period, the media with resveratrol were removed and the cells were fixed in 10% formaldehyde (Sigma, F8775) (15 min at 37 °C). After fixation, the coverslips were washed three times with phosphate buffer (PBS) (Sigma, D5652). Cells were incubated with Triton X-100 (0.2%) (Sigma, T8787) for 5 min and then rinsed three times with PBS. After the cells were stained with hematoxylin-eosin, they were covered with a water-based mounting medium. The photographs of the coverslips were captured under a light microscope.

Cell migration assay

Equal numbers of PANC-1 cells (3x105 cells) were seeded in 6-well plates. After the cells covered the base, cells were scraped in a straight line at a certain point on the base of the plate with a 200 μL pipette tip (Eppendorf, 0030000870). Mediums containing doses of resveratrol (40, 50, 100, 150 and 189 μM) were added to the wells. Images of the striped area were taken at 0 and 48 hours with a 10X objective under an inverted microscope (IX71 mikroskop ve DP70 kamera, Olympus).

Statistical analysis

The experiments were conducted in triplicate, and data analysis was performed using statistical package software (IBM SPSS Statistics 21). Significance was determined at p<0,05. The normal distribution of data was assessed using the Shapiro–Wilk test. For normally distributed data, one-way ANOVA was applied, followed by Tukey's multiple comparison test.

RESULTS

Viability Results

In the MTT experiment, the 24-hour effectiveness of resveratrol on PANC-1 cells was examined. Resveratrol increases cell viability at low doses. Cell viability decreases with increasing doses. When we examine in terms of dose, the highest cell viability was determined at 10 μM (p<0.05). Cell viability at 50 μM was similar to the control group (p>0.05). Cell viability in the 100, 150 and 200 μM treated groups was as follows: 81%, 73%, 36%, respectively (p<0.05). The 24-hour IC50 dose of resveratrol in PANC-1 cells was determined as 189.5 μM. (Figure 1).

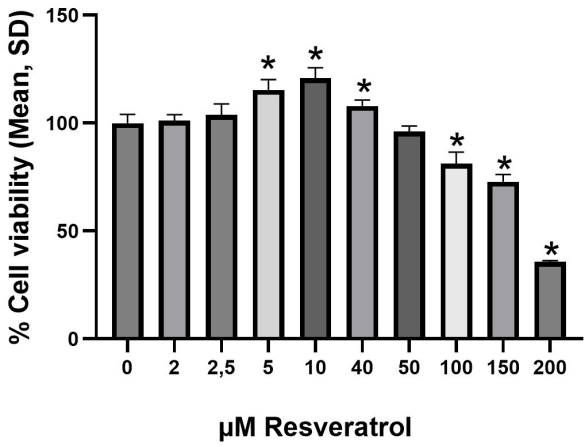

Figure 1. MTT results. Resveratrol increases cell viability up to certain doses (10μM). After 10μM, viability decreases in a dose-dependent manner. After 40μM, viability falls below the control group (p<0.05).
*Significantly different from untreated cells (Control group).

Effects of resveratrol on PANC-1 cell morphology

When the effects of resveratrol on PANC-1 cell morphology were examined, it was observed that resveratrol had no effect on cell morphology and density at 40 and 50 μM, and the cells had euchromatic nuclei and eosinophilic cytoplasm. The cells had similar cell sizes. At these two doses, cell morphology was similar to the control group. It was determined that cell morphology was impaired in a dose-dependent manner at 100, 150 and 189 μM. It was determined that the cells physically shrank and contracted, their nuclei became heterochromatin and cell density decreased (Figure 2).

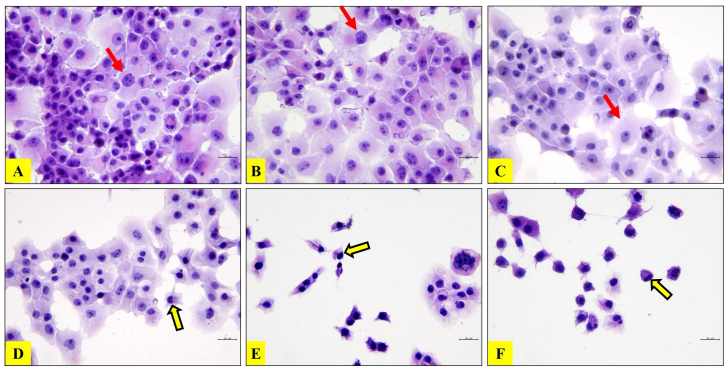

Figure 2. HE staining of PANC-1 cancer cells treated with resveratrol at different deses. The groups in the figure are as follows: A:Control, B:40μM, C:50μM, D:100μM, E:150μM and F:189μM resveratrol treated. Cells treated with 40 and 50 μM resveratrol were similar to the control group in terms of morphology, and cells were observed to have normal appearance (Thin arrow). Cells treated with 100, 150 and 189 μM were found to have shrunk, and in terms of nuclear appearance, the formation of pyknotic nuclei (Thick arrow) increased in a dose-dependent manner. Scale bars are 30.0 μm in all figures.

Cell migration assay

We evaluated cell movement of PANC-1 cells in a resveratrol environment with a wound closure experiment. This is a cheap, effective, and easy-to-apply method. When we evaluated our results; it was calculated that after 48 hours, the cells in the group without any application had closed the wound area by 73.37% and showed rapid movement. The wound closure amounts in the groups with 40 and 50 μM doses were as follows; 34.77% and 8.76%, respectively. It was determined that the movement of the cells stopped in the groups with 100, 150 and 189 μM doses. (Figure 3).

DISCUSSION

This study showed that resveratrol, which is found in many plants, especially grapes, and has many beneficial physiological properties, has cytotoxic and anti-metastatic properties on PANC-1 pancreatic cancer cells.

Pancreatic cancer is considered one of the most malignant cancer types in terms of cancer types. Despite advances in surgical techniques and the use of local and systemic adjuvant treatments, the mortality rate of patients with pancreatic cancer is still very high. In order to determine treatment technologies and strategies, the process from the beginning of the cancer type to its growth and metastasis must be understood in detail. In addition to existing ones, natural compounds must also be considered in the creation of new treatment strategies. Every day, a new compound is found in this field, and we can say that resveratrol is one of the oldest and most biologically active in this field (9, 10).

Resveratrol is a polyphenolic compound found naturally in many plants, including grapes, peanuts, and blueberries. Resveratrol has a wide range of biological functions, including antioxidant, anti-inflammatory, anti-diabetic, and

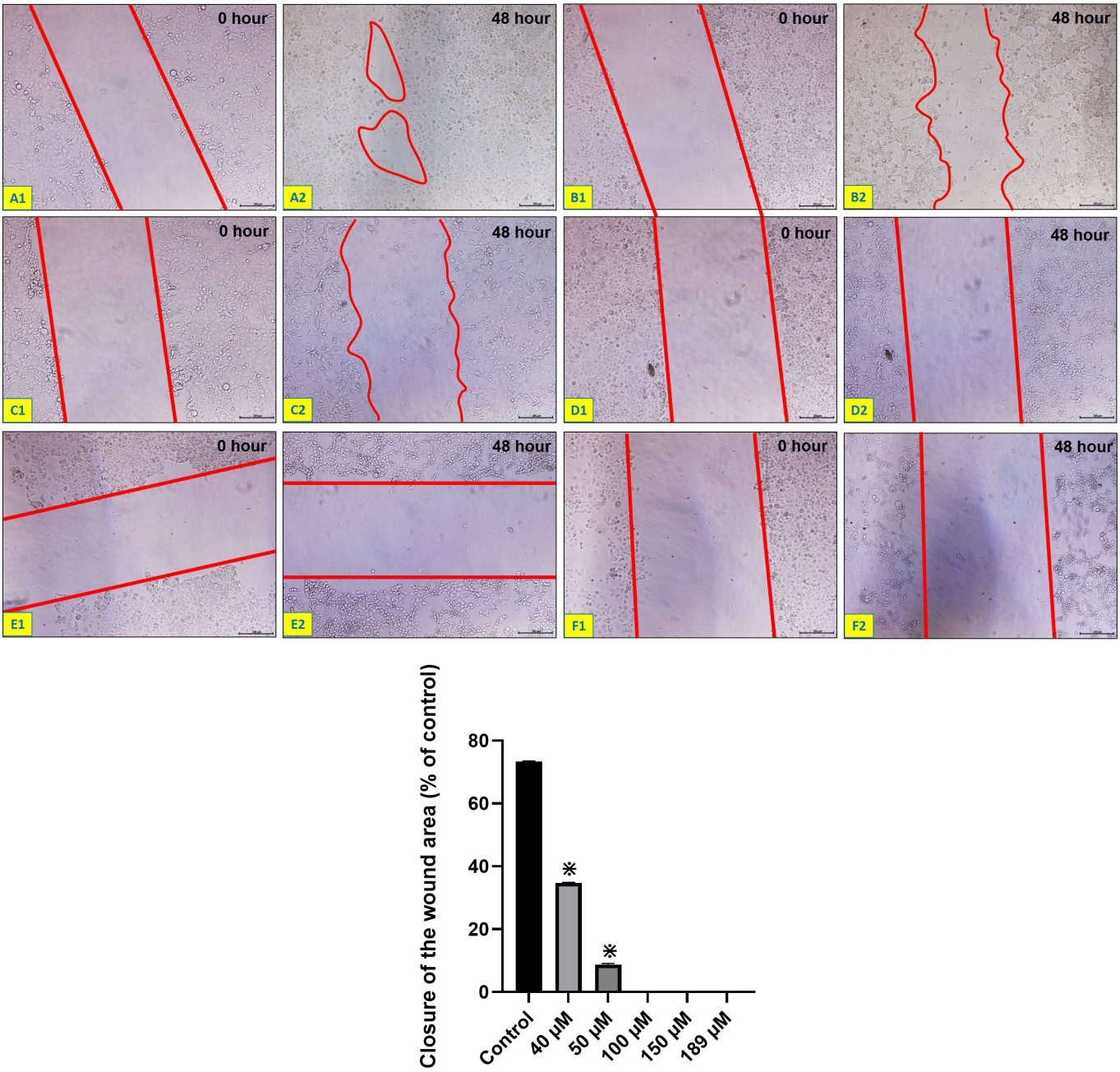

Figure 3. Results of cell migration of PANC-1 cancer cells. At 48 hours, the cells in the control group (A1-A2) covered 73% of the wound area, while the area covered by the cells in the 40 (B1-B2) and 50 µM (C1-C2) applied groups was 34% and 8%, respectively. It was determined that the movement of the cells stopped at other doses. Other doses: 100 µM (D1-D2), 150 µM (E1-E2) and 189 µM (F1-F2). *Significantly different from untreated cells (Control group). Scale bars are 200 µm in all figures.

anti-cancer (11). With the discovery of resveratrol, many studies have been conducted and are still being conducted to investigate its biological activity on different cancer cell types and lines and its detailed mechanism of action. Its activity on cancer in particular is a very important topic of curiosity (12). With developing diagnostic methods, cancer types are diversifying. Although there are many studies on resveratrol on cancer types, this issue needs to be detailed and resolved in the finest detail. We tested increasing doses of resveratrol in PANC-1 cancer cell lines in an in vitro experimental setting to demonstrate the effects of resveratrol on pancreatic cancer. We examined possible changes in cell viability, cell morphology and cell invasion. We observed that resveratrol increased cell viability in cells at low doses compared to control. The fact that low doses of resveratrol support viability show how important dose studies are in cancer treatments. Viability was above control up to 40 µM. Several studies have indicated that antioxidants can enhance the viability of cancer cells by mitigating oxidative stress, which may inadvertently support tumor growth and resistance to treatment (13-15). In the studies in the literature studying resveratrol and pancreatic cancer, it is generally emphasized that viability is below control at these doses (16, 17). Such differences may be due to many

reasons. For example, the cell used may be affected by the experimental conditions and experimental materials used and may produce different results. Cancer cells are dynamic cells that are highly adaptable to different environments. These characteristics may be the source of differences between experiments. We can consider cell morphology as an important end product that provides information about the internal dynamics of the cell. We can obtain information about the cell's membrane, cytoplasm, nucleus and cell volume through morphology examinations. In our experiment, we made the cell examinable in terms of cell compartments with hematoxylin-eosin staining. We observed that the cell lost its volume with increasing resveratrol doses, the cell shrank and its nucleus became pyknotic. Studies in the literature have shown that resveratrol disrupts cell morphology (18, 19). We differed with other studies in the literature regarding the dose. Molecules like resveratrol can affect almost all physiological processes of the cell. Cell movement is the most important weapon of cancer cells. Cancer cells are carried to nearby and distant organs, disrupting, slowing down and eventually stopping the normal functioning of the organism. When pancreatic cancer is diagnosed, it is usually found to have metastasized. As in other types of cancer, focusing on cell movement in pancreatic cancer is very important in the treatment of this disease. In our study, resveratrol first slowed down and then stopped PANC-1 cells with increasing doses. It was seen that cell migration was completely inhibited at doses of 100 µM and above. Similar results were also seen in the literature at different doses (7, 20, 21).

Like other types of cancer, pancreatic cancer is a major health problem that needs to be solved in society. There are many clinical, in vivo and in vitro studies on pancreatic cancer in the literature. We approached this field from the PANC-1 pancreatic cancer cell and resveratrol window. We contributed to the literature with new doses we detected in cell viability, morphological changes of cells and cell movement experiments. If our study had been supported by intracellular pathways, experimental animal models and additional molecular techniques, we could have explained the result with sharper boundaries.

CONCLUSION

As a result, we determined with this study that resveratrol reduces cell viability, disrupts cell morphology and first slows down and then stops cell movement in a dose-dependent manner. Finding anti-cancer molecules that are easily obtainable, highly beneficial, competitive with commercial equivalents and less damaging to normal tissue and determining their effectiveness are of great importance today in terms of both the country's economy and patient welfare. Resveratrol, on which thousands of studies have been conducted for years, is a strong candidate in this sense. There are many drug studies of resveratrol that are preclinical and candidates to reach clinical levels. We wanted to contribute to this pool with this study we conducted. Of course, as long as a disease is not completely cured, researching treatment options is an important goal. Although the path to the treatment of this cancer disease is difficult and long, the goal will be closer as long as we work with determination.

Ethics Committee Approval: This study is an in vitro study. Since no human or animal material was used in this study, an ethics committee report is not required.

Informed Consent: Since this study was not conducted on humans, we do not have an informed consent form.

Authorship Contributions: Idea/Concept:ES, Design:ES, Supervision:ES, Data Collection and Processing:ES, Analysis or Interpretation:ES, Literature Search:ES, Writing:ES, Critical Review:ES, References and Fundings: - , Materials:ES.

Conflict of Interest: No conflict of interest was declared by the author.

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Original Article / Araştırma Makalesi

USING 16S rRNA-SPECIFIC PCR ON PREOPERATIVE AMNIOTIC FLUID SAMPLES TO PREDICT SUCCESS OF EMERGENCY CERCLAGE PLACEMENT

ACIL SERKLAJ BAŞARISINI ÖNGÖRMEK IÇIN PREOPERATIF AMNIYOTIK SIVI ÖRNEKLERINDE 16S rRNA-SPESIFIK PCR KULLANIMI

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ABSTRACT

Introduction: The success of emergency cerclage in the presence of incompetent cervix and protruding fetal membrane is a matter of debate. Theories suggest that the microbiome may play an important role in influencing the structural integrity of the cervix. Hence, understanding the microbiome status before cerclage operation may have value in predicting the success rate of the procedure. This study aimed to examine the existence of microbial organisms in amniotic fluid through polymerase chain reaction (PCR) detection of 16S rRNA species and assess its correlation with perinatal outcomes in mid-trimester emergency cerclage cases.

Methods: Nineteen patients were scheduled for amniodrainage and emergency cerclage due to cervical insufficiency and they formed the cerclage group. The control group was formed by 56 patients who were seeking consultation for routine control and underwent karyotyping and physical examination at our clinic.

Results: In the cerclage group, PCR results were negative in all control group patients, whereas nine patients tested positive for PCR ($p<0.001$). Chorioamnionitis was observed in five PCR-positive patients but not in PCR-negative patients ($p<0.001$), and three newborns had sepsis in PCR-positive patients, while none were observed in PCR-negative patients ($p=0.01$). Neonatal mortality was significantly higher in PCR-positive patients compared to PCR-negative patients ($p=0.011$), with all infants born to PCR-positive patients succumbing. Conversely, 60% of the infants of PCR-negative patients in the cerclage group were discharged in good health.

Conclusions: In this study, the presence of a potential link between the microbiome status and the success of emergency cerclage procedures was underlined.

Keywords: Cervical insufficiency, emergency cerclage, perinatal outcome, polymerase chain reaction, prolapsed amniotic membranes.

ÖZET

Giriş: Servikal yetmezlik ve prolabe amniyon membranı varlığında acil serklajın başarısı tartışma konusudur. Teoriler, mikrobiyomun serviksin yapısal bütünlüğünü etkilemede önemli bir rol oynayabileceğini öne sürmektedir. Bu nedenle, serklaj operasyonundan önce mikrobiyom durumunun anlaşılması, prosedürün başarı oranını tahmin etmede değerli olabilir. Bu çalışma, 16S rRNA türlerinin polimeraz zincir reaksiyonu (PCR) tespiti yoluyla amniyon sıvısında mikrobiyal organizmaların varlığını incelemeyi ve orta trimester acil serklaj vakalarında perinatal sonuçlarla korelasyonunu değerlendirmeyi amaçlamaktadır.

Yöntemler: Servikal yetmezlik nedeniyle amniodrenaj ve acil serklaj planlanan 19 hasta serklaj grubunu oluşturdu. Kontrol grubu, rutinde yapılan perinatal testlerde risk saptanması üzerine amniyosentez yapılması planlanan 56 hastadan oluşturuldu.

Bulgular: Tüm kontrol hastalarında PCR negatifti, serklaj grubundaki dokuz hastanın ise PCR sonucu pozitif idi ($p<0,001$). PCR-pozitif 5 hastada koryoamniyonit tespit edilirken PCR-negatif hastalarda tespit edilmedi ($p<0,001$). PCR-pozitif hastalar arasında üç yenidoğanda sepsis gelişti, ancak PCR-negatif hastaların yenidoğanlarında gelişmedi ($p=0,01$). PCR-pozitif hastalarda neonatal mortalite PCR-negatif hastalara kıyasla önemli ölçüde daha yüksekti ($p=0,011$), PCR-pozitif hastalardan doğan tüm bebekler kaybedildi. Serklaj grubundaki PCR-negatif hastaların %60'ının bebekleri ise sağlıklı olarak taburcu edilmiştir.

Sonuç: Bu çalışmada, mikrobiyom durumu ile acil serklaj prosedürlerinin başarısı arasında olası bir bağlantının varlığı vurgulanmıştır.

Anahtar Kelimeler: Servikal yetmezlik, acil serklaj, perinatal sonuçlar, polimeraz zincir reaksiyonu, prolabe amniyon membranı.

INTRODUCTION

Two cases per 1000 births are complicated by amniotic membrane prolapse. The main cause of amniotic membrane prolapse is cervical insufficiency (1). Hassan et al reported that 9% of asymptomatic women with a shortened cervix have microbiologically proven intraamniotic infection, suggesting that these infections may precede the development of acute cervical insufficiency with bulging membranes (2). The clinical value of cerclage has been the subject of several studies (3-5). In addition to dilatation and effacement of the cervix, the fetal membranes are bulging into the vagina, making imminent delivery or cerclage failure more likely. Although the incidence of infection-related complications may be high with emergency cerclage (EC),

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pregnancy can be prolonged in most cases of cervical insufficiency and may improve perinatal outcomes (6,7).

Investigators have shown that many of the infection markers increase in the amniotic fluid (AF) when pregnancies are complicated by preterm delivery (8,9). However, only ~45% of pregnancies with elevated interleukin (IL-6, IL-1α, IL-1β, prostaglandin-E2, tumor necrosis factor (TNF)-α) concentrations have microbial infections in AF cultures (10,11). It is therefore possible that a higher percentage of AF samples may have microbial infection but the infection cannot be detected due to limitations in standard culturing techniques (3). Of the 116 patients, 87 (63%) were classified as infected and 52 (37%) as uninfected. In the infection group, 13 cultures were negative but PCR-positive (12). Mays et al reported that evaluation of AF for infection markers before EC placement may identify patients with subclinical chorioamnionitis who would not benefit from cerclage (13). However, assessment of the levels of these infection markers in AF is time-consuming and costly process creating a need for a simple and quick test i.g., PCR.

PCR allows for the detection of trace amounts of deoxyribonucleic acids. Bacterial DNA sequences e.g., those coding for 16S rRNA, which are not found in mammalian cells are rational targets for the PCR and could potentially be used to detect microbial invasion of the amniotic cavity in women with cervical dilatation and amniotic membrane prolapse. To date, it remains unclear whether it is possible to detect bacterial 16S rRNA in AF samples collected via amniodrainage before EC in improving obstetric and perinatal outcomes for decision-making regarding EC. The purpose of this study was to test the feasibility of using the 16S rRNA PCR to detect bacteria in AF samples and compare perinatal and obstetric outcomes in women undergoing EC for amniotic membrane prolapse.

METHODS

This study was approved by the Kocaeli University Faculty of Medicine Human Ethics Committee (KAEK 2011/160), financed by the Scientific Research Unit (BAP), and was designed and carried out by the Declaration of Helsinki. All subjects signed an informed consent. 138 patients who applied to the Kocaeli University Faculty of Medicine's Department of Gynecology and Obstetrics with a diagnosis of cervical insufficiency between 2010 and 2012 were included in the study. Nineteen patients with prolapsed amniotic membranes from the cervix to a more distal position, beyond the urethral entrance of the bladder were included in the study as the cerclage group (Figure 1). Emergency cerclage was applied between 14+0 and 28+0 weeks of gestation. Informed consent was obtained after providing detailed information to the patients about the pros and cons of amniodrainage and EC. The inclusion criteria for the cerclage group were: 1. Presence of a live intrauterine fetus without detected anomalies, 2. Advanced cervical change and herniated amniotic membranes, are defined as protrusion and prolapse from the cervix to a more distal position than the urethral entrance of the bladder (Figure 1). The exclusion criteria for the cerclage group were: 1. Placental ablation, 2. Vaginal bleeding, 3. Uterine contractions, 4. Amniorrhexis with positive amnisure test results (AmniSure® Qiagen N.V.), 5. Multiple pregnancies, 6. Fetuses with growth retardation, and 7. Clinical findings such as fever and/or the presence of infection markers suggestive of chorioamnionitis.

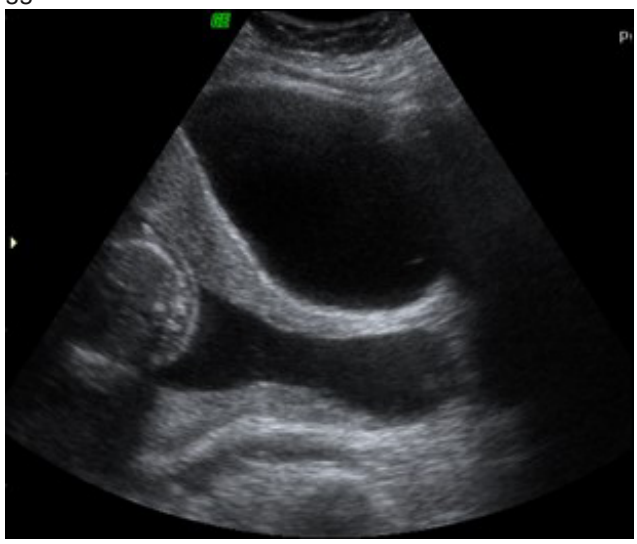

Figure 1. Transabdominal ultrasonography shows that herniated amniotic membranes, defined as advanced cervical change and protrusion, protrude from the cervix more distally than the urethra entrance of the bladder.

Fifty-six women with a high risk for chromosomal abnormalities in a first-trimester screening test and who required amniocentesis between 16-18 gestational weeks were selected as the control group. This group had four times more patients with no signs of infection and inflammation and allowed assessment of highly reliable negative predictive value for the 16S rRNA PCR approach. The diagnosis of advanced cervical change and protrusion of the amniotic membranes was confirmed by speculum and sterile digital examinations and ultrasonography (Figures 1, 2a). Uterine activity was monitored using conventional tocography and vital signs were evaluated. Betamethasone was administered to patients in the cerclage group if the gestational age exceeded 24 weeks. Cervicovaginal, urine, blood, and AF cultures, along with blood samples were collected. Prophylactic tocolysis with indomethacin suppositories was used for all patients selected for cerclage. Calcium channel blockers were preferred in cases where indomethacin could not be administered. Despite the absence of clinical signs of infection in any of the patients in the cerclage group, intravenous antibiotics (1g ampicillin, 2x1, and 500mg aminoglycoside, 2x1) were administered before the cerclage procedure and continued for up to one

week, even if the culture results were negative. Before undergoing EC all patients in the cerclage group underwent amniodrainage. 1–2 cc of AF was sent for PCR evaluation of 16S rRNA.

The PCR results from cultures were not available at the time of EC placement because the collected AF samples were stored at -80oC for later analysis. The PCR and culture results were evaluated afterward to assess any potential association between microbial infection and the need for EC. In cases requiring prompt intervention, patients were monitored for six hours before cerclage placement to ensure that cervical dilatation was not caused by active labor, placental abruption, or clinical signs of infection. The intervention was performed immediately after the necessary preparations for anesthesia were completed.

At the time of amniocentesis or amniodrainage, to prevent skin flora contamination, the skin was cleansed using an antiseptic solution. With an amniocentesis needle guided by an ultrasound device (Voluson®Dawei, PRC), 2 cc of AF was aspirated and immediately discarded to mitigate the risk of contamination. For the control group, 20 cc of AF was collected and sent to the genetics laboratory, while 1–2 cc of AF was sent to the PCR laboratory and stored frozen at -80°C until use (n=56).

In the cerclage group (n=19), varying amounts of fluid, ranging from 110 to 230 cc were extracted through amniodrainage. Following amniodrainage, each patient in the cerclage group received one gram of ampicillin intraamniotically. Sterile aliquots of AF were collected into samples for the BacT/Alert blood culture system (Bactec®Becton Dickinson, USA), Gram staining, and AF culture analysis. Additionally, sterile 1–2ml samples of AF were frozen at -80°C for subsequent PCR analysis. After completion of these procedures, patients underwent EC in the operating room (Figure 2b).

The protruding membranes were gently guided back into the cavity under general anesthesia and in the Trendelenburg position, using sterile moist gauze with gentle pressure. Emergency cerclage was then performed using the McDonald procedure with 5-mm Mersilene tape (Mersilene®Ethicon, USA). A second cerclage suture was placed at the distal end of the Mersilene tape using 1.0 Vicryl (Figure 2c). Postoperative tocolysis was continued for 48 hours. If no complications arose within 72 hours after surgery, patients were discharged with instructions for bed rest, avoiding strenuous activity, and refraining from sexual intercourse.

AF samples stored at -80°C were thawed on ice and centrifuged at 1500 × g for 10 minutes at 4°C. PCR was performed with a long PCR enzyme mix (Fermentas, USA). The sense and antisense primers used were 5'-TGGCTCAGATTGAACGCTGGCGGC and 5'-TACCTTGTTACGACTTCACCCCA, respectively. A 25 µL PCR reaction mixture consisted of 1 ×PCR buffer, 0.2 mM of each dNTP, 0.5 µM of each primer, 1.25 mM MgCl2, 1.5 units of PCR enzyme mix, and 2 µL of AF. An initial 5-minute denaturation at 94°C was followed by 35 cycles of 30 seconds denaturation at 94°C, 1-minute annealing at 57°C, and 1.5 minutes elongation at 72°C. PCR reactions were ended with a 10-minute final elongation at 72°C. PCR products were analyzed by agarose gel electrophoresis, cleaned with a PCR purification kit (Qiagen, USA) and sequenced (Iontek Inc., Istanbul, Turkey).

All statistical analyses were performed using IBM SPSS for Windows version 20.0 (IBM Corp., Armonk, NY, USA). Shapiro-Wilk's test was used to assess the normality assumption. Continuous variables were presented with mean±standard deviation or median (IQR: Interquartile range). Categorical variables were summarized as counts and percentages. Comparisons between groups were carried out using the Mann-Whitney U test. The association between two categorical variables was examined using the Chi-square test. A p-value of <0.05 was considered statistically significant.

RESULTS

All control patients tested negative for PCR. PCR results were positive in 9 cases and negative in 10 cases in the cerclage group (p<0.001). The demographic and obstetric data for the cerclage group were compared between PCR-positive and PCR-negative patients (Table 1). In PCR-positive patients, the parity rate and the rate of having live children were lower (p< 0.05).

Table 1. Demographic and obstetric data of the cerclage group between PCR-positive and PCR-negative patients.

Cerclage group (n = 19)	PCR-positive (n = 9)	PCR-negative (n = 10)	p value*
Age (years), mean ±SD	29.6 ± 5.5	30.7 ± 5.4	0.48
BMI† (kg/m²), mean ±SD	26.5 ± 5.5	25.1 ± 3.0	0.09
Smoking, n	2	1	N/A
Gravida, mean ±SD	1.8 ± 1.1	2.4 ± 1.7	0.16
Parity, mean ±SD	0.42 ± 0.9	1.0 ± 1.1	0.03
Miscarriage, mean ±SD	0.37 ± 0.76	0.27 ± 0.67	0.58
Live healthy child, mean ±SD	0.16 ± 0.5	1.0 ± 1.0	< 0.0001
Preterm birth history, n	2	0	N/A
Cerclage at previous pregnancy, n	1	0	N/A
History of intrauterine fetal death, n	1	0	N/A
Infertility treatment at current pregnancy, n	2	1	N/A
Iron supplement, n	5	8	0.350
Multivitamins supplement, n	5	9	0.141
Threatened miscarriage, n	5	2	0.170

†: BMI: Body mass index; *p< 0,05 significant; N/A: not available.

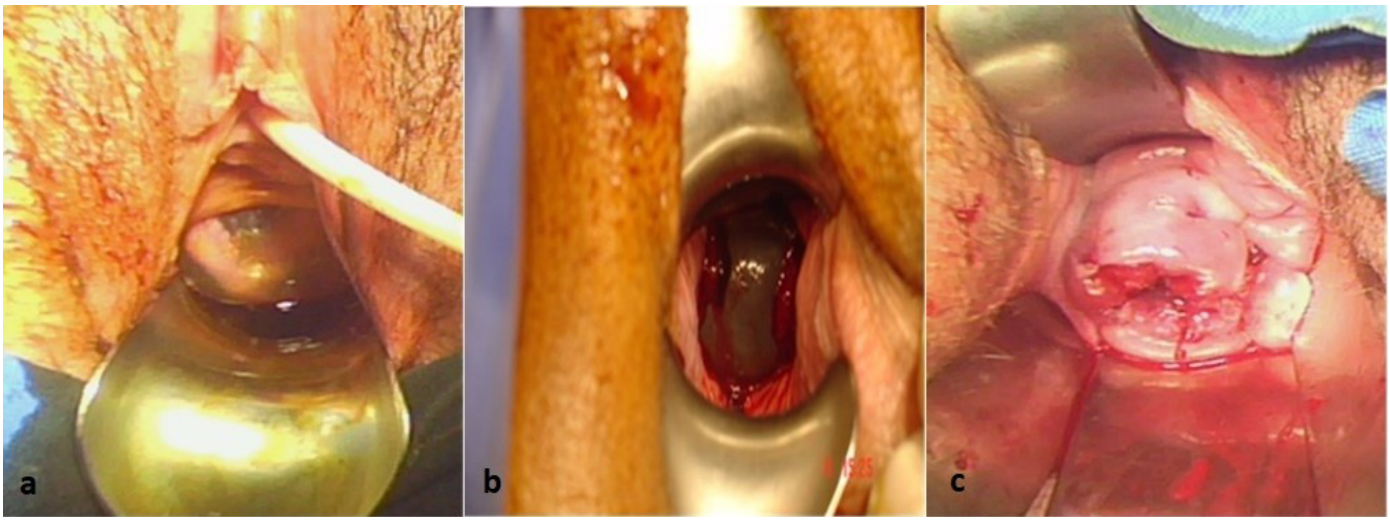

Figure 2. a) During examination with a speculum, the "hourglass-shaped" amniotic membrane is observed to be intact and tense before the amniodrainage process. The left foot of the fetus is observed just behind the highly protruded amniotic membrane, at the level of the hymen.
b) In the pelvic examination of the same patient, it is observed that the tension of the amniotic membrane decreases and shifts proximally after amniodrainage. c) In the same patient, the cervix is observed after emergency cerclage.

Table 2. Perinatal outcome in PCR-positive and PCR-negative patients in the cerclage group.

Cerclage group (n = 19)		**PCR-positive (n= 9)**	**PCR-negative (n= 10)**	**p value***
Positive PCR, n		9	0	< 0.001
Gestation time at cerclage (day), mean ±SD		140 ± 15	143 ± 21	0.78
Mode of Delivery, n	**Vaginal birth**	2	6	N/A
	Cesarean (C/S)	1	4	
	Abortion	6	0	
Chorioamnionitis†, n (%)		5 (26.3%)	0	= 0.001
< 7 1. minute Apgar score, n		8	9	N/A
< 7 5. minute Apgar score, n		8	6	0.303
Birth weight (g) ‡, mean ±SD		383.8 ± 193.6	1245.6 ± 981.5	0.022
NICU§ necessity, n		2	8	0.023
Lenght of NICU§ stay (day), median (IQR)		0 (0 - 1)	7.5 (0.75 - 50.25)	0.010
Newborn with sepsis, n		3	0	= 0.01
Neonatal mortality‖, n		9	4	0.011

†: Patients were diagnosed with chorioamnionitis with the presence of two or more of these fever (> 37.5 ˚C), uterine sensitivity, abdominal pain, malodorous vaginal discharge, maternal and/or fetal tachycardia, and leukocytosis (> 15,000). ‡: grams. §: NICU: Neonatal intensive care unit. ‖: Live-born neonates who died postpartum. *p< 0,05 significant. N/A: not available.

Perinatal outcomes were compared between PCR-positive and PCR-negative patients in the cerclage group (Table 2). The gestational ages of PCR-positive and PCR-negative patients at the time of cerclage were not statistically significant (p>0.05), suggesting that there was no significant difference in the gestational ages between the two groups at the time of cerclage. Five cases of chorioamnionitis were detected in PCR-positive patients but not in PCR-negative patients, and this difference was statistically significant (p<0.001). The mean birth weight of all live or stillborn infants was statistically significant (p=0.022). However, it was not determined whether this difference was between PCR-positive and PCR-negative patients or within each group. In PCR-positive patients, three newborns developed sepsis, and this difference was statistically significant (p=0.01). Neonatal mortality in PCR-positive patients was significantly higher than in PCR-negative patients (p=0.011). Overall, these findings suggested that PCR-positive patients in the cerclage group had a higher incidence rate of chorioamnionitis, neonatal sepsis, and neonatal mortality compared to PCR-negative patients. In addition, there were no significant differences in gestational ages at cerclage placement, fifth-minute APGAR scores, or the necessity and length of neonatal intensive care unit (NICU) stay between the two groups.

The clinical data and prognosis of PCR-positive and PCR-negative patients in the cerclage group were compared in Table 3. The presence of postoperative uterine contractions in PCR-positive patients was found to be significantly higher than in the PCR-negative patients (p<0.01). No significant difference was detected in white blood cell (WBC) count, C-reactive protein (CRP) value, and postoperative fever (p>0.05). Infants of all PCR-positive patients died while six out of 10 patients with negative PCR results (60%) were discharged from the NICU as healthy (p<0.008). Compared

Table 3. Evaluation of clinical data and prognosis of PCR-positive and PCR-negative patients in the cerclage group.

Cerclage group (n = 19)	PCR-positive (n = 9)	PCR-negative (n = 10)	p value*
Fever (> 37.5 °C)‡, n (%)	2 (22%)	0	0.21
The presence of uterine contraction[b], n (%)	8 (88%)	3 (30%)	< 0.01
CRP (> 1) elevation†,‡, n (%)	8 (88%)	8 (80%)	0.81
Leukocytosis (> 15.000)‡, n (%)	3 (33%)	3 (30%)	0.63
Cerclage success (More than a week), n (%)	6 (66%)	7 (70%)	0.63
The take-home baby alive, n (%)	0	6 (60%)	< 0.008
Gestational age at birth (day), mean ±SD	148 ± 16	189 ± 47	< 0.02
The mean extended gestation period (day), mean ±SD (the shortest – longest number of extended days, n)	8 ± 9.1 (1 - 28)	45 ± 53.3 (2 - 142)	= 0.13

*p< 0,05 significant, †: normal CRP level: 0 - 0.05 mg/dl; CRP level in advanced inflammation: > 1 mg/dl; ‡: Presence of fever, CRP elevation, leukocytosis, uterine contractions starting within the first 48 hours postoperatively.

to PCR-negative patients, the gestational age at birth was significantly lower in PCR-positive patients.

The organisms detected by PCR were Enterococcus faecalis (n=2), Streptococcus agalactiae GY102 (n=2), Klebsiella HaNA22 (n=2), Bacterium NLAE-zl-H51 (n=1), Staphylococcus spp clone JPL-53 (n=1), Escherichia fergusonii ATCC 35469 (n=1), and Escherichia sp. ASG34 (n=1). Only one patient was positive for Klebsiella pneumoniae when an AF culture was performed, which was concordant with the bacteria detected by PCR. The AF Gram stain was also positive for this culture. In another patient, whose AF PCR was also positive for Klebsiella, the organism was detected only in urine and cervicovaginal cultures, but not in blood and AF cultures.

DISCUSSION

Due to the heterogeneous nature of this disorder, there are contradictory reports in the literature. Patients with different stages of cervical dilatation, various etiologies, and different management strategies were examined, within the same study (4,5,14-16). To avoid these limitations, only patients with cervical insufficiency (dilated to at least 6 cm) accompanied by amniotic membrane protrusion were included in this study. The amniotic membranes of these patients protruded from the cervix to a position distal to the urethral entrance of the bladder. These patients showed no clinical signs of inflammation, and their vital signs were stable before cerclage. Many studies have emphasized that the success of cerclage is higher when the appropriate patient is selected (5,17). The results of this study demonstrated that PCR amplification and subsequent sequencing of the 16S rRNA species from AF had a strong predictive value for the success of cerclage. In a similar study by Satılmış et al.,16S rRNA PCR was shown to be effective for the diagnosis of sterile body site infections, especially in cases of meningitis and infective endocarditis where routine cultures fail (12).

The primary objective of employing EC for managing amniotic sac protrusion during the second trimester is to extend the duration of the pregnancy as much as possible (14). We assessed the efficacy of EC based on the prolonged duration of pregnancy post-cerclage, perinatal mortality rates, and birth weight as primary outcomes. A systematic review conducted by Ehsanipoor et al. compared the effectiveness of cervical cerclage in second-trimester pregnancies with cervical dilatation and membrane prolapse identified by physical examination. The researchers found a significant improvement in neonatal survival rates (71% compared to 43%; RR 1.65, 95% CI 1.19-2.28) and a prolongation in the gestational period (mean difference 33.98 days, 95% CI 17.88-50.08) (18).

In cases of protruding amniotic sac, the prognosis for pregnancy is often reported to be poor (7,14). During the placement of EC, the goal of amnioreduction is to relieve the pressure on the prolapsed amniotic membranes, thereby facilitating the cerclage procedure and reducing the risk of accidental membrane rupture. This approach also helps decrease the rates of prematurity and related neonatal morbidity (Figure 2b) (19). Depending on the patient, 110-230 cc AF is removed for this purpose. After the amniocentesis performed in this study, during 16-18 weeks of gestation, the AF should be replaced within 14-30 hours, as it typically takes about 3-4 hours to replace 20-25 cc of fluid. Therefore, during follow-up examinations, AF was replaced within 14-30 hours, and no permanent oligohydramnios was observed in our patients. Genetic amniocentesis for karyotyping in pregnant women is widely recognized as reliable. Consequently, the potential adverse effects of amniocentesis on maternal and fetal outcomes are considered negligible.

In a study, pleura, cerebrospinal fluid, peritoneal fluid, and synovial fluid were cultured using the BacT/Alert blood culture system. Reproduction was detected in 18 (95%) of the samples, while only 11 (58%) were detected using the classical culture method (20). This finding led us to use the BacT/Alert blood culture system in our experiments for isolating microorganisms from AF, while routine culture procedures were employed for culturing urine, blood, and cervicovaginal samples.

Despite the existing scientific consensus suggesting challenges in treating intra-amniotic infection or

inflammation in the presence of cervical insufficiency (10), successful treatment of both intra-amniotic infection and inflammation with antimicrobial agents is still possible (10,14,19,21). The use of indomethacin in the treatment of protruded amniotic membranes should be limited to 48 hours to allow time for corticosteroid therapy while minimizing neonatal complications (22). It has been noted that the use of prophylactic tocolytics suppresses uterine contractions and reduces intrauterine pressure, thereby preventing the protrusion of the amniotic membrane. Indomethacin reduces AF production in addition to its anti-inflammatory effects. With cerclage, the inflammatory-like process responsible for the initiation of contraction is also reduced (17). In this study, rectal indomethacin was administered for 48 hours as part of prophylactic tocolysis.

It has been demonstrated that applying double cerclage with two separate sutures to the cervix can improve the success rate, particularly in cases involving amniotic membrane protrusion (1). Among various cerclage techniques with similar success rates (14), the McDonald procedure was chosen due to its ease of implementation, especially in emergencies. To further enhance the success of the cerclage, a specific modification was employed, which involved the use of 1.0 Vicryl suture material placed more distally as the second suture following the initial placement of Mersilen tape. This modification was likely intended to provide additional reinforcement and support to the cervix, thereby improving the effectiveness of the cerclage procedure in preventing premature cervical dilation and its associated complications.

The potential contribution of infection in cervical insufficiency during the second trimester has been suggested, highlighting the importance of screening for infection before cerclage placement as a way to predict prognosis (17). Various preoperative procedures, such as cervicovaginal cultures, urine and blood cultures, Gram staining, and evaluation of inflammatory status in the endocervix, have been proposed to identify patients who would benefit from cerclage, while also recognizing those in whom this intervention might be harmful (3).

However, these laboratory procedures can be time-consuming, and expensive, and may delay surgical intervention. Cerclage placement in emergencies for amniotic sac prolapse is not fundamentally different from prophylactic cerclage The critical distinction between EC and prophylactic cerclage lies in the timing of the procedure. To address this challenge, cervicovaginal, urine, blood, and AF cultures, along with AF Gram staining and PCR analysis, were evaluated. The goal was to identify a diagnostic method that would facilitate rapid patient selection without compromising effectiveness. Despite the uncertain timing of cerclage (23), delaying the procedure could increase the risk of infection, as protruding membranes are more exposed to vaginal bacteria (17).

Emergency cerclage is an effective procedure for reducing the rate of premature birth in patients with cervical insufficiency and amniotic membrane protrusion challenges, favorable outcomes have been reported (15). Despite the challenges, favorable outcomes have been reported. Caruso et al. conducted a study involving 23 patients, finding an average gestational prolongation of 28 days and a survival rate of 46%. The average gestational age at birth was 25 weeks, with a mean birth weight of 700 grams, which was considered a successful outcome (24). Shivani et al. reported that EC, used as a salvage measure for pregnancies at high risk of preterm delivery or mid-trimester miscarriage, extended pregnancy duration by up to 71.2 days following rescue cerclage placement (6). Similarly, Ciancimino et al. reported an average pregnancy prolongation of 89.9 days and a newborn survival rate of 83.3% after EC in 12 patients (25). In another study by Rius et al., EC in 39 patients resulted in a mean gestational prolongation of 49.1 days, with a mean gestational age at birth of 28.6 weeks and a neonatal survival rate of 82.4% (26). A literature review by Cockwell et al., spanning 10-years, suggested that EC significantly prolonged pregnancy. In 25 studies involving 638 patients who underwent EC, the average pregnancy extension was 7 weeks and 1 day, with an average neonatal survival rate exceeding 70%. These findings underscore the efficacy of EC in extending pregnancy and improving neonatal outcomes in cases of cervical insufficiency and protrusion of amniotic membranes (27).

In the study, variables such as age, BMI, gravidity, WBC, CRP values, as well as gestational age at cerclage and birth, and birth weight were not found to be statistically significant ($p>0.05$) (14). However, gestational age at birth was significantly lower in PCR-positive patients. Although most PCR-positive patients experienced miscarriage and had shorter gestational periods, statistical significance was not observed. No significant difference was found in the gestational age at the time of cerclage between PCR-positive and PCR-negative patients. However, significant differences were noted in gestational age at birth, with PCR-positive patients having a mean of 148 days and PCR-negative patients having a mean of 189 days. The mean extended gestation period was 8 days for PCR-positive patients and 45 days for PCR-negative patients. Emergency cerclage extended the gestational period by an average of 45 days in PCR-negative cases. Significant differences were observed in the rates of chorioamnionitis, neonatal sepsis, and neonatal mortality between PCR-positive and PCR-negative patients. Unfortunately, all infants born to PCR-positive patients died, while 60% of infants born to PCR-negative patients survived. A 60% live birth rate should be considered a successful outcome for mid-trimester EC in cases with protruding membranes.

CONCLUSION

Our findings underscore the complexity of identifying patients who would benefit from EC based solely on conventional clinical evaluations such as gynecological examination, genitourinary cultures, WBC count, or CRP levels. This study suggests that women undergoing EC due to amniotic membrane prolapse may harbor subclinical intra-amniotic or extra-amniotic inflammation, reflecting the advanced stages of a process where avoiding cerclage placement could potentially reduce maternal morbidity.

A significant portion of cases in this study (47.3%) were PCR-positive, indicating microbial invasion of the membranes and suggesting a poor prognosis for cerclage in patients with acute cervical insufficiency and subclinical intra-amniotic infection or inflammation (28). We propose that a combination of gynecological examination, history of previous cervical surgeries, prior preterm deliveries, and the absence of intraamniotic inflammation -confirmed by AF PCR- may help identify patients who genuinely require EC placement.

The patients in this study benefited from EC, which potentially improved perinatal outcomes. Despite the anticipated poor prognosis in some cases, proper selection based on these criteria can lead to successful results. We suggest that the success of the cerclage procedure could be predicted based on 16S rRNA analysis PCR results.

Although the number of patients in the cerclage group was limited, preventing definitive conclusions, a larger sample size of the patients who underwent EC would have yielded more statistically reliable results. Additionally, measuring the levels of inflammatory markers, such as interleukins could have provided valuable insights into the changes associated with EC and its impact on inflammation.

This study emphasizes the importance of integrating molecular diagnostic techniques, such as PCR analysis of AF for microbial infection, alongside clinical parameters to more accurately identify candidates for EC placement and ultimately improve perinatal outcomes.

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Original Article / Araştırma Makalesi

SURGICAL ONCOLOGY-BASED CHECK-UP PROGRAM AND DETERMINING OF ASYMPTOMATIC PATHOLOGIES

CERRAHİ ONKOLOJİ TEMELLİ CHECK-UP PROGRAMI VE ASEMPTOMATİK PATOLOJİLERIN BELİRLENMESİ

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ABSTRACT

Introduction: Our study aims to determine the effectiveness of check-up programs in the early diagnosis of benign and malignant pathologies, and evaluate their importance.

Methods: Posteroanterior chest X-ray, thyroid, and abdominal ultrasonography were performed. Breast ultrasonography was performed on all females and mammography on those over 40 years. Fecal occult blood, carcinoembryonic antigen, and prostate-specific antigen tests were performed on the cases along with hemogram and routine biochemical tests. Radiological images and reports were retrospectively scanned, and fecal occult blood, carcinoembryonic antigen, and prostate-specific antigen values were examined. Breast Imaging Reporting and Data Systems (BIRADS) in breast radiology and the Thyroid Imaging Reporting and Data System (TIRADS) in thyroid radiology were used for classification.

Results: 1411 cases were included in this study. 49.4% were female and 50.6% male. Median age was 50 years. Benign lesions in the liver were detected in 5.31% of the cases, kidney stones in 4.7%, and cholelithiasis in 5.7%. Benign Prostatic Hyperplasia was observed in 20% of the males. Thyroid gland nodule with a risk of malignancy in 3.8% and biopsy was recommended. Malignancy-suspicious lesions were detected in 0.28%, that performed breast ultrasonography and in 0.8%, that performed mammography, and biopsy was recommended. Normal lung parenchymal findings were detected in 75.4%, minor in 22%, and major in 2.6%, and thoracic computed tomography was recommended.

Conclusions: Effective check-up programs are found to be beneficial for public health as they provide diagnosis and follow-up in benign lesions and early diagnosis and treatment opportunities in malignant/premalignant lesions.

Keywords: Check-Up, Cancer, Radiology Health Screening Program

ÖZET

Giriş: Çalışmamızın amacı check-up amacıyla başvuran sağlıklı bireylerin bulgularını paylaşmak, check-up programlarının benign ve malign patolojilerin erken tanısındaki etkinliğini saptamaktır.

Yöntemler: Check-up programı dahilinde; tüm olgulara postero-anterior akciğer grafisi, tiroid ve batın ultrasonografisi yapılmıştır. Tüm kadın olgulara meme ultrasonografisi ve 40 yaşından büyüklere mamografi uygulanmıştır. Hemogram ve rutin biyokimyasal testlerle birlikte gaytada gizli kan, karsinoembriyonik antijen ve erkeklerde prostat spesifik antijen testi yapıldı. Radyolojik görüntüler ve raporlar retrospektif olarak tarandı, laboratuvar sonuçlarından ise gaytada gizli kan, karsinoembriyonik antijen ve erkeklerde prostat spesifik antijen testi değerleri incelendi. Meme radyolojisinde meme görüntüleme raporlama ve veri sistemi (BIRADS), tiroid radyolojisinde tiroid görüntüleme raporlama ve veri sistemi (TIRADS) sistemi kullanılarak sınıflandırma yapıldı. Batın ultrasonografi bulguları organ spesifik olarak ayrı ayrı değerlendirildi.

Bulgular: Çalışmaya 1411 olgu dahil edildi. Olguların %49,4'ü kadın, %50,6'sı ise erkek, median yaş 50 idi. Olguların %5,31'inde karaciğerde benign lezyon, %4,7'sinde böbrek taşı, %5,7'sinde kolelitiyazis, kadın olguların %2,72'sinde benign jinekolojik kitle tespit edildi. Erkeklerin %20'sinde benign prostat hiperplazisi görüldü. %3,8'inde tiroid bezinde malignite şüpheli nodül görüldü ve biyopsi önerildi. Meme Ultrasonu yapılan olguların %0,28'inde, mamografi yapılanların ise %0,8'inde malignite şüpheli lezyon saptandı ve biyopsi önerildi. Olguların hepsine akciğer grafisi çekildi. %75,4'ünde normal, %22'sinde minör, %2,6'sında ise majör akciğer parankim bulguları saptandı ve bu olgulara toraks bilgisayarlı tomografisi ile ileri tetkik önerildi.

Sonuç: Çalışmadaki sonuçlarımıza göre; etkin check-up programları benign lezyonlarda tanı ve takibi sağladığından, malign veya premalign lezyonlarda ise erken tanı ve tedavi şansı sunduğundan toplum sağlığı açısından yararlı bulunmuştur.

Anahtar Kelimeler: Check-up, kanser, radyoloji, sağlık tarama program

INTRODUCTION

Check-up programs are defined as general systematic checks performed to evaluate the general health status of healthy individuals without any disease, with physical examinations, preventive tests, and interventions for early diagnosis of possible health problems (1). Health screenings can be applied specifically to the person, gender, age, and risk group of diseases due to their advantages such

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as early diagnosis, allowing interventions to prevent the disease, and being able to follow the detected pathology. Organ-specific screenings such as breast, prostate, and lung are widely used (2).

One of the genders and age-specific screening programs accepted globally is breast screening. Mammography is the most widely used screening technique for early diagnosis of breast cancer and plays an important role in reducing breast cancer deaths. A spiculated, irregularly shaped, and high-density mass detected in mammography is the most suspicious finding in terms of malignancy. Calcification is a common finding in mammography. In addition, when a mass is seen in mammography, other accompanying findings such as skin or nipple retraction, skin or trabecular thickening, and axillary lymphadenopathy should also be evaluated. When a mass is seen in mammography, Ultrasonography (US) is quite useful in distinguishing between solid/cystic. Breast Imaging Reporting and Data Systems (BIRADS) is the standard reporting system (3). BIRADS has high diagnostic accuracy rates in distinguishing benign/malignant. In addition, short-term follow-up of lesions defined as BIRADS-3 as an alternative to biopsy reduces the number of biopsies performed in benign lesions (4).

The incidence of thyroid nodules is rapidly increasing, and careful risk stratification is important in preventing overdiagnosis and treatment. The first radiological modality to evaluate the thyroid gland is US criteria such as the size, borders, internal structure of the thyroid nodule, calcification in the nodule, and Doppler US is very valuable in distinguishing between benign and malignant lesions (5). To reduce unnecessary imaging and biopsies, it is necessary to evaluate the US features of thyroid nodules in a standardized manner. Thyroid Imaging Reporting and Data System (TIRADS) is currently the most useful method for thyroid screening (6-7). Postero-anterior chest X-ray (PA-Chest X-ray) is the first imaging method used for screening purposes for lung cancer. PA-Chest X-ray, which has advanced methods and settings, can detect lung cancer early. If an abnormality is detected in PA-Chest X-ray, thoracic Computed Tomography (CT) is recommended (8).

In asymptomatic individuals, abdominal US, which is used for screening purposes, can be used to identify benign pathologies such as gallstones and kidney stones, but which may cause symptoms in the future, in addition to liver cancer, kidney cancer, and other intra-abdominal cancer screenings. In recent years, it has found a place in routine screenings (9). US is the first-choice imaging method for liver masses due to its advantages such as being radiation-free, non-invasive, relatively inexpensive, and easy to apply. It is used as a first-line imaging method for focal liver lesions (10). Non-Alcoholic Fatty Liver Disease (NAFLD) is the most common liver disease with a prevalence of 20% to 46%. US is the most frequently preferred imaging technique for screening fatty liver, gallbladder, and biliary tract due to its low cost, easy accessibility, and lack of radiation (11-12).

The first medical imaging for urinary tract is US. The most common renal lesion is simple cysts. Most are benign and follow-up is sufficient. Some benign renal cysts may be complicated by bleeding or infection or may become calcified. It may be difficult to distinguish them from heterogeneous, semisolid cystic renal tumors such as cystic Renal Cell Carcinoma (RCC), multilocular cystic nephroma, and mixed epithelial and stromal tumors with US. When such US findings are present, further examination with contrast-enhanced Magnetic Resonance Imaging (MRI) is necessary (13).

Both adrenal glands can be evaluated with US. Various masses such as adenoma, pheochromocytoma, and metastasis can be seen in the adrenal region. When a solid mass is seen in the adrenal region on US, a differential diagnosis can be made with contrast-enhanced fat-suppressed MRI (14).

First method for diagnosing female genital system is US to define polycystic ovary syndrome and endometrial pathologies. Doppler US also contributes to the differential diagnosis. In addition to US, MRI is important in distinguishing benign/malignant ovarian masses (15).

While the head and uncinate process of the pancreas are relatively easier to image with US, imaging the body and tail is difficult due to intra-abdominal gas and obesity. Pancreatic echogenicity may appear hypoechoic or hyperechoic (16). Solid or cystic lesions may be seen in the pancreas. Additional cross-sectional examinations are required for a clearer differential diagnosis (17). US is the most common radiological imaging for spleen. Spleen dimensions can be measured. Congenital anomalies such as accessory spleen, polysplenia, wandering spleen, and contour lobulation can be distinguished. Cysts are seen as anechoic, while hemangiomas are seen as hyperechoic. Abscesses are seen as thick-walled, hypoechoic lesions containing dense fluid. Primary spleen lymphoma is seen as millimetric hypoechoic nodular zones with unclear boundaries in the spleen parenchyma. Metastatic tumors are seen as isoechoic nodular lesions with hypoechoic halos (18).

Tumor markers such as Carcinoembryonic Antigen (CEA), Prostate Specific Antigen (PSA), α-fetoprotein (AFP), CA 19-9, CA 125, and CA 15-3 are included in various health screening programs because they are non-invasive and less expensive than interventional diagnostic methods. CA-125 predicts ovarian cancer, and CA 15-3 breast cancer and provides patients with an early-stage diagnosis and surgery (19). CA 125 and CA 15-3 are screening tests specific to females, and PSA is specific to males. With the use of PSA for check-ups, the incidence of advanced prostate cancer has decreased. Prostate cancer can now be detected at an early stage and provides patients an opportunity for surgery (20).

When the upper intestinal system is to be examined, gastroscopy is the best option for upper intestinal system. Even small lesions can be seen with a gastroscope and provide an opportunity for diagnosis. Benign and malignant lesions can be easily separated with the advantage of biopsy (21). The most important screenings are Fecal Occult Blood (FOB) and colonoscopy to prevent colorectal cancer. Colonoscopy allows early diagnosis of colorectal cancer with its real-time imaging capability and biopsy advantage. Colonoscopy is necessary for patients with FOB positivity (22).

In normal healthy individuals, those with a family history of cancer, and individuals who are concerned about themselves, early diagnosis can be made in health screenings performed with non-invasive, radiation-free methods. This can provide relief for healthy individuals who are concerned about their illness. For this reason; check-up programs are applied in many health institutions.

Our aim in this study is to share our radiological and laboratory findings in the health screening program that healthy adult cases have voluntarily performed in our university hospital, to determine the effectiveness of this program in possible early cancer diagnosis.

METHODS

Healthy adults who voluntarily had a check-up at our institution were included in the study. All cases underwent PA-Lung X-rays, thyroid, and abdominal US. All female cases underwent breast US and those over 40 years of age mammography in addition. Hemogram and biochemical laboratory tests, FOB, CEA, and male cases underwent PSA (males) tests were performed.

Radiological findings were evaluated retrospectively by a single radiologist (D.D.). All verified radiology reports and laboratory results included in the study were evaluated retrospectively by a single surgical oncologist (K.G.).

Ethics committee approval (İstanbul Okan University non-invasive clinical research ethics Received No:169/35, dated 18-10-2023) before initiating the research. The study complies with the principles of the Declaration of Helsinki and relevant legislation was carried out appropriately.

Mean, standard deviation, minimum, maximum, frequency, and ratio values were used in the descriptive statistics of the data. The distribution of variables was measured by Kolmogorov Smirnov, and Shapiro-Wilk tests. Mann-Whitney U test was used in the analysis of quantitative independent data with non-normal distribution. Chi-Square test was used in the analysis of qualitative independent data. SPSS 28.0 program was used in the analyses. Descriptive information is expressed in numbers (n) and percentages (%). The Chi-Square test was used in intergroup comparisons. Significance was assumed when the p-value was <0.05.

RESULTS

1411 healthy individuals, females n=697 (49.4%), and males n=714 (50.6%) included in this study who voluntarily had a check-up at our institution between January 2021 and July 2022. Median age was 50, mean age 50.3±13.2, and range between 18-87. There was no significant difference between male and female cases in terms of gender and age ($p>0.05$).

Grade-3 hepatosteatosis was detected with liver US in 34 patients (2.4%) and these patients were referred to the gastroenterology clinic to be evaluated for pre-cirrhotic processes. Hepatomegaly and hepatosteatosis rates in the liver were significantly higher in men than women ($p<0.05$). One or more liver hemangiomas were detected in 31 patients (2.2%). The rate of liver hemangiomas was significantly higher in females than in males ($p<0.05$). The rates of liver cysts and additional pathology did not differ significantly between males and females ($p>0.05$). One or more simple liver cysts were detected in 44 patients. Sludge in the gallbladder was detected in 41 patients (2.9%), and cholelithiasis in 81 patients (5.7%) and laparoscopic cholecystectomy was recommended. Sludge and polyps in gallbladder were significantly higher in males than females ($p<0.05$).

Stones were detected in the right kidney in 29 patients (2%) and the left kidney in 38 patients (2.7%). Stones in both kidneys did not differ significantly between males and females ($p>0.05$). Cysts in both kidneys were significantly higher in males than females ($p<0.05$).

Gastric wall thickening was detected in 15 cases (1.1%), and gastroscopy was recommended. FOB was positive in 42 (3%), and gastroscopy/colonoscopy were recommended. FOB positivity rate between males and females ($p=0.961$) did not show a significant difference. Splenomegaly was detected in 23 (1.6%) and hematology control was planned. Cystitis was detected in 22 (1.6%) and they were followed up under medical treatment. Cystitis was significantly lower in males than females ($p<0.05$). BPH was seen in 143 (20%) males. Uterine myoma was detected in one female, and ovarian cyst was detected in 18 females. These patients were followed up in the gynecology clinic (Tables 1 and 2).

In thyroid gland examinations, TIRADS-4 lesions were found in 53 cases (3.8%) and fine needle biopsy was planned. In breast examinations, one of each BIRADS-4A, and BIRADS-4C lesions are found. Mammographic examinations revealed BIRADS-4A in one female, BIRADS-4B in one, BIRADS-4C in one, and BIRADS-5 in one. Breast MRI and TRU-CUT biopsy were recommended for these cases (Table 3).

Age did not differ significantly ($p>0.05$) between males and females. CEA levels were significantly lower in males than females ($p<0.05$) (Table 4).

The degree of liver hepatosteatosis and hepatomegaly were significantly higher in males than females ($p<0.05$).

Table 1: Sonographic findings of the liver and gallbladder

		n	%
Liver Hepatosteatosis	(-)	600	42.5%
	Grade I	588	41.7%
	Grade II	189	13.4%
	Grade III	34	2.4%
Liver Hepatomegaly	(-)	1107	78.5%
	(+)	304	21.5%
Liver Hepatomegaly	(-)	1380	97.8%
	One	25	1.8%
	More than one	6	0.4%
Liver Cyst	(-)	1367	96.9%
	One	30	2.1%
	More than one	14	1.0%
Gallbladder Stone	(-)	1330	94.3%
	(+)	81	5.7%
Gallbladder Polyp	(-)	1323	93.8%
	One	71	5.0%
	More than one	17	1.2%
Gallbladder Sludge	(-)	1370	97.1%
	(+)	41	2.9%

The rate of liver hemangioma was significantly lower in males than females ($p<0.05$). The rate of liver cysts did not differ significantly ($p>0.05$) between males and females (Table 4).

The presence of gallbladder stones did not differ significantly ($p>0.05$) between males and females. The presence of gallbladder polyps was significantly ($p<0.05$) higher in males than females. The presence of gallbladder sludge was significantly ($p<0.05$) lower in males than females (Table 4).

There was no significant difference ($p>0.05$) in the presence of stones in the right and left kidney between males and females. The presence of cysts in the kidneys was significantly higher in males than females ($p<0.05$) (Table 5).

The antral wall thickening rate was significantly lower in males than females ($p<0.05$). Cystitis was significantly lower in males than females ($p<0.05$). There was no significant difference ($p>0.05$) in the FOB positivity between males and females (Table 6).

Table 2: Sonographic findings of other intra-abdominal organs

		n	%
Stone in right kidney	(-)	1382	97.9%
	One	27	1.9%
	More than one	2	0.1%
Cyst in right kidney	(-)	1256	89.0%
	One	154	10.9%
	More than one	1	0.1%
Right kidney Additional pathologies	(-)	1323	93.8%
	Pelvic Ectasia	12	0.9%
	Other benign findings	76	5.4%
Stone in left kidney	(-)	1373	97.3%
	One	38	2.7%
Cyst in left kidney	(-)	1263	89.5%
	One	146	10.3%
	More than one	2	0.1%
Left kidney Additional pathologies	(-)	1328	94.1%
	Pelvic Ectasia	15	1.1%
	Other benign findings	68	4.8%
Pancreas	Normal	1403	99.4%
	Steatosis	7	0.5%
	Other benign findings	1	0.1%
Stomach	Normal	1396	98.9%
	Antral wall thickening	15	1.1%
Spleen	Normal	1378	97.7%
	Splenomegaly	23	1.6%
	Other benign findings	10	0.7%
Bladder	Normal	1367	96.9%
	Cystitis	22	1.6%
	Other Benign Findings	22	1.6%
Uterus	Normal	626	89.8%
	Myoma	57	8.2%
	Other Benign Findings	14	2%
Over	Normal	654	93.8%
	Cyst	40	5.7%
	Other Benign Findings	3	0.5%
Prostate	Normal	571	80%
	BPH	143	20%
FOB	(-)	777	55.1%
	(+)	42	3.0%
	Not performed	592	42.0%

Table 3. Thyroid, Breast Ultrasonography and Mammography Findings

		n	%
Thyroid US	Normal	55	3.8%
	TIRADS-1	523	37.1%
	TIRADS-2	676	47.9%
	TIRADS-3	87	6.2%
	TIRADS-4	53	3.8%
	Total Thyroidectomy	17	1.2%
Breast US	BIRADS-0	6	0.9%
	BIRADS-1	326	46.8%
	BIRADS-2	272	39.02%
	BIRADS-3	91	13.%
	BIRADS-4A	1	0.14%
	BIRADS-4C	1	0.14%
Mammography	Not performed	226	32.4%
Mammography	BIRADS-0	54	11.4%
	BIRADS-1	158	33.5%
	BIRADS-2	217	46.1%
	BIRADS-3	38	8.2%
	BIRADS-4	1	0.2%
	BIRADS-4A	1	0.2%
	BIRADS-4B	1	0.2%
	BIRADS-5	1	0.2%

In PA-Chest X-rays; minor findings were detected in 312, and major findings were in 36 cases, 15 of them had nodules in the lung parenchyma, 11 had suspected hilar LAP, 2 had suspected mediastinal LAP, 5 had infection findings, 3 had suspected aortic aneurysm and thoracic CT was recommended.

DISCUSSION

The advantages of radiological examinations performed for screening in healthy individuals can be listed as providing the chance of early diagnosis and treatment of cancer and psychological relief when no pathology is detected (9-23).

Abdominal US is the gold standard for the diagnosis of gallstones and also provides additional information about stone mobility, gallbladder size, and wall thickness (24). Polyps appear as echogenic lesions with regular contours that are located adjacent to the wall, do not have posterior dark shadowing, and change position (25).

The most appropriate method for spleen imaging and screening, including malignancy and hematological diseases, is US. To evaluate spleen diseases, it is necessary to know the normal spleen size specifically according to race, gender, and community characteristics. The average accessory spleen incidence rate in Turkish society is 2.5%. The average spleen size in Turkish society is 10.76 cm. Above this size can be evaluated as splenomegaly. Detection of a mass, cyst, and splenomegaly in the spleen requires evaluation in terms of hematological and malignant diseases (26). The rate of splenomegaly in our cases was 1.6%.

CT and MRI examinations are quite effective in imaging the pancreas, but the pathological images that can be detected on US allow switching to these advanced imaging techniques and enable the detection of cystic and solid lesions of the pancreas at an early stage (27).

US is the first and gold standard examination in liver imaging. NAFLD is divided into two subtypes: simple steatosis and nonalcoholic steatohepatitis. Most cases of simple steatosis are not progressive, while nonalcoholic steatohepatitis can cause chronic liver damage and progressive fibrosis. Liver biopsy is the gold standard in the diagnosis of NAFLD, but noninvasive imaging methods are rapidly developing and can replace biopsy in some cases. These include newer imaging technologies such as US, US elastography, CT and MRI, and MRI-based fat quantification techniques (28). Fatty infiltration is seen as increased echogenicity in B-mode US. The degree of steatosis in the liver is usually reported as normal, mild steatosis, moderate steatosis, and severe steatosis after 8 hours of fasting (29). The most common benign liver tumor is hemangioma. It is typically seen as a hyperechogenic focus compared to the liver parenchyma. Cystic liver lesions are detected as anechoic lesions on US. However, cystic or necrotic malignant tumors or metastases should be considered for differential diagnosis. Hydatid cysts or cystadenomas should be considered in the differential diagnosis of simple-looking cysts. Benign liver tumor origin includes adenomas and Focal Nodular Hyperplasia (FNH). Adenomas developing due to estrogen-progesterone therapy can lead to intratumoral or intraperitoneal hemorrhage or, more rarely, Hepatocellular Carcinoma (HCC) degeneration. Therefore, early diagnosis is important. They are usually seen as hypoechoic masses with regular contours on US. For differential diagnosis, patient history and dynamic MRI examination are very useful. However, imaging cannot provide a definitive positive diagnosis of adenoma (30). Typical FNH is often seen on US as foci with unclear boundaries and slight echogenicity changes compared to the liver parenchyma. The lesion may be observed as slightly hypoechoic, isoechoic, or slightly hyperechoic. A hypoechoic halo may be observed due to compression of the parenchyma around FNH, especially in the setting of fatty liver. Doppler US application provides additional data on the vascularity of lesions suspected of FNH. It is very important to diagnose FNH to avoid unnecessary surgery. The

Table 4: Distribution of Liver and Gallbladder Findings by Gender

		Male (n:714)				Female (n:697)				p	
		Mean±ss/n-%			Median	Mean±ss/n-%			Median		
Age		50.8	±	13.2	51.0	49.8	±	13.2	49.0	0.128	m
CEA		2.1	±	1.5	1.8	1.9	±	1.8	1.4	0.000	m
Liver Hepatostetosis	(-)	241		33.8%		359		51.5%		0.000	X²
	Grade I	335		46.9%		253		36.3%			
	Grade II	118		16.5%		71		10.2%			
	Grade III	20		2.8%		14		2.0%			
Liver Hepatomegaly	(-)	528		73.9%		579		83.1%		0.000	X²
	(+)	186		26.1%		118		16.9%			
Liver Hemangioma	(-)	702		98.3%		678		97.3%		0.044	X²
	One	12		1.7%		13		1.9%			
	More than one	0		0.0%		6		0.9%			
Liver Cyst	(-)	694		97.2%		673		96.6%		0.073	X²
	One	17		2.4%		13		1.9%			
	More than one	3		0.4%		11		1.6%			
Gallbladder Stone	(-)	681		95.4%		649		93.1%		0.067	X²
	(+)	33		4.6%		48		6.9%			
Gallbladder Polyps	(-)	659		92.3%		664		95.3%		0.026	X²
	One	47		6.6%		24		3.4%			
	More than one	8		1.1%		9		1.3%			
Gallbladder Sludge	(-)	684		95.8%		686		98.4%		0.003	X²
	(+)	30		4.2%		11		1.6%			

[m] Mann-Whitney u test / [X²] Ki-square test

Table 5: Distribution of kidney findings by gender

		Male (n:714)		Female(n:697)		p	
		n	%	n	%		
Right Kidney Stone	(-)	698	97.8%	684	98.1%	0.619	X²
	One	16	2.2%	11	1.6%		
	More Than one	0	0.0%	2	0.3%		
Right Kidney Cyst	(-)	616	86.3%	640	91.8%	0.001	X²
	One	97	13.6%	57	8.2%		
	More than one	1	0.1%	0	0.0%		
Left Kidney Stone	(-)	694	97.2%	679	97.4%	0.800	X²
	One	20	2.8%	18	2.6%		
Left Kidney Cyst	(-)	622	87.1%	641	92.0%	0.003	X²
	One	90	12.6%	56	8.0%		
	More than one	2	0.3%	0	0.0%		

X² Ki-square test

sensitivity and specificity values of contrast-enhanced MRI in the diagnosis of FNH have been reported as 70% and 98% (31-32).

The first method used in the evaluation of the adrenal gland, kidney, bladder, and prostate is US. Bladder wall thickness, trabeculation, presence of diverticula, internal structure, pathologies such as stones, and intraluminal hematoma mass can be distinguished with US in a full bladder. Prostate dimensions and volume are measured to give an idea about prostate growth (33).

If a solid mass detected in the kidney on US does not contain fat and is seen to have a heterogeneous complex internal structure, RCC, oncocytoma and fat-poor adenoma should be considered in the diagnosis. The fat content of a mass can be easily distinguished in cross-sectional imaging methods such as MRI and CT. The most helpful modality in differential diagnosis is MRI (34).

US, CT, and MRI are used as imaging methods in the evaluation of gynecological pathologies. In gynecological oncological cases, US is the first-choice imaging method (35).

Table 6: Distribution of other intra-abdominal findings and FOB(+) status according to gender

		Male(n:714)		Female(n:697)		p	
		n	%	n	%		
Pancreas	Normal	709	99.3%	694	99.6%	0.500	X²
	Steatoz	5	0.7%	2	0.3%		
	Other benign findings	0	0.0%	1	0.1%		
Stomach	Normal	711	99.6%	685	98.3%	0.147	X²
	Antral wall thickening	3	0.4%	12	1.7%		
Spleen	Normal	693	97.1%	685	98.3%	0.147	X²
	Splenomegaly	13	1.8%	10	1.4%		
	Other benign findings	8	1.1%	2	0.3%		
Bladder	Normal	693	97.1%	674	96.7%	0.023	X²
	Cystitis	6	0.8%	16	2.3%		
	Other benign findings	15	2.1%	7	1.0%		
FOB	(-)	404	94.8%	373	94.9%	0.961	X²
	(+)	22	5.2%	20	5.1%		

X² Ki-square test

Lung cancer is the leading cause of cancer-related death in the United States. Chest radiography is important in the initial evaluation. The National Lung Screening Trial showed that Low-Dose CT (LDCT) can reduce lung cancer deaths by 20% in high-risk patients compared with chest radiography. Patients with suspicious lesions on chest radiography may continue their evaluation with LDCT (36).

BIRADS has high diagnostic accuracy rates in distinguishing benign/malignant breast lesions (37). In our study, BIRADS-4/5 lesions were detected in approximately 0.28% of the cases that underwent breast US and 0.8% who underwent mammography, and biopsy was recommended.

TIRADS has high diagnostic accuracy rates in distinguishing benign/malignant thyroid nodules. Accordingly, biopsy should be performed in TIRADS-4 lesions. Studies comparing thyroid nodules reported according to the TIRADS with histopathological evaluation results have determined high diagnostic accuracy rates (38). In our study, the TIRADS-4 lesion rate was 3.8%.

The fact that 7% of cases who underwent colonoscopy due to FOB positivity had colorectal malignancy, and 19% had adenomatous polyps shows the importance of FOB positivity. Thanks to screening programs, colorectal cancers and precancerous lesions can be diagnosed early, and survival rates can be increased (39). In our study, FOB positivity was 3%.

The risk of developing colorectal cancer is higher in healthy individuals, especially those with anemia and CEA levels of 5 ng/ml and above than those with low CEA levels.

Anemia is an independent predictive factor in this case (40). In our cases, no significant increase in CEA level was detected.

CONCLUSION

Check-ups are not routinely implemented. Our study includes individuals who voluntarily participated in a health screening program. Although the sociocultural positions of these people do not represent the entire society, the results indicate that health screening programs can be effective in preventing complications that may occur due to benign lesions while indicating that premalignant lesions can be diagnosed early before malignancy develops. Therefore, check-ups significantly increase the quality of life and will be used more widely as health awareness increases in society.

Ethics Committee Approval: The approval of this study in accordance with the ethical rules of the Declaration of Helsinki was approved by the ethics committee of İstanbul Okan University at the meeting numbered 169 on 18.10.2023 with decision number 35.

Informed Consent: The study was conducted retrospectively.

Authorship Contributions: Concept: DD, KG, Design: DD, KG, Supervising: DD, Data collection and entry: DD, Analysis, and interpretation: KG, Literature search: DD, KG, Writing: DD, KG, Critical review: KG.

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Original Article / Araştırma Makalesi

IS PROGESTERONE SUPPLEMENTATION NECESSARY FOR LUTEAL PHASE SUPPORT IN MODIFIED NATURAL CYCLE FROZEN EMBRYO TRANSFERS?

MODIFIYE DOĞAL SIKLUS DONDURULMUŞ EMBRIYO TRANSFERLERINDE LUTEAL FAZ DESTEĞI IÇIN PROGESTERON TAKVIYESI GEREKLI MIDIR?

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ABSTRACT

Introduction: This study aimed to evaluate the effect of progesterone supplementation as luteal phase support on pregnancy outcomes in women under thirty-eight years of age undergoing modified natural cycle frozen embryo transfer.

Methods: A retrospective analysis was conducted on 2216 modified natural cycle frozen embryo transfers performed at Sisli Memorial Hospital, Assisted Reproductive Technology (ART), and Reproductive Genetics Centre between 2011 and 2023. The study included women under thirty-eight who transferred a single embryo, classified as top quality or good quality. Cycles involving medium-quality or poor-quality embryos, double embryo transfers, and preimplantation genetic testing for aneuploidy were excluded. Participants were categorised into three groups: Group A (n=493) with no luteal phase support, Group B (n=1327) receiving 200 mg of vaginal micronised progesterone twice daily, and Group C (n=396) receiving 200 mg of vaginal micronised progesterone plus 25 mg of subcutaneous progestin daily. Statistical analysis was performed using SPSS 22.

Results: Demographic and fresh cycle characteristics were similar among groups. There were no statistically significant differences in pregnancy outcomes: live birth rates were 58.4% (A), 60.8% (B), and 60.1% (C) (p=0.650); clinical pregnancy rates were 65.9% (A), 69.1% (B), and 68.2% (C) (p=0.432); biochemical abortion rates were 4.5% (A), 6.6% (B), and 5.3% (C) (p=0.186); and clinical abortion rates were 6.3% (A), 6.7% (B), and 5.3% (C) (p=0.828).

Conclusions: Modified natural cycle frozen embryo transfers in women under 38 years of age showed similar pregnancy outcomes regardless of using progesterone for luteal phase support.

Keywords: Modified natural cycle frozen embryo transfer (mNC-FET), Luteal phase support, Progesterone supplementation

ÖZET

Giriş: Bu çalışmanın amacı, modifiye doğal siklusta dondurulmuş embriyo transferi yapılan otuz sekiz yaş altı kadınlarda luteal faz desteği olarak progesteron takviyesinin gebelik sonuçları üzerindeki etkisini değerlendirmektir.

Yöntemler: Şişli Memorial Hastanesi, Üremeye Yardımcı Teknoloji (ÜYTE) ve Üreme Genetiği Merkezinde 2011-2023 yılları arasında gerçekleştirilen 2216 modifiye doğal siklusta yapılmış dondurulmuş embriyo transfer siklusu retrospektif olarak analiz edildi. Çalışmaya, otuz sekiz yaşın altında, en iyi kalite veya iyi kalite olarak sınıflandırılan tek embriyo transferi yapılan kadınlar dahil edildi. Orta kaliteli veya düşük kaliteli embriyoları içeren sikluslar, çift embriyo transferleri ve anöploidi için preimplantasyon genetik testi yapılan sikluslar hariç tutuldu. Vakalar üç gruba ayrılarak incelendi. Luteal faz desteği almayan Grup A (n=493), günde iki kez 200 mg vajinal mikronize progesteron alan Grup B (n=1327) ve günde 200 mg vajinal mikronize progesteron artı 25 mg subkutan progesteron alan Grup C (n=396). İstatistiksel analiz SPSS 22 kullanılarak gerçekleştirilmiştir.

Bulgular: Demografik ve siklus özellikleri gruplar arasında benzerdi. Gebelik sonuçlarında istatistiksel olarak anlamlı bir fark yoktu: canlı doğum oranları %58,4 (A), %60,8 (B) ve %60,1 (C) (p=0,650); klinik gebelik oranları %65,9 (A), %69,1 (B) ve %68,2 (C) (p=0,432); biyokimyasal düşük oranları %4,5 (A), %6,6 (B) ve %5,3 (C) (p=0,186); ve klinik düşük oranları %6,3 (A), %6,7 (B) ve %5,3 (C) (p=0,828).

Sonuç: Modifiye edilmiş doğal siklusta dondurulmuş embriyo transferi yapılan 38 yaşın altındaki kadınlarda luteal faz desteği için progesteron kullanılmasına bakılmaksızın benzer gebelik sonuçları olduğu görülmüştür.

Anahtar Kelimeler: Modifiye doğal siklusta dondurulmuş embriyo transferi, luteal faz desteği, progesteron takviyesi.

INTRODUCTION

Progesterone is a crucial hormone in regulating the female reproductive system, significantly contributing to maintaining the luteal phase and the early stages of pregnancy (1). Progesterone, predominantly synthesised by the corpus luteum after ovulation, is crucial for conditioning the endometrium and embryo implantation (2). Inadequate progesterone levels during the luteal phase can lead to implantation failure or early miscarriage (3-5). In an artificial or hormone replacement cycle, which is one of the endometrial preparation protocols for frozen embryo transfer

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cycles, exogenous progesterone is routinely administered because there is no endogenous progesterone production. However, progesterone-secreting corpus luteum supports the luteal phase in a natural or modified natural cycle frozen embryo transfer (mNC-FET). Therefore, the need for additional progesterone supplementation in these cases is unclear. Numerous studies suggest that exogenous progesterone supplementation may be unnecessary in women undergoing mNC-FET cycles, as the corpus luteum is expected to produce adequate endogenous progesterone (6-9). Nonetheless, some clinicians persist in prescribing progesterone throughout the luteal phase due to concerns about luteal phase insufficiency, believed to be a possible contributor to embryo implantation failure and early pregnancy loss (10,11). Different methods in clinical practice perform progesterone administration as luteal phase support. One method typically involves the use of vaginal progesterone, which is preferred because of its simple administration and the high local concentrations achieved in the endometrium. However, alternative procedures combine vaginal progesterone with subcutaneous progestins to improve systemic progesterone concentrations. In some cases, especially in patients who do not respond adequately to vaginal therapy, intramuscular progesterone is preferred because of its reliable absorption and prolonged release into the bloodstream.

We aimed to assess whether progesterone supplementation is essential for luteal phase support (LPS) in patients undergoing mNC-FET cycles. This research has important clinical implications. In mNC-FET cycles, if the corpus luteum produces sufficient endogenous progesterone to sustain the luteal phase, it may be possible to streamline treatment protocols by eliminating unnecessary progesterone supplementation. It may be possible to simplify treatment protocols by eliminating unnecessary progesterone supplementation. Such an approach could improve patient comfort, reduce healthcare costs, and facilitate ART procedures without compromising pregnancy outcomes. Furthermore, determining whether progesterone supplementation is necessary for mNC-FET cycles may contribute to improving clinical guidelines and developing individualised treatment strategies for women undergoing FET.

METHODS

Ethical approval: The Institutional Review Board of Istanbul Memorial Sisli Hospital, Istanbul, Turkey, accepted this study. (Approval number: 28.06.2024/003).

This retrospective study examines 2216 mNC-FET cycles between 2011 and 2023. It was conducted at Sisli Memorial Hospital, Assisted Reproductive Technology (ART), and Reproductive Genetics Centre. This investigation aimed to ascertain whether progesterone supplementation is essential to the luteal phase in mNC-FET cycles. For analysis purposes, the mNC-FET cycles were categorised into three distinct groups: The luteal phase was not supported for participants in Group A, which consisted of 493 cycles. In Group B, consisting of 1327 cycles, a vaginal dose of 200 mg micronised progesterone was administered two times a day. Group C, consisting of 396 participants, received 25 mg progesterone subcutaneous injection in addition to progesterone vaginal capsules two times daily. The cycle characteristics and pregnancy outcomes of the groups were compared. The study focused on women under the age of 38 who underwent single embryo transfer (SET) in mNC-FET cycles, specifically involving embryos of top quality (TQ) or good quality (GQ). Embryo transfers involving medium-quality (MQ) or poor-quality (PQ) embryos were excluded from the analysis. The study excluded cases of double embryo transfer, cycles employing preimplantation genetic testing, and women with Müllerian abnormalities. Furthermore, patients with untreated endocrine disorders or with endometrial thickness below 7 mm on the day ovulation was triggered were excluded from the study.

Controlled ovarian hyperstimulation was initiated on the second day of the cases' menstrual cycle. Starting dosages were based on patient characteristics. Ovarian stimulation was performed as described in our previous study (12). 250 mcg of recombinant human chorionic gonadotropin (r-hCG) (Ovitrelle; Merck, Switzerland) or GnRH analog (Gonapeptyl®, lucrin®) was administered to trigger ovulation. Thirty-six hours after administering the trigger medication, the oocyte collection procedure was performed using transvaginal ultrasound (TVUSG) guidance.

mNC/ FET cycle

Patients were checked with TVUSG on the 2nd day of menstruation for mNC-FET cycle preparation. In cases with normal ultrasound findings, i.e., no hormone-secreting cyst or any pathology was found to affect the endometrial cavity, follicle follow-up was started to determine the time of ovulation. E2 and LH levels were analysed when the follicle size reached when LH reached 15 IU/L and above a specific level, a single subcutaneous dose of r-hCG was administered to trigger it. In cases where LPS was recommended, progesterone treatment was started 2 days after triggering. Blastocyst transfer was performed 6 days after trigger. Following blastocyst transfer, a pregnancy test was administered after 9 days. For patients who tested positive, LPS was maintained through the 10th week of gestation.

Embriyo grading

Embryo morphological evaluation was performed using the classification protocol established by Gardner et al. Embryos with 3AA-4AA-5AA-6AA were classified as TQ, and embryos with 3AB-4AB-5AB-6AB-3BA-4BA-4BA-5BA,6BA were classified as GQ. This study excluded low or medium-

quality embryos. Freezing followed the manufacturer's guidelines utilising Kitazato Vitrification Medium (Kitazato, Japan). Kitazato Warming Medium was used to thaw the blastocysts. Any thawed embryos that exhibited a decrease in grade were excluded from the study.

Pregnancy outcomes

Beta-human chorionic gonadotropin (β-hCG) level equal to or exceeding 20 IU/L was utilised as the threshold for defining a biochemical pregnancy. The occurrence of a biochemical pregnancy loss was characterised by the detection of serum β-hCG levels that did not progress to a clinically recognisable pregnancy. Ultrasonography was used to detect a fetal heartbeat, which indicated a clinical pregnancy. The absence of a fetal heartbeat in a pregnancy that had been previously confirmed as clinical was considered a clinical pregnancy loss. The live birth rate (LBR) was calculated as the number of live births per embryo transfer cycle.

Statistical Analysis

SPSS 22 was used for statistical analysis. Results were provided as mean ± standard deviation for variables with a normal distribution. However, numerical variables without a normal distribution were reported as medians with minimum and maximum values. Categorical variables were shown as frequencies and percentages to simplify the statistical presentation. The descriptive statistical methods were evaluated using the Shapiro-Wilk test and boxplot diagrams. Non-normally distributed metric variables were analysed using the Kruskal-Wallis test. Categorical data were evaluated among groups utilising the chi-square test and a post hoc Bonferroni adjustment. Statistical significance was determined as a p-value of < 0.05.

RESULTS

The study comprised three groups based on the type of luteal phase support: group A without progesterone (n = 493, 22.2%), group B receiving vaginal progesterone tablets (n = 1327, 59.9%), and group C administered a combination of vaginal progesterone tablets and subcutaneous progesterone (n = 396, 17.9%) Figure 1. Patient demographics and clinical features are shown in Table 1. No substantial difference was observed among the three groups regarding, male age, female age, body mass index (BMI), duration of infertility, anti-Müllerian hormone levels, number of frozen embryos, number of collected oocytes, number of Metaphase II (MII) oocytes, and number of fertilised oocytes (PN2) (p > 0.05). When the groups were compared in terms of endometrial thickness, the mean endometrial thickness in the vaginal progesterone group was 10.77 ± 3.24 mm. In the subcutaneous progesterone group, the mean endometrial thickness was 10.72 ± 1.88 mm, and a statistically significant difference was found (p = 0.047). The study did not identify any significant differences in the rate of biochemical pregnancy, clinical pregnancy, biochemical pregnancy loss, clinical pregnancy loss, ongoing pregnancy, and live births among the three groups (p values>0.05).

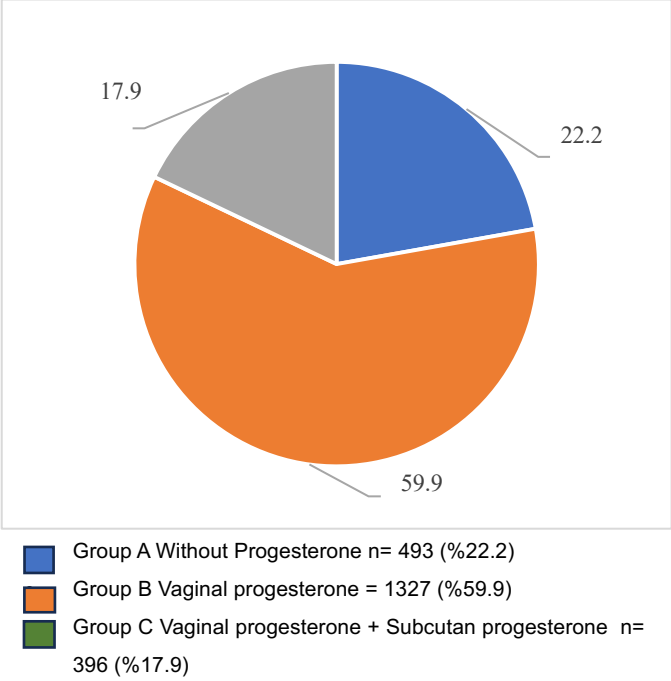

Figure 1. Cycle distribution based on luteal phase support

DISCUSSION

This study assessed the necessity of LPS with progesterone in mNC-FET cycles and its effect on pregnancy outcomes. Our findings suggest that progesterone supplementation, whether administered vaginally or in combination with subcutaneous progestin, does not significantly improve clinical outcomes compared to cycles without progesterone support. Notably, live birth rates and clinical pregnancy rates remained statistically similar across all groups, indicating that LPS with progesterone may not be essential in mNC-FET cycles.

The evidence for the need for progesterone supplementation for LPS in-modified natural cycle frozen embryo transfer is mixed. Some studies support its use to improve live birth rates, while others have shown that progesterone production by the corpus luteum is sufficient, and progesterone supplementation is not required. A randomised controlled trial by Horowitz et al. showed that vaginal progesterone supplementation did not significantly enhance clinical pregnancy rates compared to no supplementation in mNC-FET cycles, suggesting that the need for exogenous progesterone may be less critical in these particular conditions(6). In a systematic review and meta-analysis based on randomised controlled trials, the authors showed that moderate-quality evidence indicated that progesterone supplementation for LPS was associated with increased live birth rates and clinical pregnancy rates in

Table 1. Comparison of demographics and cycle characteristics of patients

	Group A		Group B		Group C		p
	Mean ± SD	Med.	Mean± SD	Med.	Mean± SD	Med.	
Female age (years)	30.93 ± 3.73	31	30.91 ± 3.72	31	30.96 ± 3.88	31	0.884
Male age (years)	30.93 ± 3.73	34	34.44 ± 4.68	34	34.54 ± 4.47	34	0.697
BMI (kg/m^2)	24.38 ± 4.23	23.6	24.29 ± 4.34	23.6	24.72 ± 4.89	23.9	0.490
Duration of infertility (years)	3.84 ± 3.08	3	3.53 ± 2.80	3	3.90 ± 3.10	3	0.080
AMH (ng/ml)	3.26 ± 2.41	2.80	3.26 ± 2.37	2.70	3.26 ± 2.28	2.68	0.929
Endometrial thickness (mm)	10.49 ± 1.83	10	10.77 ± 3.24	10.50	10.72 ± 1.88	10.65	0.047
Number of embryos frozen	5.54 ± 3.64	5	5.85 ± 3.72	5	6.09 ± 4.13	5	0.102
Number of retrieved oocytes	14.93 ± 8.22	13	14.71 ± 8.05	13	14.74 ± 7.92	13	0.847
Number of Metaphase II oocytes (MII)	12.58 ± 6.82	11	12.56 ± 6.79	12	12.64 ± 6.89	11	0.991
Number of Fertilized oocytes (PN2)	10.40 ± 5.80	9	10.31 ± 5.81	9	10.46 ± 5.91	9	0.886

AMH= anti Mullerian hormone, BMI=body mass index, SD: Standard Deviation, Med.: Median.

true NC-FET cycles. However, they noted that the efficacy of progesterone supplementation in mNC-FET cycles needs to be further validated by conducting large, randomised controlled trials (10). Recent studies suggest that LPS via additional progesterone supplementation may be unnecessary even in natural cycles where ovulation occurs without exogenous r-hCG administration. Li et al. showed that the pregnancy outcomes of NC FET with or without LPS were similar. The authors stated that the women's age was the most critical factor affecting the clinical pregnancy rates (13). Waldman et al. also found that using progesterone to support the luteal phase in cryopreserved blastocyst transfers for true natural cycles did not significantly affect the number of ongoing pregnancies. This suggests that natural cycles may not necessitate additional progesterone support (14).

Some studies contradict our results. In a retrospective study of 231 cases, Schwartz et al. compared the groups with and without progesterone as LPS in mNC/FET cycles. They reported higher live birth rates in the progesterone group. However, in this study, both cleavage periods and blastocyst transfers were performed and without any information about embryo quality. Since the existing literature shows the effect of embryo quality on pregnancy outcomes (12,15,16), this may have affected the results. Our study compared only the 2216 TQ/GQ blastocyst transfer results to avoid bias and evaluate progesterone supplementation's effectiveness.

Most studies recommending progesterone support as LPS are true natural cycles, not mNC/FET cycles (4,8,17). In a mNC/FET cycle, the administration of human chorionic gonadotropin (hCG) serves a dual purpose: it not only triggers ovulation but also enhances serum progesterone (P4) production during the early and mid-luteal phases. support (18). Therefore, progesterone support in mNC/FET cycles is unnecessary, especially in cases under the age of thirty-eight. Luteal phase defects may occur at older age (19). Therefore, we compared the groups by including younger patients in our study.

One of the study's strengths is the large sample size and well-defined inclusion criteria, particularly the inclusion of only TQ and GQ embryos under the age of 38. Nonetheless, the study’s retrospective design limits the ability to establish causal relationships definitively.

CONCLUSION

In conclusion, our study suggests that in mNC-FET cycles among women under 38, the natural luteal support provided by hCG-triggered ovulation and corpus luteum activity may suffice, making additional progesterone supplementation unnecessary. Additionally, progesterone supplementation can cause physical discomfort. Therefore, the decision to use progesterone should be individualised based on patient-

Table 2. Comparison of pregnancy outcomes

Outcomes of FET cycles	Group A	Group B	Group C	Test	P value
Biochemical Pregnancy	347 (70.4)	1005 (75.7)	291 (73.5)	5.474	0.065
Clinical Pregnancy	325 (65.9)	917 (69.1)	270 (68.2)	1.678	0.432
Biochemical Pregnancy Loss	22 (4.5)	88 (6.6)	21 (5.3)	3.361	0.186
Clinical Pregnancy Loss	31 (6.3)	89 (6.7)	29 (7.3)	0.377	0.828
Ongoing Pregnancy	294 (59.6)	828 (62.4)	241 (60.9)	1.243	0.537
Live Birth	288 (58.4)	807 (60.8)	238 (60.1)	0.861	0.650

specific factors and clinical judgment. To support these findings further, prospective randomised controlled trials are essential, as they would provide more evidence on the necessity of progesterone LPS in mNC-FET cycles. Future studies might also investigate potential subgroups that could benefit from LPS or explore different LPS regimens in modified natural cycle protocols.

Ethics Committee Approval: The Institutional Review Board of Istanbul Memorial Sisli Hospital, Istanbul, Turkey, accepted this study. (Approval number: 28.06.2024/003).

Informed Consent: No need.

Authorship Contributions: SO, and GO conceptualised and designed the study. SO performed the analyses while GO prepared the initial manuscript draft. SO also contributed to data collection result interpretation and supported further data analysis. All authors were involved in drafting, revising, and approving the final version of the manuscript.

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

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Original Article / Araştırma Makalesi

SELECTIVE FETAL REDUCTION; PROCEDUREL FACTORS ASSOCIATED WITH ADVERSE PREGNANCY OUTCOME

SEÇİCİ FETAL REDÜKSİYON; OLUMSUZ GEBELİK SONUÇLARIYLA İLİŞKİLİ PROSEDÜREL FAKTÖRLER

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ABSTRACT

Introduction: The aim of this study was to investigate the factors associated with selective fetal reduction (SFR) procedures that result in adverse pregnancy outcomes.

Methods: The study cohort comprises all multiple pregnancies that underwent SFR during the period of six years. The SFR procedure has been performed for two main indications: first, in cases of fetal anomaly; and secondly, electively to reduce the number of fetuses in triplet and higher-order pregnancies. Preterm birth or preterm premature rupture of the membranes prior to 34 weeks of gestation, placental abruption, pregnancy loss before 24 weeks of gestation, and intrauterine fetal death defined as adverse pregnancy outcomes. Procedural factors associated with adverse pregnancy outcomes were evaluated.

Results: A total of 39 SFR procedures were performed on 33 multiple pregnancies, with 31 resulting in live birth. A higher rate of adverse pregnancy outcomes was observed in pregnancies that underwent elective SFR, more than one procedure , were having triplets or higher-order pregnancies prior to the procedure, or were having twin or higher-order pregnancies post-procedure. Elective SFR procedures and multiple procedures have been demonstrated to be associated with an 8-fold and a 13.3-fold increased risk of adverse pregnancy outcomes. The risk ratio of triplet or higher-order pregnancies prior to the procedure and twin or higher-order pregnancies post-procedure was found to be 6.5 and 5.8, respectively, for adverse pregnancy outcomes.

Conclusions: Instead of considering SFR as the first option in the management of higher order pregnancies, it is recommended that assisted reproductive technologies be used in a way that does not lead to high-order pregnancies. In cases where the prevention of a higher order pregnancy has not been possible, SFR should be considered in terms of its risks and benefits as a method of reducing adverse pregnancy outcomes.

Keywords: Selective fetal reduction, multifetal pregnancy, higher order multiple pregnancy, multifetal pregnancy reduction, perinatal outcome

ÖZET

Giriş: Bu çalışmanın amacı, seçici fetal redüksiyon (SFR) uygulamalarında olumsuz gebelik sonuçlarına yol açan prosedürel faktörleri araştırmaktır.

Yöntemler: Çalışma kohortu, altı yıllık süre boyunca SFR uygulanan tüm çoğul gebelikleri içermektedir. SFR prosedürü iki ana endikasyon için uygulanmıştır: birincisi, fetal anomali vakalarında; ikincisi ise, üçüz ve daha yüksek dereceli gebeliklerde fetüs sayısını azaltmak için elektif olarak. 34. gebelik haftasından önce preterm doğum veya preterm prematür membran rüptürü, plasental abrupsiyon, 24. gebelik haftasından önce gebelik kaybı ve intrauterin fetal ölüm olumsuz gebelik sonuçları olarak tanımlanmıştır. Olumsuz gebelik sonuçları ile ilişkili prosedürel faktörler değerlendirilmiştir.

Bulgular: 33 çoğul gebeliğe toplam 39 SFR işlemi uygulanmış ve bunların 31'i canlı doğumla sonuçlanmıştır. Elektif endikasyon ile SFR uygulanan, birden fazla işlem uygulanan, işlem öncesinde üçüz veya daha yüksek dereceli veya işlem sonrasında ikiz veya daha yüksek dereceli gebeliklerde daha yüksek oranda olumsuz gebelik sonuçları gözlenmiştir. Elektif SFR prosedürlerinin ve birden fazla işlemin olumsuz gebelik sonuçları riskinde 8 kat ve 13,3 kat artışla ilişkili olduğu gösterilmiştir. İşlem öncesinde üçüz veya daha yüksek dereceli gebeliğe ve işlem sonrasında ikiz veya daha yüksek dereceli gebeliğe sahip olmak olumsuz gebelik sonuç riskini sırasıyla 6,5 ve 5,8 kat arttırmaktadır.

Sonuç: Seçici fetal redüksiyon işleminin yüksek dereceli gebeliklerin yönetiminde ilk seçenek olarak görülmesi yerine, yardımcı üreme teknolojilerinin yüksek dereceli gebeliklerin oluşmasına yol açmayacak şekilde kullanılması önerilmektedir. Yüksek dereceli gebeliğin önlenmesinin mümkün olmadığı durumlarda, olumsuz gebelik sonuçlarını azaltmanın bir yöntemi olarak seçici fetal redüksiyon risk ve faydaları açısından değerlendirilmelidir.

Anahtar Kelimeler: Seçici fetal redüksiyon, multifetal gebelik, yüksek dereceli çoğul gebelik, multifetal gebelik redüksiyonu, perinatal sonuçlar

INTRODUCTION

Monozygotic twining occurs at a constant rate of approximately 4 per 1000 (1/250). Conversely, dizygotic twining rates have been demonstrated to vary according to a number of individual characteristics, including race (low in Asians, high in blacks), increases with advanced maternal age and parity, and family history, especially on the maternal side (1). Recent decades have seen a notable increase in the incidence of multiple pregnancies, largely attributable to the advanced materanal age and increased utilisation of assisted reproductive technologies such as in vitro

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fertilization and ovulation induction (2). Despite the implementation of some measures have been taken to reduce multiple pregnancy rates, such as restricting the number of embryos transferred, this increase could not be completely prevented due to gonadotropin use and embryo splitting (3).

It is an established fact that the perinatal outcomes of multiple pregnancies are not equivalent to those of singleton pregnancies. In addition to higher perinatal mortality and morbidity due to increased preterm birth rates, maternal complications such as preeclampsia, gestational diabetes, and postpartum haemorrhage are also more common than in singleton pregnancies (4). The afformentioned risks are more pronounced in higher-order multiples, with one third of triplets expected to give birth prior to 32 weeks (5). Due to the reduced viability of the aneuploid fetus chromosomal anomalies are less prevalent in multiple pregnancies than in singleton pregnancies. However, structural anomalies are more common in multiples and usually affect a single fetus (6). In the cases of multiple pregnancies accompanied by an anomaly in one of the fetuses, the healthy fetus is more prone to elevated risks such as low birth weight and preterm birth.

Fetal reduction is an intervention employed in cases of multiple gestations with the objective of reducing the overall number of fetuses. It is achieved by inducing termination of one or more fetuses, with the intention of enhancing pregnancy outcomes (2). Selective termination is also performed in multiple gestations where one fetus exhibits severe fetal growth restriction or discordant fetal anomalies in order to improve the outcomes of the healthy fetus (7). Although the term 'selective termination' is used for fetuses of advanced gestational age, the term 'selective fetal reduction' is an overall term that includes selective termination.

The primary factor determining the method employed in selective termination is chorionicity. In pregnancies uncomplicated with monochorionicity, asystole is achieved within a short time with ultrasound-guided intracardiac, intrathoracic or umbilical vein injection of KCl. This procedure can be performed even in advanced gestational ages, with a low incidence of complications (8, 9). However, in the event of reduction being performed on a fetus with a monochorionic pair, methods such as bipolar cord coagulation, radiofrequency ablation or intrafetal laser ablation should be used to prevent neurological damage or fetal death in the healthy twin pair (10).

The present study examined the results of selective fetal reduction procedures performed by potassium chloride (KCl) injection in a tertiary perinatology center retrospectively. The objective of the research was to investigate the procedural factors that contribute to adverse pregnancy outcomes.

METHODS

This retrospective cohort study evaluated all selective fetal reduction cases in the Perinatology Clinic of Zeynep Kamil Women and Children Diseases Training and Research Hospital. The study protocol was approved by the institutional ethical committee (Approval number:168, Date:20.12.2023). All procedures were conducted in accordance with the Declaration of Helsinki, and informed consent was obtained for all examinations and procedures.

The study group consists of multiple pregnancies that underwent selective fetal reduction between January 2018 and December 2024. Patients with known uterine anomalies, cervical insufficiency or a history of preterm labour were excluded from the study. The demographic and clinical information of the patients was obtained from the electronic archives or patient files.

The selective fetal reduction procedure was performed for two primary indications. Firstly, the procedure was performed when there was a structural or genetic anomaly that could result in severe sequelae or be incompatible with life in one of the fetuses in a multiple pregnancy. Secondly, the procedure was performed electively in triplets and higher order pregnancies, with the objective of reducing the complications associated with multiple pregnancies. All potential risks associated with multiple pregnancies and selective fetal reduction procedures were thoroughly explained to the family, and informed consent was obtained from all patients prior to the procedure. The decision of the number of live fetus(es) to be preserved subsequent to the procedure was established on the basis of patient preference, technical applicability and chorionicity. The fetus(es) to be reduced was decided according to the presence of fetal anomaly or the ease of application of the procedure.

Prior to the procedure, anatomical screening was performed on all fetuses, irrespective of gestational age. All selective fetal reduction procedures were performed transabdominally by an expert perinatologist. The selective fetal reduction procedure was performed with ultrasound guidance using a 20-G spinal needle. The injection of KCl was administered either intracardiac or intrathoracic, or, if the gestational age was appropriate, into the umbilical vein. Fetal cardiac activity was observed for a minimum of two minutes; if this persisted, an additional KCl injection was administered. The procedure was terminated after ensuring asystole. If more than one fetus was to be reduced, the procedure was repeated for each fetus. Following the procedure, a re-evaluation of all patients was conducted after a 2-hour observation period. The determination of asystole in the fetus(es) that underwent reduction and continuation of cardiac activity in the other fetus(es) was deemed to be indicative of procedural success. In the period of one week following the procedure, pain more than

expected, vaginal bleeding and amniotic fluid leakage were considered as acute complications.

The mode of conception, timing and number of the procedure, indication, number of procedures, development of acute complications, multiple pregnancy feature and reducted pregnancy feature were evaluated in terms of their effects on adverse pregnancy outcomes. Adverse pregnancy outcomes are defined as follows: preterm birth or preterm premature rupture of the membranes prior to 34 weeks of gestation, placental abruption, pregnancy loss before 24 weeks of gestation, and intrauterine fetal death. The present study categorised pregnancies resulting from ovulation induction, with or without intrauterine insemination, and in vitro fertilisation as assisted pregnancies. In the timing of the procedure, the first trimester corresponds to the period up to the 14th week of gestation and the second trimester corresponds to the period between 14-28 weeks of gestation. Multiple pregnancy feature prior to selective fetal reduction procedure was categorised as twin and triple or higer-order, the reduced pregnancy feature was categorised as single and twin or higher-order.

The primary outcome of the study was to identify the factors that affect the outcome of the pregnancy. The data for the continuous variables was expressed in terms of mean ± standard deviation or median, together with minimum and maximum values, according to the distribution of the data. Categorical variables were expressed as numbers and percentages. For the comparison of categorical variables Fisher exact test was used due to low expected counts. The relative risk for an adverse pregnancy outcome is subsequently calculated. The statistical analysis was conducted using IBM SPSS Statistics version 22.0 (IBM Corporation, Armonk, New York, United States). The level of statistical significance was established as $p < 0.05$.

RESULTS

Selective fetal reduction procedures were performed on 33 multiple pregnancies over a 6-year period. The total number of procedures carried out was 39. The earliest and most advanced gestational weeks at which the procedure was performed were 100/7 and 260/7 weeks, respectively. Ten complications arising from the procedure itself was observed. None of the acute complications resulted in pregnancy loss. A total of 10 patients experienced adverse pregnancy outcomes. Preterm birth occurred in four patients prior to the 34th week of gestation. Preterm premature rupture of the membranes was observed in three patients before the 34th week of gestation. Abruptio placentae was diagnosed in one patient in the 35th week of pregnancy. Only in one pregnancy that was reduced from quintuplets to triplets at 14 weeks of gestation, pregnancy loss occurred 3 weeks after the procedure. IUMF occurred in a fetus reduced from DKDA twin pregnancy at 23 weeks of gestation due to unknown reason. 43 newborns were born from 31 pregnancies that resulted in live births. Neonatal death occurred in 1 newborn born at 24 weeks of gestation due to severe prematurity. The clinical characteristics of the patients are shown in Table 1.

Table 1. Clinical characteristics of the patients (n=33)

Age (years)	33.9±7.3
Parite (n)	0 [0-3]
Mode of conception	
Spontaneous	8 (24.2)
Ovulation induction	7 (21.1)
In vitro fertilisation	18 (54.5)
Multifetal pregnancy feature	
Dichorionic-diamniotic twin pregnancy	17 (51.5)
Trichorionic-triamniotic triplet pregnancy	8 (24.2)
Dichorionic-triamniotic triplet pregnancy	3 (9.1)
Quadruplet and above multiple pregnancy	5 (15.2)
Fetal Reduction Feature	
Reduction to singleton	20 (60.6)
Reduction to twin	12 (36.4)
Reduction totriplet	1 (3.0)
Gestational week at the procedure	15.2±4.0
Number of performed procedures (needle insertions)	1 [1-3]
Procedural complication	
No	23 (69.7)
Pain	4 (12.1)
Bleeding	1 (3.0)
Amnion leakage	5 (15.2)
Indication	
Fetal anomaly	18 (54.5)
Elective	15 (45.5)
Adverse Pregnancy Outcome	10 (30.3)
Preterm delivery<34th gestational week	4
PPROM<34th gestational week	3
Ablatio placenta	1
Pregnancy loss before 24th gestational week	1
Still birth	1
Pregnancies resulted in live birth	31 (93.9)
Number of live born fetuses	43 (91.5)
GA at birth (weeks)	35,36±3.96
Birth weight (gram)	2458.2±924.9
Way of birth*	
Vaginal	6 (18.8)
Ceserean	26 (81.3)
APGAR 1	7 [3-9]
APGAR 5	9 [5-10]
pH	7.31±0.18
NICU admission**	16 (37.2)
Neonatal death**	1 (2.3)

*one pregnancy loss was excluded **one pregnancy loss and one intrauterine fetal death were excluded. The data was shown as mean±standard deviation; median [min–max]; n (%)

Table 2. Comparison of clinical characteristics associated with multifetal reduction in terms of adverse pregnancy outcomes (n=31)

	Adverse Pregnancy Outcome		
	No (n=21)	Yes (n=10)	p
Mode of conception			0.116
Spontaneous	8 (88.9)	1 (11.1)	
Assisted	13 (59.1)	9 (40.9)	
Reduction timing			0.597
First trimester	12 (66.7)	6 (33.3)	
Second trimester	9 (69.2)	4 (30.8)	
Reduction indication			0.019
Elective	7 (46.7)	8 (53.3)	
Fetal anomaly	14 (87.5)	2 (12.5)	
Acute Complication	7 (77.8)	2 (22.2)	0.375
Multiple procedures (multiple needle insertions)	1 (20.0)	4 (80.0)	0.027
Pregnancy feature before the procedure			0.035
Twin	13 (86.7)	2 (13.3)	
Triplet or higher order	8 (50.0)	8 (50.0)	
Pregnancy feature after the procedure			0.036
Reduction to single	15 (83.3)	3 (16.7)	
Reduction to twin or higher order	6 (46.2)	7 (53.8)	

*one pregnancy loss was excluded **one pregnancy loss and one intrauterine fetal death were excluded. The data was shown as mean±standard deviation; median [min–max]; n (%)

In two out of 33 SFR performed pregnancies, labour occurred before 34 weeks of gestation due to severe pre-eclampsia. Following the exclusion of these two cases, a comparison was made of the clinical characteristics of the remaining 31 patients in terms of adverse pregnancy outcome (Table 2). A higher rate of adverse pregnancy outcomes was observed in pregnancies that underwent elective SFR, had undergone multiple procedures, were having triplets or higher-order pregnancies prior to the procedure, or were having twin or higher-order pregnancies after the procedure ($p<0.05$). The study found that the mode of conception, trimester of multifetal reduction performed, and the development of procedural complications were not significant factors in the occurrence of an adverse pregnancy outcome ($p>0.05$).

The findings of the study indicated that there was an 8-fold and a 13.3-fold increased risk of adverse outcomes when the SFR procedure was performed electively and multiple times. As expected, risk of adverse outcomes was elevated in triplet or higher-order pregnancies before the procedure, and in twin or higher-order pregnancies after the procedure. The risk ratio was determined to be 6.5 and 5.8, respectively. The frequency of adverse pregnancy outcomes was found not to be significantly affected by the mode of conception, the timing of the procedure, or the occurrence of acute procedural complications.

Table 3. Adverse pregnancy outcome risk based on clinical characteristics (n=31)

	RR (95% confidence interval)	Frequency
Assisted pregnancy	2.595 (0.586 – 52.331)	70.9%
Fetal reduction at first trimester	1.125 (0.243 – 5,207)	58.1%
Elective procedure	8.000 (1.328 – 48.183)	48.4.%
Acute Complication	0.500 (0.083 – 3.011)	29.0%
Multiple procedures	13.333 (1.242 – 143.151)	16.1%
Triplet or higher order before the procedure	6.500 (1.094 – 38.633)	51.6%
Twin or higher order after the procedure	5.833 (1.119 – 30.403)	41.9%

RR, Risk ratio

DISCUSSION

It is widely documented that multiple pregnancies are associated with an elevated risk of perinatal complications, including fetal anomalies, hypertensive disorders, and gestational diabetes, compared with singleton pregnancies. The primary issue relating to infant morbidity and mortality is preterm birth. The Royal College of Obstetricians and Gynaecologists and the American College of Obstetricians and Gynecologists (ACOG) recommend the discussion of selective fetal reduction as a potential procedure for the reduction of perinatal complications in higher-order pregnancies (11, 12).

A previous systematic review reported that reducing trichorionic triamniotic (TCTA) multiple pregnancies to twins resulted in a decrease in preterm birth rates, but an increase in miscarriage rates when compared to expectant management (13). Chaveeva et al also confirmed the current

findings in their study, and also found that miscarriage rates increased and preterm birth rates decreased even more in TCTA multiple pregnancies reduced to singleton pregnancies. (14). A more recent review has shown that reducing TCTA multiple pregnancies to twin pregnancies reduces preterm birth rates without significantly increasing miscarriage rates (15). In this study, no instances of miscarriage were observed among patients who underwent reduction of triplet pregnancies to singleton or twin pregnancies, thereby corroborating the existing findings. Moreover, a meta-analysis of 769 patients who underwent reduction from triplet to singleton pregnancies revealed a decline in preterm birth, neonatal mortality, hypertensive diseases of pregnancy, antenatal hospitalization rates, and cesarean section rates, along with an increase in birth weights. These findings emphasise the significance of counselling families about the risks and benefits of selective fetal reduction in triplet and higher-order pregnancies (16).

In twin pregnancies where there are no maternal or fetal complications, selective fetal reduction depending on the family's request is controversial (11). In the present study, all fetal reduction procedures performed in twin pregnancies were carried out due to the presence of fetal anomalies that were deemed to be incompatible with life or capable of causing serious sequelae. Selective fetal reduction performed in the second trimester of twin pregnancies has been reported to be associated with an elevated risk of miscarriage and preterm birth when compared with the first trimester (17). Although it is possible to diagnose most of the major structural malformations with first trimester fetal anatomical screening, this is not possible for all fetal anomalies. Sometimes, the time required for the completion of fetal genetic studies that will provide an indication for selective fetal reduction necessitates that the procedure be performed in the second trimester. Contrary to the findings reported in the existing literature, our results did not reveal any statistically significant differance in adverse perinatal outcomes based on the trimester of procedure performed.

The acute complication rate of selective fetal reduction in the present study was 30.3%. This appears to be slightly higher than the complication rates reported in the extant literature (18, 19). The most prevalent acute complication was amniotic fluid leakage, which occurred in 5 cases (15.2%). It has been reported that selective termination with KCl injection can result in the occurrence of pain or amniotic fluid leakage. The primary cause of amnion fluid leakage is usually the reduced fetal sac. However, vaginal bleeding is not a common complication (10). In the present study, postprocedural vaginal bleeding was observed in only one patient (3.0%). As the origin of amniotic fluid cannot be definitively ascertained, all observed amniotic fluid leakages in this study were defined as acute complications. This may have resulted in an increased incidence of complications. No pregnancy loss occurred in any patient following the observed complications. After the complications were observed, no patient experienced pregnancy loss before the 24th week of gestation. In addition, since no significant increase in the risk of adverse pregnancy outcomes was found, it can be concluded that the occurrence of acute complications does not have a negative effect on long-term outcomes.

The results of the study demonstrate that selective fetal reduction, performed in triplet or higher-order pregnancies before the procedure and in twin or higher-order pregnancies post-procedure, is associated with increased adverse pregnancy outcomes. It is evident that the incidence of adverse perinatal outcomes in multiple pregnancies is higher than in singleton pregnancies. The rates of twin and higher-order pregnancies have increased in conjunction with the utilisation of assisted reproductive techniques (5, 10). The present study found that pregnancies resulting from assisted methods, procedures performed with elective indications, and multiple procedures were associated with an increased risk of adverse pregnancy outcomes. Performing selective fetal reduction electively is considered as an option to avoid perinatal complications in higher order pregnancies. However, the increased adverse perinatal risk in pregnancies with assisted methods, elective procedures, and multiple procedures can be mainly attributed to the risks associated with higher order pregnancies. In this context, instead of considering selective fetal reduction as the first option in the management of higher order pregnancies, single embryo transfer should be the priority in patients undergoing in vitro fertilization (20). Single embryo transfer has been shown to reduce the incidence of multiple pregnancies, though not entirely prevent it (21). In circumstances where the prevention of a higher order pregnancy has not been possible, selective fetal reduction should be considered as a method of reducing the risk of adverse pregnancy outcomes.

It must be accepted that this study is not free from certain limitations. These include the retrospective design and the relatively low number of patients. Furthermore, the heterogeneity of multiple gestations that undergo selective fetal reduction is another limitation of this study. Consequently, further research comprising a larger patient cohort and ensuring uniformity with the type of multiple pregnancy could provide more accurate and detailed data.

CONCLUSION

Triplets and higher-order pregnancies, electively indicated procedures requiring multiple interventions have been shown to be associated with an increased risk of adverse pregnancy outcomes. Assisted reproductive technologies (ART) should be used in a manner that does not lead to higher order pregnancies, rahter than the utilisation of SFR as a primary option.

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Ethics Committee Approval: The study protocol was approved by Zeynep Kamil Women and Children Diseases Training and Research Hospital Clinical Investigations Ethical Committee (Approval number:168,Date:20.12.2023).

Informed Consent: An informed consent form was obtained from the patient/patient's representative to collect and publish the patient's clinical information.

Authorship Contributions: Idea/Concept:ÖGE, MG, Design: ÖGE, MEÖ, Supervision: OD, Data Collection and Processing: ÖGE, MG, ÜT, Analysis or Interpretation: MEÖ, ÜT, Literature Search:MG, ÜT, Writing: ÖGE, Critical Review: MEÖ, ÜT, OD, References and Fundings: -, Materials: -.

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Original Article / Araştırma Makalesi

THE EFFECTS OF AVNRT ABLATION ON THE CONDUCTION SYSTEM

AVNRT ABLASYONUNUN İLETİ SISTEMİ ÜZERINE ETKİLERİ

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ABSTRACT

Introduction: Atrioventricular nodal reentrant tachycardia (AVNRT) is one of the most common types of narrow QRS complex supraventricular tachycardias. Catheter ablation is considered one of the most effective treatment methods for AVNRT due to its high success rates and low complication risks. However, it is crucial to evaluate the changes in the atrioventricular conduction system following ablation. In this study, electrocardiographic changes in patients who underwent AVNRT ablation were analyzed.

Methods: A total of 148 patients who were presented with palpitations and were diagnosed with AVNRT through an electrophysiological study between January 2023 and December 2024 were included in the study. The minimum required sample size to detect a 1 msn difference between the two groups was calculated as at least 44 individuals with 90% power and a 0.05 Type I error using the open-source program R 3.0.1. Descriptive statistics of the data included mean, standard deviation, ratio, and frequency values. The Kolmogorov-Smirnov test was used to assess the normality of continuous variable distributions. The McNemar test was used for the analysis of dependent variables. The Student's t-test was used for the analysis of parametric data, while the Mann-Whitney U test was used for nonparametric data. Statistical significance was defined as $p < 0.05$.

Results: After ablation, heart rate increased significantly, while the QRS duration also showed a statistically significant prolongation. A slight but significant prolongation was observed in the PR interval as well. However, no significant change was detected in the corrected QT interval.

Conclusions: Following AVNRT ablation, slight but significant changes were observed in heart rate, QRS duration, and PR interval. These changes are thought to be associated with the modification of the slow pathway of the atrioventricular node. The absence of a significant change in the QTc interval suggests that ablation does not have a negative impact on ventricular repolarization. These electrocardiographic changes following successful AVNRT ablation may serve as potential predictors of success, particularly in patients without well-defined endpoints.

Keywords: AVNRT, cardiac conduction system, cathether ablation.

ÖZET

Giriş: Atriyoventriküler nodal reentrant taşikardi (AVNRT), dar QRS kompleksli supraventriküler taşikardiler arasında en sık görülenlerden biridir. Kateter ablasyonu, yüksek başarı oranları ve düşük komplikasyon riskleri nedeniyle AVNRT'nin en etkili tedavi yöntemlerinden biri olarak kabul edilmektedir. Ancak ablasyon sonrası atriyoventriküler iletim sistemindeki değişikliklerin değerlendirilmesi önemlidir. Bu çalışmada, AVNRT ablasyonu yapılan hastaların elektrokardiyografik değişiklikleri incelenmiştir.

Yöntemler: Ocak 2023 ile Aralık 2024 tarihleri arasında çarpıntı şikayetiyle başvuran ve yapılan elektrofizyolojik çalışma sonucunda AVNRT tanısı konulan toplam 148 hasta çalışmaya dahil edilmiştir. İki grup arasındaki 1 msn farkı tespit etmek için gereken minimum örneklem büyüklüğü, açık kaynaklı R 3.0.1 programı kullanılarak %90 güç ve 0.05 tip I hata ile en az 44 birey olarak hesaplanmıştır. Birincil etki değişkeni ± 0,18 olarak hesaplanmıştır. Verilerin tanımlayıcı istatistiklerinde ortalama, standart sapma, oran ve frekans değerleri kullanıldı. Sürekli değişkenlerin dağılımının normal olup olmadığını değerlendirmek için Kolmogorov-Smirnov testi kullanıldı. Bağımlı değişkenlerin analizinde McNemar testi kullanıldı. Parametrik verilerin analizinde Student t-testi kullanıldı. Nonparametrik verilerin analizinde Mann-Whitney U testi kullanıldı. İstatistiksel anlamlılık p<0,05 olarak tanımlandı.

Bulgular: Ablasyon sonrası kalp hızı istatistiksel olarak anlamlı bir şekilde artmış olup QRS süresi de anlamlı düzeyde uzamıştır. PR süresinde ise hafif fakat anlamlı bir uzama gözlenmiştir. Bununla birlikte, düzeltilmiş QT süresi açısından anlamlı bir değişiklik saptanmamıştır.

Sonuç: AVNRT ablasyonu sonrası kalp hızı, QRS süresi ve PR süresinde hafif fakat anlamlı değişiklikler gözlenmiştir. Bu değişikliklerin, atriyoventriküler düğüm yavaş yolunun modifikasyonu ile ilişkili olduğu düşünülmektedir. QTc süresinde belirgin bir değişiklik olmaması, ablasyonun ventriküler repolarizasyon üzerine olumsuz bir etkisi olmadığını göstermektedir. Başarılı AVNRT ablasyonu ile oluşan bu değişikliklerin, özellikle sonlanım noktaları belirli olmayan hastalarda başarıyı öngördürebileceği düşünülmektedir.

Anahtar Kelimeler: AVNRT, kardiyak ileti sistemi, kateter ablasyon.

INTRODUCTION

Atrioventricular nodal reentrant tachycardia (AVNRT) is one of the most common types of narrow QRS complex tachycardias. Although it generally follows a benign course, it can significantly impact on the quality of life in symptomatic patients. While pharmacological agents can be used in the treatment of AVNRT, catheter ablation is the most effective

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treatment method for recurrent cases and symptomatic patients.

Catheter ablation, the standard treatment for AVNRT, has minimal effects on the conduction system, though the risk of complications is present in every case (1). Changes in electrocardiographic (ECG) parameters following ablation provide valuable insights into the physiological responses of the AV conduction system.

Catheter ablation aims to eliminate the tachycardia circuit by targeting the dual pathway phenomenon within the AV node. During this procedure, slow pathway modification is typically performed, interrupting the reentrant tachycardia circuit. Post-ablation side effects such as PR interval prolongation, QRS duration changes due to bundle branch block formation, and, in rare cases, complete AV block may occur (2,3). Therefore, postoperative ECG evaluations are of great importance in assessing the effects of ablation on the conduction system.

After ablation, mild to moderate prolongation of the PR interval may be observed. This results from changes in AV conduction due to slow pathway modification. However, clinically significant PR prolongation is rare and usually does not cause symptoms. Since the ablation procedure does not directly affect the His-Purkinje system, the QRS duration generally remains unchanged. With advancements in technology, selective lesion formation has become more precise, further reducing the risk of AV block (4). Nevertheless, transient widening may occasionally occur during ablation due to changes in the autonomic tonus. Additionally, conduction disturbances such as bundle branch blocks may arise due to conductive thermal damage affecting the AV node, AV nodal artery, or the right and/or left bundle branches (5,6).

In our study, preoperative and postoperative ECGs of patients who underwent AVNRT ablation in our clinic were compared to evaluate potential ECG changes.

METHODS

A total of 148 patients who presented with palpitations and were diagnosed with AVNRT through an electrophysiological study (EPS) between January 2023 and December 2024 were included in the study. Among these patients, 56 were male, and 92 were female. Electrophysiological study and radiofrequency (RF) ablation were performed in patients who had experienced at least two or more episodes of supraventricular tachycardia (SVT). Electrophysiological data obtained during the procedure and any potential complications were recorded.

Approval of this study was obtained from the local clinical research ethics committee (decision no. 297, dated 27.10.2022). The study was conducted in accordance with the International Conference on Harmonization (ICH) guidelines, Good Clinical Practice (GCP) principles, and the Declaration of Helsinki.

Due to the non-experimental and retrospective design, patient consent was not obtained; instead, approval for the retrospective clinical study was obtained.

Body weight, height (cm), and waist circumference (cm) measurements were taken in accordance with proper measurement techniques, and body mass index (BMI) was calculated based on these measurements. BMI was determined by dividing body weight by the square of height (m^2).

Hypertension was defined as a systolic/diastolic blood pressure of 140/90 mmHg or higher and/or the use of antihypertensive medication. Diabetes mellitus was defined as a fasting plasma glucose level of ≥126 mg/dL or active use of oral antidiabetic medication and/or insulin. Hyperlipidemia was defined as a total cholesterol level of ≥200 mg/dL or the use of antihyperlipidemic medication.

Patients with active infections, tachycardia due to secondary causes, congestive heart failure, symptomatic congenital heart disease, symptomatic valvular heart disease, or a diagnosed psychiatric disorder were excluded from the study.

Anti-arrhythmic treatment was discontinued in all patients two weeks before the procedure. None of the patients were on treatment with Amiodarone, which has significantly prolonged half-life elimination time.

Ablation Procedure

Patients underwent the procedure under local femoral anesthesia without sedation after completing a 12-hour fasting period. Coronary sinus catheter, diagnostic catheter, and a 4 mm tip ablation catheter (Marinr RF, Medtronic, Minneapolis, USA) were used. The procedure was performed under fluoroscopic guidance with right and left anterior oblique views. When tachycardia could not be induced under baseline conditions, patients received a bolus of either atropine (0.01–0.02 mg/kg) or isoproterenol (0.02–0.06 mg) to facilitate stimulation-induced tachycardia. The diagnosis of AVNRT was based on electrophysiological study (EPS) criteria, including a sudden prolongation of the AH interval by 50 ms or more ("jump"), followed by the observation of an echo beat and subsequent tachycardia induction. Additionally, a VA interval of less than 70 msn on the HIS catheter during tachycardia was considered a key diagnostic finding. Patients were monitored for 12 to 24 hours following the ablation procedure. Before discharge, patients were started on a daily oral dose of 81 mg acetylsalicylic acid. No other antiarrhythmic medication was administered.

Statistical Methods

All statistical analyses were performed using SPSS for Windows version 19.0 (SPSS, Chicago, IL). The minimum required sample size to detect a 1 ms difference between the two groups was calculated as at least 44 individuals with

90% power and a 0.05 Type I error using the open-source program R 3.0.1.

Descriptive statistics included mean, standard deviation, ratio, and frequency values. The Kolmogorov-Smirnov test was used to assess the normality of continuous variable distributions. The McNemar test was used for the analysis of dependent variables. The Student's t-test was applied for the analysis of parametric data, while the Mann-Whitney U test was used for nonparametric data. Statistical significance was defined as $p < 0.05$.

Due to the reason that anti-arrhythmic drugs were stopped at least 2 weeks prior to the ablation procedure, logistic regression analysis on anti-arrhythmic drug usage wasn't performed.

RESULTS

The demographic characteristics, comorbid conditions, and medication use of the patients included in the study are presented in Table 1. Beta-blockers were used for tachyarrhythmia control in 54.1% of the patients, and there was no statistically significant difference in other medication use or comorbid conditions compared to the general population.

Compared to pre-ablation values, the heart rate increased significantly (76.15 ± 7.65 vs. 77.58 ± 7.72 bpm, $p < 0.001$). A statistically significant prolongation was observed in QRS duration (90.97 ± 6.90 ms vs. 93.48 ± 9.39 ms, $p < 0.001$). Similarly, PR interval increased significantly after ablation (156.65 ± 13.85 ms vs. 159.86 ± 21.15 ms, $p = 0.022$). However, no significant difference was detected in the corrected QT interval. (Table 2).

DISCUSSION

AVNRT is one of the most common forms of supraventricular tachycardia and is typically observed in individuals without structural heart disease. Due to its high success rates and low complication risks, catheter ablation is the first-line treatment option for AVNRT.

In our study, the demographic, clinical, and electrocardiographic characteristics of patients who underwent AVNRT ablation were analyzed. Approximately 42% of the patients were female, supporting the existing literature indicating that AVNRT is more frequently observed in women (7). As AVNRT is a supraventricular tachyarrhythmia that typically occurs in individuals without structural heart disease, the normal ejection fraction levels and the low prevalence of coronary artery disease in our study cohort align with this finding.

When evaluating cardiovascular risk factors, the smoking rate was notably high at 45.9%, highlighting a significant cardiovascular risk factor in this patient population. Given its effects on the autonomic nervous system, smoking is thought to enhance arrhythmogenic potential and may contribute to the development of AVNRT (8).

Table 1. Demographic, Clinical, Echocardiographic, and Laboratory Characteristics of the Patients

Variables	(n=148)
Age (years)	52.98 ± 5.07
Female Sex, n(%)	21 (42.0%)
Body Mass Index, kg/m^2	27.92 ± 2.78
Diabetes Mellitus, n(%)	24 (16.2%)
Hypertension, n(%)	18 (12.2%)
Dyslipidemiae, n(%)	27 (18.2%)
Smoking, n(%)	68 (45.9%)
Coronary Artery Disease, n(%)	10 (6.8%)
Left Ventricular Ejection Fraction (%)	61.96 ± 3.94
Systolic Blood Pressure, mmHg	118.96 ± 11.02
Diastolic Blood Pressure, mmHg	76.55 ± 7.85
Serum Glucose Levels, mg/dl	111.47 ± 36.76
Serum Kreatinin Levels, mg/dl	0.79 ± 0.17
LDL, mg/dL	106.30 ± 33.35
HDL, mg/dL	50.77 ± 9.33
Triglyceride, mg/dL	138.77 ± 73.36
White Blood Cell Count, 10^3/mm^3	7.11 ± 2.11
Neutrophil Count, 10^3/mm^3	5.00 ± 2.30
Lymphocyte Count, 10^3/mm^3	1.75 ± 0.55
Hemoglobin, g/dL	13.21 ± 1.65
Thrombocyte Count , 10^3/mm^3	275.2 ± 35.67
Medication Use	
-Propafenone	4 (2.7%)
-Amiodarone	0 (0%)
-Beta-blockers	80 (54.1%)
-Non-dihydropyridine Calcium Channel Blockers	4 (2.7%)
-Acetylsalicylic Acid	36 (24.3%)
- Clopidogrel	8 (5.4%)
- ACE inhibitor/ARB	18 (12.2%)
- Statins	27 (18.2%)
- Other Medications	36 (24.3%)
Post-procedural Complications	
-Complete AV Block	1 (0.7%)
-Access Site Hematoma	7 (4.7%)
-Inappropriate Sinus Tachycardia	3 (2%)
-Symptomatic Premature Atrial Contractions	4 (2.7%)

Data are presented as mean ± standard deviation or as percentages [n (%)]. LDL: low-density lipoprotein, HDL: high-density lipoprotein, ACE: angiotensin-converting enzyme, ARB: angiotensin receptor blocker.

When comparing pre- and post-ablation ECG parameters, a slight but significant increase in heart rate was observed. This change can be explained by the reorganization of AV conduction and a decrease in vagal tone following ablation (9,10). Additionally, a small but statistically significant prolongation in QRS duration was detected. Since AVNRT ablation involves modifying the slow pathway of the AV node, it may lead to minimal intraventricular conduction

Table 2. Pre- and post-ablation ECG parameters.

Variables	Pre-Ablation	Post-Ablation	P value
Heart Rate, BPM	76.15 ± 7.65	77.58 ± 7.72	**<0.001**
QRS duration, msn	90.97 ± 6.90	93.48 ± 9.39	**<0.001**
PR duration, msn	156.65 ± 13.85	159.86 ± 21.15	**0.022**
Corrected QT Interval, msn	410.65 ± 21.76	412.81 ± 25.31	0.751

Data are presented as mean ± standard deviation or as percentages [n (%)].

changes, which could be associated with the observed QRS prolongation (11). Moreover, potential thermal damage to the AV nodal artery may alter AV node conduction properties (12).

The slight prolongation of the PR interval also supports the slow pathway modification within the AV node (13-16). However, the absence of a significant change in QTc duration post-ablation suggests that the procedure does not have a negative impact on ventricular repolarization.

The ablation of AVNRT has become safer and more effective in recent years due to technological advancements and procedural optimizations. In addition to conventional RF ablation, alternative energy sources such as cryoablation are increasingly used, particularly in young patients, as they offer a significant option for reducing the risk of AV block. Electroanatomic mapping systems and high-resolution catheter technologies enhance the accuracy of ablation by improving the localization of target tissue and increasing success rates. Furthermore, studies on emerging energy modalities, such as non-contact ablation and pulsed field ablation (PFA), promise a safer approach by minimizing the risk of complications in AVNRT treatment. These advancements contribute to improving both the short- and long-term efficacy of AVNRT ablation, representing a significant step forward in patient management.

Some data in the literature report that AV block is more common in older ages during conventional AVNRT ablations. Transitioning from conventional methods to modern techniques may enhance protection against potential conduction system damage, as observed in our study (17).

CONCLUSION

Overall, the ECG changes observed after AVNRT ablation are expected and clinically acceptable outcomes of slow pathway modification and can be considered indicators of a successful ablation. In cases where a clear endpoint for successful ablation is not definitively achieved, these changes may serve as markers of effective slow pathway modification. However, further long-term studies in a larger population are needed to confirm this relationship.

LIMITATIONS

First, the number of patients included in the study is relatively low. Second, edema occurring in atrial tissue during the acute phase may cause ECG changes, but we do not yet have long-term ECG follow-up results. Finally, the study has a retrospective nature; therefore, there may be standardization issues.

Ethics Committee Approval: Approval of this study was obtained from the local clinical research ethics committee (decision no. 297, dated 27.10.2022).

Informed Consent: Due to the non-experimental and retrospective nature of the study, informed consent from patients was not obtained. Instead, approval for retrospective clinical study was obtained from local ethics committee and the hospital administration.

Authorship Contributions: Idea/Concept: MHU, MSK, Design: MSK, Supervision: MSK, Data Collection and Processing: MHU, Analysis or Interpretation: MSK, Literature Search: MSK, Writing: MHU, Critical Review: MSK.

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Original Article / Araştırma Makalesi

A LOOK TO THE RELATIONSHIP BETWEEN AUTOPHAGY AND EMBRYONAL CARCINOMA FROM A DIFFERENT PERSPECTIVE

OTOFAJİ VE EMBRİYONEL KARSİNOM ARASINDAKİ İLİŞKİYE FARKLI BİR PERSPEKTİFTEN BAKIŞ

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ABSTRACT

Introduction: Testicular embryonal carcinoma is a type of testicular cancer that affects the germ cells which are the precursors of sperm cells that will eventually develop into sperm. These carcinoma cells are often aggressive. In such cells, autophagy may promote the survival and proliferation of cancer cells, as autophagy can help cells survive under stress. Autophagy may also allow cancer cells to develop resistance to their environment and to metastasize. ATG4 (Autophagy related 4) is a protein that plays a role in the initiation of autophagy. JAB1 (Jun activation domain binding protein 1) is a protein involved in cellular signaling and regulates many biological processes such as cell cycle, apoptosis, and gene expression. JAB1 is also involved in the regulation of the p27 protein, and its high expression can be observed in cancer cells. NEDL2 (Neuroepithelial differentiation marker like 2) is involved in cellular growth and developmental processes. It is also a protein that is thought to play a role in cancer progression. The aim of the study was to demonstrate expression and localization of ATG4, JAB1 and NEDL2 in testicular embryonal carcinoma cells.

Methods: The morphological examination of testis embryonal carcinoma cells were performed by using hematoxylin eosin (H-E) staining. Then, using the immunohistochemical technique, cellular expression and location of ATG4, JAB1 and NEDL2 in testis embryonal carcinoma cells were examined.

Results: According to immunohistochemistry results, ATG4, JAB1 and NEDL2 expression was detected in human testicular embryonal carcinoma cells.

Conclusions: By determining the expression levels of these three proteins, more information can be obtained about how important processes such as autophagy, cell cycle regulation and cellular development are affected in testicular embryonal carcinoma. This information could be an important step in understanding the biology of cancer and developing treatment strategies.

Keywords: Testis embryonal carcinoma, autophagy, ATG4, JAB1, NEDL2.

ÖZET

Giriş: Testis embriyonal karsinom, sperme dönüşecek olan sperm hücrelerinin öncüleri olan germ hücrelerini etkileyen bir testis kanseri türüdür. Bu karsinom hücreleri genellikle agresiftir. Bu hücrelerde otofaji, hücrelerin stres altında hayatta kalmasına yardımcı olabileceğinden, kanser hücrelerinin hayatta kalmasını ve çoğalmasını destekleyebilir. Otofaji ayrıca kanser hücrelerinin çevrelerine karşı direnç geliştirmesine ve metastaz yapmasına izin verebilir. ATG4 (Autophagy related 4), otofajinin başlatılmasında rol oynayan bir proteindir. JAB1 (Jun activation domain binding protein 1), hücresel sinyalizasyon yer alan ve hücre döngüsü, apoptoz ve gen ifadesi gibi birçok biyolojik süreci düzenleyen bir proteindir. JAB1 ayrıca p27 proteininin düzenlenmesinde de yer alır ve yüksek ifadesi kanser hücrelerinde görülebilir. NEDL2 (Neuroepithelial differentiation marker like 2), hücresel büyüme ve gelişim süreçlerinde yer alır. Ayrıca kanser ilerlemesinde rol oynadığı düşünülen bir proteindir. Çalışmanın amacı, testis embriyonal karsinom hücrelerinde ATG4, JAB1 ve NEDL2'nin ekspresyonunu ve lokalizasyonunu göstermektir.

Yöntemler: Testis embriyonal karsinom hücrelerinin morfolojik incelemesi hematoksilen eozin (H-E) boyama kullanılarak yapıldı. Daha sonra, immünohistokimyasal teknik kullanılarak, testis embriyonal karsinom hücrelerinde ATG4, JAB1 ve NEDL2'nin hücresel ekspresyonu ve lokalizasyonu incelendi.

Bulgular: İmmünohistokimya sonuçlarına göre, insan testis embriyonal karsinom hücrelerinde ATG4, JAB1 ve NEDL2 ekspresyonu tespit edildi.

Sonuç: Bu üç proteinin ekspresyon seviyelerinin belirlenmesiyle, otofaji, hücre döngüsü regülasyonu ve hücresel gelişim gibi önemli süreçlerin testis embriyonal karsinomunda nasıl etkilendiği hakkında daha fazla bilgi elde edilebilir. Bu bilgi, kanser biyolojisinin anlaşılması ve tedavi stratejilerinin geliştirilmesinde önemli bir adım olabilir.

Anahtar Kelimeler: Testis embriyonal karsinomu, otofaji, ATG4, JAB1, NEDL2.

INTRODUCTION

Human testicular embryonal carcinoma is a rare type of cancer found in the testicles and usually occurs in young men. Embryonic carcinoma is a type of germ cell tumor and is one of the most aggressive and fast-growing types of testicular cancer (1). Embryonal carcinoma cells can divide and grow rapidly, similar to cells seen during embryonic development. These cells have characteristics that accelerate the proliferation of the cancer (2).

ATG4 plays important roles in the initiation of autophagy. This protein is involved in a series of modifications required for the functionalization of autophagic vesicles. In particular, ATG4 is involved in the activation of autophagy-related

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proteins such as LC3 (Microtubule-associated protein 1A/1B-light chain 3) and contributes to the progression of the autophagy process (3). In addition, it has been suggested that the expression levels of ATG4 in cancer cells, and especially in testicular embryonal carcinoma cells, may affect the sensitivity of cells to autophagy, thus determining tumor growth and response to treatment. The role of ATG4 in testicular embryonal carcinoma cells may be important in understanding the effects of autophagy on tumor progression and resistance to treatment. Some studies have suggested that inhibiting ATG4 may support cancer treatment because autophagy may help cells survive and develop resistance to cancer treatments (4,5). Therefore, ATG4 may be a potential target for cancer treatment. In testicular embryonal carcinoma cells, ATG4 may play an important role in the modulation of autophagy processes and this protein may be a molecule that should be evaluated as a target for cancer treatment strategies.

JAB1 (Jun activating binding protein 1), also known as CSN5 (COP9 Signalosome Subunit 5), is a protein that plays important roles in various cellular processes. JAB1 is part of a protein complex known as the COP9 (Constitutive Photomorphogenic 9) signaling complex (CSN) and has multifaceted effects on cellular functions (6). Jun family proteins are important proteins that regulate processes such as cellular growth and differentiation (7). JAB1 also plays a role in protein degradation by the proteasome. As part of the COP9 signaling complex, it helps the function of the proteasome to degrade and properly control cellular proteins. JAB1 interacts with some proteins that control the cell cycle. In particular, it can accelerate the cell cycle by reducing the stability of the cell cycle regulator called p27. This feature may be related to cancer because the irregular progression of the cell cycle can lead to cancer development (8). The relationship between JAB1 and cancer has been widely studied. In many types of cancer, overexpression of JAB1 and uncontrolled reduction of cell cycle regulators such as p27 can lead to excessive proliferation of cancer cells and tumor formation (9,10). JAB1 also has an effect on the migration and metastasis potential of cells in some types of cancer. Therefore, JAB1 is thought to be directly related to cancer progression (11). JAB1 can also affect cell differentiation. This may be especially important in processes such as embryonal development or tissue regeneration. JAB1 is a versatile protein that plays a role in a number of important biological processes, such as cellular growth, differentiation, apoptosis, cell cycle regulation, and protein degradation. Due to its association with cancer, a better understanding of JAB1's functions is of great importance for cancer treatment and modulation of cellular processes.

NEDL2 (Neuroepithelial differentiation marker-like 2) is a protein discovered in humans and plays a role in neural development and cellular processes. This protein may play an important role in various biological processes associated with cellular signaling, differentiation, and some diseases (12). NEDL2 can function as an E3 ubiquitin ligase. Ubiquitin ligases are enzymes that facilitate the recognition and degradation of cellular proteins by proteasomes via marking them with ubiquitin (13). This is important in maintaining cellular control and homeostasis. NEDL2 regulates the degradation of proteins through ubiquitination. This plays a critical role in processes such as the cell cycle, gene expression, and cellular signaling. Ubiquitination ensures that proteins are degraded at the right time at the end of their life cycle. NEDL2 function as an E3 ubiquitin ligase may be critical in ensuring proper transitions in the cell cycle. However, some studies suggest that NEDL2 regulates the cell cycle while ensuring that certain proteins are destroyed. The relationship between E3 ubiquitin ligases and cancer is quite common, because they directly affect the growth and survival processes of cells (14). NEDL2 may be involved in processes such as neural tube development and neural cell differentiation. This role of NEDL2 may be important in embryonal development and some aspects of nervous system diseases (15). Abnormal expression of NEDL2 in some types of cancer may cause uncontrolled cell growth.

Demonstrating the expression of ATG4, JAB1, and NEDL2 proteins in testicular embryonal carcinoma cells is an important step towards a deeper understanding of how these proteins play a role in cancer cells and contribute to the molecular biology of cancer.

MATERIALS AND METHODS

In this study, human testicular embryonal carcinoma cell line CRL-2073 was used (NCCIT/ATCC). This cell line consists of adherent germ cell tumors isolated from a male patient with pluripotent embryonal carcinoma in adult testicular tissue. The pluripotent cell line has the ability for somatic and extraembryonic differentiation. The undifferentiated cells were equivalent to the intermediate stage between seminoma and embryonal carcinoma.

Cell Culture:

The CRL-2073 (NCCIT / ATCC) cell lines were obtained. Embryonal carcinoma cells were cultured in RPMI-1640 (with L-glutamine, HEPES, Gibco Capricorn, 21875-034) supplemented with 1% antibiotics (penicillin/streptomycin, Gibco, 15140-122) and 5% fetal bovine serum (FBS, Capricorn, 10270-106) under 5% CO2 in a 37 °C humidified incubator.

Histologic Evaluation:

Hematoxylin and eosin staining was performed for morphological analyses of CRL-2073 cells. Cells were suspended in 2 ml of cell medium on round coverslips placed in 6-well cell plates. The media was removed from the cells adhering to the coverslips and washed with Phosphate Buffered Salt Solution (PBS). 4% Paraformaldehyde (PFA) was added to the cells and waited for 10 minutes to fix the cells. After fixation, the cells were washed with PBS. Then, the round coverslips were placed on the slide. The cells were kept in 100%, 96% and 75% alcohol series for 2 minutes, respectively, and washed with distilled water. It was kept in Hematoxylin (Sigma-Aldrich, 105174) for 5 minutes. After washing with distilled water, it was kept in Eosin (Sigma Aldrich, 109844) for 1 minute. It was quickly passed through 75%, 96% and 100% alcohol series. It was kept in Xylene for 30 minutes and covered with entellan. Imagery was performed with a light microscope (Nicon, Eclipse E100).

Immunocytochemistry:

It is a microscopic method based on examining the localization and expression of proteins within the cell. We also examined the localization and expression of ATG4, JAB1 and NEDL2 proteins with this technique. For immunocytochemistry staining, cells were cultured on round coverslips (12x12mm, Nest) placed in 6 well cell plates.

When the cells reached a certain density, the medium of the cells was withdrawn and the cells were washed with PBS. Then, the cells were fixed with 4% paraformaldehyde (PFA) for 15 minutes. The coverslips in the wells were placed on the slide and then were drawn with Pap pen. They were incubated with blocking solution (Abcam) for 1 hour to prevent nonspecific binding. After incubation, they were washed again with PBS. Primary antibodies ATG4 (Abcam, ab108322), JAB1 (Abcam, ab12323) and NEDL-2 (Abcam, ab236784) prepared at a certain dilution (1:100) were added to cover the coverslips and incubated overnight at +4°C. The next day, the primary antibodies were withdrawn and washed with PBS. Then, they were incubated with Biotin-containing secondary antibody for 1 hour. After washing with PBS, they were incubated with Streptavidin solution for 30 minutes. In order for the reaction to become visible, AEC solution (Patholab, PL-125-HA) was prepared and added to them and the incubation time was adjusted by checking under a microscope. After washing, they were incubated with Mayer's Hematoxylin for 30 seconds to stain the nucleus and covered with mounting medium. Evaluations were made with a light microscope (Nicon, Eclipse E100).

RESULTS

Histomorphological result:

The morphological appearance of human testicular embryonal carcinoma cells under the light microscope typically has certain characteristics that can help in the identification of this tumor. Embryonic carcinoma is included in the category of germ cell tumors and usually shows fast-growing, aggressive and heterogeneous cellular structures. Embryonic carcinoma cells were usually large, round or oval in shape. Nuclei were usually dark, large and irregularly shaped. Hyperchromatic nuclei had a denser chromatin structure and can be clearly seen under the light microscope. The nuclei of embryonal carcinoma cells were usually irregular. The nuclei were large and can be of various shapes; some are round, while others were more oval or elliptical. Testicular embryonal carcinoma cells tend to be invasive and metastatic potential of the tumor. These cells were often found in large, heterogeneous clusters. The clusters can be regular or irregular in shape (Figure 1).

Immunocytohemistry results:

ATG4, JAB1 and NEDL2 protein expression has not been examined in human testicular embryonal carcinoma cell lines before. In this study, firstly, in order to understand whether there is protein expression, the presence of ATG4 (Figure 2), JAB1 (Figure 3) and NEDL2 (Figure 4) was demonstrated in CRL-2073 cells by immunocytochemistry method. For ATG4; high expression is observed in testicular embryonal carcinoma cells (Figure 2). With JAB1 immunostaining high expression is observed (Figure 3). At NEDL2 stained preparations high expression is observed (Figure 4).

DISCUSSION

Testicular embryonal carcinoma is a malignant type of cancer that originates from germ cells in the testis. Embryonal carcinoma contains cancer cells that can grow and metastasize rapidly. (16). This type of cancer can use autophagy as a survival mechanism. Autophagy provides proteins that support cancer cell proliferation, while also clearing toxic substances and damaged organelles within

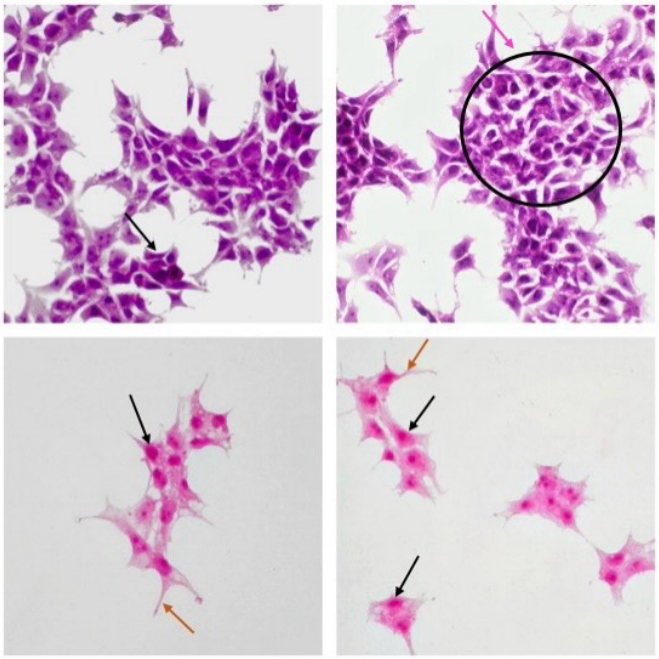

Figure 1. Light microscopic image of human testicular embryonal carcinoma cells (CRL-2073) (Stain: Hematoxylin Eosin, Magnification: 400X). Black arrows show the nuclei of cells. Orange arrows indicate the extension of the cells. Pink arrow and circle show the clusters of cells.

the cell, allowing cancer cells to survive (17). This process can support the rapid division and growth of cells in rapidly growing cancers such as testicular embryonal carcinoma. Autophagy can enable cancer cells to develop resistance to chemotherapy and radiotherapy. When cancer treatments damage cells, cells can repair this damage and survive thanks to autophagy. Testicular embryonal carcinoma cells can also develop resistance to treatment, and autophagy may form the basis of these resistance mechanisms.

Demonstrating the expression of ATG4, JAB1 and NEDL2 proteins is an important step towards understanding the biological and molecular basis of testicular embryonal carcinoma. All three proteins are involved in cellular process, especially cell cycle, cell survival, apoptosis and protein degradation. ATG4 is a protease that plays an important role in the autophagy process. Autophagy is a process that helps cells survive under stress and destroys damaged or unnecessary organelles and proteins within the cell. ATG4 enables the conversion of LC3 and ATG8 proteins into their active forms during autophagy. Since testicular embryonal carcinoma cells usually exhibit high proliferation and aggressive growth characteristics. ATG4 expression may help these cells survive and cope better under stress. In this case, ATG4 expression is expected to be high because autophagy contributes to the survival of cancer cells and balances metabolic imbalances. In addition, it has been shown that ATG4 may be associated with resistance to chemotherapy and cell death.

JAB1 is an E3 ubiquitin ligase involved in the regulation of the cell cycle, control of transcriptional activity, and differentiation of cells. It leads to cell cycle progression and acceleration of cell proliferation by increasing the destruction of cell cycle regulators such as p27. JAB1 can be highly expressed in testicular embryonal carcinoma cells as a factor that accelerates the cell cycle and increases the proliferation of cancer cells. High JAB1 expression may

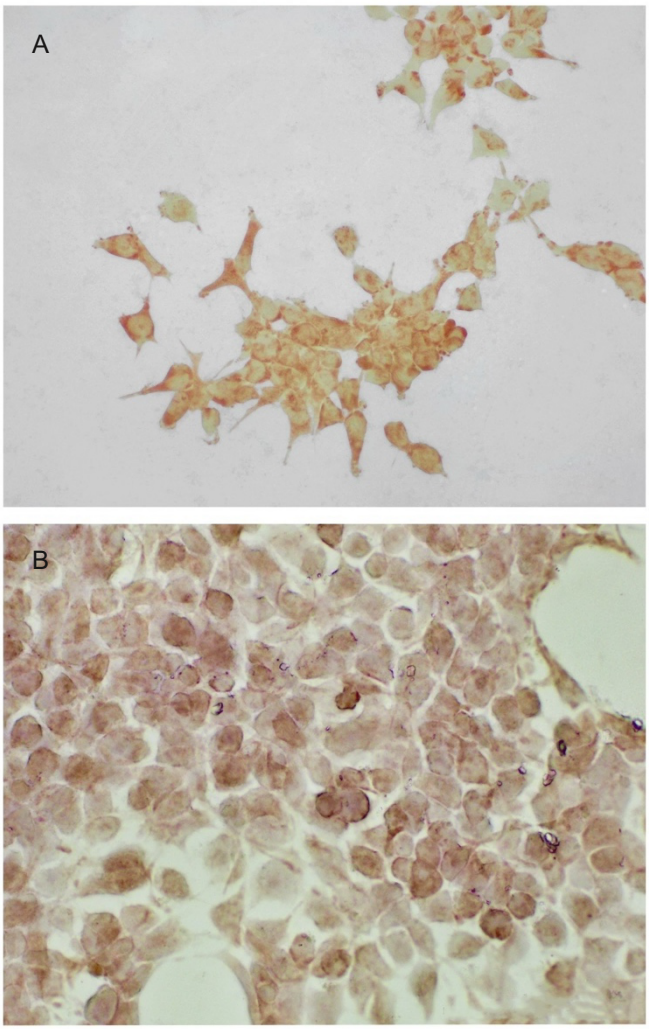

Figure 2. Immunocytochemical demonstration of ATG4 protein in human embryonal carcinoma cells (Magnification A; 200X B; 400X and 1:100 dilution). A higher expression was observed in the cytoplasm.

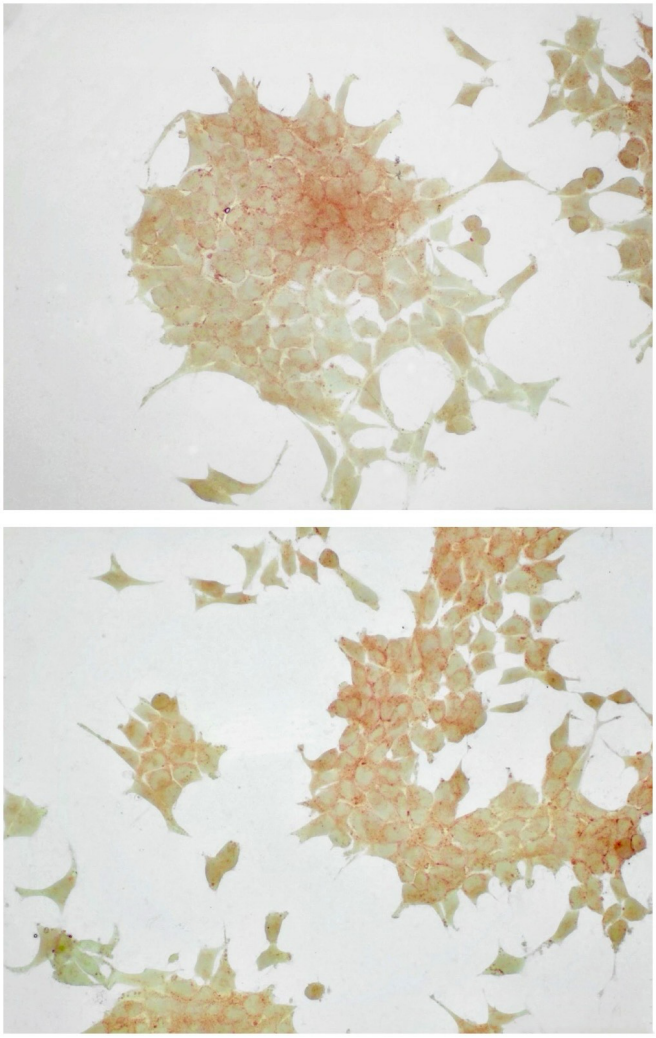

Figure 3. Immunocytochemical demonstration of JAB1 protein in human embryonal carcinoma cells (Magnification: 400X and 1:100 dilution). A higher expression was observed in the cytoplasm.

support the growth and metastasis potential of cancer cells, because JAB1 has an important role in regulating the cell cycle. JAB1's promotion of p27 degradation accelerates the G1/S transition of the cell cycle, which allows cancer cells to divide rapidly. In our previous study on human testicular cancers (18), the highest JAB1 protein was seen in the embryonal carcinoma group among testicular cancers. In this study, the high JAB1 expression in CRL-2073 cells is consistent with the literature. Consequently, high JAB1 expression is expected, because this contributes to faster division of testicular embryonal carcinoma cells and tumor growth.

NEDL2 acts as an E3 ubiquitin ligase and is associated with the cell cycle. It also plays a role in the modulation of some signaling pathways. NEDL2 is important for the correct timing of cell differentiation and survival. NEDL2 is likely to be highly expressed in testicular embryonal carcinoma cells because this protein may play a role in promoting cell proliferation. In cancer cells, overexpression of E3 ligases generally regulates factors that control the cell cycle and prevent cell death. This may contribute to tumor growth.

Abnormal differentiation of cancer cells can lead to the cell becoming malignant and acquiring metastatic properties. So, the expression of these three proteins can cause cancer cells to survive, grow rapidly, and develop resistance to treatment. Even it is so important to understand the relation between these proteins and cancer, until now ATG4, JAB1 and NEDL2 protein expression has not been examined in human testicular embryonal carcinoma specifically.

Although it is not possible to perform control staining on the cancer cell line, the data found with the relevant antibodies in previous studies on the testis are as follows. In our previous study (18), JAB1 antibody expression in human testicular tissue was generally detected in the cytoplasm of testicular cells. In the embryonal carcinoma group, JAB1 expression was seen in the cell nucleus and cytoplasm. According to HScore analysis, the number of JAB1 immunopositive cells increased significantly in the embryonal carcinoma group. In the literature (19), the expression of different isoforms of NEDL2 was shown in pig oocytes, embryos, spermatozoa and somatic cells. Expression of NEDL2 protein was shown in both the nucleus and cytoplasm. According to this study, NEDL2 has an important role in oocyte fertilization, especially in the decondensation of sperm DNA and in the early stages of pronuclear development. According to the information in The Human Protein Atlas (20), ATG4 expression is seen in the testis, especially in the seminiferous tubules and Leydig cells.

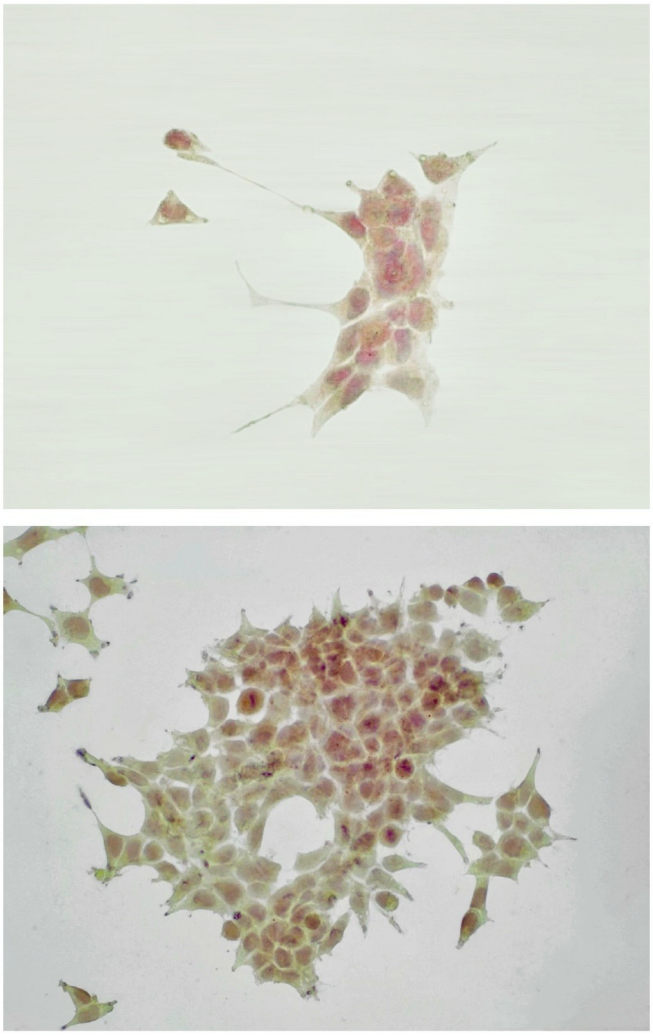

Figure 4. Immunocytochemical demonstration of NEDL2 protein in human embryonal carcinoma cells (Magnification: 400X and 1:100 dilution). A higher expression was observed in the nucleus.

Targeting these proteins can potentially help develop cancer treatment strategies. Autophagy may be an important potential target in the treatment of testicular embryonal carcinoma. Inhibiting autophagy may make it harder for cancer cells to survive and reduce resistance to treatment. In addition, activation of autophagy may lead to more aggressive cell growth. Various strategies are being investigated to inhibit autophagy in cancer treatment. Autophagy inhibitors may be an effective adjunctive therapy in cancer treatment. Such treatment may allow more effective destruction of cancer cells when combined with chemotherapy or radiotherapy. For example, inhibition of ATG4 can make it difficult for cancer cells to survive by inhibiting autophagy. In particular, inhibiting ATG4 and other autophagy-related proteins may be an important strategy to reduce resistance to treatment and more effectively destroy cancer cells. This research could open new ways to treat cancers such as testicular embryonal carcinoma. Inhibition of JAB1 can prevent tumor growth by slowing down the cell cycle. Inhibition of NEDL2 can reduce the aggressiveness of tumors by making cancer cells more differentiated. In this study, we aimed to show the expression of these three proteins firstly because of their possible roles in autophagy, cell cycle and differentiation mechanisms. In further studies, it can be examined how these mechanisms can be affected by inhibiting these proteins.

This study, which shows the expression of ATG4, JAB1 and NEDL 2 in testicular embryonal carcinoma cells for the first time, is a descriptive study. In further studies, numerical data can be obtained that compared by using inhibitors of these proteins or gene silencing. In this context, the effects of the expression of ATG4, JAB1, and NEDL2 on cancer biology can be investigated in more depth, and targeting these proteins can be an important strategy for developing cancer treatment. The relationship between testicular embryonal carcinoma and autophagy constitutes an important area in understanding the biological functioning of cancer. The relationship of autophagy with cancer survival and resistance mechanisms will shed light on future research on how these processes can be used in cancer treatment.

CONCLUSION

In summary, the expression of these proteins may contribute to the aggressive nature of testicular embryonal carcinoma and the rapid proliferation of cells. And the observation of these proteins in testicular embryonal carcinoma offers important opportunities for understanding the molecular mechanism of cancer, early diagnosis, generating therapeutic targets and biomarker discovery.

Ethics Committee Approval: Ethics committee approval was not required for this study because of there was no study on animals or humans. Commercial cell lines were used in this study and therefore ethical approval was not required.

Informed Consent: Informed consent was not required for this study because of there was no study on animals or humans.

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

Authorship Contributions: Idea/Concept:ŞK, Design:ŞK, Supervision:ŞK, HN, Data Collection and Processing:ŞK, Analysis or Interpretation:ŞK, HN, Literature Search:ŞK, Writing:ŞK, HN, Critical Review:ŞK, HN, References and Fundings: -, Materials: -.

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Case Report / Olgu Sunumu

SOLITARY FIBROUS TUMOR OF INGUINAL REGION: REPORT OF A CASE

İNGUINAL BÖLGENIN SOLİTER FİBRÖZ TÜMÖRÜ: OLGU SUNUMU

MEHMET SAIT OZSOY[1], DOGAC DEMIR[1], LEYLA ZEYNEP TİGREL[1], SELMAN CIHANGIR[1], YUSUF MELİH TAS[1], OZGUR EKINCI[1], ORHAN ALIMOGLU[1]

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ABSTRACT

Solitary fibrous tumor (SFT), previously known as hemangiopericytoma, is a fibroblastic mesenchymal neoplasm and constitutes a very small proportion of soft tissue sarcomas. It is usually recognised as a painless mass. The site of localisation is mostly the thorax. However, it can also be seen in the central nervous system, peritoneal cavity and retroperitoneal region. Inguinal region is a very rare site for SFT. In imaging computed tomography and magnetic resonance imaging are the diagnostic modalities of choice. Fine needle biopsy after radiological imaging helps histopathological diagnosis. It is characterised by STAT6/CD34 positivity immunohistochemically. En-block excision of the lesion is the main treatment due to the potential for recurrence and metastasis. Inguinal SFTs are rare lesions and should be included in the differential diagnosis of inguinal masses. In this study, we report a 46-year-old female patient with an inguinal SFT lesion who underwent total excision.

Keywords: General surgery, Inguinal region, Soft tissue sarcoma, Solitary fibrous tumor

ÖZET

Daha önce hemanjioperisitom olarak da bilinen soliter fibröz tümör (SFT), fibroblastik mezenkimal bir neoplazmdır ve yumuşak doku sarkomlarının çok küçük bir bölümünü oluşturur. Genellikle ağrısız bir kitle olarak fark edilir. Yerleşim yeri çoğunlukla toraks bölgesidir. Ancak santral sinir sistemi, periton boşluğu ve retroperitoneal bölgede de görülebilir. İnguinal bölge SFT için çok nadir bir bölgedir. Görüntülemede bilgisayarlı tomografi ve manyetik rezonans görüntüleme tercih edilen tanı yöntemleridir. Radyolojik görüntüleme sonrası ince iğne biyopsisi histopatolojik tanıya yardımcı olur. İmmünohistokimyasal olarak STAT6/CD34 pozitifliği ile karakterizedir. Rekürrens ve metastaz potansiyeli nedeniyle lezyonun blok eksizyonu ana tedavidir. İnguinal SFT'ler nadir görülen lezyonlardır ve inguinal kitlelerin ayırıcı tanısında yer almalıdır. Bu çalışmada, inguinal SFT lezyonu olan ve total eksizyon uygulanan 46 yaşında bir kadın hasta sunuldu.

Anahtar Kelimeler: Genel cerrahi, İnguinal bölge, Soliter fibröz tümör, Yumuşak doku sarkomu

INTRODUCTION

Solitary fibrous tumors (SFTs) are rare fibroblastic mesenchymal neoplasms, historically referred to as hemangiopericytomas (1). While they predominantly occur in the thoracic region, up to 70% of cases are extrapleural, with the central nervous system being the second most common site (2). SFTs are generally painless, slow-growing tumors, typically presenting in the fifth to seventh decades of life. Imaging modalities such as computerized tomography (CT) and magnetic resonance imaging (MRI) provide crucial information about tumor characteristics and relationships to surrounding structures. Histopathological diagnosis is facilitated by various biopsy techniques. The cornerstone of treatment is complete surgical resection with negative margins. Given the rarity of SFTs, case reports are vital for expanding the knowledge base. This case is particularly unique due to its inguinal localization being a rare site for SFTs, and its clinical presentation as a painless mass without systemic symptoms. Such characteristics add to the understanding of the diverse presentations of SFTs and highlight diagnostic challenges in atypical locations.

CASE REPORT

A 46 year-old female patient with no medical history presented to our general surgery outpatient clinic with a painless mass on left groin that has been slowly growing for the past two years. Patient denies any associating syptoms including pain, tenderness, weight loss, loss of range of movement or instestinal passage obstruction. All vital signs were normal. Physical examination revealed a painless, mobile, non-reducible mass in the left inguinal region. No palpable lymphadenopathy in inguinal region and other possible lymph node areas has been noted.

The laboratory parameters were unremarkable. An ordered CT scan, lesion revealed that there was an irregular defined, lobulated 6.5x5cm solid tumor with necrotic components in the center (Figure 1). No specific vessel supply has been noted. Regional lymph nodes showed no

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Cite as: Ozsoy MS, Demir D, Tigrel LZ, et al. Solitary Fibrous Tumor of Inguinal Region: Report of a Case.Eskisehir Med J. 2025; 6(1): 67-69. doi: 10.48176/ esmj.2025.183

signs of metastasis. The patient was evaluated in the multidisciplinary surgical council and thick needle biopsy was not recommended due to the vascularity density of the mass. It was advised that the lesion be completely removed.

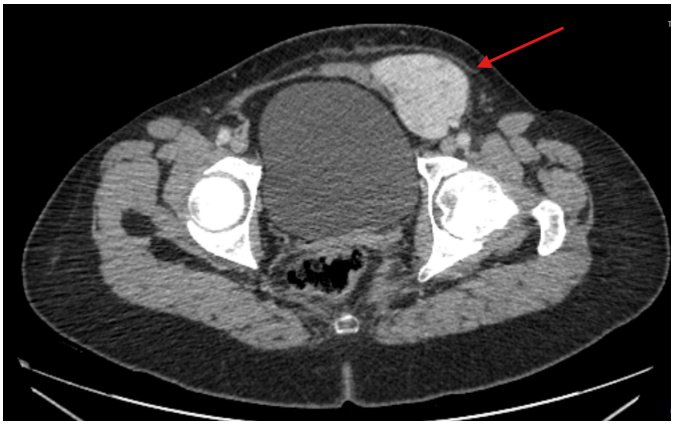

Figure 1. Axial view computerized tomography scan showing a lesion on the left groin marked with red arrow.

The patient was operated under general anesthesia. General anesthesia was preferred because of the possibility of intraabdominal or vascular intervention. A left inguinal incision has been made. On exploration, a 10x5x3 cm solid lesion with a smoothly circumscribed cystic component arising from the external inguinal ring and adjacent to the femoral vein was observed. The lesion had minimal adhesion to the surrounding tissues, and multiple vessels originating from the femoral veins supplied the lesion. The tumor was totaly removed in consideration with safe surgical margins (Figure 2). In the removed specimen, the lesion was marked with sutures in the superior and lateral directions.

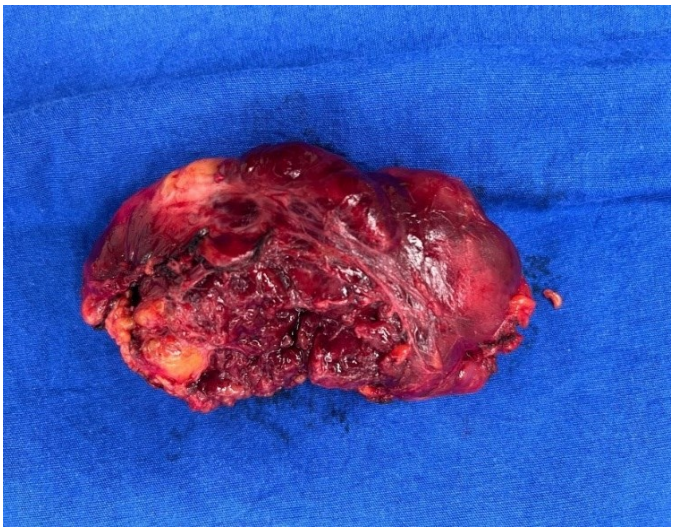

Figure 2. Macroscopic view of tumor

No complications such as surgical site infection and hemorrhage were observed in the postoperative follow-up period and the patient was discharged on postoperative day 1. The lesion was also examined pathologically. Gross examination of the lesion revealed a tumor size of 10x5x3 cm with one irregular border. Cream colored solid nodule was seen in sections. Multiple sections were examined. It was revealed that the surgical margins of the lesion were safe on pathological examination. The lesion was histopathologically reported as solitary fibrous tumor. On immunohistochemical examination was positive for signal transducer and activator of transcription 6 (STAT6) and CD34. ETS-related gene (ERG) was positive in vessels. Ki67 was 4%. Metastatic/recurrence risk scoring was 3/7 (Mitosis 1/2, size 2/3). No necrotic tissue was reported.

The patient was evaluated by the surgical-oncology-pathology board of our institution together with the pathology results. No additional treatment was recommended. The patient was scheduled with laboratory and CT scans at 3, 6 and 12 months. The third and sixth month follow-ups revealed no evidence of local recurrence

Informed consent was obtained from the patient and legal representative for the collection and publication of the patient's clinical information.

DISCUSSION

Initially known as fibrous mesothelioma in the 1930s and later as hemangiopericytoma, tumors involving fibroblastic mesenchymal tumors were defined as SFT in the 2002 World Health Organization Classification (3). They account for 3.7% of all soft tissue sarcomas and mesenchymal tumors. The majority of SFTs are located intrathoracic; however, they can also be found in various locations outside of the thorax. The fifth to seventh decades are the age group in which the disease is more common. Our patient was also in the fifth decade. SFTs of intraabdominal origin are more common in the young population, whereas pleural SFTs are more common in the elderly population. The male to female ratio is similar (4, 5). Studies have shown also that another 30% of SFTs originate from the peritoneal cavity, retroperitoneal tissue or pelvis (6). Another 20% of cases originate from the head and neck region. The remaining cases originate from the soft tissues of the trunk and extremities. SFTs originating from superficial tissues are rare (3, 7). In our case, the site of the tumor was the inguinal region as an atypical localization.

The most common presenting symptom of SFTs is a painless mass. These types have a tendency to grow slowly, sometimes for decades. Our patient presented with a slow-growing painless mass in the inguinal region.

Imaging options for diagnosis are CT and MRI, which are useful in determining tumor size, margins, vascular relationships and cystic/solid differentiation. SFTs are usually homogeneous but may contain cystic components or haemorrhagic areas. Invasion to surrounding tissues is rare (6, 8). In our patient, radiological diagnosis was made with CT. The recommended treatment for SFT is surgical excision with negative surgical margins (R0).

Adjuvant therapy in the postoperative period is not recommended because it is generally ineffective and the metastatic potential of SFTs is low. Adjuvant radiation therapy can be used on a case-by-case basis. Local recurrent cases should be treated with reresection and adjuvant radiotherapy (9). As a result of the evaluations of the surgical-oncology-pathology board of our institution, no additional treatment was recommended to our case.

Although the prognosis is excellent in patients with SFT in whom the mass is completely excised, local recurrences and metastases have been reported. Therefore, follow-up after treatment should be continued. Previous studies have shown that recurrence can be seen in the early period at 4-6 months CT imaging (10). Appropriate interval imaging contributes to early detection of possible recurrence. The post-treatment follow-up program for soft tissue SFTs is the same as the National Comprehensive Cancer Network (NCCN) soft tissue sarcoma guidelines (11).

CONCLUSION

Inguinal SFTs are rare and should be considered in the differential diagnosis of inguinal masses. Imaging modalities like CT and MRI are valuable diagnostic tools, but definitive diagnosis relies on histopathological and immunohistochemical findings. Complete surgical excision with negative margins is the treatment of choice. Long-term follow-up is essential to monitor for recurrence or metastasis.

Informed Consent: Since the patient died after clinical follow-up, consent for the case presentation was obtained from his family.

Authorship Contributions: Idea/Concept:OA, OE, MSO, Design: MSO, LZT, DD, Supervision: OA, OE, Data Collection and Processing: DD, SC, YHT, Analysis or Interpretation: MSO, LZT, DD, Literature Search:DD, SC, YMT, Writing: MSO, LZT, DD, Critical Review: OA, OE, References and Fundings: -, Materials: -.

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

Financial Disclosure: The authors declare that they have no relevant financial.

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Original Article / Araştırma Makalesi

EVALUATION OF THE PREDICTIVE ABILITY OF LABORATORY PARAMETERS IN FIBROMYALGIA SYNDROME COMPARED TO INDIVIDUALS WITH LOCAL MUSCULOSKELETAL PAIN

LOKAL KAS-İSKELET AĞRILI BIREYLERLE KARŞILAŞTIRILDIĞINDA FIBROMİYALJİ SENDROMUNDA LABORATUVAR PARAMETRELERININ ÖNGÖRÜ YETENEĞİNİN DEĞERLENDİRİLMESİ

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ABSTRACT

Introduction: Fibromyalgia syndrome (FMS) is a chronic pain syndrome characterized by widespread pain and various somatic symptoms. This study aims to investigate the differences between FMS patients and individuals with localized musculoskeletal disorders by evaluating inflammatory and metabolic markers that may play a role in FMS pathogenesis.

Methods: This retrospective study included 43 patients diagnosed with FMS and 43 patients with localized musculoskeletal pain, aged between 30 and 65 years. White blood cell count (WBC), red cell distribution width (RDW), mean corpuscular haemoglobin concentration (MCHC), mean platelet volume (MPV), platelet distribution width (PDW), albumin, creatinine, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) values of the participants were recorded. Additionally, CRP/albumin ratio (CAR), neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), monocyte/lymphocyte ratio (MLR), and systemic inflammatory indices (SII) of the participants were calculated.

Results: The mean ages were similar between the two groups in our study (Group 1 mean age: 46.76±8.94 years and Group 2 mean age: 46.3±8.67 years). MPV levels were found to be statistically significantly higher in the FMS group compared to the group with localized pain (p=0.02). No statistically significant difference was detected between the two groups in terms of the other evaluated laboratory parameters.

Conclusions: According to our research, we found a statistically significant difference only in MPV levels between FMS patients and controls. This suggests that it may help facilitate the diagnosis of FMS patients.

Keywords: Fibromyalgia, inflammation, laboratory parameters, mean platelet volume, pain

ÖZET

Giriş: Fibromiyalji sendromu (FMS) yaygın ağrı ve çeşitli somatik semptomlarla karakterize bir kronik ağrı sendromudur. Bu çalışmanın amacı, FMS patogenezinde rol oynayabilecek inflamatuar ve metabolik belirteçleri değerlendirerek FMS hastaları ile lokalize kas-iskelet sistemi rahatsızlığı olan bireyler arasındaki farklılıkları araştırmaktır.

Yöntemler: Bu retrospektif çalışmaya 30-65 yaş arası 43 FMS tanılı hasta ve 43 lokalize kas-iskelet bozukluğu olan hasta dahil edildi. Katılımcıların beyaz kan hücresi sayımı (WBC), kırmızı hücre dağılım genişliği (RDW), ortalama eritrosit hemoglobin konsantrasyonu (MCHC), ortalama trombosit hacmi (MPV), trombosit dağılım genişliği (PDW), albümin, kreatinin, eritrosit sedimantasyon hızı (ESR) ve C-reaktif protein (CRP) değerleri not edildi. Ayrıca katılımcıların CRP/albümin oranı (CAR), nötrofil/lenfosit oranı (NLR), trombosit/lenfosit oranı (PLR), monosit/lenfosit oranı (MLR) ve sistemik inflamatuar indeksleri (SII) hesaplandı.

Bulgular: Çalışmamızda yaş ortalamaları her iki grupta da benzerdi (1. grup yaş: 46,76±8,94 yıl ve 2. grup yaş: 46,3±8,67 yıl). FMS grubunda MPV düzeyleri lokalize ağrısı olan gruba göre istatistiksel olarak anlamlı farkla daha yüksek bulundu (p=0,02). Değerlendirdiğimiz diğer laboratuvar parametreleri açısından iki grup arasında istatistiksel anlamlı fark tespit edilmedi.

Sonuç: Araştırmamıza göre FMS hastaları ile kontroller arasında sadece MPV düzeylerinde istatistiksel olarak anlamlı fark bulduk. FMS hastalarının tanısını kolaylaştırmada faydalı olabileceğini düşündürmektedir.

Anahtar Kelimeler: Fibromiyalji, inflamasyon, laboratuvar parametreleri, ortalama trombosit hacmi, ağrı

INTRODUCTION

Fibromyalgia (FMS) is a common cause of chronic pain that affects the muscles and soft tissues. FMS is characterized by a variety of symptoms, including fatigue, cognitive dysfunction, psychiatric issues, and various physical complaints (1, 2). The mechanisms that contribute to the development of FMS are not yet fully understood. However, according to the current mechanism, it is thought to be related to central sensitization, where sensitivity to pain increases due to dysfunction of neural circuits involved in the perception and processing of pain (3). The diagnosis is still made clinically and there are no objective markers that can be used.

Although FMS is considered a non-inflammatory disease, some studies have reported that patients have

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increased levels of C-reactive protein (CRP). This observation suggests the potential presence of low-grade chronic inflammation (4-6). CRP and ESR (erythrocyte sedimentation rate) are laboratory parameters frequently used to assess the presence and severity of inflammation. Although these markers are fast and widely applicable, they have some limitations such as cost, accessibility problems and fluctuations in their results. More stable and reliable indicators are needed to overcome these problems. In this context, the relevance of negative acute-phase reactants like albumin is becoming increasingly significant. CRP-albumin ratio (CAR) is considered a more sensitive and specific marker than CRP levels alone and stands out as a low-cost and accessible biomarker in clinical practice (7).

On the other hand, parameters such as red blood cell distribution width (RDW), platelet distribution width (PDW), and mean platelet volume (MPV), which are routinely reported in the complete blood count (CBC) report, are also associated with inflammation (8-10). The association of RDW with inflammation has been linked to the effects of oxidative stress on erythropoiesis and the release of immature and differently sized erythrocytes into the circulation (11). Studies in different patient groups have shown that inflammatory responses trigger platelet activation via proinflammatory cytokines, which may lead to changes in platelet indices. The role of platelets in inflammation has been associated with the release of cytokines and chemokines, attracting leukocytes and facilitating adhesion to the endothelium at the site of injury (12).

CBC is a widely used and inexpensive test, and subclinical markers of inflammation such as platelet-lymphocyte ratio (PLR), neutrophil-lymphocyte ratio (NLR), monocyte-lymphocyte ratio (MLR), and systemic inflammation index (SII) can be easily obtained (13). These rates have been emphasized as predictors for disease activity in rheumatoid arthritis and ulcerative colitis, survival times of cancer patients, and major cardiac events in diabetic patients (14-18).

In addition to inflammation markers, the relationship between mean corpuscular haemoglobin concentration (MCHC) levels, which reflect tissue oxygenation, and chronic fatigue is also being investigated (19). In this respect, MCHC may be an important indicator for understanding fatigue and other symptoms in FMS patients. Creatinine levels, a product of muscle metabolism, may be affected by environmental and psychological stress as a result of sympathetic nervous system activity (20, 21).

FMS is a chronic musculoskeletal disorder characterized by widespread body pain, in which central sensitization and systemic inflammation are thought to play a role (22, 23). In local musculoskeletal disorders, there is no central sensitisation, but it is caused by injury, inflammation or strain in a specific area of the joint nerve connective tissue (24). We planned to investigate whether there would be a significant difference in FMS evaluation parameters in favour of systemic inflammation by selecting disorders with short-term localised inflammation, which is different from FMS, as a control group.

Despite significant progress in understanding FMS, the lack of reliable biomarkers continues to hinder accurate diagnosis and management strategies. Assessing both inflammatory markers and metabolic changes may enhance the understanding and management of FMS. This study aimed to assess the predictive capacity of laboratory parameters to distinguish FMS patients from individuals with localized musculoskeletal pain.

METHODS

Subjects

A total of 86 female patients aged between 30 and 65 years (FMS group N=43, localized musculoskletal disorders (LMD) N=43) were included in our study. Participants were recruited from the Physical Medicine and Rehabilitation outpatient clinic of Osmangazi University Faculty of Medicine between January 2022 and September 2023. Patients were selected through a cross-sectional file search.

The diagnosis of FMS was established based on the 2016 criteria of the American College of Rheumatology (25). Patients with widespread pain scale scores of 7 and above and symptom severity scale scores of 5 and above were included in the study. The LMD group consisted of patients with a symptom duration of less than one month and a single diagnosis from the following: mild carpal tunnel syndrome, rotator cuff syndrome, knee sprain or strain, or Kellgren & Lawrence grade 1 gonarthrosis. Exclusion criteria included: age below 30 or above 65 years, chronic inflammatory diseases (e.g., Hashimoto's thyroiditis, Crohn's disease, ulcerative colitis, multiple sclerosis), inflammatory rheumatic diseases (e.g., rheumatoid arthritis, Sjögren's disease, ankylosing spondylitis), acute or subacute infections, diabetes mellitus, malignancies, thrombocytopenic conditions associated with bleeding disorders, anticoagulant use, pregnancy, smoking, or incomplete data.

Clinical assessment and laboratory data

The patients' ages and laboratory values were recorded. The serum parameters were obtained from blood test results taken during the outpatient clinic visit, immediately before the diagnosis was made. From the laboratory parameters obtained from the CBC, the values of white blood count (WBC), neutrophils, lymphocytes, platelets, MCHC, MPV, PDW, and RDW, as well as NLR, PLR, and MLR, were calculated respectively by dividing the absolute neutrophil count by the absolute lymphocyte count, the absolute platelet count by the absolute lymphocyte count, and the absolute neutrophil count by the absolute monocyte count.

Additionally, the systemic immune-inflammation index (SII) was calculated using the formula: neutrophils × platelets / lymphocytes.

Among the biochemical parameters, CRP, ESH, creatinine and albumin values were recorded. The CRP/albumin ratio was calculated and noted.

The study adhered to the principles outlined in the Declaration of Helsinki and received ethical approval from the Local Ethics Committee on 31.10.2023 (approval number: 50).

Statistical analysis

Normality of the distribution for each continuous variable was assessed using the Shapiro-Wilk test. Variables with non-normal distributions were analyzed using the Mann-Whitney U-test and reported as medians with interquartile ranges (25th-75th percentiles). Variables that followed a normal distribution were analyzed using the independent samples t-test, with results expressed as mean ± standard deviation. A p-value of less than 0.05 was regarded as statistically significant. All statistical analyses were performed using SPSS software, version 22.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Out of a total of 110 patient records reviewed, 24 were excluded based on the study's exclusion criteria. The final study cohort comprised 43 patients diagnosed with FMS and 43 patients with LMD. All participants in the study were women. The mean age of participants was 46.7±8.9 years in the FMS group and 46.3±8.6 years in the local pain group. No notable age difference was observed between the groups (p = 0.80). MPV (p=0.02) was significantly higher in the fibromyalgia group compared to the LMD group. No statistically significant differences were identified between the two groups for other laboratory parameters (Table 1).

Table 1. Comparative analysis of laboratory parameters and calculated ratios between groups

	Fibromyalgia group (N=43)	Localized musculoskletal disorders group (N=43)	*P-value*
Age (years)[a]	46.7±8.9	46.3±8.6	0.80*
ESH (mm/h)[a]	17.02±8.5	15.1±6.4	0.25*
CRP (mg/l)[b]	1.8 (1.0-3.5)	1.4 (1.0-2.4)	0.43**
Albumin[a]	4.5±0.2	4.6±0.28	0.50*
Creatinin[b]	0.68 (0.63-0.76)	0.67 (0.61-0.73)	0.34**
PDW[a]	12.3±2.04	12.3±2.05	0.98*
MPV[a]	10.5±0.9	10.01±1.06	**0.02***
WBC (10^3)[b]	6.9 (5.8-8.1)	6.8 (5.8-7.6)	0.73**
PLT (mm^3)[b]	290 (262-334)	301 (255-351)	0.77**
RDW[b]	13.4 (12.8-14.1)	13 (12.5-13.5)	0.051**
MCHC (%)[b]	33.1 (32.8-33.7)	33.3 (32.4-34)	0.63**
NLR[b]	1.7 (1.4-1.9)	1.9 (1.4-2.1)	0.52**
PLR[b]	129.9(113.2-147.2)	138.04(109.2-161)	0.42**
MLR[b]	0.22 (0.19-0.25)	0.21 (0.17-0.29)	0.51**
SII[b]	496.1 (407.4-616.4)	518.7 (375.8-642.8)	0.74**
CAR[b]	0.38 (0.19-0.79)	0.32 (0.22-0.52)	0.45**

[a]: Mean ±SD, [b]: Median (25-75%), *Analyzed by independent samples t-test; **Analyzed by Mann-Whitney U test.

Abbreviations: ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, PDW: Platelet distribution width, MPV: Mean platelet volume, WBC: White blood count, PLT: Platelets, RDW: Red blood cell distribution, NLR: Neutrophil/Lymphocyte Ratio, PLR: Platelet/Lymphocyte Ratio, MLR: Monocyte/ Lymphocyte Ratio, SII: Systemic immune-inflammation index, CAR: CRP/albumin

DISCUSSION

This study aims to analyze the differences between FMS patients and individuals with LMD by evaluating inflammatory and metabolic markers that may contribute to FMS pathogenesis. Central sensitization is considered to be the primary cause of changes in pain perception in FMS. In particular, neuroplastic changes in nerve cells in the spinal cord and brain lead to exaggerated processing of pain signals. Inflammation is thought to play a role in this processing (23). Despite this, no marker has been shown to predict disease severity or activity in FMS in routine use. Metya et al. defined a FMS subgroup with an increased inflammatory response (4). Pro-inflammatory cytokines, including IL-6, IL-8, and TNF-α which mediate inflammatory processes, have been implicated in the pathogenesis of FMS in various studies. Increased levels of cytokines may predispose to hyperalgesia through the increase of substance P (6, 26). Using these cytokines as biomarkers in FMS patients is not possible in clinical practice. Therefore, we investigated whether the systemic inflammatory response markers, blood parameters and ratios (CAR, NLR, PLR, MLR and SII) which are cheaper, and easily accessible, could be used in the diagnosis of fibromyalgia syndrome. Among the laboratory parameters we evaluated in our study, we found only the MPV value to be higher in FMF patients compared to the LMD group.

CRP is a proinflammatory marker whose levels are elevated in chronic inflammatory and rheumatic diseases. Hıra et al. reported higher CRP levels in the FMS group compared to the control group, suggesting that inflammation may contribute to the pathogenesis of FMS (27). However, in some studies, similar to our study, no statistical difference was found in CRP and ESR values when fibromyalgia patients were compared with the control group (28-30). Another suggested marker of inflammation is the CRP-

albumin ratio. The fact that high CRP and low albumin are associated with inflammatory processes suggests that this index may be a better indicator than CRP alone (7). In our study, both groups had similar CAR values. This was also observed by Pamukcu et al. in their study, where no significant difference was found between the control and FMS groups in terms of the CAR parameter (31).

Our findings are consistent with previous studies reporting increased MPV levels in FMS patients (5, 32). MPV is a sensitive measurement of platelet size obtained from CBC. It is considered an indicator of platelet activity, and increased MPV is associated with greater granule content and higher reactivity (33). MPV is an indicator of vascular risk factors and has been found to be increased in some diseases such as diabetes mellitus, acute ischemic stroke, and myocardial infarction (33, 34). In recent years, MPV has been suggested to be used as a marker of rheumatoid arthritis disease activity (10, 35). Proinflammatory cytokines, especially IL-6, may contribute to the increase in TPO and the induction of megakaryopoiesis (36, 37). Haliloglu et al. detected higher MPV in FMS compared to controls, independently of smoking, obesity and inflammation, and emphasized that there may be an increased cardiovascular risk in these patients (28). However, platelet count and MPV levels can be influenced by physiological and pathological conditions (Table 2) (31, 38, 39). Further research and detailed standardization are needed to understand their roles in diseases.

Table 2. Factors Influencing Mean Platelet Volume (MPV)

1. Age
2. Gender
3. Race/ethnicity
4. Genetic variants
5. Body mass index
6. Diet, smoking and alcohol consumption
7. Physical activity
8. Hormonal profile
9. Antiplatelet drugs
10. Preanalytical and analytical procedures

NLR, PLR, MLR and SII are novel inflammatory biomarkers used in various diseases as prognostic factors (11-14). Although it is found to be very useful in determining both disease activity and prognosis in many rheumatic and proliferative diseases, such a relationship was not found in our study. Contrary to our study, Al-Nimer et al. found that NLR and PLR levels of patients with FMS can predict the severity and prognosis of the disease (40). Researchers reported that these indexes were independent predictors of fibromyalgia diagnosis through regression analysis. Similar to our study, Karatas et al. did not find a relationship between NLR, PLR, MLR and FMS (32). A study by Uysal et al. found that the systemic immune-inflammation index (SII) was significantly higher in patients with lateral epicondylitis than in healthy controls (41). Although this finding indicates that the Systemic Immune-Inflammation Index (SII) may be a valuable inflammatory marker for disorders of the musculoskeletal system, our study revealed no significant difference in SII values between the two groups.

One of the primary limitations of our study is its retrospective design. For this reason, the relationship between the patients' pain levels, disease activity and laboratory parameters could not be explained. Another limitation is that our study group was small. Furthermore, the sensitivity, specificity and cut-off values for MPV were not subjected to analysis.

CONCLUSION

MPV levels showed a significant difference between individuals with fibromyalgia and the control group. While this finding suggests a potential association, MPV alone cannot be considered a definitive diagnostic marker for FMS. Further multicenter studies with larger sample sizes are necessary to validate the hematological and biochemical indices, determine their cut-off values, and gain deeper insights into the mechanisms underlying FMS.

Ethics Committee Approval: The study adhered to the principles outlined in the Declaration of Helsinki and received ethical approval from the Eskisehir Osmangazi University Ethics Committee on 31.10.2023 (approval number: 50).

Informed Consent: Not applicable

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