Journal of Ankara University Faculty of Medicine

E-ISSN: 1307-5608

Ankara Üniversitesi Tıp Fakültesi mecmuası

Cilt-Volume: 76 Sayı-Issue: 1 Mart-March 2023

Özgün Makaleler / Research Articles

ANKARA TIP 75 yıl

galenos yayınevi

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Journal of Ankara University Faculty of Medicine

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Journal of Ankara University school of medicine is an official journal of the Ankara University Faculty of Medicine.

Yayınevi İletişim/Publisher Contact
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Telefon/Phone: +90 (530) 177 30 97
E-posta/E-mail: info@galenos.com.tr/yayin@galenos.com.tr **Web:** www.galenos.com.tr
Yayıncı Sertifika No: 14521

Yayın Tarihi/Publishing Date: Mart 2023 / March 2023

ISSN: 0365-8104 **E-ISSN:** 1307-5608

Üç ayda bir yayımlanan süreli yayındır.
International scientific journal published quarterly.

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AMAÇ VE KAPSAM

Ankara Üniversitesi Tıp Fakültesi Mecmuası, Ankara Üniversitesi Tıp Fakültesi'nin resmi yayınıdır. Bağımsız, çift-kör hakemli ve açık erişimli bir dergidir. Yılda 4 sayı olmak üzere Mart, Haziran, Eylül, Aralık aylarında yayınlanır. Mecmua; Temel, Dahili ve Cerrahi alanlarda araştırma makaleleri, davetli derleme ve olgu sunumları yayınlar.

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Dergi Adı (İngilizce): Journal of Ankara University Faculty of Medicine

Dergi Adı (Türkçe): Ankara Üniversitesi Tıp Fakültesi Mecmuası

Resmi Kısaltma: J Ankara Univ Fac Med

E-ISSN: 1307-5608

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The aim of the journal is to publish articles at the highest scientific and clinical value on current topics in medicine for all researchers, physicians, specialists and students.

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Journal of Ankara University Faculty of Medicine is indexed in **Tübitak/Ulakbim TR Dizin, Ebsco, Index Copernicus, Gale, ProQuest, CABI, Idealonline, J-GATE, Hinari, GOALI, ARDI, OARE, AGORA, Türk Medline** and **Türkiye Atıf Dizini**.

English Title: Journal of Ankara University Faculty of Medicine

Turkish title: Ankara Üniversitesi Tıp Fakültesi Mecmuası

Official abbreviation: J Ankara Univ Fac Med

E-ISSN: 1307-5608

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Publisher Info

Galenos Publishing House

Address: Molla Gürani Mahallesi Kaçamak Sokak No: 21 34093 Fındıkzade - İstanbul/Turkey

Phone: +90 (212) 621 99 25

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YAZARLARA BİLGİ

Ankara Üniversitesi Tıp Fakültesi Mecmuası, Ankara Üniversitesi Tıp Fakültesi tarafından üç ayda bir (Mart, Haziran, Eylül, Aralık) yayınlanır. Temel, Dahili, ve Cerrahi Tıp Bilimleri konusunda araştırma, davetli derleme ve olgu sunumları yayınlamayı amaçlar.

Ankara Üniversitesi Tıp Fakültesi Mecmuası bağımsız, önyargısız ve çift-kör hakemlik ilkeleri çerçevesinde yayın yapan süreli bir yayın organıdır. Makale baş editöre ulaşınca bilimsel kalitesi değerlendirilir ve ön değerlendirmeyi geçen yazılar yardımcı editöre gönderilir. Bölüm editörü makaleyi 2 hakeme gönderir. Hakemler 21 gün içinde kararlarını belirtmelidirler. Yardımcı editör hakem kararlarına kendi değerlendirme ve önerisini ekleyerek baş editöre gönderir ve son kararı baş editör verir. Hakemlerin kararları çatışıyorsa dergi editörü yeni hakem atayabilir.

Derginin editöryal ve yayın süreçleri ile etik kuralları the International Committee of Medical Journal Editors (ICMJE), World Association of Medical Editors (WAME), Council of Science Editors (CSE), Committee on Publication Ethics (COPE), European Association of Science Editors (EASE), ve National Information Standards Organization (NISO) gibi uluslararası kuruluşların kurallarına uygun olarak şekillenmektedir. Dergimiz, şeffaf olma ilkeleri ve "akademik yayıncılıkta en iyi uygulamalar ilkeleri" ile uyum içindedir.

Dergiye yayınlanmak amacıyla gönderilen ve etik kurul onayı alınması zorunluluğu olan deneysel, klinik ve ilaç araştırmaları için uluslararası anlaşmalara ve Helsinki Bildirisine uygun etik kurul onay raporu gereklidir. Etik kurul onayı ve "bilgilendirilmiş gönüllü onam formu" alındığı araştırmanın "Hasta ve Yöntem" bölümünde belirtilmelidir. Deneysel hayvan çalışmalarında ise yazarlar Guide for the Care and Use of Laboratory Animals doğrultusunda hayvan haklarını koruduklarını belirtmeli ve kurumlarından etik kurul onay raporu almalıdır.

Makale türlerinin gönderimi, araştırma raporlama kılavuzlarına uygun olarak tasarlanmalıdır:

İnsan araştırmaları: Helsinki Declaration as revised in 2013

Sistematik incelemeler ve meta-analizler: PRISMA guidelines

Vaka raporları: Cthe CARE case report guidelines

Klinik denemeler: CONSORT

Hayvan çalışmaları: ARRIVE ve Guide for the Care and Use of Laboratory Animals

Araştırmalara yapılan her türlü yardım ve diğer desteklerin alındığı kişi ve kuruluşlar beyan edilmeli ve çıkar çatışmasıyla ilgili durumları açıklamak amacıyla Çıkar Çatışmaları Bildirim Formu doldurulmalıdır.

Yayın, direkt ya da indirekt ticari bağlantı içeriyorsa veya çalışmaya materyal desteği veren bir kuruluş varsa, yazarlar kullanılan ticari ürün, ilaç, firma vs. ile ticari hiçbir ilişkisinin olmadığını ya da var ise nasıl bir ilişkisinin olduğunu (konsültan, diğer anlaşmalar), editöre sunum sayfasında belirtmek zorundadır.

İncelemeye sunulan araştırmada olası bir bilimsel hata, etik ihlal şüphesi veya iddiasıyla karşılaşılırsa, bu dergi verilen yazıyı destek kuruluşların veya diğer yetkililerin soruşturmasına sunma hakkını saklı tutar. Bu dergi sorunun düzgün biçimde takip edilmesi sorumluluğunu kabul eder ancak gerçek soruşturmayı veya hatalar hakkında karar verme yetkisini üstlenmez.

Genel Kurallar

Yazılar sadece çevrim-içi olarak kabul edilmektedir. Yazarların makale gönderebilmesi için Journal Agent web sayfasına kayıt olup, şifre almaları gerekmektedir.

Türkçe yazılarda Türk Dil Kurumu'nun Türkçe Sözlüğü ve Yazım Kılavuzu temel alınmalıdır.

Sayfa düzeni: Makaleler, A4 sayfasınının iki yanında 2.5 cm boşluk bırakacak şekilde, Arial yazı stilinde, 12 font büyüklüğünde, 1.5 satır aralığıyla, Microsoft Word programında yazılmalıdır.

Kısaltmalar: Kelimenin ilk geçtiği yerde parantez içinde ve tüm metin boyunca kullanılır. Uluslararası kullanılan kısaltmalar için "Bilimsel Yazım Kuralları" kaynağına başvurulabilir.

Editöre sunum sayfası: Gönderilen makalenin kategorisi, daha önce başka bir dergiye gönderilmemiş olduğu, var ise çalışmayı maddi olarak destekleyen kişi ve kuruluşlar ve bu kuruluşların yazarlarla ilişkileri, makale İngilizce ise; İngilizce yönünden kontrolünün ve araştırma makalesi ise biyoistatistiksel kontrolünün yapıldığı belirtilmelidir.

Yazı Çeşitleri

Sisteme yüklenen tüm makaleler aşağıdaki kurallara uygun olmalıdır:

Başlık sayfası: Makalenin başlığı, kısa başlık, yazar isimleri ve yazar bilgilerini kapsayan sayfadır. Sırasıyla şu tanımlar yapılmalıdır:

1. Makalenin başlığı (Türkçe ve İngilizce) mümkün olduğunca kısa ve açıklayıcı olmalı, kısaltma içermemeli ve 12 kelimeyi aşmamalıdır.
2. Kısa başlık (Türkçe ve İngilizce) en fazla 60 karakterden oluşmalıdır.
3. Yazar isimleri (yazarların isimleri tam olarak kısaltılmadan yazılmalıdır, yazarın akademik görevi yazılmamalıdır) ve bağlı bulunduğu kurumlar.
4. İletişim kurulacak yazarın ismi, adresi, telefon ve faks numarası ile e-posta bilgileri.
5. Bilimsel toplantılarda sunulan ve özeti kongre kitabında yer almış eserlerin toplantı yeri ve tarihi.

Özet: Makalenin özeti Türkçe ve İngilizce yazılmalıdır. Özet 250 kelimeyi aşmamalıdır. Kaynaklara atıf yapılmamalıdır. Mümkün olduğunca kısaltmalar kullanılmamalıdır; kullanıldığı halde ana metinden bağımsız olarak ele alınmalıdır.

Araştırma makalelerinde, özet aşağıdaki başlıklardan oluşmalıdır:

Amaç: Çalışmanın amacı açıkça belirtilmelidir.

Gereç ve Yöntem: Çalışma tanımlanmalı, standart kriterleri, randomize olup olmadığı, retrospektif veya prospektif olduğu ve varsa istatistiksel yöntem belirtilmelidir.

Bulgular: Çalışmanın detaylı sonucu verilmeli, istatistik anlamlılık derecesi belirtilmelidir.

Sonuç: Çalışmanın sonuçlarını yansıtmalı, klinik uygulanabilirliği tanımlamalı, olumlu ve olumsuz yönleri gösterilmelidir.

Anahtar Kelimeler: En az 3, en çok 5 anahtar kelime özetin sonunda yer almalıdır. İngilizce anahtar kelimeler "Medical Subject Headings'e (MESH)" uygun olarak verilmelidir. Türkçe anahtar kelimeler ise MESH terimlerinin aynen çevirisi olmalıdır.

Özgün Araştırmalar

Klinik araştırma, klinik gözlem, yeni teknikler, deneysel ve laboratuvar çalışmalarını kapsar. Özgün araştırmalar; başlık, öz, yazının ana konusu ile ilgili anahtar kelimeler, giriş, hasta/gereç ve yöntem, bulgular, tartışma, kaynaklar, tablolar/şekiller ve teşekkür bölümlerini içermelidir. Başlık, öz ve anahtar kelimeler hem Türkçe hem İngilizce yazılmalıdır. Yazı yukarıdaki kurallara uygun olarak düzenlenmeli ve 3000 kelimeyi aşmamalıdır.

Giriş: Konu hakkında kısa ve öz bilgi verilmeli, çalışmanın amacı belirtilmeli, bunlar literatür bilgisi ile desteklenmelidir.

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Gereç ve Yöntem: Çalışma planı verilmeli, randomize olup olmadığı, retrospektif veya prospektif olduğu, denek sayısı, özellikleri, çalışmaya dahil edilme ve dışlanma kriterleri, kullanılan istatistiksel yöntem belirtilmelidir.

Bulgular: Elde edilen sonuçlar belirtilmeli, tablolar ve şekiller numara sırasıyla verilmeli, sonuçlar uygulanan istatistiki analiz yöntemine göre değerlendirilmelidir.

Tartışma: Elde edilen değerler olumlu ve olumsuz yönleriyle tartışılmalı, literatür ile karşılaştırılmalı, çalışmadan elde edilen sonuç vurgulanmalıdır.

Teşekkür: Her türlü çıkar çatışması, finansal destek, bağış ve diğer editöryal (istatistik analiz, İngilizce/Türkçe değerlendirme) ve/veya teknik yardım var ise metnin sonunda sunulmalıdır.

Kaynaklar: Kaynak sayısı 40'ı aşmamalıdır. Kaynakların gerçekliğinden yazarlar sorumludur. Kaynakların yazım kuralları hakkında gerekli bilgi "Kaynaklar" bölümünde bulunmaktadır.

Olgu Sunumları

Nadir görülen, tanı ve tedavide farklılık gösteren, mevcut bilgilerimize yenilerini ekleyip, katkı sağlayan olguları içermelidir. Türkçe ve İngilizce başlık, 250 kelimeyi aşmayan, yapılandırılmamış öz ve anahtar kelimeler ilk sayfada yer almalıdır. Ana metin 1500 kelimeyi aşmamalı ve giriş, olgu sunumu, tartışma ve 20'yi aşmayan kaynaklardan oluşmalıdır.

Derleme

Dergi sadece davetli derleme yayınlar. Bir bilgi ya da konunun klinikte kullanılması için vardığı son düzeyi anlatan, tartışan, değerlendiren ve gelecekte yapılacak olan çalışmalara yön veren bir formatta hazırlanmalıdır ve güncel bir konuyu, bağımsız, hiçbir farklı görüşü öne çıkarmadan derinlemesine incelemelidir. Yazının ilk sayfasında Türkçe ve İngilizce başlık, yapılandırılmamış öz, anahtar kelimeler bulunmalıdır. Konuda geçen her alıntının kaynağı mutlak gösterilmeli ve 100'ü geçmemelidir. Ana metin 5000 kelimeyi aşmamalıdır.

Kaynaklar

Yazarlar kaynakların gerçekliğinden ve metin içindeki doğru kullanımından sorumludur. Tüm kaynaklar aşağıdaki kurallara göre düzenlenmelidir:

Metin içinde: Kullanılan kaynaklar, ilgili cümlenin sonunda noktadan önce parantez içinde belirtilmelidir. Eğer, kullanılan kaynağın yazar/yazarları cümle başında belirtiliyorsa, kaynak isimden hemen sonra gelecek şekilde parantez içinde yazılmalıdır. Türkçe kaynak var ise, belirtilmesine özen gösterilmelidir.

Kaynaklar bölümünde: Kaynaklar metin içerisinde geçiş sırasına göre numaralandırılmalıdır. Üçten fazla yazar ismi olduğu durumlarda, üç yazardan sonra Türkçe yayınlarda "ve ark.", yabancı dildeki yayınlarda "et al." kullanılmalıdır. Dergilerin isimleri Index Medicus'ta kullanılan stillere göre kısaltılmalıdır.

Kaynak yazımı için örnekler

Dergi: Vargün R, Özkan-Ulu H. Nörolojik problemli çocuklarda beslenme problemleri ve tedavisi. J Ankara Univ Fac Med. 2004;4:181-185.

Kitap: Çakmak M. Ortopedik muayene. 2nd ed. Istanbul: Nobel Tıp Kitapevleri; 1991.

Editörlü kitap: Kurt N, editör. Yetişkinlerde ve Çocuklarda Ameliyat Öncesi Değerlendirme. Istanbul: Nobel Tıp Kitapevleri; 2002.

Kitap içinden bir bölüm: Rowe JS. Liver. In: Skandalakis JE, Gray SW, Rowe JS, editörler. Anatomical Complications in General Surgery. 1st ed. New York: McGraw-Hill Book Co.; 1986. s. 103-124.

Bilimsel toplantıda yapılan sunumlar: Seyhan F. Kalça ekleminde yüzey değiştirme artroplastisinin (Wagner protezi) geç sonuçları. In: Ege R, editör. X. Milli Türk Ortopedi ve Travmatoloji Kongre Kitabı; 17-20 Mayıs, 1987; Mersin, Türkiye. Ankara: Emel; 1989. s. 494-6 (Yayınlanmamış toplantı sunumlarının kaynak gösterilmemesi gerekir)

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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

DOI: 10.4274/atfm.galenos.2023.22590
Ankara Üniversitesi Tıp Fakültesi Mecmuası 2023;76(1):1-10

TEMEL TIP BİLİMLERİ / BASIC MEDICAL SCIENCES

Diyabetik Kardiyomiyopatide Kronik Ilımlı Egzersizin İskemi - Reperfüzyon Hasarına Etkisi

Effect of Chronic Moderate Intensity Exercise on Ischemia - Reperfusion Injury in Diabetic Cardiomyopathy

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Öz

Amaç: Diyabet hiperglisemi ile karakterize yaygın metabolik bir rahatsızlıktır. Diyabetiklerde kalp, iskemi/reperfüzyon (I/R) hasarına karşı daha hassas hale gelmektedir. Kalpte meydana gelen bu bozulmanın nedeninin diyabete bağlı oksidatif stres artışı olduğu düşünülmektedir. Uygun şiddet ve sıklıkta uygulanan egzersiz oksidatif stresi azaltmakta ve I/R hasarına karşı koruyucu etki göstermektedir. Çalışmamızda kronik ılımlı egzersizin diyabette I/R hasarı ve oksidatif stres üzerindeki etikleri araştırılmıştır.

Gereç ve Yöntem: Çalışmada 10 haftalık erkek Wistar albino sıçanlar kullanılmıştır (n=36). Hayvanlar rastgele dört gruba ayrılmıştır: Kontrol (K), Egzersiz (EX), Diyabet (DM), Diyabet+Egzersiz (DM+EX). Tip 1 diyabet streptozotosin (50 mg/kg) enjeksiyonu ile oluşturulmuştur. Hayvanlara artımlı yük testi uygulanarak maksimum egzersiz kapasiteleri (MEK) belirlenmiştir. Hayvanlar MEK değerlerinin %70'ine denk gelecek şekilde günde 45 dakika haftada 5 gün olacak şekilde 12 hafta boyunca egzersiz yapmışlardır. Hayvanların kalpleri Langendorff düzeneğine alınmış 30 dakika global iskemi/120 dakika reperfüzyon uygulanmıştır. Sol ventrikül gelişimsel basıncı, kalp hızı, hız-basınç çarpımı parametreleri ölçülmüştür. Alınan plazma ve sol ventrikül örneklerinde total oksidan ve antioksidan status, tiyol disülfit düzeyi ölçümleri yapılmıştır.

Bulgular: Kalp ağırlığı/Vücut ağırlığı verileri incelendiğinde diyabetik hayvanlarda diyabete bağlı hipertrofi geliştiğini, egzersizin hipertrofiyi engelleyemediği anlaşılmıştır. DM grubunda kontrole göre iskemi sonrası toparlanma cevabında bozulma görülmüştür. EX grubunda iskemiye tolerans K grubuna göre artarken, DM+EX grubunda K ve DM grubuna göre anlamlı derecede daha kötü toparlanma cevabı gözlenmiştir. Hem plazma hem de sol ventrikül dokusunda ölçülen oksidatif stres verilerinde herhangi bir anlamlı değişime rastlanmamıştır.

Sonuç: Kullanılan egzersiz protokolünün diyabetin yol açtığı I/R hassasiyetini daha da ilerlettiği anlaşılmaktadır. Non-diyabetik hayvanlarda uygulanan egzersizin kardiyoprotektif etkinliği gözlenirken, diyabetik hayvanlara uygun bir protokol olmadığı anlaşılmıştır. Oksidatif streste anlamlı değişiklikler gözlenmemiştir. İlerleyen çalışmalarda örneklem genişliğinin büyütülmesi ve daha düşük egzersiz şiddeti ile çalışılması ile değerli sonuçlar elde edilebilir.

Anahtar Kelimeler: Diyabet, Egzersiz, Oksidatif Stres, İskemi/Reperfüzyon

Abstract

Objectives: Diabetes is a common metabolic disorder characterized by hyperglycemia. In diabetics, the heart becomes more susceptible to ischemia/reperfusion (I/R) injury. The reason for this deterioration in the heart is oxidative stress increase due to diabetes. Exercise applied at appropriate intensity and frequency reduces oxidative stress and has a protective effect against I/R damage. In our study, the effects of chronic moderate exercise on I/R damage and oxidative stress in diabetes were investigated.

Materials and Methods: Ten-week-old male Wistar albino rats were used in the study (n=36). Animals were randomly divided into four groups: Control (K), Exercise (EX), Diabetes (DM), Diabetes+Exercise (DM+EX). Type I diabetes was induced by injection of streptozotocin (50 mg/kg). The

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Geliş Tarihi/Received: 25.02.2022 **Kabul Tarihi/Accepted:** 12.04.2023

Abstract

exercise capacity (MEC) was determined by applying the incremental load test. Animals exercised 45 minutes/day, 5 days/week, for 12 weeks, corresponding to 70% of their MEC. Hearts were removed and placed in the Langendorff apparatus and 30 minutes of global ischemia/120 minutes of reperfusion was applied. Left ventricular developmental pressure, heart rate, rate-pressure product parameters were measured. Total oxidant and antioxidant status, thiol disulfide levels were measured in the plasma and left ventricular samples.

Results: According to heart weight/body weight data, diabetes-induced hypertrophy developed in diabetic animals, and exercise could not prevent hypertrophy. The recovery after ischemia was impaired in the DM group, while it was increasing in the EX group compared to the K. A significantly worse recovery response was observed in the DM+EX group compared to the K and DM. No significant changes were found in the oxidative stress data both in the plasma and left ventricle samples.

Conclusion: Current exercise protocol increased diabetes-induced I/R sensitivity. While there was a cardioprotective effect in EX, the protocol was not a suitable protocol for diabetics. No significant change in oxidative stress was observed. In future studies, valuable results can be obtained by enlarging the sample size and prefering lower exercise intensity.

Key Words: Diabetes, Exercise, Oxidative Stress, Ischemia/Reperfusion

Giriş

Diabetes mellitus glikoz metabolizmasındaki bozukluklar, insülin etkinliği ve/veya salgısında meydana gelen değişimler nedeniyle kronik hiperglisemi ile kendini gösteren, sık rastlanan, metabolik bir hastalıktır (1). Günümüzde hareketsiz yaşam tarzı ve kötü beslenme alışkanlıkları nedeniyle diyabet hastalığı oldukça büyük bir toplum sağlığı problemi haline gelmiştir. 2030 yılı itibariyle dünya çapında 366 milyon insanın diyabetik olacağı öngörülmektedir (2).

Diyabette kardiyovasküler komplikasyonlar en önemli morbidite ve mortalite nedenleri arasındadır (3). Diyabetik bireylerde kalp, iskemi/reperfüzyon (I/R) hasarına karşı daha hassas hale gelmekte ve iskemi sonrası toparlanma cevabında azalmalar görülmektedir (4-7). Literatürde, I/R uygulaması sonrası, diyabetik hayvanların infarkt alanlarının kontrol hayvanlarına göre daha büyük olduğu gözlenmiştir (8). Streptozotosin (STZ) enjeksiyonu ile diyabet yapılan kemirgenlerde oksidatif stres artışına paralel I/R hasarı artışı saptanmış, antioksidan etkinliği bilinen maddelerin uygulanması I/R hasarında azalmaya neden olmuştur (7,9,10). Diyabetiklerde görülen I/R hasarındaki artışın en önemli nedeni artmış kronik, bazal oksidatif strestir (11).

Oksidatif stres artışı, reaktif oksijen türevleri (ROS) adı verilen, hücrelerde oksidatif metabolizma sonucunda ortaya çıkan serbest radikallerin artması veya bu radikalleri tamponlama görevi gören antioksidan enzim sistemlerinde meydana gelen bozulmalar nedeniyle gerçekleşir. Artmış oksidatif stres hücrenin yaşamsal faaliyetleri için kritik önemde olan proteinler, nükleik asitler gibi moleküllerin hasar görmesine neden olur (12). Diyabetik insan ve hayvan çalışmalarında oksidatif stresin hem doku düzeyinde hem de plazmada artış gösterdiği bildirilmektedir (6,13-16).

Düzenli yapılan egzersizin insan sağlığı üzerindeki yararlı etkileri bilinmektedir. Aerobik egzersiz, görece düşük şiddette sürdürülen ve enerji ihtiyacının temelde oksidatif süreçlerden karşılandığı, anaerobik egzersiz ise maksimale yakın efor gerektiren, genellikle glikolitik metabolizmanın baskın olduğu egzersiz tipidir (17). Literatürde egzersizin I/R hasarını iyileştirici etkisi hem insan çalışmalarında hem de deney hayvanı modellerinde defalarca gösterilmiştir (18-22). Her ne kadar egzersizin I/R hasarı üzerindeki yararlı etkileri bilinse de sağlıklı veya kronik hastalığı olan (diyabetik, dislipidemik, hipertansif, vb.) gruplarda doğru egzersiz protokolünün belirlenmesi hala üzerinde araştırma yapılan bir alandır. Her ne kadar anaerobik egzersiz önkoşullama etkisi ile kardiyak ve metabolik süreçlerde potent bir etkiye sahip olsa da, kronik hastalığı olan gruplarda olumsuz etkilere neden olabilmektedir (23). Bu nedenle çalışmamızda orta şiddette egzersize karşılık gelen bir egzersiz protokolü seçilmiştir.

Çalışmamızın amacı STZ enjeksiyonu ile diyabet oluşturulmuş sıçanlarda egzersizin kardiyak I/R hasarı üzerindeki etkilerini araştırmaktır. Çalışmamızda, 12 haftalık kronik, orta şiddette yapılan ve hayvanların maksimal egzersiz kapasitesine (MEK) göre planlanan koşu egzersizinin diyabetin yaratacağı kardiyak I/R hassasiyetini engelleyeceğini hipotezlenmektedir.

Egzersizin (egzersiz şiddeti, tipi ve yoğunluğuna bağlı olarak) genellikle antioksidan savunma sistemlerini güçlendirip, ROS'lerin üretimini azaltarak oksidatif stresi azalttığı bilinmektedir (24-26). Seçilen egzersiz protokolünün sıçanlarda oksidatif stresi azaltacağını ve bu azalmanın da I/R hasarında koruyucu bir etki göstereceği öngörülmektedir.

Gereç ve Yöntem

Deney Hayvanları ve Etik Kurul İzni

Çalışmada erkek, yetişkin (10 haftalık), Wistar albino (*Rattus norvegicus*) cinsi sıçanlar kullanılmıştır (n=36). Hayvanlar, Ankara Üniversitesi Deney Hayvanları Üretim ve Araştırma Laboratuvarı'ndan temin edilmiş, sabit sıcaklık (22-24 ˚C), ve nemin (%50-55), korunduğu hayvan laboratuvarımızda 12 saat aydınlık/12 saat karanlık döngüsünde barındırılmıştır.

Hayvanların standart sıçan yemine ve musluk suyuna sınırsız (*ad libitum*) erişimleri sağlanmıştır. Hayvan kullanım izni ve deney protokollerinin etik ilkelere uygunluğu Ankara Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu tarafından onaylanmıştır (karar no: 2017-4-25, tarih: 19/02/2016). Hayvanlara yapılan tüm işlemler ve verilerin sunumu ARRIVE Guidelines (Guide for the Care and Use of Laboratory Animals, 8th edition) bildirgesine uygun olarak yapılmıştır (27).

Laboratuvara gelen hayvanlar bir haftalık adaptasyonun ardından hayvanlar rastgele dört gruba ayrılmışlardır. Gruplar:

1. Kontrol (K) (n=9).
2. Egzersiz (EX) (n=9).
3. Diyabet (DM) (n=9).
4. Diyabet + Egzersiz (DM+EX) (n=9).

Deneysel Tip 1 Diyabetin Oluşturulması

STZ (Sigma-Aldrich, Missouri, ABD, S0130-1G) 0,1 M sitrat tamponu (pH 4,5) içerisinde çözüldükten sonra tip 1 diyabet yapılacak hayvanlara 50 mg/kg dozunda intraperitoneal (*i.p.*) olarak enjekte edilmiştir (28). Enjeksiyonu takip eden 1, 3 ve 7. günlerde kuyruk ucundan ufak bir kesi yapılarak glikometre (On Call Plus Glucometer, Acon Labs Inc. ABD) ile kan glikozu ölçümü yapılmış, her üç ölçümü de 250 mg/dL ve üstü olan hayvanlar diyabet olarak kabul edilerek çalışmaya dahil edilmiştir. Bu düzeyi sağlayamayan hayvanlar çalışma dışı bırakılmışlardır. Hayvanlar diyabet tanısını aldıktan bir hafta sonra (enjeksiyondan sonraki 7. gün) egzersiz programına alınmışlardır. Hayvanların kan şekeri ve vücut ağırlıkları düzenli olarak takip edilmiştir.

Artımlı Yük Testi ve Antrenman Programı

Antrenman programının başlangıcında egzersiz yapacak hayvanların MEK değerlerini belirlemek için artımlı yük testi uygulanmıştır (29). Artımlı yük testine hayvanlar düşük iş yükünde başlatılmış (5 m/dak ve 0° eğim), her üç dakikada bir hız 3 m/dakika veya eğim 2° artırılarak iş yükü artışı sağlanmıştır. Hayvanın elektriksel ve mekanik uyarıya rağmen devam etmeyi reddettiği iş yükü ilgili hayvanın MEK değeri olarak kabul edilmiştir. Antrenman programındaki egzersiz şiddeti her hayvan için kendi MEK değerinin %70'ine karşılık gelecek şekilde belirlenmiştir. Artımlı yük testi antrenman programının 3, 6 ve 9. haftalarında tekrarlanmış ve egzersiz şiddetleri yeni değerlere göre tekrar ayarlanmıştır. Antrenman programı süresince koşu bandının eğimi hep sabit tutulmuş (10°), egzersiz şiddetini değiştirmek için hayvanların koşu hızları artırılıp veya azaltılmıştır. Antrenman programı günde 45 dakika, haftada 5 gün olacak şekilde 12 hafta boyunca sürdürülmüştür. Günlük antrenmanın başında eğim ve hızın yavaş yavaş artırıldığı 7,5 dakikalık bir ısınma periyodu, antrenmanın sonunda ise aksine yavaş yavaş azaltıldığı 7,5 dakikalık bir soğuma periyodu uygulanmıştır.

Hayvanların Sakrifikasyonu

Son egzersiz seansından 24 saat sonra, ağırlık ve kan şekeri ölçülen hayvanlar sodyum tiopental (i.p; 50 mg/kg) ile anestezi altına alınmış. Parmak kıstırma yanıtlarının kaybolması ile anestezi derinliğinin sağlandığı anlaşılmış, ardından, hayvanların göğüs kafesleri hızlıca açılarak kalpleri izole kalp fonksiyonu ve I/R çalışmasında kullanılmak üzere çıkartılmıştır. Göğüs kafesine dolan kan toplanarak kırmızı kapaklı tüpe alınmış, 5000 g, +4 °C'de 10 dakika boyunca santrifüj edilmiştir. Elde edilen serum ileri analizler için -80 °C'de saklanmış, izole kalp fonksiyonu çalışmasında kullanılan kalp dokuları, çalışma sonrasında sıvı azot ile dondurularak -80 °C'de saklanmıştır.

İzole Kalp Fonksiyonu ve I/R

Langendorff düzeneğinde yapılan deneyler literatürdeki veriler ışığında tasarlanmıştır (30). Kısaca özetlemek gerekirse, hızlıca çıkartılan kalpler önceden karbojen ile gazlandırılmış (%95 O_2 ve %5 CO_2), buz üzerinde bekleyen, soğuk Krebs-Henseleit (mM: 119 NaCl, 4,8 KCl, 1,6 $CaCl_2$, 1,2 $MgSO_4$, 1,2 KH_2PO_4, 20 $NaHCO_3$, ve 10 glukoz, ve pH 7,4) çözeltisi içerisine alınmış, böylece soğuğun yarattığı kardiyoplejik etkiden yararlanılarak kalplerin Langendorff düzeneğine bağlanana kadar geçen sürede gördüğü zarar en aza indirilmiştir. Kalp ağırlığı, düzeneğe bağlanmadan önce solüsyon içerisinde tartılmıştır. Ardından kalpler Langendorff düzeneğindeki ilgili kanüle aorttan bağlanmıştır. Langendorff sistemi içerisinde bulunan Krebs-Henseleit solüsyonu deney süresince karbojen (%95 O_2 ve %5 CO_2) ile gazlandırılmış ve sıcaklığı sabit sıcaklıkta (37 °C) tutulmuştur. Kalpler deney boyunca belirli bir hızda aorttan bağlanan kanül ile ters yönlü perfüze edilmişlerdir (retroperfüzyon).

Perfüzyon hızı kalbin yüzey alanına göre ilgili formül kullanılarak hesaplanmıştır (perfüzyon hızı=7,43 x kalp ağırlığı^{0,56}). Sol atrium dokusu kesilerek uzaklaştırılmış ve açılan yarıktan sol ventrikülün içerisine içi su dolu, ince bir tüpe bağlı elastik bir balon yerleştirilmiştir. Balon sol ventrikülün içerisinde belirli bir basınç değerine kadar (=diastolik basınç ≈8-10 mmHg) şişirilerek, ventrikül duvarını sarması sağlanmıştır. Balona bağlı içi su dolu ince tüp ile elektronik bir basınçölçere (transducer) iletilen basınç değişimleri Biopac MP35 (Biopac Syst. CA, USA) sistemi ile dijital ortamda kayıt altına alınmıştır. Deneylere başlamadan önce kalp dokusunun stabilize olması için 20 dakika beklenmiştir.

Bazal (iskemi öncesi) kalp fonksiyonu kayıtlarının elde edilmesinin ardından kalbin perfüzyonu 30 dakika boyunca durdurularak global iskemi uygulaması yapılmıştır. Global iskeminin ardından 120 dakika boyunca reperfüzyon uygulaması yapılarak deney tamamlanmıştır. Elde edilen basınç değişimi grafiklerinde hem iskemi öncesi hem de iskemi sonrası çeşitli zaman noktalarında (15', 30', 45', 90' ve 120') veri analizler

yapılmıştır. Öncelikle grafiklerden sol ventrikül gelişimsel basıncı (LVDP) değerleri hesaplanmış, ardından bu değer o anki kalp hızıyla (HR) çarpılarak hız-basınç çarpımı (RPP) değeri elde edilmiştir. Bu işlemler hem iskemi öncesi hem de iskemi sonrasındaki (reperfüzyondaki) zaman noktaları için tekrarlanmıştır.

Oksidatif Stres Analizleri

Serum

Elde edilen serumlardan toplam antioksidan durumu (TAS), toplam oksidan durumu (TOS) ve Tiyol disülfit parametreleri analiz edilmiştir.

Oksidatif stres indeksi (OSI) değerleri %OSI = [(TOS, μmol H_2O_2 equivalent/L)/(TAS, μmol Trolox equivalent/L)] × 100 formülü ile hesaplanmıştır. Analizler kolorimetrik prensiplere göre çalışan kitler ile yapılmış, analizlerde üreticinin talimatnamesi takip edilmiştir (Rel Assay Diagnostics Gaziantep, Türkiye).

Kalp dokusu

Kalp dokusu örnekleri sol ventrikülün lateral duvarının orta 1/3'lük kısmından alınmıştır. Donuk olarak saklanan dokulardan alınan yaklaşık 100 mg'lik, bir parça içerisi azot dolu havanın içerisine konulmuş. çözülmesine izin verilmeden tokmak ile ezilerek küçük parçalara ayrılmıştır. Ardından doku parçaları özel bir tüpün içerisine alınarak üzerlerine 1:5 oranında homojenizasyon tamponu (0,1 M Phosphate-Buffered Saline) eklenmiştir. Karışım teflon uçlu özel bir homojenizatör (Glas-Col LLC, Homogenizer; IN, USA) yardımıyla homojenize edilmiş, ardından 5000 g, +4 °C'de 5 dakika boyunca santrifüj edilmiştir. Süpernatant alınarak saklanmış pellet atılmıştır. Ayrılan homojenizatlar hücre zarlarının ileri düzeyde parçalanabilmesi için son bir aşama olarak sonikatör (Fisher Scientific Sonic Dismembrator 550 New Hampshire, USA) yardımıyla buz üzerinde degrade edilmiştir. Son ürünlerde TAS ve TOS parametreleri yukarıda belirtilen yöntemlerle incelenmiş ve OSI değerleri hesaplanmıştır.

İstatistiksel Analiz

İstatistiksel analiz için GraphPad Prism (GraphPad Prism for Windows v5 2007) yazılımı kullanılmıştır. Öncelikle tüm parametreler için veri setlerine Shapiro-Wilk testi uygulanarak verilerin normal dağılım gösterip göstermediği tespit edilmiş, elde edilen sonuçlar ışığında parametrik test varsayımlarının sağlandığı kanaati oluştuğu için çoklu karşılaştırmalarda One Way ANOVA testi tercih edilmiştir. Farkın tespiti halinde post-hoc analizler için Tukey testi kullanılmıştır. Reperfüzyon boyunca elde edilen RPP değerleri iki yönlü varyans analizi (Repeated Measures ANOVA) ile karşılaştırılmıştır. Veriler ortalama ($\bar{x}$) ± standart sapma (SS) olarak sunulmuş, $p<0.05$ düzeyi istatistiksel olarak anlamlı kabul edilmiştir.

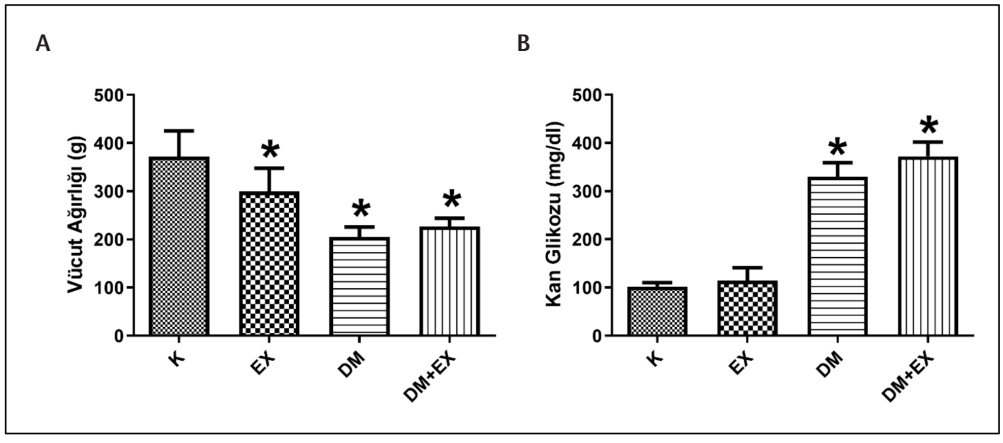
Bulgular

Deney Hayvanı Takip Verileri

Hayvanların sakrifikasyon öncesi vücut ağırlığı ve kan glikozu değerleri Şekil 1 ve Tablo 1'de verilmiştir.

Vücut ağırlığı gruplar arasında fark göstermektedir [$F_{(3,24)}$=23,20; p<0,0001]. EX, DM ve DM+EX grubundaki hayvanların vücut ağırlıkları kontrol grubuna göre anlamlı derecede düşük bulunmuştur (p<0,05). DM ile DM+EX grubu arasında istatistiksel olarak anlamlı bir fark gözlenmemiştir (p>0,05).

Kan glikozu değerleri gruplar arasında anlamlı fark göstermektedir [$F_{(3,17)}$=164,7; p<0,0001]. Diyabetik gruplardaki (DM ve DM+EX) kan glikozu değerleri kontrole göre anlamlı derecede yüksek bulunmuştur (p<0,05). DM ve DM+EX grupları arasında anlamlı fark gözlenmemiştir (p>0,05).



Şekil 1: Hayvan takip parametreleri. Sakrifikasyonun hemen öncesinde yapılan, **A)** vücut ağırlığı ölçümü (n: K=7; EX=9; DM=6; DM+EX=6), **B)** Kan glikozu ölçümü (n: K=5; EX=6; DM=5; DM+EX=5) değerleri (*: p<0,05 vs. kontrol) ($\bar{x}$ ± SS)

K: Kontrol, EX: Egzersiz, DM: Diabetes mellitus, DM+EX: Diyabet + Egzersiz, SS: Standart sapma

Kardiyak Hipertrofi (KA/VA)

Hayvanların ıslak kalp ağırlıkları ve kardiyak hipertrofiyi gösteren kalp ağırlığı/vücut ağırlığı x 10^3 (KA/VA) değerleri Şekil 2'de verilmiştir. Kalp ağırlığı ve KA/VA değerleri gruplar arasında fark göstermektedir [sırasıyla $F_{(3,17)}$=6.365; p=0,0043 ve $F_{(3,17)}$=7.768; p=0,0018]. Diyabetik gruplardaki (DM ve DM+EX) hayvanların kalp ağırlıkları kontrole göre düşük bulunurken, KA/VA değerleri kontrol grubuna göre istatistiksel olarak anlamlı derecede yüksektir (p<0,05). Her iki parametre için de hem DM ve DM+EX grupları arasında hem de K ve EX grupları arasında anlamlı fark bulunmamıştır (p>0,05).

İzole Kalp Fonksiyonu ve İskemi-Reperfüzyon

Hayvanların bazal fonksiyon (iskemi öncesi) parametreleri incelenmiş Tablo 2'de sunulmuştur. LVDP, HR ve RPP değerleri gruplar arasında anlamlı fark göstermemiştir [sırasıyla, $F_{(3,16)}$=0,95; p=0,4399 $F_{(3,16)}$=2,84 p=0,0712; $F_{(3,15)}$=1.147 p=0,3624].

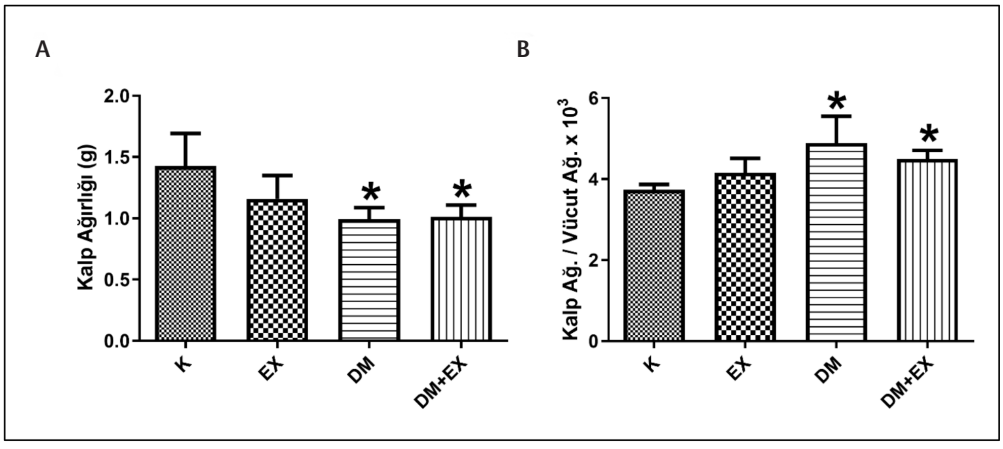
Deney boyunca kaydedilen hız-basınç çarpımı (RPP) değerleri Şekil 3'te verilmiştir.

RPP değerleri gruplar arasında iki yönlü varyans analizi ile karşılaştırılmıştır. Analiz sonucuna göre gruplar arasında anlamlı fark tespit edilmiştir [$F_{(3,16)}$=13,25; p=0,0001]. EX grubu ortalamaları K grubuna göre yüksek seyretmekle birlikte, bu fark sadece 15. dakikada anlamlılık göstermiştir (p<0,05). DM grubu kontrol grubu ortalamalarına yakın seyretmiş ancak 120. dakikada kontrol grubuna göre anlamlı derecede düşük RPP değerleri kaydedilmiştir (p<0,05). DM+EX grubu RPP değeri açısından tüm zaman noktalarında en kötü grup olmuştur. 30, 45, 90 ve 120. dakikalarda kontrole göre anlamlı derecede düşük fonksiyon değerlerine sahiptir (p<0,05). Ayrıca, reperfüzyonun ilk yarısında (10, 15 ve 30. dakikalarda) DM+EX grubu DM grubundan da istatistiksel olarak anlamlı derecede düşük RPP değerleri göstermiştir (p<0,05).

Tablo 1: Deney hayvanı takip verilerini içeren tablo ($\bar{x}$ ± SS)

	K	EX	DM	DM+EX
Vücut Ağırlığı (g)	372,10±52,98	299,60±48,36	204,70±48,36	226,80±17,29
Kalp Ağırlığı (g)	1,43±0,27	1,16±0,19	0,99±0,01	1,01±0,09
Kalp/Vücut Ağırlığı*	3,74±0,13	4,15±0,36	4,89±0,66	4,50±0,21
Kan glikozu (mg/dL)	101,80±8,23	114,30±26,59	329,60±29,36	372,40±29,59

*Kalp/Vücut Ağırlığı=KA/VA x 10^3
K: Kontrol, EX: Egzersiz, DM: Diabetes mellitus, DM+EX: Diyabet + Egzersiz, SS: Standart sapma



Şekil 2: Kardiyak hipertrofiyi takip etmek için yapılan ölçümler. **A)** Kalp ağırlığı (ıslak), **B)** Hayvanlarının ıslak kalp ağırlıklarının terminal vücut ağırlığına bölünmesi ile elde edilen (KA/VA) değerleri (*: p<0,05 vs. Kontrol) (n: K=5, EX=6, DM=5, DM+EX=5) ($\bar{x}$ + SS)

KA/VA: Kalp ağırlığı/vücut ağırlığı, K: Kontrol, EX: Egzersiz, DM: Diabetes mellitus, DM+EX: Diyabet + Egzersiz, SS: Standart sapma

Tablo 2: Hayvanların bazal (iskemi öncesi) kalp fonksiyonu parametreleri ($\bar{x}$ ± SS)

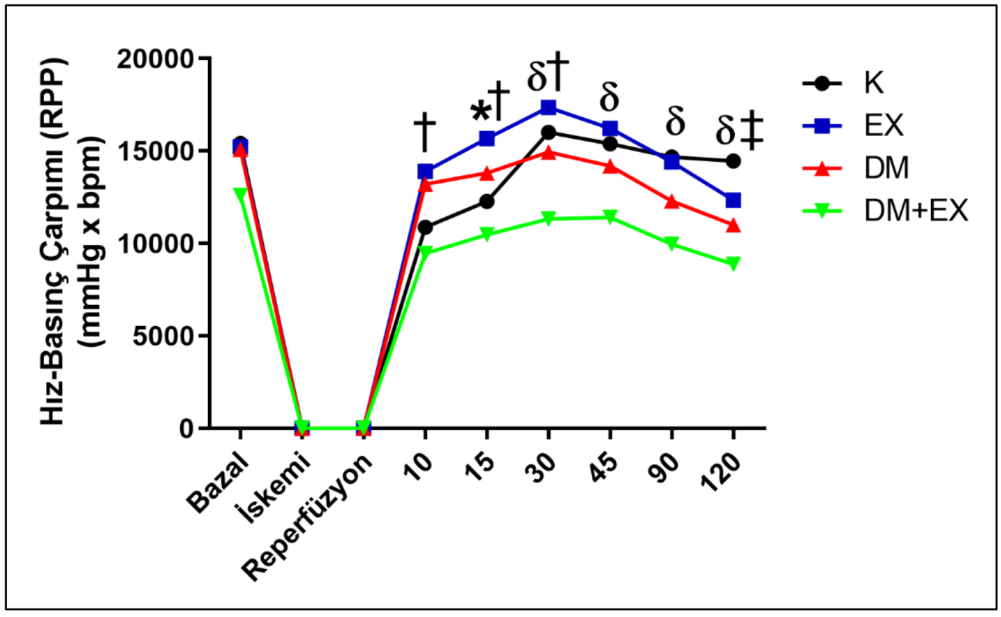
	K	EX	DM	DM+EX
LVDP (mmHg)	70,05±16,65	75,74±15,76	62,35±12,87	64,56±8,64
HR (mmHg)	223,20±30,39	197,20±28,62	244,80±36,66	197,3±24,27
RPP x 10^{-3} (mmHg/sn)	15,41±2,66	15,23±2,89	15,10±2,89	12,60±0,86

LVDP: Sol ventrikül gelişimsel basıncı, HR: Kalp hızı, RPP: Hız-basınç çarpımı, K: Kontrol, EX: Egzersiz, DM: Diabetes mellitus, DM+EX: Diyabet + Egzersiz, SS: Standart sapma

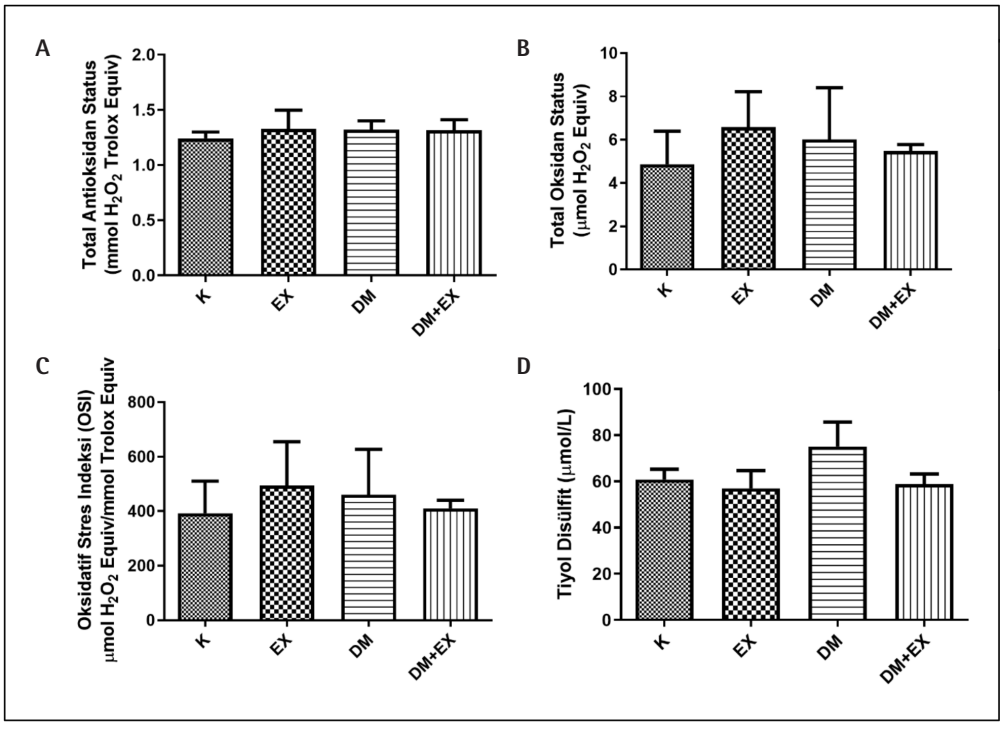
Oksidatif Stres Analizleri

Hayvanların plazma TAS, TOS, OSI ve plazma tiyol disülfit değerleri Şekil 4'te, sol ventrikülde doku örneklerindeki TAS, TOS ve OSI değerleri Şekil 5'te verilmiştir.

Plazma verileri incelendiğinde TAS, TOS, OSI ve tiyol disülfit parametreleri açısından gruplar arasında herhangi bir fark gözlemlenmemiştir [sırasıyla $F_{(3,24)}$=0,8429; p=0,4838; $F_{(3,20)}$=1.357; p=0,2846; $F_{(3,20)}$=0,8077; p=0,5044 ve $F_{(3,21)}$=1.150; p=0,3520].



Şekil 3: Tüm deney boyunca kaydedilen RPP verileri (p<0,05 *: K vs. EX; ǂ: K vs. DM; δ: K vs. DM+EX; †: DM vs. DM+EX) (n: K=5; EX=5; DM=5; DM+EX=4)
K: Kontrol, EX: Egzersiz, DM: Diabetes mellitus, DM+EX: Diyabet + Egzersiz



Şekil 4: Plazmada ölçülen oksidatif stres parametreleri. **A)** Toplam antioksidan durumu (n: K=7, EX=9, DM=6, DM+EX=6), **B)** toplam oksidan durumu (n: K=7, EX=7, DM=5, DM+EX=5), **C)** Oksidatif stres indeksi (n: K=7, EX=7, DM=5, DM+EX=5), **D)** Tiyol disülfit (n: K=7, EX=8, DM=5, DM+EX=5) ($\bar{x}$ + SS)
K: Kontrol, EX: Egzersiz, DM: Diabetes mellitus, DM+EX: Diyabet + Egzersiz, SS: Standart sapma

Sol ventrikül doku örneklerinde TAS, TOS ve OSI parametreleri açısından gruplar arasında herhangi bir fark gözlemlenmemiştir [sırasıyla $F_{(3,17)}$=3.091; p=0,0549; $F_{(3,16)}$=1.438; p=0,2687 ve $F_{(3,15)}$=2.995; p=0,0641].

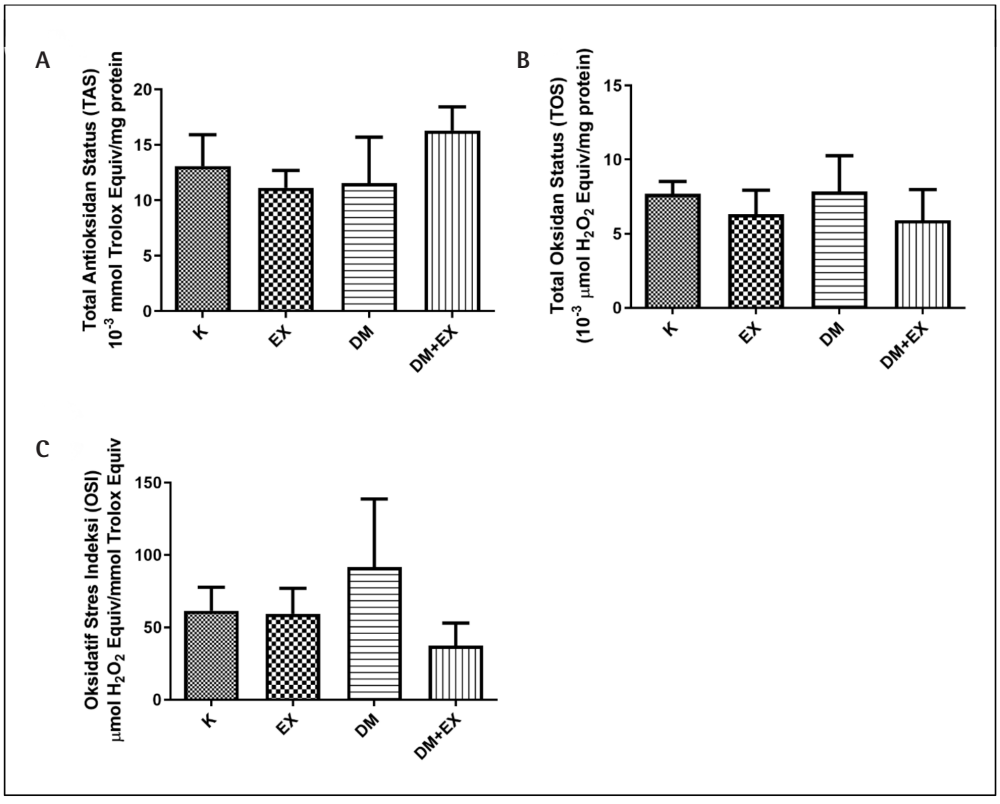
Tartışma

Çalışmamızda, diyabetik hayvanlara 12 hafta boyunca uygulanan, orta şiddette, kronik egzersizin diyabetik kalpte gelişen I/R hassasiyetini azaltmadığı, aksine iskemi sonrası toparlanma cevabında istatistiksel olarak anlamlı derecede bozulmaya neden olduğu gösterilmiştir. Egzersiz diyabetik olmayan hayvanlarda I/R'ye karşı direnç artışına neden olurken diyabetiklerde tam tersi yönde bir etki yapmıştır. Elde edilen sonuçlar, uygulanan egzersiz protokolünün diyabetli popülasyon için uygun olmadığını düşündürmektedir.

Fonksiyonel değişimlerin altında yatan mekanizmaları açıklamak adına hayvanlara ait kalp dokusu ve plazmalarda, diyabetteki I/R hasarı artışının ve aynı zamanda egzersizin sağladığı kardiyoproteksiyonun en önemli modülatörü olan oksidatif stres parametreleri incelenmiş gruplar arasında herhangi anlamlı bir değişime rastlanmamıştır.

Uyguladığımız antrenman programı sağlıklı hayvanlarda vücut ağırlığında azalmaya neden olmuştur. Egzersizin enerji tüketimini artıracağı düşünüldüğünde elde edilen sonuçların genel bilgi ile uyumlu olduğu düşünülmektedir. Önceki çalışmalarda elde edilen veriler ışığında, tip 1 diyabet modelinde hayvanların hızlı bir şekilde kilo kaybetmeleri beklenir (31). Diyabetik gruplarda (DM ve DM+EX) kontrole göre anlamlı bir kilo kaybı gözlenmektedir. Kan glikozu verileri incelendiğinde diyabetik hayvanların (DM ve DM+EX) kan glikozu düzeylerinin diyabetik olmayanlara göre anlamlı düzeyde yüksek olduğu görülmektedir. Kan glikozu ve vücut ağırlığı verileri birlikte değerlendirildiğinde tip 1 diyabet modelinin sorunsuz şekilde gerçekleştirildiği anlaşılmaktadır. Egzersiz uygulaması diyabetik hayvanlarda herhangi bir ağırlık değişimine neden olmamıştır ayrıca hem sağlıklı hem de diyabetik hayvanlarda kan glikozu düzeylerine önemli bir etki yapmamıştır. Seçtiğimiz diyabet modelinde STZ uygulamasının pankreasta kalıcı hasara neden olduğu bilinmektedir (32). Bu nedenle hasar oluştuktan sonra uygulanan egzersiz protokolü kan şekeri veya vücut ağırlığı düzeylerini etkilememiştir.

Devereux ve ark.'nın (33) 2000 yılında çok sayıda katılımcı ile gerçekleştirdikleri Strong Heart Study isimli epidemiyolojik çalışma, diyabetik bireylerde sol ventrikül kütlesinin ve duvar kalınlığının sağlıklı kontrollere göre anlamlı derece fazla olduğu göstermiştir. Çok sayında katılımcı ile gerçekleştirilen bir diğer çalışma Framingham Heart Study isimli epidemiyolojik çalışma



Şekil 5: Sol ventrikül doku örneklerinde ölçülen oksidatif stres parametreleri. A) Toplam antioksidan durumu (n: K=6, EX=6, DM=5, DM+EX=4), B) toplam oksidan durumu (n: K=6, EX=5, DM=5, DM+EX=4), C) Oksidatif stres indeksi (n: K=5, EX=5, DM=4, DM+EX=4) ($\hat{x}$ + SS)

K: Kontrol, EX: Egzersiz, DM: Diabetes mellitus, DM+EX: Diyabet + Egzersiz, SS: Standart sapma

da aynı sonuçlara ulaşmış, bozulmanın diyabetin derecesi ile ilişkili olduğunu bildirmiştir (34). Benzer şekilde STZ uygulaması ile diyabet yapılan sıçanlarda da kardiyak hipertrofinin gelişimi çeşitli yayınlarda, çeşitli tekniklerle gösterilmiştir (35-37). Çalışmamızda diyabet gruplarında görülen KA/VA oranındaki anlamlı artış diyabetik hayvanlarda kardiyak hipertrofinin gelişimine işaret etmektedir, bu bulgu literatür ile uyumludur. Öncül hipotezimizin aksine, egzersiz uygulaması diyabetiklerde hipertrofiyi engelleyici bir etkide bulunmamıştır.

Diyabetin kalpte I/R hasarına karşı hassasiyeti artırdığı bilinmektedir (4,5). Ek olarak, deneysel çalışmalarda da benzer veriler elde edilmiştir. Qiu ve ark. (8) STZ enjeksiyonu ile diyabet oluşturdukları sıçanlarda koroner arter ligasyonu ile I/R uygulaması yapmışlar ve diyabetik hayvanlarda infarkt alanın kontrollere göre daha büyük olduğunu göstermişlerdir. Sunulan sonuçlar benzer tasarımla gerçekleştirilen hayvan çalışmaları ile desteklenmektedir (6,7).

Çalışmamızda diyabet ile kontrol arasındaki fark reperfüzyonun sonunda anlamlılık kazanmıştır. Uyguladığımız iskemi süresi ılımlı iskemi olarak kabul edilen bir uzunluktadır. DM ve K arasında toparlanma cevabının tüm zaman noktalarında belirgin bir şekilde ayrışmaması iskeminin düzeyi ile açıklanabilir. Literatürde tersi yönde sonuçlara da rastlamak mümkündür. Gurel ve ark. (38) 1 aylık tip 1 diyabet uygulamasını takiben uyguladıkları global I/R protokolünde diyabetin kardiyoprotektif bir fenotip ortaya çıkardığını öne sürmüşlerdir. İlerleyen çalışmalarda iskemi süresinin artırılması veya global iskemi yerine koroner arter ligasyonu protokollerinin uygulanması iki grup arasındaki farkı belirginleştirebilir. Ancak yine de seçtiğimiz diyabet modelinin miyokart düzeyinde yol açtığı I/R hassasiyet artışının reperfüzyonunun ilerleyen dönemlerine yansıdığı anlaşılmaktadır.

Uygulanan egzersiz protokolü diyabetik olmayan hayvanlarda I/R hasarına karşı direnci artırmıştır. Bu sonuçlar uyguladığımız protokolün kardiyoprotektif karakterine işaret etmektedir. Literatürde de çeşitli şiddet ve sürelerde uygulanan egzersizin I/R hasarına karşı koruyucu etkileri gösterilmiştir (39-41). Bununla birlikte, seçilen egzersiz protokolünün kişisel özelliklere uygun olması egzersizin olumlu etkilerinin gözlenmesi için olmazsa olmaz bir şarttır. Yanlış planlanan egzersiz protokolü istenen yararlı etkileri göstermekten ziyade zarar verici olabilir. Komatsu ve ark. (42) diyabetik bireylerin sağlıklı insanlara göre daha düşük egzersiz kapasitesine sahip olduğunu bildirmişlerdir. Bu nedenle, çalışmamızda tüm hayvanlara aynı egzersiz protokolünü uygulamak yerine, hayvanların MEK değerleri ölçülerek egzersiz programı bu değerlere göre düzenlenmiştir. Ancak MEK değerleri açısından diyabetik ve diyabetik olmayan hayvanlar arasında anlamlı bir farka rastlanmamıştır (veriler sunulmamıştır). Çalışmamızda MEK değerinin %70'ine denk gelecek ılımlı bir egzersiz protokolü seçilmiştir. Ancak diyabetik hayvanlarda bu uygulamanın iskemi sonrası toparlanma cevabını daha da kötü hale getirdiği anlaşılmaktadır. Seçilen egzersiz protokolünün sağlıklı hayvanlara uygulanması yarar sağlarken, aynı protokolün diyabetli hayvanlara zarar verdiği ortaya çıkmaktadır. İlerleyen çalışmalarda diyabetli hayvanlara daha düşük şiddetli bir egzersiz protokolünün uygulanmasının daha doğru olacağını düşünmekteyiz. Ek olarak, çalışmamızda kullanılan egzersiz kapasitesi ölçüm yöntemi dolaylı (indirekt) bir yöntemdir. Teknik yetersizliklerden dolayı egzersiz kapasitesi ölçümlerinde hayvanların solunum gazları ve kalp hızlarını takip etmeye olanak sağlayan metabolik koşu bantları kullanılamamıştır. Bu koşu bantlarının kullanımı ile ölçülen maksimum oksijen tüketim kapasitesi (Vo_{2max}) değeri egzersiz şiddetinin belirlenmesinde kullanılan altın standarttır. İlerleyen çalışmalarda bu tarz cihazların kullanımı ile diyabetik ve diyabetik olmayan hayvanların arasında kapasite farklarına rastlanabilir ve daha doğru egzersiz protokolleri planlanabilir.

Oksidatif stres ile ilgili ölçülen parametreler incelendiğinde istatistiksel açıdan gruplar arasında anlamlı bir fark görülmediği anlaşılmaktadır. Her ne kadar kalp dokusundaki OSI değerleri ve plazmadaki tiyol disülfit parametrelerinde diyabet grubunda bir artış trendi görülse de grup içi varyasyonun yüksekliği nedeniyle bu farklar anlamlılık kazanmamıştır. ROS oldukça hızlı bozunmaya uğrayan moleküller olduğundan örnekler arasındaki ihmal edilebilecek düzeyde küçük farklılıklar sonuçlara geniş varyasyonlar olarak yansımaktadır. İlerleyen çalışmalarda diyabet süresinin değiştirilmesi ve başka oksidatif stres parametrelerinin eklenmesi yerinde olacaktır. Bu nedenle ROS ile yapılan ölçümlerde örnek sayısının fazlalığı diğer ölçümlere göre daha kritik hale gelmektedir. Ancak çalışmamızda bu ölçümlerdeki örnek sayısı görece az sayıda kalmaktadır. Örnek sayısının azlığı çalışmamızın en önemli kısıtlılıklarından birisini teşkil etmektedir. İlerleyen çalışmalarda aynı testlerin daha büyük örneklemlerde çalışılmasının daha güçlü veriler ortaya çıkaracağını düşünmekteyiz.

Çalışmanın Kısıtlılıkları

Elbette diyabet ve egzersiz organizmanın tamamına yayılan etkileri ile birçok moleküler yolağı aynı anda, oksidatif stresten bağımsız olarak etkilemektedir. Diyabetin yol açtığı proenflamatuvar etkilenin I/R hasarını artırdığı çeşitli çalışmalar ile gösterilmiştir (8). Diyabette kalp dokusunda hücre içi kalsiyum homeostazisi parametrelerinde meydana gelen değişimler de I/R hassasiyetinin artışında önemli yere sahiptir (43). Son olarak, egzersizin kalp dokusunda damarlanmayı artırdığı ve bu adaptasyonun I/R hasarında koruyucu etki yaptığı bilinmektedir (39). Çalışmamızda bu parametrelerin incelenmemiş olması çalışmamızın kısıtlılıkları arasındadır.

Sonuç

Sağlıklı ve hasta popülasyonlar için planlanan antrenmanların şiddet, süre ve sıklığının belirlenmesi çalışmaları toplum sağlığı açısından oldukça önemli bir araştırma alanıdır. Aynı zamanda ortaya çıkan yararlı veya zararlı etkilerin altında yatan moleküler mekanizmaların belirlenmesi egzersiz fizyolojisi bilgimize değerli katkılar yapmaya adaydır. Gelecekteki çalışmalarda Vo_{2max} değerlerinin ölçümü ile daha kapsamlı çalışmaların yapılması bu alanın gelişimi için önem arz etmektedir.

Teşekkür

Belirtilmesi gereken herhangi bir çıkar çatışması bulunmamaktadır. Sunulan çalışma Ankara Üniversitesi Bilimsel Araştırma Projeleri (AÜ BAP) tarafından desteklenmiştir (proje no: 17L0230013).

Etik

Etik Kurul Onayı: Hayvan kullanım izni ve deney protokollerinin etik ilkelere uygunluğu Ankara Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu tarafından onaylanmıştır (karar no: 2017-4-25, tarih: 19/02/2016).

Hasta Onayı: Hayvan deneyi çalışmasıdır.

Hakem Değerlendirmesi: Editörler kurulu içinden ve dışından olan kişiler tarafından değerlendirildi.

Yazarlık Katkıları

Konsept: F.A., G.Ö., H.F., H.Ç., M.B., Dizayn: F.A., G.Ö., H.F., H.Ç., M.B., Analiz veya Yorumlama: F.A., G.Ö., H.F., H.Ç., M.B., Literatür Arama: F.A., G.Ö., H.F., H.Ç., M.B., Yazan: F.A., G.Ö., H.F., H.Ç., M.B.

Çıkar Çatışması: Yazarlar arasında herhangi bir çıkar çatışması bulunmamaktadır.

Finansal Destek: Sunulan çalışma Ankara Üniversitesi Bilimsel Araştırma Projeleri (AÜ BAP) tarafından desteklenmiştir (proje no: 17L0230013).

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RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ

DOI: 10.4274/atfm.galenos.2023.58815
Ankara Üniversitesi Tıp Fakültesi Mecmuası 2023;76(1):11-16

BASIC MEDICAL SCIENCES / TEMEL TIP BİLİMLERİ

Baicalein Has Protective Effect in H_2O_2-induced L929 Cell Damage

Baicalein, H_2O_2 Kaynaklı L929 Hücre Hasarında Koruyucu Etkiye Sahiptir

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Abstract

Objectives: In this study, it was aimed to investigate the possible protective effects of baicalein in H_2O_2-induced L929 fibroblast cell model by determining the parameters of oxidative stress (SOD) and inflammation process (TNF-alpha, TGF-beta, IL-10).

Materials and Methods: Baicalein was applied at different doses (200, 100 and 80, µM) to L929 fibroblast cells. After 23 hours of incubation, 1 mM H_2O_2 was added and cell viability was test by CVDK8. Then, fluorescent diacetate (FDA)-propidium iodide (PI) staining was performed to determine the ratio of live and dead cells. The levels of TNF-alpha, IL10, TGF-beta and the activity of SOD were determined by ELISA.

Results: After 1 h incubation with 1 mM H_2O_2, the viability decreased by approximately 77%. It was observed that cell viability increased the most in the B100 group, with/without H_2O_2. The cell viability increased after 24 h of baicalein and H_2O_2 co-incubation. The 100 µM baicalein application reduced the damage of H_2O_2 up to 63%. When the levels of TNF-alpha were examined, a significant increase was observed in the H_2O_2-damage group compared to control group. Additionally, the levels of IL-10 and TGF-beta increased in H_2O_2-damage group compared to control group; however, the levels of those cytokines were decreased in baicalein application groups.

Conclusion: Pretreatment with baicalein (especially 200 µM and 100 µM) results in significant findings including the suppression of inflammatory cytokines, TNF-alpha, TGF-beta, IL-10 and increase of SOD parameter, SOD, in L929 cells. Taking everything into account, baicalein which is a natural product may be an alternative therapeutic option for prevention of cellular SOD and inflammatory processes. To better understand the potential beneficial effects of baicalein, molecular mechanisms and cellular targets should be investigated.

Key Words: Baicalein, Hydrogen Peroxide, Inflammation, Oxidative Stress, L929

Öz

Amaç: Mevcut çalışmada, Baicalein'in H_2O_2 ile indüklenmiş L929 fibroblast hücre hasarı modelinde oksidatif stres (SOD) ve enflamasyon süreci (TNF-alfa, TGF-beta, IL-10) parametreleri üzerine olan koruyucu etkilerinin araştırılması amaçlanmıştır.

Gereç ve Yöntem: L929 fibroblast hücrelerine farklı dozlarda (200, 100 ve 80 µM) Baicalein uygulandı. Yirmi üç saatlik inkübasyonun ardından 1 mM H_2O_2 eklendi ve hücre canlılığı CVDK8 ile test edildi. Daha sonra canlı ve ölü hücrelerin oranını belirlemek için floresan diasetat (FDA)-propidyum iyodür (PI) boyaması yapıldı. TNF-alfa, IL10, TGF-beta seviyeleri ve SOD aktivitesi ELISA ile belirlendi.

Bulgular: 1 mM H_2O_2 ile 1 saatlik inkübasyondan sonra hücre canlılığı yaklaşık %77 oranında azaldı. Hasarsız ya da H_2O_2 hasarlı gruplarda, hücre canlılığında artışın en fazla B100 grubunda olduğu görüldü. Baicalein ve H_2O_2'nin birlikte inkübasyonundan 24 saat sonra hücre canlılığının arttığı tespit edildi. 100 µM Baicalein uygulaması, H_2O_2'nin hücreleri öldürme oranını %63'e kadar azalttı. TNF-alfa düzeyleri incelendiğinde H_2O_2-hasarlı grupta kontrol grubuna göre anlamlı artış gözlendi. Ek olarak, IL-10 ve TGF-beta seviyeleri H_2O_2-hasarlı grupta kontrol grubuna göre arttı; ancak bu sitokinlerin seviyeleri Baicalein uygulama gruplarında azaldı.

Sonuç: Baicalein (özellikle 200 µM ve 100µM) uygulaması sonucunda L929 hücrelerinde enflamatuvar sitokinler olan TNF-alfa, TGF-beta, IL-10'un baskılanması ve SOD parametresi olan SOD'nin artması gibi önemli bulgular elde edilmiştir. Tüm veriler ışığında, doğal bir etken madde olan baicalein, hücresel SOD ve enflamatuvar süreçlerin önlenmesi için alternatif bir tedavi seçeneği potansiyeline sahiptir. Baicalein'in potansiyel yararlı etkilerini daha iyi anlamak için moleküler mekanizmalar ve hücresel hedeflerin araştırılmasına yönelik yeni çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Baicalein, Hidrojen Peroksit, Enflamasyon, Oksidatif Stres, L929

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Received/Geliş Tarihi: 08.01.2023 **Accepted/Kabul Tarihi:** 04.04.2023

Introduction

Wound may be the result of an accident; however, it can also occur intentionally for reasons such as surgery. Wound healing following trauma is described as the normalization of cellular, biochemical and systemic processes with the formation of new tissue. It is also an important physiological process that preserves the integrity of the tissue (1). Wound healing consists of three phases; (i) the hemostasis/inflammation phase, (ii) the proliferation phase, and (iii) the remodeling phase (2). Chronic and difficult to heal wounds causes a significant economic burden because of reducing the patient's quality of life (3).

There are many factors that affect wound healing such as infection, oxygenation defects, stress, aging, and smoking. At low concentrations, reactive oxygen species (ROS) are involved in the wound healing process, defense against invading microorganisms, and signals necessary for cell survival. Production of high concentrations of ROS or impaired ROS detoxification is the main cause of chronic non-healing wounds (4). One of the causes of cellular oxidative stress (SOD) is the overproduction of hydrogen peroxide, a kind of ROS. Reducing oxidative balance is very important for the functioning of fibroblasts (5). On the other hand, the inflammatory response that begins after tissue injury accompany with the growth factor, cytokine, and chemokine expressions and those are necessary for wound healing. In the early phase of wound healing, cytokines and chemokines such as interleukine-1-alpha (IL-1α), IL-1-β, transforming growth factor-β (TGF-β), vascular endothelial growth factor, tumor necrosis factor alpha (TNF-α) and IL-8 are substantial and play important roles (6).

Scutellaria baicalensis Georgi. (Lamiaceae), contains baicalein as a major component, is a flowering plant species and its dried roots are used in traditional Chinese medicine for the treatment of cold, diarrhea, dysentery, hypertension, bleeding, insomnia, inflammation and respiratory tract infections (7). Baicalein has lots of beneficial effects including anti-oxidant, anti-inflammatory, anti-cancer, cardioprotective, neuroprotective, and hepatoprotective properties (8-10).

Fibroblasts are one of the key factors in wound healing and remain at the wound site until epithelialization occurs. First, they migrate to the wound site, then proliferate there, after that promote normal wound healing to form new extracellular matrix, and finally induces collagen structures to support other cells associated with effective wound healing (11).

In this study, it was aimed to investigate the possible protective effects of baicalein in H_2O_2-induced L929 fibroblast cell model by determining the parameters of SOD and inflammation process (TNF-alpha, TGF-beta, IL-10).

Materials and Methods

Cell Culture and Viability Test

The mouse fibroblast cell line L929 was purchased from American Type Culture Collection (ATCC, USA). It was cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco, Thermo Fisher Scientific) containing 10% fetal bovine serum (FBS, Gibco, Thermo Fisher Scientific), 1% antibiotic (Penicillin, Streptomycin, Amphotericin, Gibco, Thermo Fisher Scientific), under 95% humidity and 5% CO_2, and 37 °C temperature. Baicalein (MedChemExpress, Cat.No: HY-N0196), dissolved in 10% DMSO.

Baicalein Administration

For determine the baicalein doses, L929 cells incubated with varying doses (800 µM, 400 µM, 200 µM, 100 µM, 80 µM and 40 µM, 20 µM) for 24 hours. It was observed that the 800 µM dose reduced the viability by 26% and the 400 µM dose by 17% and 20 µM dose by 13%. The 40 µM dose increased the viability by only 5%. Therefore, these doses were not included in the experiment. 200 µM (B200), 100 µM (B100), 80 µM (B80) doses were used in the experiment.

H_2O_2 Administration

L929 cells were incubated for 24 hours with varying doses of H_2O_2 (1 mM, 0.5 mM, 0.4 mM, 0.3 mM, 0.2 mM, 0.1 mM) while determining H_2O_2 damage. It was observed that cell viability decreased by 77% at 1 mM, 78% at 0.5 mM, 78% at 0.4 mM, 83% at 0.2 mM and 88% at 0.1 mM. So, 1 mM dose was chosen for the experiment. The groups with H_2O_2 were named as H+B200, H+B100 and H+B80.

CVDK-8 Cell Viability Test

Cell viability test was performed to see the effect of baicalein on cell proliferation. Cells were first seeded into 96-well plates, 2 µL of baicalein was applied at the specified doses (200 µM, 100 µM and 80 µM) after 70% confluence was detected. Then, 1 mM hydrogen peroxide (H_2O_2) was applied to related wells 23 hours after baicalein application. After 1 hour, 10 µL of CVDK-8 was added to each well. It was incubated at 37 °C for 4 hours for the formation of formazan crystals and the optical density (OD) value was detected with a spectrophotometer (Thermo, MultiSkan GO) at a wavelength of 450 nm.

Semi-Quantitative ELISA Technic

Cells ($2x10^4$) were seeded 48-well plates in 200 µL medium for each well plate. The medium was collected 24 hours after the H_2O_2 damage and the addition of the specified doses of baicalein. In this study, we aimed to measure the levels of following parameters; TGF-beta ELISA Kit (BT Laboratory, Shanghai, China), TNF-alpha ELISA Kit (BT Laboratory, Shanghai,

China), IL-10 ELISA Kit (BT Laboratory, Shanghai, China), SOD ELISA Kit (BT Laboratory, Shanghai, China). The ELISA technic was applied according to the manufacturer's protocol. At the end of the experiments, the microplate was read at a wavelength of 450 nm with ELISA reader (Thermo, MultiSkan GO). The OD values were noted and the calculations were performed.

Immunofluorescence Assay

Cells were seeded 24-well plates in 400 µL medium for each well plate. After the H_2O_2 and baicalein co-incubation, the medium was removed and washed twice with PBS. For fixation, 400 mL of 4% formaldehyde was added to per well and after 4 minutes formaldehyde was removed and cells were washed with PBS. For permeabilization of cells, they were incubated with 99.9% methanol for 20 minutes at room temperature and then washed with PBS. Five ml each of fluorescent diacetate (FDA, ThermoFisher, Cat.No: F1303) and propidium iodide (PI, ThermoFisher, Cat.No: P1304MP) were added to per well, visualized with an inverted microscope (Invitrogen Evos FL) with fluorescence attachment after 10 minutes of incubation.

Statistical Analysis

The results were expressed as means ± SEM. Statistical significance was evaluated by One-Way ANOVA followed by Tukey post-test. All data were analyzed using GraphPad Prism, version 5.0 for Windows (Graph Pad Software, San Diego, California, USA). The probability level of $p<0.05$ was considered statistically significant.

Results

Baicalein Increased Cell Viability with/without H_2O_2-Induced

To test whether baicalein has a cytotoxic effect on healthy L929 fibroblast cells, a viability test was performed. It was observed that high doses of baicalein (200 µM, 100 µM, 80 µM) did not decrease cell viability, on the contrary, it induced cell proliferation (18%, 21%, 13%, respectively). The highest cell proliferation was observed at the dose of 100 µM.

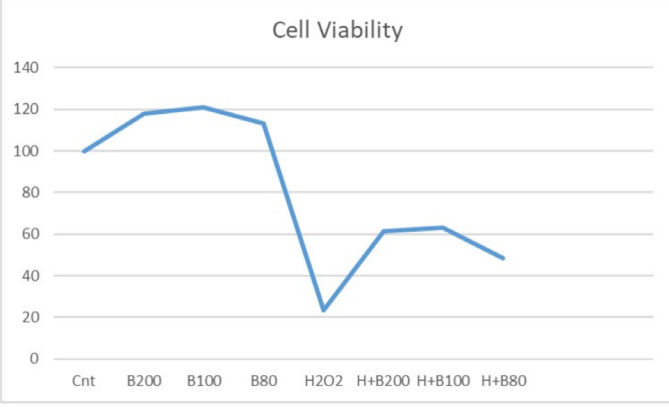

Figure 1: The cell viability assay of experimental groups. The viability of the control group was accepted as 100% and the averages of the experimental groups were calculated

Cnt: Control

After 1 h incubation with 1 mM H_2O_2, the viability decreased by approximately 77%. The cell viability increased after 24 hours of baicalein H_2O_2 co-incubation in all doses especially 100 µM. The 100 µM baicalein application reduced the damage of H_2O_2 up to 63% (Figure 1).

Baicalein Had Anti-Apoptotic Effect on H_2O_2 Damaged Cells

One hundred and ninety-six immunofluorescence assay (IFA) findings to show the anti-proliferative and anti-apoptotic effects of baicalein on L929 cells were similar with the cell viability assay findings. Baicalein given to healthy cells did not seem to decrease cell viability (Figure 2). It was observed that the number of apoptotic cells increased in H_2O_2 damaged group. The number of apoptotic cells decreased due to the increasing dose of baicalein, and the groups with the highest number of viable cells were 200 µM and 100 µM doses (Figure 3). Baicalein has been shown to have an anti-apoptotic effect with/without H_2O_2 in a dose-dependent manner.

Baicalein Effects the Levels of Inflammatory Cytokine

When the levels of TNF-alpha, a pro-inflammatory cytokine, were examined, a significant increase was observed in the H_2O_2 damage group compared to control group. Application of baicalein reduced H_2O_2 damage (Figure 4). Additionally, the

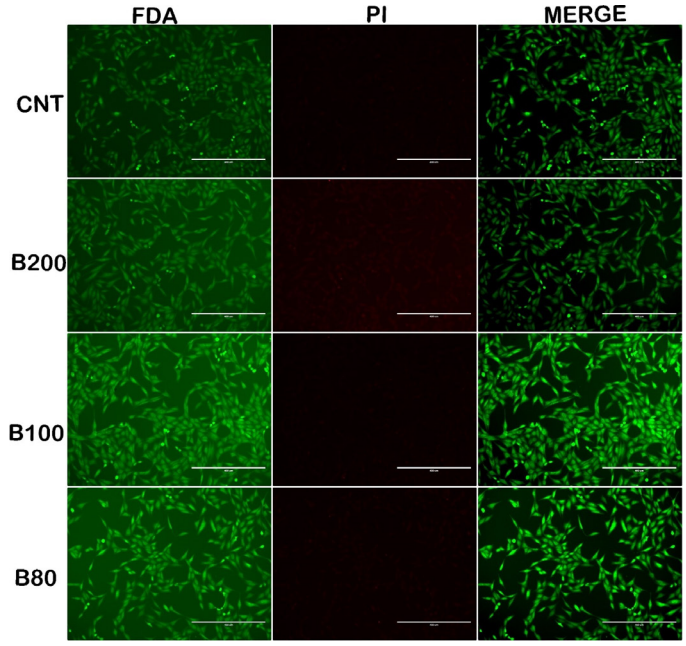

Figure 2: FDA and PI immunofluorescence labeling in L929 cells with baicalein. Red cells (PI positive) indicate dead cell, green cells (FDA positive) indicate live cells

FDA: Fluorescent diacetate, PI: Propidium iodide, CNT: Control

levels of IL-10 and TGF-beta increased in H_2O_2 damage group compared to control group; however, the levels of those anti-inflammatory cytokines were decreased in the baicalein application groups (Figure 5). The activity of SOD enzyme showed a significant decrease in H_2O_2 damage group compared to control. An increase in the activity of SOD enzyme was observed in H_2O_2+baicalein application groups. In B200, B100, and B80 groups, the activity of SOD enzyme increased when compared to H_2O_2 group (Figure 6).

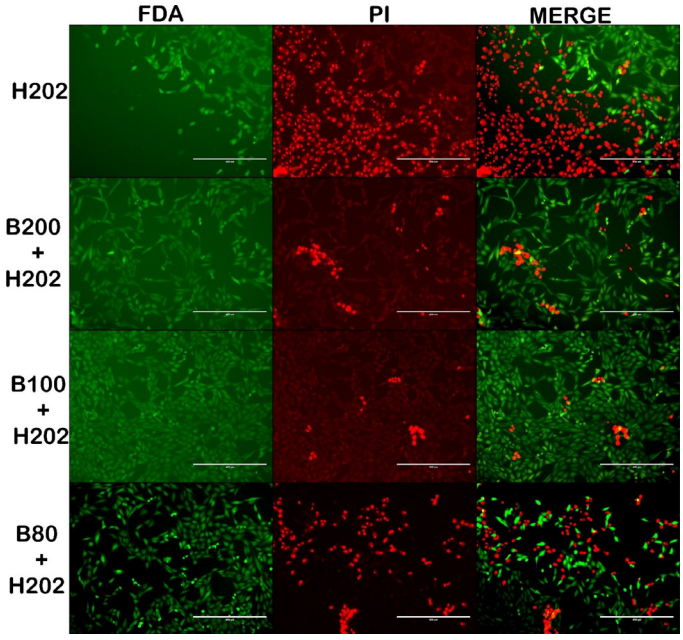

Figure 3: FDA and PI immunofluorescence labeling in L929 cells with H_2O_2 and baicalein. Red cells (PI positive) indicate dead cell, green cells (FDA positive) indicate live cells

PI: Propidium iodide, FDA: Fluorescent diacetate

Discussion

To our knowledge, it was shown for the first time that baicalein has a protective effect on H_2O_2-induced L929 cell damage by effecting two important cellular process: SOD and inflammation. In current literature, it is well-known that the anti-inflammatory, antioxidant, anti-angiogenesis, and immunomodulatory effects of baicalein have been proven (12). A study reported that baicalein decreased cell viability in human lung fibroblast MRC-5 cell line (13). In this study, baicalein increased cell viability in L929 fibroblast cells, while it was shown that cell viability decreased in H_2O_2 damage group (5,14,15).

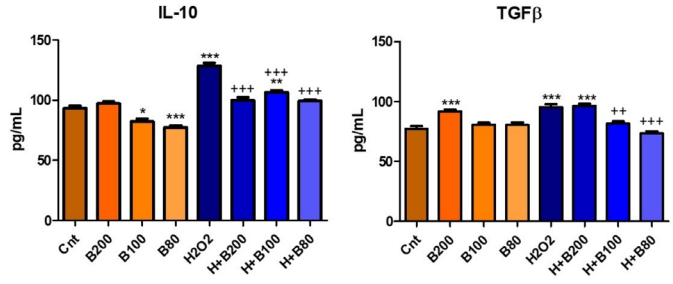

Figure 5: The levels of anti-inflammatory cytokines

Control versus all experimental groups; + H_2O_2 versus all experimental groups; +,=p<0.05, ++,**= p<0.01, +++,***= p<0.001

IL-10: Interleukin 10, TGFB: The transforming growth factor beta, Cnt: Control

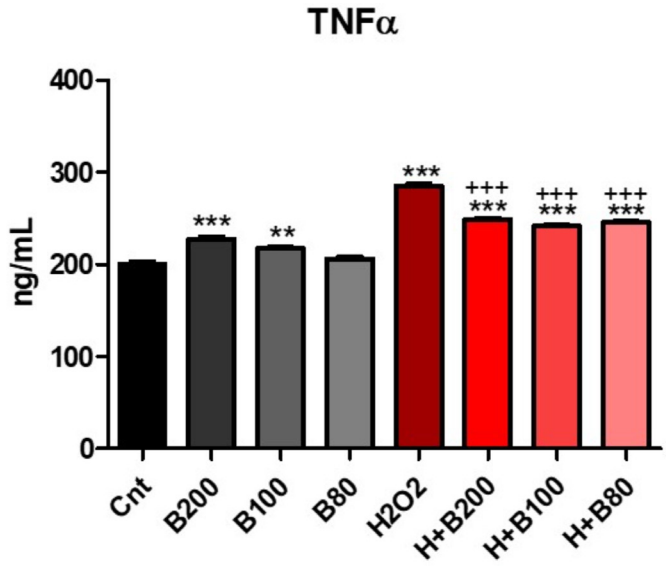

Figure 4: The level of pro-inflammatory cytokine

Control versus all experimental groups; + H_2O_2 versus all experimental groups; +,=p<0.05, ++,**=p<0.01, +++,***=p<0.001

TNFa: Tumor necrosis factor alpha, Cnt: Control

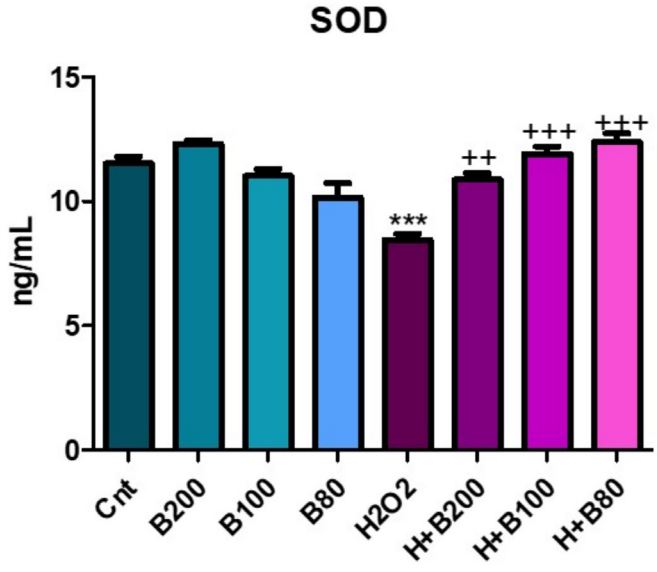

Figure 6: The activity of SOD enzyme

Control versus all experimental groups; + H_2O_2 versus all experimental groups; +,=p<0.05, ++,**=p<0.01, +++, ***=p<0.001

SOD: Superoxide dismutase, Cnt: Control

IFA findings were compatible with the cell viability assay findings. The ratios of FDA, which gives green fluorescent color to living cells, and PI to DNA helix of dead cells, have shown that baicalein has no toxic effects on healthy cells. A study reported similar findings with our study that baicalein did not cause death in Chinese hamster lung fibroblast cells (V79-4) (16). After H_2O_2 penetrates the cells, it produces a highly reactive hydroxyl radical that attacks cellular components including lipids, proteins and DNA (17,18). Membrane lipid peroxidation induced by H_2O_2 is one of the most important damages responsible for the loss of cell viability. It was detected that there were less death cells in H_2O_2 + baicalein treatment groups when compared to control. Consistent with the literature, it can be said that baicalein has a protective effect on H_2O_2 damaged cells (17,19).

SOD is one of the important factors inducing organ damage and associated with over-released of reactive oxygen species such as hydrogen peroxide (20). On the other hand, SOD is an important reactive oxygen species (ROS) scavenger, which can transform superoxide radicals into H_2O_2 and prevent ROS attack to important organs (21). It is known that SOD has therapeutic effects in physiological and pathological cellular conditions including inflammatory diseases, cancer, and neurodegenerative diseases (22). In our study, it was observed that high-dose baicalein application increased the SOD level, and low-dose baicalein slightly decreased the activity of SOD enzyme in related experiment groups. Tan et al. (23) performed a study and reported that pretreatment of baicalein increased the activity of SOD. Additionally, Ye et al. (24) also reported similar findings that baicalein activated apoptosis through induced intracellular ROS generation, and SOD apparently inhibited intracellular ROS production. These information shows us that baicalein has a potential to be effective on cellular SOD parameters.

On the other hand, all kinds of cellular damage including H_2O_2 can cause an inflammation in tissue and cytokine release. In our study, H_2O_2 caused an increase in the levels of TNF-alpha, TGF-beta, and IL-10; while different doses of baicalein application decreased the levels of mentioned cytokines. In current literature, it can be seen that baicalein has anti-inflammatory effects of some experimental models. Lin et al. (25) reported that TNF-alpha and IL-6 were suppressed by baicalein pretreatment; however, IL-10 level was significantly elevated by baicalein in contrast to our study. Generally, studies in literature support the current data about the inflammatory cytokine levels (26-28).

Study Limitations

The study have some limitations. First, I should have used more than one cell culture to compare the exact effects of baicalein on hydrogen-peroxidase damage. Second, I should have tried to determine more inflammatory and oxidative stress parameters. For example, to better explain the anti-inflammatory and anti-oxidant effects, additional parameters including catalase (CAT), lipid peroxidase (LPO), Malondialdehyde (MDA), Interleukin-1-beta (IL-1-B), Interleukin-6 (IL-6), Interleukin-17 (IL-17), and Interleukin-22 (IL-22) should have been determined. Third, seconder cellular signaling pathways related with the inflammation and oxidative stress processes should have been checked.

Conclusion

As a conclusion, pretreatment with baicalein results in significant findings including the suppression of inflammatory cytokines, TNF-alpha, TGF-beta, IL-10 and increase of SOD parameter, SOD, in L929 cells. Taking everything into account, baicalein which is a natural product may be an alternative therapeutic option for prevention of cellular SOD and inflammatory processes. To better understand the potential beneficial effects of baicalein, molecular mechanisms and cellular targets should be investigated.

Ethics

Ethics Committee Approval: This study is a cell culture study and therefore ethics committee approval was not obtained.

Informed Consent: Cell culture study.

Peer-reviewed: Externally peer-reviewed.

Financial Disclosure: The author declared that she did not receive any financial support during this article.

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RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ

DOI: 10.4274/atfm.galenos.2023.46873
Ankara Üniversitesi Tıp Fakültesi Mecmuası 2023;76(1):17-23

BASIC MEDICAL SCIENCES / TEMEL TIP BİLİMLERİ

Immunoreactivity of NOS2 and NF-κB in Kidney Tissue in Experimental Alcohol Consumption Model

Deneysel Alkol Tüketimi Modelinde Böbrekteki NOS2 ve NF-κB'nin İmmünreaktivitesi

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Abstract

Objectives: The negative effects of alcohol on health have attracted attention in recent years. The most devastating complications of alcoholism, such as kidney damage, can be seen due to the continuous consumption of alcohol. Possible mechanisms by which alcohol may increase renal dysfunction have been expressed in the literature. Among these mechanisms, oxidative stress is thought to be a potential mechanism that affects kidney function. Nitric oxide synthase (NOS) and nuclear factor kappa B (NF-κB) levels, which have roles in oxidative stress and inflammation, may be at abnormal levels in the kidney in alcohol use disorder. This study aimed to evaluate the role of NOS and NF-κB molecules in the mechanism of kidney damage caused by alcohol use.

Materials and Methods: The immunoreactivity of NOS2 and NF-κB in kidney tissue was evaluated in an experimental model of acute and chronic alcohol intake in male and female rats (n=56). Groups, control female, control male, sham female, sham male, acute male model, acute female model, chronic female model and chronic male model. The acute and chronic model groups were given ethanol to induce alcohol intake. Immunohistochemical analyzes were performed for NOS2 and NF-κB expressions along with histopathological analysis in kidney tissues.

Results: It was observed that glomerulation degeneration, bleeding, vacuolization, and inflammation were increased in kidney tissues in all groups compared to control groups. In addition, NF-κB and NOS2 expressions were found to be significantly higher in the acute and chronic model groups compared to the control groups.

Conclusion: The presented findings reveal that the expression of NOS2, which is involved in oxidative stress, and NF-κB, which is involved in inflammation, increases kidney damage in acute and chronic alcohol intake. Therefore, NFκB and NOS2 proteins, which play a role in tissue damage, inflammation, and oxidative stress response, may be associated with alcohol-induced renal damage.

Key Words: Alcohol Consumption Model, NF-κB, NOS2, Kidney, Oxidative Stress

Öz

Amaç: Alkolün sağlık üzerindeki olumsuz etkileri son yıllarda dikkatleri üzerine çekmektedir. Kronik alkol tüketimi nedeniyle böbrek hasarı gibi yıkıcı komplikasyonlar görülebilir. Alkolün böbrek fonksiyon bozukluğunu artırabileceği olası mekanizmalar literatürde ifade edilmiştir. Bu mekanizmalar arasında oksidatif stresin böbrek fonksiyonlarını etkileyen potansiyel bir mekanizma olduğu düşünülmektedir. Oksidatif stres ve enflamasyonda rolü olan nitrik oksit sentaz (NOS) ve nükleer faktör kappa B (NF-κB) seviyeleri, alkol kullanım bozukluğunda böbrekte anormal düzeylerde görülebilmektedir. Bu çalışmada alkol kullanımına bağlı böbrek hasarının mekanizmasında NOS ve NF-κB moleküllerinin rolünün değerlendirilmesi amaçlanmıştır.

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Received/Geliş Tarihi: 01.04.2023 **Accepted/Kabul Tarihi:** 13.04.2023

Öz

Gereç ve Yöntem: Böbrek dokusunda NOS2 ve NF-κB'nin immünoreaktivitesi, erkek ve dişi sıçanlarda (n=56) deneysel bir akut ve kronik alkol alımı modelinde değerlendirildi. Gruplar, kontrol dişi, kontrol erkek, sham dişi, sham erkek, akut erkek model, akut dişi model, kronik dişi model ve kronik erkek model olarak ayrılmıştır. Akut ve kronik model gruplarına alkol alımını teşvik etmek için etanol verildi. Böbrek dokularında histopatolojik analiz ile birlikte NOS2 ve NF-κB ekspresyonları için immünohistokimyasal analizler yapıldı.

Bulgular: Kontrol grubuna göre tüm gruplarda böbrek dokularında glomerülasyon dejenerasyonu, kanama, vakuolizasyon ve enflamasyonun arttığı görüldü. Ayrıca NF-κB ve NOS2 ekspresyonları akut ve kronik model gruplarında kontrol gruplarına göre anlamlı olarak yüksek bulunmuştur.

Sonuç: Sunulan bulgular, akut ve kronik alkol alımında oksidatif streste yer alan NOS2 ve enflamasyonda rol oynayan NF-κB ekspresyonunun böbrek hasarını artırdığını ortaya koymaktadır. Bu nedenle, doku hasarı, enflamasyon ve oksidatif stres yanıtında rol oynayan NF-κB ve NOS2 proteinleri, alkolün neden olduğu böbrek hasarı ile ilişkili olabilir.

Anahtar Kelimeler: Alkol Tüketim Modeli, NF-κB, NOS2, Böbrek, Oksidatif Stres

Introduction

Alcohol consumption is one of the leading causes of preventable deaths worldwide, with 3 million deaths per year attributable to alcohol. Alcohol-related morbidity and mortality are largely due to the high rates of alcohol use disorder in the population (1). Chronic alcohol consumption can often be associated with clinical diseases such as breast and colon cancer, pancreatic disease, liver cirrhosis, diabetes, osteoporosis, arthritis, kidney disease, immune system dysfunction, and hypertension (2). In addition, a relationship between kidney diseases due to alcohol use disorder has been shown in the literature (3).

Studies in the literature report that the incidence of kidney disease is lower in heavy alcohol drinkers (more than 210 g/week alcohol consumption) compared to moderate alcohol drinkers (70-210 g/week alcohol consumption) (4,5). The possible relationship between alcohol consumption and kidney damage has not yet been fully elucidated. In the literature, it has been suggested that oxidative stress resulting from increased production of reactive oxygen species (ROS) may play a role in pathogenesis. Increased ROS can trigger excessive amounts of free radicals, triggering tissue damage in the kidney. In addition, the effect on other major organs (liver, heart, intestines and skeletal muscle) in alcohol use disorder can trigger adverse pathological processes that damage the kidneys (3).

A clinical study conducted in 2018 showed its association with alcohol use disorder due to the development of chronic kidney disease. An approximately two-fold increase in the incidence of newly diagnosed chronic kidney disease has been reported in alcohol use disorder (6). Alcohol addiction may be a risk factor for the development of chronic kidney disease, especially in the younger population. A study in 2022 also investigated the mortality rate, disease progression, and disease burden of acute kidney injury in the alcohol use disorder population. It was stated that the subpopulation of patients with alcohol use disorder had a higher number of patients with acute kidney injury and a higher mortality rate than the subpopulation without alcohol use disorder (7). Therefore, determining the pathogenesis of kidney damage in individuals with alcohol use disorder may be important in preventing alcohol-related mortality.

Nitric oxide synthase (NOS), a molecule associated with oxidative stress, may be involved in alcohol dependence (8). Nitric oxide (NO), the smallest known signaling molecule, is produced by three isoforms of NOS. All use l-arginine and molecular oxygen as substrates (9). The NO pathway is involved in the regulation of glomerular hemodynamics. The net effect in the kidney is to support natriuresis and diuresis, to adapt to changes in dietary salt intake, and to contribute to the maintenance of normal blood pressure (10). In a study, the effect of the NOS cofactor tetrahydrobiopterin during chronic alcohol consumption was investigated. Sprague-Dawley rats fed alcoholic or non-alcoholic liquid diets for 2-3 months have demonstrated impaired NOS-induced vasodilation during alcohol consumption (11). In this context, NOS in chronic alcohol consumption may affect kidney damage about oxidative stress.

NF-κB, which is involved in tissue damage and inflammatory processes, are inducible transcription factors (12). NF-κB stimulates two major signaling pathways, canonical and non-canonical (or alternative) that are responsible for regulating immune and inflammatory responses (13). The most important function of NF-κB is the regulation of inflammatory responses. NF-κB, mediates the induction of various proinflammatory genes in innate immune cells (14). Activation of the NF-κB signaling pathway is associated with the expression of NOS and cyclooxygenase-2 enzymatic proteins (15,16). In this context, NF-κB may act by triggering ROS and oxidative stress in alcohol dependence. In a study conducted in 2020, an agent trial was conducted that affects the NF-κB signaling pathway in chronic alcoholic liver disease. It has been shown that andrographolide can reduce alcohol-induced liver pathological damage and oxidative stress by reducing the expression of NF-κB and TNF-α in mice exposed to ethanol (17). Thus, the NOS-mediated NF-κB signaling pathway may be activated in the liver and kidney in alcohol-induced tissue injury.

In this study, the immunoreactivity of NOS and NF-κB molecules was investigated for the mechanism of kidney damage in alcohol dependence. NOS and NF-κB expressions were determined by immunohistochemistry in rats with alcohol dependence through ethanol. In addition, the pathology of kidney damage in alcohol dependence was evaluated by H&E staining.

Materials and Methods

Experimental Groups

This study was carried out with ethical approval from Erciyes University Animal Experiments Local Ethics Committee (HAYDEK) (approval no: 21/059, date: 07.04.2021). Experimental groups were designed as follows.

Control Group (n=7): Animals in this group were not treated.

Sham Group (n=7): Animals in this group were given only the volume (2 mL) of distilled water given to the animals in the acute model and chronic model by oral gavage.

Acute Model Group (n=7): Animals in this group were given 18% v/v ethanol (prepared in distilled water) in a total volume (2 mL) and a total dose of 1 g/kg body weight by oral gavage (18). It is planned to have at least two days between two gavage applications. In other words, it is aimed to create a model of alcohol exposure for a period of approximately two weeks in total. Thus, it was intended to represent a consumption habit reported in the majority of rats, typically referred to as acute, low-moderate alcohol intake (19,20).

Chronic Model Group (n=7): Animals in this group were administered 20% v/v ethanol (prepared in distilled water) in a total volume (2 mL) by oral gavage at a total dose of 4.5 g/kg body weight (21).

Gavage was applied to the rats every day between 9.00-10.00 in the morning (daylight). Body weights were measured every week and it was observed whether they gained weight or lost weight. No restrictions were applied on feed and water consumption. After the experimental protocol was completed, the experimental animals were decapitated and the kidney tissue was taken.

Histological Analysis

After 10% formaldehyde fixation, kidney tissue samples were cleared in xylene and embedded in paraffin after passing through increasing alcohol series (70%, 80%, 96%, and 100%). Sections were taken as 5 µm for hematoxylin and eosin (H&E) staining and immunohistochemical staining (22). It was examined under a light microscope at different magnifications. Lesions in glomerular degeneration and tubulointerstitial injury (such as vacuolization, bleeding, and infiltration of inflammatory cells) were evaluated separately for each animal and were graded from 0-3: 0 absent, 1 mild, 2 moderate, and 3 severe in each category. Quantification was performed on 30 glomeruli randomly selected by two investigators and randomly selected cortical areas per 20 sections, and statistical analysis was performed on mean values from each animal group, n=7 rats per group (23).

Immunohistochemical Analysis

Immunoreactivities of NF-κB (BT-MCA1291, Bioassay Technology Laboratory) and NOS2 (E-AB-70051, Elabscince) proteins were determined by immunohistochemical analysis in sections taken from kidney tissues of experimental groups using the Avidin-Biotin peroxidase method (22). In summary, after deparaffinization of 5µm thick sections, citrate buffer was used to open epitopes (pH: 6.0; Thermo Fischer Scientific, UK, AP-9003-500). Then slides were placed in a 3% hydrogen peroxide solution in methanol to inhibit endogenous peroxidase activity. Ultra V block solution (Thermo Fischer Scientific, UK, TA-125-UB) was applied to prevent non-specific staining. Then, it was incubated with primary antibodies (NF-kB was used at dilution ratios of 1:50 and NOS2 at 1:300 dilution ratios) overnight at 4 °C. After, it was incubated with biotinylated goat anti-polyvalent secondary antibody (Thermo Fisher Scientific, UK, TP-125-BN) for 40 minutes in a 37 °C oven. After washing several times with PBS, it was incubated with streptavidin peroxidase (Thermo Fisher Scientific, UK, TS-125-HR) for 30 minutes in a 37 °C oven. The antibody complex was visualized by incubation with diaminobenzidine (DAB) chromogen (Thermo Fisher Scientific, UK, TA-125-HD). Sections were then counterstained with Gill III Hematoxylin (Merck, Germany, 1.05174.1000). It was dehydrated by passing through a series of increasing alcohol and covered with a sealant called entellan. Sections were examined with an Olympus BX53 light microscope. Evaluation of immunoreactivity levels was done with ImageJ Version 1.46 (National Institutes of Health, Bethesda, Maryland).

Statistical Analysis

GraphPad Prism 8.0 software program was used for all statistical analysis and graph drawing. Two-way ANOVA analysis of variance and Tukey's multiple comparison tests were applied to analyze the numerical data obtained from the observed damage rates in the kidney. $P<0.05$ was considered statistically significant.

Results

Histological Results

It was observed that glomerulation degeneration, bleeding, vacuolization, and inflammation were increased in kidney tissues in acute and chronic models of both sexes compared to control groups (Figure 1, Table 1). Especially in the acute male

group, glomerular degeneration, bleeding, and vacuolization were found to be significantly increased compared to the acute female group (**p<0.001, *p<0.05; Table 1). While there was a significant decrease in vacuolization in the chronic male group compared to the chronic female group, an increase in inflammation was observed (***p<0.0001; Table 1).

Immunohistochemical Results

NF-κB and NOS2 expressions were significantly higher in the acute and chronic model groups compared to the control groups (Figure 2). While NF-κB expression increased 3.6 times in the acute female group and 4.9 times in the chronic female group compared to the control female group, it was observed that it increased 4.2 times in the acute male group and 4.4 times in the chronic male group compared to the control male group. While the NF-κB expression observed in the chronic female group was found to be significantly higher than in the acute female group, no difference was observed between the acute male and chronic male groups.

While NOS2 expression increased 3.08-fold in the acute female group and 3.1-fold in the chronic female group compared to the control female group, it was observed that it increased 3.6-fold in the acute male group and 4.3-fold in the chronic male group compared to the control male group. It was determined that there was no difference between the acute and chronic model groups.

When the female and male acute models were compared within themselves, no difference was observed in terms of NF-κB and NOS2 expression. While NOS2 expression increased significantly in the chronic male group compared to the chronic female group, there was no difference in NF-κB expression.

Discussion

In recent years, the negative effects of alcohol on health have reached remarkable dimensions. Mortality and morbidity rates resulting from alcohol consumption are increasing every year. Complications such as kidney damage and liver failure can often be seen due to the continuous consumption of alcohol. Chronic alcohol consumption can seriously affect kidney damage. In our study, we found that the immunoreactivity of NF-κB and NOS2 in the kidney was increased in both acute and chronic alcohol intake models compared to control groups. While the NF-κB expression observed in the chronic female group was significantly higher than in the acute female group, no difference was observed between the acute male and chronic

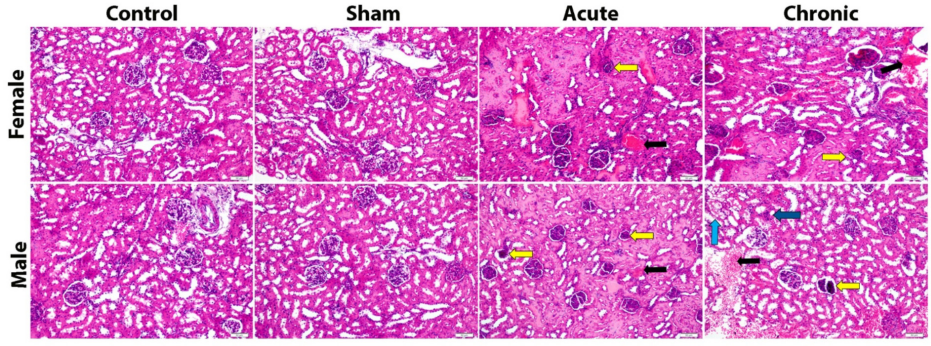

Figure 1: H&E staining images of kidney tissues of the experimental groups. The black arrow indicates hemorrhage, the yellow arrow indicates glomerular degeneration, the light blue arrow vacuolization, and the dark blue arrow inflammation. Magnification 20X, scale bar= 50 µm

Table 1: Kidney damage rates by groups

		Tubulointerstitial lesions		
Groups	**Glomerular degeneration**	**Hemorrhage**	**Vacuolization**	**Inflammation**
Control female	0.38±0.05	0.34±0.05	0.44±0.06	0.20±0.05
Sham female	0.36±0.04	0.31±0.05	0.50±0.08	0.25±0.03
Acute female	1.21±0.12^{ab}	1.40±0.32^{ab}	1.06±0.18^{ab}	0.73±0.04^{ab}
Chronic female	1.98±0.18^{abc}	2.01±0.26^{abc}	2.09±0.39^{abc}	0.80±0.07^{ab}
Control male	0.40±0.07	0.35±0.14	1.10±0.22	0.33±0.08
Sham male	0.41±0.06	0.23±0.08	0.40±0.04	0.37±0.18
Acute male	1.62±0.16^{de**}	1.84±0.31^{de**}	1.42±0.29^{de*}	1.08±0.30^{de}
Chronic male	2.20±0.13^{def}	2.06±0.19^{de}	1.55±0.40^{de***}	1.49±0.30^{def***}

Two-way analysis of variance and Tukey's multiple comparison tests were applied. Data shown in the table are expressed as mean ± standard deviation. ^{a}: p<0.05 compared to the control female group; ^{b}: p<0.05 to the sham female group; ^{c}: p<0.05 compared to the acute female group; ^{d}: p<0.05 compared to the control male group; ^{e}: p<0.05 compared to the sham male group; ^{f}: p<0.05 shows that there is a statistical difference compared to the acute male group, *p<0.05, **p<0.001, ***p<0.0001

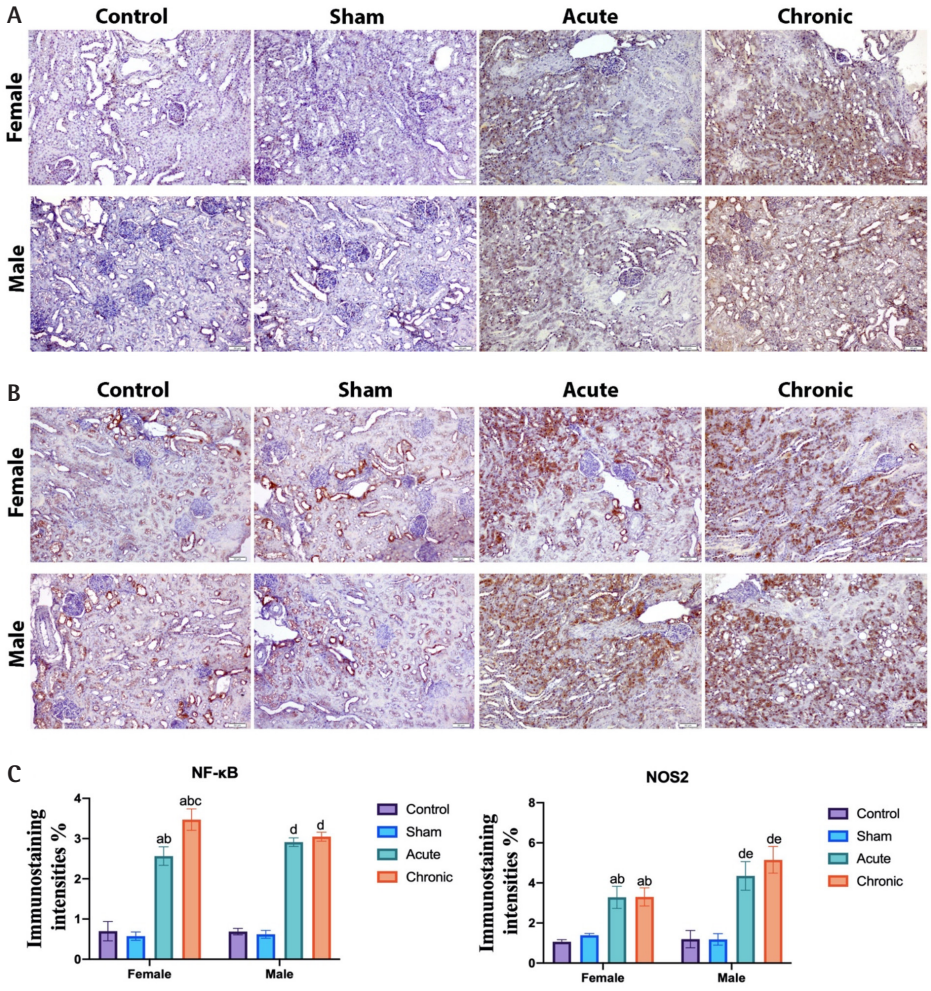

Figure 2. NF-κB and NOS2 immunostaining images in the kidney tissues of the experimental groups. A) NF-κB immunostaining images. B) NOS2 immunostaining images. Magnification 20X, scale bar=50 µm. C) Bar graphs showing immunostaining intensities. Data shown in bar graphs are expressed as mean ± standard deviation. Two-way analysis of variance and Tukey's multiple comparison tests were applied. a: means p<0.05 from the control female group, b: p<0.05 from the sham female group, c: p<0.05 from the acute female group, d: p<0.05 from the control male group, e: from the p<0.05 sham male group, and f: p<0.05 from the acute male group

male groups. When the female and male acute models were compared within themselves, no difference was observed in terms of NF-κB and NOS2 expression. While NOS2 expression increased significantly in the chronic male group compared to the chronic female group, there was no difference in NF-κB expression. Therefore, NF-κB and NOS2 proteins, which play a role in tissue damage, inflammation, and oxidative stress response, may be associated with alcohol-induced renal damage.

Chronic alcohol consumption can affect kidney damage to a certain extent. A 2017 study showed that acute alcohol poisoning exacerbated acute renal failure due to rhabdomyolysis in rats. The renal tissue injury parameters NF-κB and inducible NOS (iNOS) were evaluated by giving rats intravenous injections of 5 g/kg ethanol for 3 hours (24). In this context, acute alcohol intoxication may exacerbate renal failure through pro-oxidant and inflammatory effects such as iNOS and NF-κB. In correlation with our results, NF-κB and NOS2 expression increases due to damage to the mechanism of alcoholic nephropathy. In addition, new studies on kidney damage due to acute and chronic alcohol intake are needed in the literature.

iNOS mediates ethanol-induced redox imbalance and upregulation of inflammatory cytokines in the kidney. Renal damage was investigated by administering ethanol (20% v/v) to C57BL/6 wild-type and iNOS gene-deficient (iNOS-/-) mice for 10 weeks (25). iNOS may play a role in ethanol-induced oxidative stress and proinflammatory cytokine production in the kidney. In another study, it was determined that inhibition of NF-κB reduced glycerol-induced kidney damage. Wistar rats were given 8 ml/kg of 50% glycerol intramuscularly and their renal parameters were evaluated (26). In the context of these data, decreased NF-κB expression may reduce alcohol-induced kidney damage.

In the literature, the role of CYP2E1 in the regulation of NOS and NF-κB is emphasized. Therefore, a 2015 study observed that CYP2E1 was induced in the renal tubules of mice on a chronic alcohol diet (27). Therefore, it can be stated that kidney damage due to chronic ethanol intake can be induced by CYP2E1 in the liver. In addition, induction of CYP2E1 can lead to the generation of ROS and oxidative stress (28). NOS, a molecule involved in the oxidative stress mechanism and shown to be induced by CYP2E1 (29), has been reported to stimulate the production of proinflammatory cytokines through the activation of redox-sensitive NF-κB by exerting the pathological effects of CYP2E1 in alcohol damage by increasing nitroxidative stress and lipid peroxidation (30). Therefore, enzymes such as CYP2E1 may have a role in the mechanism that triggers the activation of NOS2 and NF-κB in acute and chronic alcohol consumption-induced kidney damage. Determination of molecules that trigger oxidative stress and inflammatory processes in renal damage due to alcohol dependence is considered important in pathogenesis.

Study Limitations

Our data show increased expression of NOS2 and NF-κB in acute and chronic alcohol consumption-induced kidney damage. However, only the demonstration of immunohistochemical changes can be considered as the limitation of the study. In future studies, a comprehensive characterization can be revealed by investigating the protein and mRNA expressions of NOS2, NF-κB and other inflammatory mediators in the same tissues.

Conclusion

Our data show increased expression of NOS2 and NF-κB in the kidney in the acute and chronic alcohol intake model induced in rats. On the other hand, only the demonstration of immunohistochemical changes can be considered a limitation of our study. In future studies, a comprehensive characterization can be revealed by examining the protein and mRNA expressions of NOS2, NF-κB, and other inflammation and oxidative stress parameters in similar tissues.

Ethics

Ethics Committee Approval: This study was carried out with ethical approval from Erciyes University Animal Experiments Local Ethics Committee (HAYDEK) (approval no: 21/059, date: 07.04.2021).

Informed Consent: Animal experiment study.

Peer-reviewed: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: A.C.U., S.Y., Z.D., Concept: A.O., A.C.U., O.Ö., S.Y., Z.D., Design: A.O., A.C.U., S.Y., Z.D., Data Collection and Processing: A.O., A.C.U., O.Ö., E.E., S.Y., Z.D., Analysis or Interpretation: A.O., A.C.U., O.Ö., S.Y., Z.D., Literature Search: A.O., A.C.U., O.Ö., E.E., S.Y., Z.D., Writing: A.O., A.C.U., O.Ö., E.E., S.Y., Z.D.

Conflict of Interest: The authors declared that there was no conflict of interest during the preparation and publication of this article.

Financial Disclosure: The authors declared that they did not receive any financial support during the research and authoring of this article.

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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

DOI: 10.4274/atfm.galenos.2023.17136
Ankara Üniversitesi Tıp Fakültesi Mecmuası 2023;76(1):24-29

DAHİLİ TIP BİLİMLERİ / MEDICAL SCIENCES

Kazanılmış Faktör VIII ve IX İnhibitörü Olan Hastalarda İmmünosüpresif Tedavi Etkinlik ve Güvenilirliğinin Değerlendirilmesi

Evaluation of the Efficacy and Safety Immunosuppressive Therapy in Patients with Acquired Factor VIII and IX Inhibitor

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Öz

Amaç: Mortalitesi yüksek kanamalara yol açabilen ve nadir gözlemlenen kazanılmış faktör inhibitörü olan hastalarda, immünosüpresif tedavi etkinlik ve güvenilirlik sonuçlarının değerlendirilmesidir.

Gereç ve Yöntem: Kasım 2018-Mart 2022 tarihleri arasında merkezimizde kazanılmış faktör inhibitörü ile takip ve tedavi edilmiş hastalar çalışmaya dahil edildi.

Bulgular: Çalışmaya ortanca yaşı 65 (aralık, 43-76) dördü erkek toplam yedi hasta dahil edildi. Faktör inhibitör gelişim nedenleri; postpartum dönem (n=1), lupus antikoagülan (n=1), ileri yaş (n=2) ve malignite (n=3) olup, tüm hastalarda ortanca takip süresi 9 ay (aralık, 2-46 ay) idi. Dört hastada tanıda yüksek titreli inhibitör vardı [ortanca: 7,06 BÜ/mL (aralık, 5,76-100)] ve tümü immünosüpresif tedavi (metilprednizolon ve siklofosfamid kombinasyonu, rituximab) almıştı. Hastaların tamamı immünosüpresif tedaviye yanıt verirken (toplam yanıt oranı %100; tam yanıt %50, kısmi yanıt %50), postpartum dönemde faktör inhibitörü gelişmiş bir hastada rituximab tedavisine yanıt alınmıştır. Hastaların ikisinde kanama sırasında rekombinant aktive-faktör VII (rFVIIa) ve aktive protrombin kompleks konsantreleri (aPCC) hemostazı sağlamıştır. Düşük titrede faktör inhibitörü saptanan üç hasta ise kanama olmadığından immünosüpresif tedavi almamış, hastalarda ihibitör gelişiminden sorumlu neden ortadan kalktığında koagülasyon testleri düzelmiştir.

Sonuç: Kazanılmış yüksek titreli faktör inhibitörü olan hastalarda bilinen tedavi seçeneklerinin birbirine üstünlüğü kanıtlanmamıştır. Dirençli faktör inhibitör tedavisinde rituximab alternatif bir seçenek olabilir ve hastalarda hemostazı sağlamada rFVIIa ve aPCC kullanımı etkindir. Düşük titrede faktör inhibitörü olan hastaların tedavisinde ise klinik durum ve altta yatan hastalık göz önünde bulundurularak tedavi planı yapılmalıdır.

Anahtar Kelimeler: Faktör İnhibitör, İmmünosüpresif Tedavi, Hemostaz

Abstract

Objectives: Acquired factor inhibitor is a rare disorder and can cause life-threatening bleeding. Consequently, we report the efficacy and safety of our patients with acquired factor inhibitor treated with immunosuppressive drugs.

Materials and Methods: We retrospectively investigated acquired factor inhibitor patients who were followed and treated in our center between November 2018 and March 2022.

Results: A total of seven patients, four of whom were male, with a median age of 65 (43-76) were included. Acquired factor inhibitor was associated with malignancy (n=3), advanced age (n=2), postpartum period (n=1) and lupus anticoagulant (n=1). Median follow-up time was 9 months (range, 2-46). High inhibitor titres were detected in four patients [median: 7.06 BU/mL (range, 5.76-100)] and all of them were treated with immunosuppressive drugs (metilprednisone in combination with cyclophosphamide, rituximab). The overall response rate was 100% (n=4) with

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Geliş Tarihi/Received: 19.12.2022 **Kabul Tarihi/Accepted:** 17.03.2023

Abstract

50% complete response and 50% partial response and one patient who developed the inhibitor in the postpartum period responded to single agent rituximab. To achieve the hemostatic target, recombinant activated clotting factor VII (rFVIIa) and activated prothrombin complex concentrate (aPCC) were used in two out of four patients with active bleeding. Hemostasis was achieved successfully. Three patients developed a low-titre factor inhibitor and immunosuppressive drugs were not started for inhibitor elimination. Coagulation assays disappeared when the underlying condition was self-limited.

Conclusion: Clinical presentation of factor inhibitors is highly variable and optimal treatment is not clearly established. Rituximab might be a promising treatment for therapy-resistant factor inhibitor and bleeding control was achieved with rFVIIa and aPCC. Treatment plan of patients with low-titre factor inhibitor should be provided considering the clinical situation and underlying disease.

Key Words: Factor Inhibitor, Immunosuppressive Theraphy, Hemostasis

Giriş

Edinsel faktör inhibitör otoantikorları oldukça nadir olup, hayatı tehdit eden kanamalara yol açabilir. Kanamaların yaklaşık %80'den fazlası deride geniş hematom veya ekimoz alanlarını içermekle birlikte, ayrıca intramüsküler alan, yumuşak doku, eklem ve mukozalarda da (epistaksis, gastrointestinal kanama, hematüri) görülebilir (1). Edinsel faktör inhibitör meydana gelme nedeni çoğunlukla idyopatik olup, otoimmün hastalık, malignite (en sık sırasıyla prostat adenokarsinom, akciğer kanseri, kolon kanseri; hematolojik malignite en sık sırasıyla lenfoma, kronik lenfositer lösemi, plazma hücreli diskrazi), enfeksiyon, ilaç ilişkili ve postpartum dönemde gelişebilir (2-4).

Kazanılmış inhibitörler genellikle faktör VIII'e (FVIII) karşı gelişmekte olup, diğerleri çok daha nadirdir (2). Kanser nedenli kazanılmış FVIII inhibitörü olan hastalar, idyopatik olanlara karşılaştırıldığında tekrarlayan kanamaların daha sık görüldüğü ve inhibitör eradikasyon tedavisine tam yanıtın daha az oranda olduğu gözlemlenmiştir (5,6). Bununla birlikte, kanamaların faktör seviyesi ve inhibitör titresiyle ilişkili olmadığı bilinmektedir (7). Faktör inhibitörü gelişen hastalarda en uygun tedavi seçeneği henüz bilinmemekle birlikte, tedavide uzun süreli immünosüpresyon ve maliyetli faktör replasmanı uygulanır. Tedavide amaç, kanamayı engellemek veya kontrol altına almak, inhibitör eradikasyonu, sekonder gelişimli faktör inhibitöründe altta yatan hastalığı kontrol altına almaktır. Tedavi genellikle merkezin klinik deneyimine göre verilmekte olup, bilinen tedavi seçeneklerinin birbirine üstünlüğü kanıtlanmamıştır (8,9). Biz bu çalışmada kendi merkezimizde kazanılmış faktör inhibitörü nedeniyle immünosüpresif tedavi alan hasta sonuçlarını paylaştık.

Gereç ve Yöntem

Kasım 2018-Mart 2022 tarihleri arasında merkezimizde kazanılmış FVIII inhibitörü ile tedavi edilmiş hastalar çalışmaya dahil edildi. Hasta dosyaları geriye dönük olarak değerlendirildi. Çalışmada inhibitör tanısı ile takip edilen hastaların tanı ve/veya takip sürecinde kanaması olup olmadığı, kanaması olan hastalarda kanama bölgesi, hastaların koagülasyon testlerinde uzama, faktör inhibitör gelişim nedeni, faktör inhibitör titre düzeyi, koagülasyon testlerinde uzamaya ve dirençli faktör inhibitör oluşumun eşlik edebilecek lupus antikoagülan tespiti amacı ile antinükleer antikor (ANA), anti-DsDNA, antikardiyolipin antikorları ve lupus antikoagülan immün belirteç varlığı değerlendirildi. İnhibitör titresi modifiye Bethesda testi ile saptandı. Bethesda testi ile >0,6 BÜ/mL olan titreler pozitif olarak kabul edildi. İzlem sırasında titre 5 BÜ/mL'yi aşmıyorsa "düşük titreli inhibitör" adı verildi (10). İmmünosüpresif tedavi uygulanan bu hastalarda faktör inhibitör eradikasyon yanıtı, yanıt devamlılık süresi, tedavi ilişkili toksisite, kanama kontrolü (kanama durumunda) değerlendirildi. Tam yanıt (TY) normal sınırlarda faktör aktivitesi (%70-140) ve faktör inhibitör titresinin saptanmaması, kısmi yanıt (KY) faktör aktivitesinin %30'a kadar görülmesi, faktör inhibitör titresinin <5 BÜ/mL olması olarak tanımlandı (11). Çalışmaya katılan tüm hastalara hastanın yaşı, altta yatan hastalığı, önceki kanama alanı-şiddeti ve inhibitör düzeylerine göre bireyselleştirilmiş tedavi uygulandı.

Çalışmamız, Ankara Üniversitesi Tıp Fakültesi, İnsan Araştırmaları Etik Kurulu tarafından onaylandı (tarih: 10.12.2021, karar no: İ11-696-21) ve Helsinki Deklarasyonu'nda belirtilen etik standartlara uygun olarak yürütüldü.

Çalışmanın birincil amacı, ilgili dönemlerde merkezimizde kazanılmış faktör inhibitörü tanısı ile takip edilen hastalarımızda, immünosüpresif tedavi etkinlik ve güvenilirlik sonuçlarının değerlendirilmesidir (faktör inhibitör eradikasyon yanıtı, yanıt devamlılık süresi, tedavi ilişkili toksisite, kanama kontrolü). Çok nadir gözlemlenen kazanılmış faktör inhibitör tanı farkındalığını artırmak ve etkin olarak kullanılabilecek alternatif tedavi seçeneklerinin literatüre kazandırılması ise çalışmanın ikincil amaçlarıdır.

İstatistiksel Analiz

Çok nadir görülen bir hastalık olması nedeni ile çalışma sonuçları olgu bazında değerlendirilerek sunuldu. Normal dağılmayan sürekli değişkenler için ortanca, minimum ve maksimum değerler verildi. Sağkalım hesaplamaları için Kaplan-Meier yöntemi kullanıldı. İstatistiksel analizler, "Statistical

Package for Social Sciences (SPSS) for Windows 21.0 (SPSS Inc, Chicago, IL)" yazılımı kullanılarak yapıldı.

Bulgular

Çalışmaya ortanca yaşı 65 (aralık, 43-76; 3 kadın, 4 erkek) olan toplam yedi hasta dahil edildi. Hastaların özellikleri Tablo 1'de verilmiştir. Faktör inhibitör gelişim nedenleri; postpartum dönem (n=1), lupus antikoagülan (n=1), ileri yaş (n=2) ve malignite (n=3) idi. Hastaların dördü (%57) nedeni bilinmeyen kanama, eşlik eden anemi öyküsü ve aktive parsiyel tromboplastin zamanı (aPTT) uzaması ile başvurmuştu. Bu hastalarda (n=4) tanıda ANA, antikardiyolipin antikorları ve lupus antikoagülan immün belirteçleri negatif olup, tanıda faktör inhibitör titresi >5 BÜ/mL'nin üzerindeydi [ortanca: 7,06 BÜ/mL (aralık, 5,76-100)] ve tümü immünosüpresif tedavi almıştı. İki hastada kanama sırasında hemostaz kontrolü için bypass edici ilaçlar (rFVIIa, aPCC) kullanılmıştı. Faktör inhibitör titresi <5 BÜ/mL (hesaplanamayacak kadar düşük miktarda) olan 3 (%43) hastada ise aktif kanama olmayıp, operasyon öncesi yapılan rutin tetkiklerinde saptanan izole aPTT yüksekliği nedeniyle faktör inhibitörü saptanmıştı. Bu hastaların birine birlikte eşlik eden lupus antikoagülanı nedeniyle immünosüpresif tedavi verilmişti.

Bir no'lu hastada (43 yaşında kadın) doğum sonrası 5. ayda gelişmiş hem FVIII hem de FIX; FXI, FXII ye karşı edinsel inhibitör vardı ve aktif kanama ile hastanemize başvurmuştu. Tedavide Bonn Malmö (immünadsorbsiyon, metilprednizolon, siklofosfamid, IVIG, FVIII) rejimi verilmişti. Bonn Malmö bitimi sonrası FVIII %1,7 olurken, aPTT 67,3 sn, FVIII inhibitör titresi 33,3 BÜ/mL'ye gerilemişti. Siklofosfamid idame tedavisi altındayken sağ el bilek hemartrozu ve kompartman sendromu gelişen hastada, rFVIIa ve aPCC ardışık olarak kullanımı ile hemostaz sağlandı. Takipte 8 kür rituximab ile FIX inhibitörü için TY, FVIII inhibitörü için KY yanıt elde edildi. Klinik olarak ise kanamaları tamamen düzeldi ve tekrarlamadı. Günümüzde düşük de olsa halen FVIII inhibitörü devam etmektedir (Şekil 1).

Diğer üç hasta (70, 76 ve 65 yaşında erkek) 1 mg/kg/gün metilprednizolon ve siklofosfamid (50-100 mg oral doz) ile tedavi edildi. İki no'lu hastada faktör inhibitörü ileri yaşta (70 yaşında) gelişmişti. Tedavi ile 2. ayda tamamen düzeldi ve hastalığı tekrarlamadı. Üçüncü hastada altta akciğer malignitesi vardı. Tedavi ile inhibitör 1. ayda düzelmiş, ancak hasta malignitesi nedeni ile 4 ay içinde ölmüştür. Dördüncü hastada iliopsoas kas, toraks duvarı hematomu ve kraniyal kanama nedeni ile başvuruda hemostaz kontrolünde ardışık olarak rFVIIa ve aPCC kullanılmıştı.

Tablo 1: Hasta özellikleri

Hasta no	Tanı yaşı	Cinsiyet	Altta yatan neden	Kanama bölgesi	Hb (g/dL)	aPTT (sn)	Faktör düzeyi (%)	Karışım testi (sn)	İnhibitör titresi (BÜ/mL)	Takip süresi & Sonlanım
1	43	K	Postpartum	Diş eti kanaması, tedavi sırası hemartroz	11,8	94,8	FVIII 0,1 FIX 50	61,3	FVIII 100 FIX 13,2	31 ay/ FVIII KY FIX TY
2	70	E	İleri yaş &komorbid hastalıklar	Alt ekstremitede hematom	10	90,6	FVIII 0,5	40,9	FVIII 7,36	16 ay/TY
3	76	E	Akciğer Ca	Alt ekstremite ve kollarda hematom	13,7	62,2	FVIII 6,2	38,6	FVIII 6,76	4 ay/TY
4	65	E	Mide Ca	Alt ekstremite, toraks ön duvar, kraniyal	4,8	152,4	FVIII 0,5	38,4	FVIII 5,76	2,5 ay/KY
5	55	K	Lupus antikoagülan (LA)	Yok	8,4	76,3	FVIII 248 FXII 27,8 LA 1,64	53,8	FVIII Hesaplanamadı	13 ay/-
6	67	K	Rektum Ca	Yok	8	61	FVIII 143 FIX 103 FXI 115 FXII 46,1	44,4	FVIII, FIX, FXI, FXII Hesaplanamadı	13 ay/-
7	76	E	İleri yaş & komorbid hastalıklar	Yok	14,5	62,3	FV 24,9 FVII 29,4 FVIII 83,6 FX 45,2 FXI 13,8 FXII 30,5	40,3	FVIII Hesaplanamadı	2 ay/-

Normal aralık: aPTT sn (25,1-36,5), karışım testi aPTT sn (25,1-36,5), LA (0-1,2), %FV (62-169), %FVII (50-129), %FVIII (50-150), %FIX (65-150), %FX (77-131), %FXI (65-150), %FXII
Hb: Hemoglobin, aPTT: Aktive parsiyel tromboplastin zamanı

Takipte immünosüpresif tedavi ile 1. ayda KY alınmış (FVIII 13,4, faktör inhibitör 3,04), ancak hasta sepsis nedenli 2. ayda ölmüştür.

Beş no'lu hastada (55 yaşında kadın) sol femur boyun kırığı nedeniyle sol total kalça protez ameliyatı planlanmaktaydı. Hastanın operasyon öncesi tetkiklerinde saptanan izole aPTT yükseklik nedeni araştırılırken, lupus antikoagülan 1,64; aralık (0-1,2), faktör XII eksikliği (%27,8) ve düşük titrede FVIII inhibitör (FVIII %248) saptandı. ANA+++ ve CENP-B+++ olan hastada romatoloji ön planda skleroderma düşündü. Hastaya 1 mg/kg/gün metilprednizolon verildi ve 3 ardışık gün immünadsorbsiyon uygulandı. Ameliyat sonrası aPTT 47,4 sn'ye geriledi. Günümüzde hastanın romatoloji bilim dalında takip ve tedavisine devam edilmektedir.

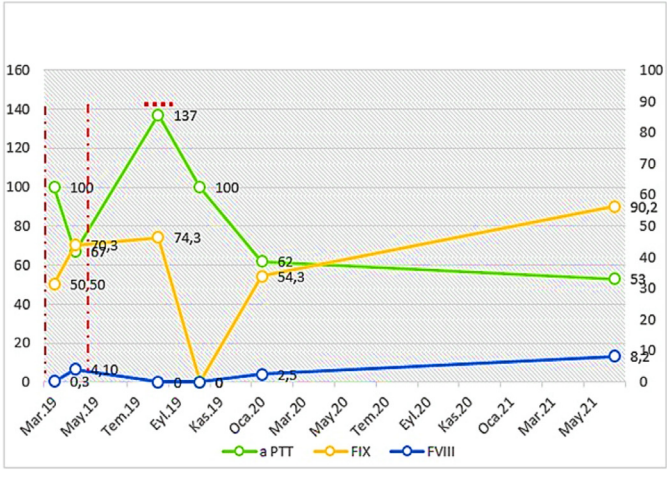
Altı no'lu hastanın (67 yaşında kadın) rektum kanseri nedeniyle ameliyatı planlanmaktaydı ve operasyon öncesi tetkiklerinde izole aPTT yüksekliği saptandı. Hastanın ANA, antikardiyolipin antikorları ve lupus antikoagülan immün belirteçleri negatif olup, tetkiklerinde hesaplanamayacak kadar düşük titrede hem FVIII hem de FIX; FXI, FXII'ye karşı edinsel inhibitörü saptandı. İmmünosüpresif tedavi planlanmayan hastada, ameliyat sonrası aPTT 36,5 sn'ye geriledi.

Yedi no'lu hastanın (76 yaşında erkek) hasta genel klinik durum bozukluğu ile bacakta şişlik, kızarıklık (nekrotizan fasiit?) ve hemotokezya nedeni ile acil servise başvurmuştu. Tetkiklerinde anemi [hemoglobin (Hb): 11,4 gr/dL] ve aPTT yüksekliği saptananan hastaya yapılan kolonoskopide rektal ülserden kanama gözlemlenmişti. Hastanın ANA, antikardiyolipin antikorları ve lupus antikoagülan immün belirteçleri negatif olup, tetkiklerinde faktör V (%24,9), VII (%29,4), IX (%29,6), X (%45,2), XI (%13,8), XII eksikliği (%30,5) ve hesaplanamayacak kadar düşük titrede FVIII inhibitör (FVIII %83,6) saptandı. İmmünosüpresif tedavi planlanmayan hasta sepsis nedenli 2. ayda öldü.

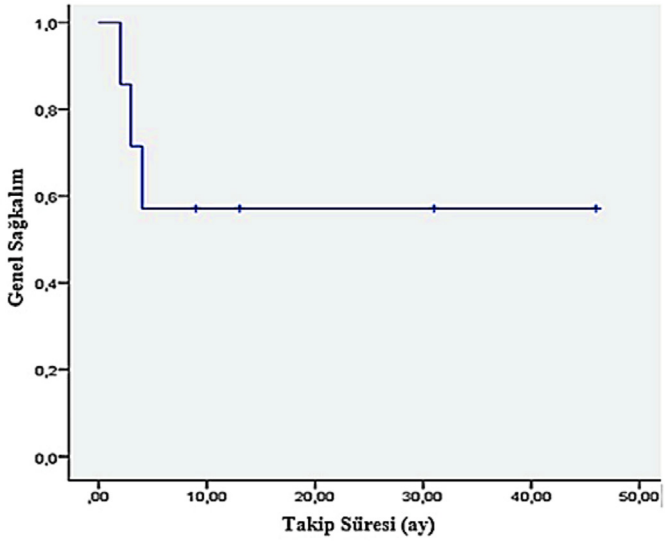
Tüm hastalarda ortanca takip süresi 9 ay (aralık, 2-46 ay) ve ortalama genel sağkalım 27,5 ay (%95 güven aralığı 11,8-43 ay) (ortanca değere ulaşılamadı) idi (Şekil 2). İmmünosüpresif tedavi verilen hastalarda (n=4) toplam yanıt oranı %100 (2 TY, 2 KY) idi ve tedavi ilişkili herhangi bir yan etki gözlenmedi. İmmünosüpresif tedavi sonrası (Bonn Malmö, metilprednizolon ve siklofosfamid kombinasyonu) sadece 1 no'lu hastada faktör inhibitör titre artışı saptandı. Kanaması olan 2 hastada rFVIIa ve aPCC kullanımının hemostazı sağlamada etkin olduğu gözlemlendi. Düşük titrede faktör inhibitörü saptanan hastalarda kanama olmadığından immünosüpresif tedavi verilmemiş, inhibitöre gelişimine yol açan neden ortadan kalktığında koagülasyon testlerinde düzelme olduğu gözlemlenmiştir.

Tartışma

Faktör inhibitör gelişen hasta sonuçlarının değerlendirildiği çalışmaların hemen hepsi geriye dönük verilere dayanmaktadır. Malign tümörlerle kazanılmış faktör inhibitör gelişimi ve klinik seyri arasındaki ilişki ile ilgili literatürde sınırlı bilgi bulunmaktadır. Kanser ilişkili kazanılmış faktör inhibitör gelişen 41 hastanın incelendiği bir çalışmada (n=25 solid tümör, n=16 hematolojik malignite) 63 hemorajik olay gözlemlenmiş olup, bu hastalarda tanı sırasında ortanca faktör inhibitör titresinin 14 BÜ/mL (aralık, 1-435 BU) olduğu saptanmıştır (4). Çalışmada hastalarda TY oranı %70 ve TY'li hastaların çoğunluğunda tümör evresinin erken evrede olduğu, ayrıca inhibitör titresinin yanıtsız hastalara göre anlamlı olarak daha düşük olduğu gözlemlenmiştir. Sallah ve Wan. (4) malignitesi olan hastalarda kemoterapi veya cerrahi tedavi ile faktör inhibitörünün eradike edilebileceğini düşünmektedir. Biz çalışmamızda rektum, akciğer ve mide kanseri tanılı faktör inhibitör gelişmiş 3 hastamızın 2'sinde immünosüpresif tedavi ile 1 TY, 1 KY gözlemledik. Dört no'lu hastada KY alınması genel klinik durum bozukluğu kaynaklı siklofosfamidi düzenli kullanmamış olması ve takip süresi kısalığı nedenli olabilir. Düşük titreli inhibitör gelişmiş bir diğer



Şekil 1: Bir no'lu hasta



Şekil 2: Genel sağkalım

hastamızda ise Sallah ve Wan'ın (4) hipoteziyle uyumlu olarak post-operatif dönemde inhibitör kısa sürede eradike oldu.

FVIII inhibitörü gelişen yaklaşık %30 hastada spontan rezolüsyon görülebilir (özellikle postpartum dönemde oluşanlar). Spontan rezolüsyon ortanca 21 ayda gerçekleştiğinden, rezolüsyonu hızlandırmak için çoğu hastada immünosüpresif tedavi kullanılır. Prednizon tek başına kullanılınca yanıt oranı yaklaşık %30 olsa da nüksü önlemek için uzun süreli idame tedavisi verilmelidir (12,13). Prednizon ve siklofosfamid kombinasyonu ise %30-70 oranında etkin olup, inhibitör eradikasyon süresi 3-37 hafta arasında değişmektedir (14,15). Ancak, hastaların 1/3'ü bu tedaviye yanıtsız ve kronik steroid kullanımının ciddi yan etkileri olduğundan, rituximab tedavide alternatif seçeneklerden biridir. Altmış beş kazanılmış FVIII inhibitörü olan hastanın değerlendirildiği olgu-kontrol çalışmasında, rituximab ile tedavi yanıt oranının %90'dan fazla olduğu gözlemlenmiştir (16). Sperr ve ark. (17) ise aynı hasta grubunda rituximab ile tedavi edilen 42 hasta ile, steroid ve siklofosfamid tedavisi alan 44 hastada tedavi yanıt oranlarının benzer olduğunu gözlemlemiştir (sırasıyla %78,6 vs %84,1). EACH2 çalışmasında ise rituximab diğer immünosüpresif tedaviler kombine edildiğinde de yanıt oranının steroid ve siklofosfamid tedavisinden istatiksel olarak anlamlı farkı olmadığı tespit edilmiştir (sırasıyla %64 vs %70) (18). Yüksek titreli inhibitörü olan hastalarda inhibitörü yok etmek için uygulanabilecek immun tolerans tedavilerinden biri Bonn Malmö protokolüdür (immünadsorbsiyon, prednizon, siklofosfamid, IVIG, yüksek doz faktör). Zeitler ve ark.'nın (19) yüksek titreli inhibitörü olan ve en az bir kez kanama (Hb<8 gr/dL) geçirmiş 60 hastanın dahil edildiği çalışmalarında, Bonn Malmö protokolü ile %90 TY, %10 KY elde edilmiştir. Hastalar ortanca 62 ay (aralık, 12-126 ay) takip edilmiş, takip süresi sonunda 55 hastada yeniden inhibitör oluşmamıştır. FVIII titresi 3 hastada üst solunum yolu enfeksiyonu sırasında %10-50 azalmış, ancak hastalarda kanama görülmemiştir. Çalışmamızda, tanıda FVIII düzeyi %0,8, FVIII inhibitör titresi 100 BÜ/mL, FIX inhibitör titresi 13,2 BU/mL hastamıza Bonn Malmö protokolü uygulanmış, ancak Zeitler ve ark.'nın (19) sonuçlarından farklı olarak inhibitör titresi tamamen yok olmamıştır. Hastamızda prednizon ve siklofosfamid idame tedavisi altında (5. ayda) kanama görülmüş, bypass edici tedaviler ve rituximab sonrası 3 yıl çok düşük titreli inhibitör ile takip edilmiştir. Bu sonuç, literatürde çoğu çalışmada belirtilen rituximab etkinliğinin steroid ve siklofosfamid kombinasyonu ile benzer olması ile çelişmektedir. Bunun nedeni, hastamızda literatürde çok nadir bulunan yüksek titreli FVIII ve FIX edinsel inhibitör ile düşük titreli FXI, FXII'ye karşı inhibitörü olmasından kaynaklanabilir.

Çalışmamızda düşük titreli inhibitör olan hastalarda aktif kanama gözlemlenmedi. Literatürde bu hasta grubunda faktör replasmanı yapılabileceği bildirilmiştir. Lupus antikoagülan varlığı ön planda tromboz eğilimi sağlamakla birlikte, dolaşımda bulunan antikorların FXII'ye de bağlanarak FXII eksikliğine neden olduğu bilinmektedir (20). Beş no'lu hastamızda düşük titreli faktör inhibitörü bulunmasına rağmen, lupus antikoagülan ve FXII eksikliği hastada ön planda tromboz gelişimini artırmıştır. Bu nedenle hastaya immünadsorbsiyon ve metilprednizolon tedavisi uygulanmıştır.

Çalışmanın Kısıtlılıkları

Çalışmaya alınan hasta sayısının az olması, hastaların faktör inhibitör oluşum nedeni ve faktör titre düzeylerinin birbirinden farklı olması ile retrospektif tasarımlı olması çalışmanın kısıtlılığı olarak kabul edilebilir.

Sonuç

Bu çalışmada, kısıtlılıklar nedeniyle sonuçların yorumlanması güç olmakla birlikte, diğer çalışmalardan elde edilen önemli bazı bulguları desteklediği görülmektedir. Çalışmamızda dirençli faktör inhibitörü olan hastalarda rituximab'ın alternatif bir tedavi seçeneği olabileceğini ve hemostazı sağlamada rFVIIa ve aPCC kullanımının etkinliğini gösterdik. Kanama ve koagülasyon parametre uzaması ile gelen hastalarda faktör inhibitörü olabileceği düşünülmesi bu nadir görülen hastalık tanısını kolaylaştırabilir. Daha geniş hasta popülasyonunda uzun dönem takip sonuçlarının değerlendirildiği çalışmalara ihtiyaç vardır.

Etik

Etik Kurul Onayı: Çalışmamız, Ankara Üniversitesi Tıp Fakültesi, İnsan Araştırmaları Etik Kurulu tarafından onaylandı (tarih: 10.12.2021, karar no: İ11-696-21).

Hasta Onayı: Retrospektif olarak hasta dosyaları değerlendirildi.

Hakem Değerlendirmesi: Editörler kurulu dışında olan kişiler tarafından değerlendirildi.

Yazarlık Katkıları

Konsept: D.K., G.C.S., S.C.B., M.Ö., P.T., Dizayn: D.K., G.C.S., P.T., Analiz veya Yorumlama: D.K., G.C.S., S.C.B., M.Ö., P.T., Literatür Arama: D.K., P.T., Yazan: D.K., G.C.S., P.T.

Çıkar Çatışması: Yazarlar arasında herhangi bir çıkar çatışması bulunmamaktadır.

Finansal Destek: Çalışma için doğrudan veya dolaylı mali destek alınmadı. Çalışma ile ilgili herhangi bir firma veya kişi ile ilgili ticari bağlantı yoktur.

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RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ

DOI: 10.4274/atfm.galenos.2023.00922
Ankara Üniversitesi Tıp Fakültesi Mecmuası 2023;76(1):30-36

BASIC MEDICAL SCIENCES / TEMEL TIP BİLİMLERİ

Effects of Interleukin-33 on Neopterin and IP-10 Production in Macrophages

Makrofajlarda Neopterin ve IP-10 Üretimine İnterlökin-33'ün Etkileri

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Abstract

Objectives: Interleukin-33 (IL-33) is a pleiotropic cytokine from the IL-1 family. It is known to cause plasticity in the Th2 direction. The macrophages, important cells of the innate immune system, express the IL-33 receptor ST2L and exhibit M2 macrophage polarity under the influence of IL-33. Macrophages produce interferon gamma-stimulated protein 10 (IP-10), an interferon-γ-induced chemokine, and neopterin which exhibits cellular immunoreaction. The aim of this study was to investigate the effects of IL-33 on neopterin and IP-10 production in unstimulated and pre-stimulated macrophages with different cytokines.

Materials and Methods: The J774.1 macrophage cell line was used. J774.1 macrophages were stimulated by IFN-γ (40 U/mL) + LPS (10 ng/mL) after pretreatment with IL-33 (100 ng/mL). Nitrit levels were measured by the Griess reaction. To examine the effect of IL-33 on polarized macrophages, J774.1 macrophages were stimulated with IL-4, IL-10 ve IL-13 cytokines to provide M1, M2a and M2c macrophage polarization. Neopterin and IP-10 measurements were carried out by ELISA method. Statistical analysis was performed with SPSS 18 software.

Results: IL-33 was found to significantly increase neopterin production in unstimulated and plasticity-affected J774.1 cells. There was no change in IP-10 levels in all experiments. No correlation was found between neopterin and IP-10 levels.

Conclusion: Our present study is the first study on the effects of IL-33 cytokine on neopterin production. IL-33 causes a significant increase in neopterin production in J774.1 cells.

Key Words: Interleukin-33, IP-10, Neopterin, Macrophage, ELISA

Öz

Amaç: İnterleukin-33 (IL-33), IL-1 ailesinden pleiotropik bir sitokindir. Th2 yönünde plastisiteye yol açtığı bilinmektedir. Doğal bağışıklık sisteminin önemli hücreleri olan makrofajlar, IL-33 reseptörü ST2L'yi ifade eder ve IL-33 etkisi altında M2 makrofaj polaritesi sergiler. Makrofajlar, interferon-γ ile indüklenen bir kemokin olan interferon gama ile uyarılan protein 10 (IP-10) ve hücresel immünoreaksiyon gösteren neopterin üretir. Bu çalışmanın amacı, uyarılmamış ve farklı sitokinlerle önceden uyarılmış makrofajlarda IL-33'ün neopterin ve IP-10 üretimi üzerindeki etkilerini araştırmaktır.

Gereç ve Yöntem: J774.1 makrofaj hücre dizisi kullanıldı. J774.1 makrofajları, IL-33 (100 ng/mL) ile ön işlemden sonra IFN-γ (40 U/mL) + LPS (10 ng/mL) ile uyarıldı. Nitrit seviyeleri Griess reaksiyonu ile ölçüldü. IL-33'ün polarize makrofajlar üzerindeki etkisini incelemek için J774.1 makrofajları, M1, M2a ve M2c makrofaj polarizasyonu sağlamak üzere IL-4, IL-10 ve IL-13 sitokinleri ile uyarıldı. Neopterin ve IP-10 ölçümleri ELISA yöntemi ile yapıldı. İstatistiksel analiz SPSS 18 yazılımı ile yapıldı.

Bulgular: IL-33'ün, uyarılmamış ve plastisitesi etkilenmiş J774.1 hücrelerinde neopterin üretimini önemli ölçüde artırdığı bulundu. Tüm deneylerde IP-10 seviyelerinde değişiklik olmadı. Neopterin ve IP-10 seviyeleri arasında bir korelasyon bulunmadı.

Sonuç: Bu çalışmamız, IL-33 sitokinin neopterin üretimi üzerindeki etkileri üzerine yapılan ilk çalışmadır. IL-33, J774.1 hücrelerinde neopterin üretiminde önemli artışa neden olmaktadır.

Anahtar Kelimeler: İnterlökin-33, IP-10, Neopterin, Makrofaj, ELİZA

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Received/Geliş Tarihi: 02.11.2022 **Accepted/Kabul Tarihi:** 25.04.2023

Introduction

Macrophages are the main effector cells of the innate immune system that participate in the inflammatory response against microbial pathogens. Tissue macrophages contribute to tissue development, metabolism, homeostasis, and repair. Tissue-resident macrophages are polarized by tissue cytokines, chemokines, and hormonal stimuli. Macrophages are classified into 2 subgroups termed classically activated macrophages (M1) and alternatively activated macrophages (M2) (1). Macrophages are also stimulated by interleukin-33 (IL-33). This interaction is mediated by the macrophage-expressed IL-33 ligand suppression of tumorigenicity 2 (ST2). IL-33/ST2 signaling is involved in alternative activation of type 2 macrophage (M2) polarization (2,3).

IL-33 was first identified in 2005 and classified as a new member of the IL-1 family. While IL-1α and IL-18 from these family members are immunomodulatory agents for Type I immune response, IL-33 leads to Th2 plasticity towards Type II response (4). IL-33 is quickly released upon tissue damage, exposure to pathogens, stress, or in necrosis-induced death, and subsequently mediates immune response as an alarmin cytokine. IL-33 plays critical roles in maintaining host homeostasis and in pathological conditions, such as allergy, infectious diseases, and cancer, by acting on multiple types of immune cells and promoting type 1 and 2 immune responses (3,5).

The C-X-C motif chemokine10, also known as interferon-γ inducible protein 10 (IP-10), is a chemokine that modulates innate and adaptive immune responses by recruiting inflammatory cells to the sites of inflammation. IP-10 is secreted by several cell types, such as leukocytes, monocytes, neutrophils, eosinophils, and epithelial cells, in response to IFN-γ (6). IP-10 is exhibits pleiotropic effects on a wide range of biological processes, including immunity, angiogenesis, and tumor metastasis (7).

Neopterin is a guanosine triphosphate metabolite with a low molecular weight, which is a member of the pteridine family. It is produced in human macrophages and dendritic cells (8,9). IFN-γ is the strongest stimulus of the GTP cyclohydrolase-I enzyme. For this reason, neopterin concentrations are an important indicator of IFN-γ activity (10). Therefore, examination of neopterin is important to detect cellular inflammatory response and Type-1 inflammatory events.

In this study, we aimed to elucidate the effect of IL-33 on neopterin and IP-10 production in the macrophage cell line J774.1. The effects of IL-33 on macrophages on neopterin production have not been investigated previously and this is the first study in this area. Therefore, mrIL-33 (recombinant murine) cytokine was added to cell cultures with or without stimulation of IFN-γ and LPS. Subsequently, neopterin, IP-10, and nitrite production of macrophages were measured. In addition, it has been investigated whether there is a relationship between these productions.

Materials and Methods

Cell and Cell Culture

J774.1 macrophage cell line (The American Type Culture Collection - ATCC) which was frozen in a liquid nitrogen tank at approximately -196 °C, in RPMI containing 10% DMSO (Gibco, USA), was used. Serial passage of J774.1 macrophage cell line was performed in RPMI-1640 (Gibco, USA) medium supplemented with 10% fetal bovine serum (FCS, Gibco, USA) and penicillin/streptomycin (Pen Strep, Gibco, USA) at 37 °C in a humidified atmosphere of 5% CO_2.

For stimulation experiments, mouse J774.1 macrophage cells grown in a 37 °C, 5% CO_2 incubator in cell culture flasks were excised with cell scrapers for fresh use in experiments. Before the experiments, the trypan blue deprivation test was performed for cell viability and the cells were counted in a thoma counting chamber. The 90% cell viability was considered sufficient for the next stage of experiments. For the experiments, dilutions were carried out to be $0.5x10^6$ viable cells per 1 mL. The cells were transferred to 24-well cell culture plates. A triplicate experiment was performed in order to evaluate the reproducibility of the data. mrIL-33 (100 ng/mL) (Ebioscience, USA), mrIL-4 (10 ng/mL) (Ebioscience, USA), mrIL-13 (10 ng/mL) (Life Sciences, USA), mrIL-10 (100 ng/mL) (Ebioscience, USA), mrIFN-γ (40 U/mL) (Ebioscience, USA), LPS (10 ng/mL) (Escherichia coli. Santa Cruz, USA) were determined as working concentrations by preliminary experiments.

Nitrite Measurement

Nitrite production was evaluated to check for stimulation in J7741 macrophages. 100 µL of cell culture supernatant was mixed with 100 µL of Griess reagent. At the end of the required study incubations, the absorbance at 540 nm was measured in a spectrophotometric microplate reader. The macrophage nitric oxide response was determined by measuring the level of nitrite accumulating in the culture supernatant with the Griess reagent (Sigma-Aldrich, USA).

Neopterin ELISA

We used a commercial neopterin enzyme immunoassay kit (Immuno Guide. Turkey) for quantitative analysis of the neopterin levels in the supernatants. Briefly, Neopterin had been pre-coated onto a microplate. Assay Buffer was added to the 96-well plate at a rate of 100 µL/well. Standards and samples were pipetted into the wells. It was covered with black adhesive film. The plate was incubated for 90 minutes at room temperature in the dark. Any Neopterin was bound by the

immobilized antibody and then any unbound substances were eliminated by washing three times. After washing, 100 µL of TMB Substrate was added to each well and incubated for 15 minutes at room temperature in the dark. Stop solution (100 µL) was added and the color development was stopped. Finally, the plate was evaluated spectrophotometrically using an ELISA reader at 450 nm wavelength within 15 minutes (BioTek ELx800, USA). The concentration in nmol/L was calculated from the standard curve. Sensitivity was 1 nmol/L.

IP-10 ELISA

Ready-to-use commercial sandwich ELISA kit was used for IP-10 (pg/mL) chemokine measurement (Mouse IP-10, YH Biosearch, China). Briefly, 120 µL of original standard + 120 µL of serial dilution from 120 µL of a standard diluent tube were prepared. Only the dilution solution was added to the blank wells. 50 µL of standard and 50 µL of conjugate were added to the standards. 40 µL of sample + 10 µL of IP-10 antibody + 50 µL of the conjugate was added to the wells of the samples. It was shaken slightly to mix and covered with adhesive film, the plate was incubated for 1 hour in an incubator at 37 °C. The plate, whose liquid content was emptied, was washed 5x with 350 µL of hand-washing solution. After washing, 50 µL of chromogen A + 50 µL of chromogen B solution was added to each well. It was incubated for 10 minutes in an incubator at 37 °C. The reaction was stopped by adding 50 µL/well stop solution to the plate and the color of the liquid in the wells turned from blue to yellow. The plate was evaluated spectrophotometrically using an ELISA reader (BioTek ELx800, USA) at 450 nm wavelength within 15 minutes. Sensitivity was 2.42 pg/mL.

Statistical Analysis

All groups were subjected to Levene's test of homogeneity within themselves, and ANOVA and Student's t-tests were performed among the groups as parametric tests if the data were homogeneous. For comparison of parametric data, ANOVA was used amongst multiple groups, and Student's t-tests were used between two groups. SPSS 18 software was used in evaluating the statistics of the data. The significance threshold value was accepted as $p<0.05$.

Results

Nitrite, neopterin, and IP-10 production of the J774.1 cell line at different stimulation doses and times

In our results, IFN-γ (4 U/mL) + LPS (10 ng/mL) and IFN-γ (40 U/mL) + LPS (10 ng/mL) stimulations increased the level of neopterin after 18 hours. A significant increase was established when the other groups were compared (15 min, 30 min, 60 min, 4 h) ($p<0.001$) (Figure 1a). It also caused an increase in neopterin production in 4 hour-treatment compared to 15 minute-treatment ($p<0.05$) (Figure 1a). There was no significant difference between IP-10 concentrations (Figure 1b). No significant correlation was observed between IP-10 production and neopterin levels ($r2=0.032$). In the high dose (1 µg/mL) LPS-treated groups, nitrite production increased during 18 hours of incubation ($p<0.05$). A significant increase in nitrite levels were observed in the IFN-γ (4 U/mL) + LPS (10 ng/mL) and IFN-γ (40 U/mL) + LPS (10 ng/mL) treatments during overnight incubation compared with the other times ($p<0.001$) (Figure 1c).

Evaluation of nitrite production stimulated by IFN-γ (40 U/mL) + LPS (10 ng/mL) and IL-33 (100 ng/mL) in J774.1 macrophages

When IL-33 was administered 18 hours before IFN-γ (40 U/mL) + LPS (10 ng/mL) stimulation, nitrite production was significantly inhibited compared to at the same time and after 4 hours (0 and +4) administration ($p<0.001$). When IL-33 was administered -1 and -4 hours before IFN-γ (40 U/mL) + LPS

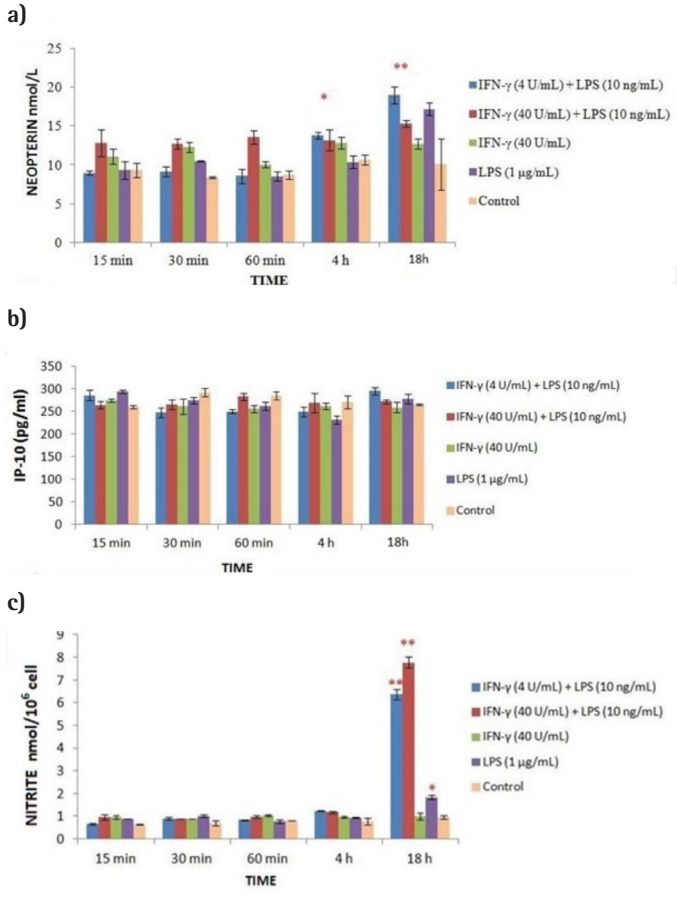

Figure 1: Neopterin, IP-10, and nitrite production in J774.1 macrophages at different stimulation doses and incubation times

a) Time-dependent neopterin concentration (*: $p<0.05$ compared with 15 min. time group, **: $p<0.001$ compared with the all-time groups) **b)** Time-dependent IP-10 concentration. **c)** Time dependent nitrit concentration (*: $p<0.05$ compared with control, **: $p<0.001$ compared with control)

(10 ng/mL) stimulation, nitrite production was significantly inhibited compared to 0 and +4 hour administrations (p<0.05). IL-33 pretreatment has been shown to inhibit nitrite production in J774.1 macrophages. It was observed that only IL-33 cytokine administration did not affect nitrite levels (Figure 2).

Neopterin production stimulated with IFN-γ (40 U/mL) + LPS (10 ng/mL), LPS (1 µg/mL), and IL-33 (100 ng/mL) after pretreatment IL-33 (100 ng/mL) in J774.1 macrophages

When IL-33 was administered 18 hours ago, it caused a significant increase in neopterin production in all groups IFN-γ (40 U/mL) + LPS (10 ng/mL), IL-33, LPS (1 µg/mL) (p<0.001) (Figure 3a). When IL-33 was administered 18 hours ago, there was no significant difference in IP-10 concentrations amongst groups in a time-dependent manner (Figure 3b). The stimulation didn't change the IP-10 level in the cells.

Neopterin production in IFN-γ (40 U/mL) + LPS (10 ng/mL)-stimulated J774.1 macrophages after IL-33 (100 ng/mL) pre-treatments

When IL-33 was treated 18 hours before IFN-γ (40 U/mL) + LPS (10 ng/mL) stimulation, it caused a significant increase in neopterin production (p<0.001) (Figure 4). This effect was observed many times in our experiments in IL-33 treatments. Therefore, we observed the neopterin-enhancing effect of IL-33 cytokine in culture for 36 hours and longer.

The effects of IL-33 on nitrite, neopterin, and IP-10 levels in the J774.1 macrophages

IL-33 treatment alone did not affect nitrite production after 2 overnight incubations (p>0.05) (Figure 5a). IL-33 cytokine showed a neopterin-increasing effect in culture treatments of 36 hours or more. It was observed that neopterin concentrations increased significantly after two overnight (42 hour) incubations (p<0.001) (Figure 5b). IP-10 production was not affected by long-term IL-33 stimulation (p>0.05) (Figure 5c).

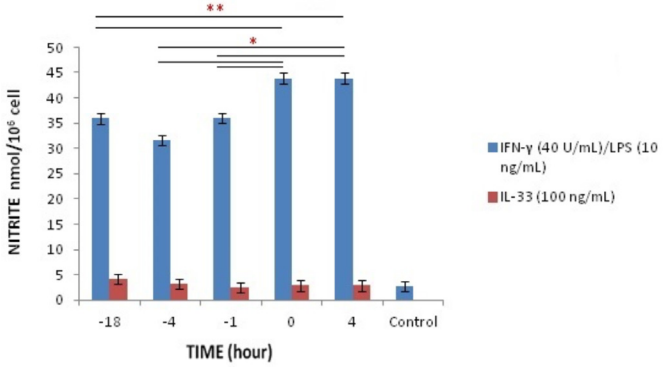

Figure 2: Nitrite production in J774.1 macrophages stimulated with IFN-γ (40 U/mL) + LPS (10 ng/mL) after preliminary administration of IL-33 (100 ng/mL) (*: p<0.05 compared with -4 and -1, time group, **: p<0.001 compared with -18 time group). Control: Untreated (-18= 18 hours before stimulation) (-4= 4 hours before stimulation) (-1= 1 hour before stimulation) (0= stimulation time) (+4= 4 hours after stimulation)

The effects of IL-4, IL-13, IL-4 + IL-13 and IL-10 on neopterin and IP-10 levels in the J774.1 macrophages

J7741 macrophages were primarily stimulated with appropriate cytokines such as IL-4, IL-10, and IL-13 so that they could be polarized towards M1, M2a, and M2c. Compared with control groups with no stimulation at 0 and 48 hours, neopterin levels were not affected by IL-4 (p>0.05). A statistically significant difference was found between 4-hour stimulation and 48-hour stimulation in neopterin production of IL-4 cytokine (p<0.001). This suggested that inhibition of the IL-4 cytokine on neopterin production might be temporary (Figure 6a). It was observed that

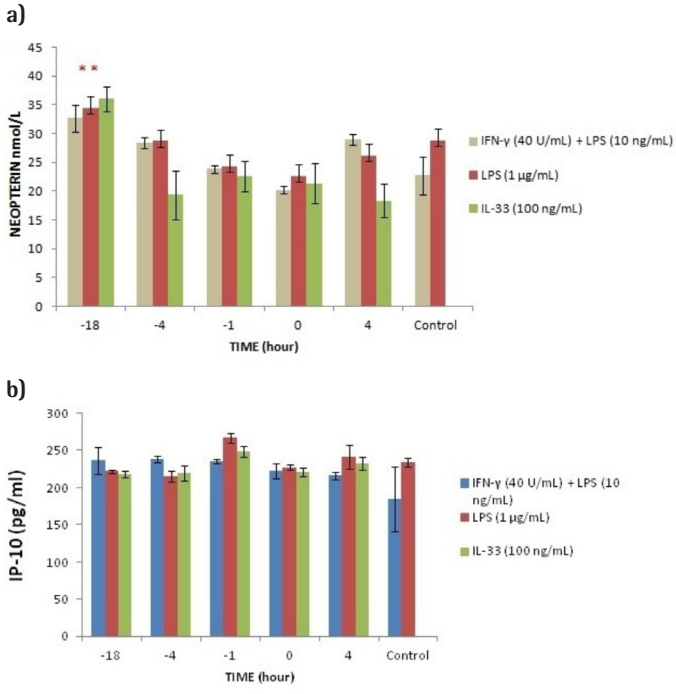

Figure 3: a) Neopterin production in J774.1 macrophages stimulated with IFN-γ (40 U/mL) + LPS (10 ng/mL) and LPS (1 µg/mL) after preliminary administration of IL-33 (100 ng/mL) (**: p<0.001 compared with control). Control: Untreated. **b)** IP-10 production in J774.1 macrophages stimulated with IFN-γ (40 U/mL) + LPS (10 ng/mL) and LPS (1 µg/mL) after preliminary administration of IL-33 (100 ng/mL) Control: Untreated

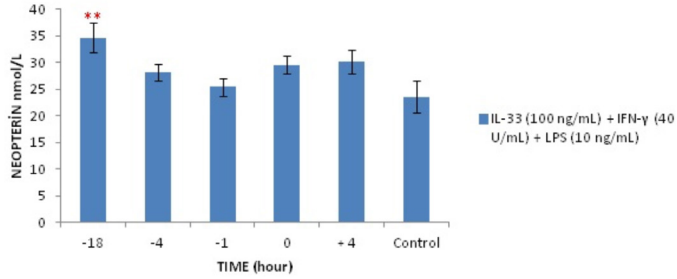

Figure 4: Neopterin production in J774.1 macrophages stimulated with IFN-γ (40 U/mL) + LPS (10 ng/mL) after preliminary administration of IL-33 (100 ng/mL). A statistically significant increase was found in the 18-hour incubation treatment when compared to the other stimulated groups (**: p<0.001 compared with control.) Control: Untreated

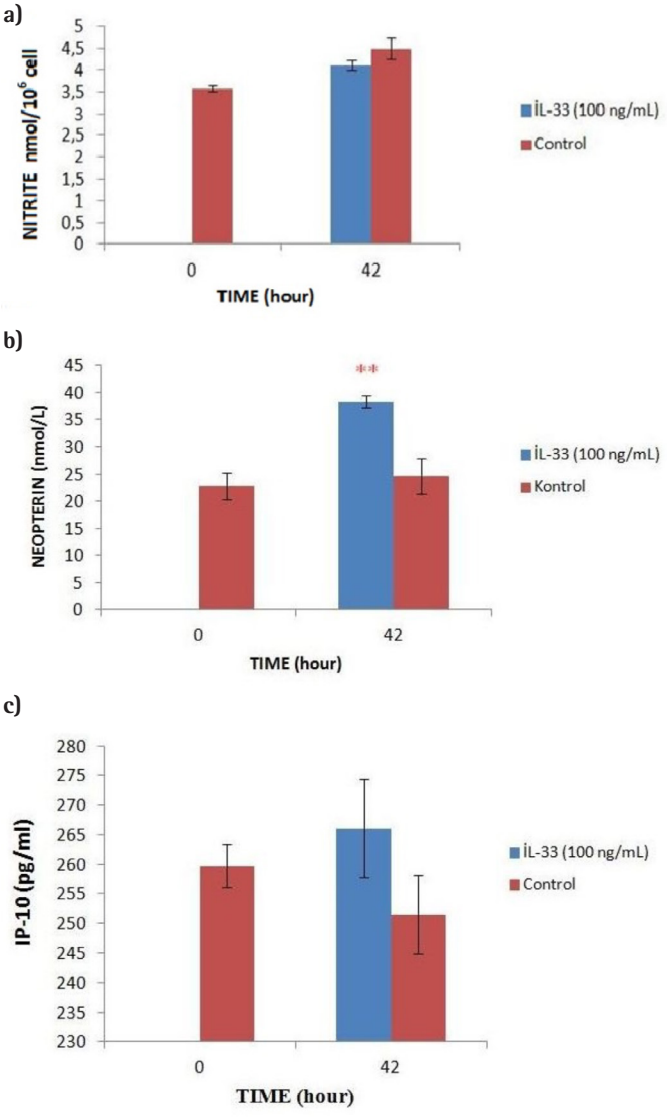

Figure 5: Nitrite, neopterin, and IP-10 production after 42 hours in J774.1 macrophages stimulated with IL-33 (100 ng/mL). **a)** Nitrit concentration. **b)** Neopterin concentration (**: p<0.001 compared with control.) **c)** IP-10 concentration

IL-10, IL-13, and IL-4 + IL-13 cytokine administrations did not affect dependent to time the neopterin levels (p>0.05). It was observed that IL-4, IL-13, IL-4 + IL-13, and IL-10 did not affect dependent to time the IP-10 levels (p>0.05).

The effects of IL-33 on neopterin and IP-10 levels in the IL-10 and IL-4 + IL-13 pre-stimulated J774.1 macrophages

When J774.1 macrophages had been pre-stimulated by IL-10 and IL-4 + IL-13 for 12 hours, they significantly increased neopterin levels compared to the control after 24 hours of stimulation with IL-33 (p<0.01). We observed that after 48 h neopterin was more elevated similar to our first observed results (p<0.001) (Figure 6b). IP-10 levels were not affected compared to the control (p>0.05).

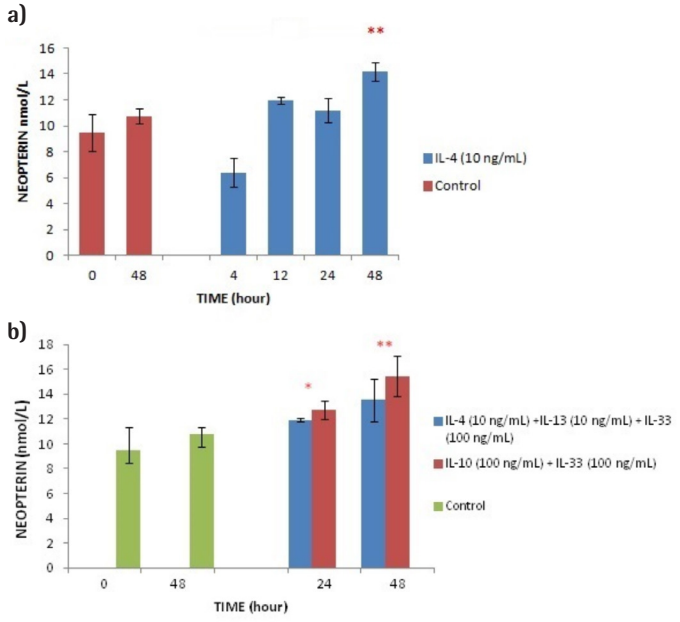

Figure 6: Neopterin production in J774.1 macrophages stimulated with IL-4, IL-10, and IL-4 + IL-13. **a)** Neopterin concentrations in J774.1 macrophages stimulated with IL-4 (10 ng/mL) (**: p<0.001 compared with 4 hours). **b)** Neopterin concentrations in J774.1 macrophages stimulated with IL-33 (100 ng/mL) after preliminary administration of IL-4 (10 ng/mL) + IL-13 (10 ng/mL) and IL-10 (100 ng/mL). (*: p<0.01 compared with control) (**: p<0.001 compared with control)

Discussion

In this study, the effect of IL-33 on neopterin and IP-10 production was investigated in macrophage cell line J774.1. Although there were many studies showing neopterin production in human macrophages, neopterin production in mouse macrophages was a controversial issue. Neopterin production was shown in the THP-1 human monocyte cell line stimulated with IFN-γ and LPS, and NO production was shown in the RAW 264.7 and J774.1 mouse macrophage cell lines (10,11). It has been reported that neopterin was produced in mouse macrophages. Production of NO and biopterin has also been demonstrated in the RAW 264.7 mouse macrophage cell line (12). In our study, it was detected that J774.1 macrophages produced neopterin. In addition, it was established that the production of neopterin increased after 18 hour-stimulation with IFN-γ + LPS of J774.1 macrophages.

IFN-γ was also known to stimulate the production of IP-10, another inflammatory marker (7,13). In a study by Tighe et al. (14), IFN-γ was found to stimulate IP-10 production in macrophages. It was also shown that treatment of LPS to peritoneal macrophages stimulated IP-10 *in vivo* as well as *in vitro*. Viral dsRNA analog poly I:C had been shown to increase IP-10 production in THP-1 cells and RAW264.7 macrophages (15). In our study, we showed that the J774.1 macrophage cell

line produced IP-10, but we found no significant difference in IP-10 concentrations amongst the groups of the time-dependent treatments. The difference might be due to the longer incubations were not used in this study.

Macrophages have been reported to constitutively express ST2, supporting the idea that IL-33 could stimulate and polarize macrophages towards M2, dependent on IL-13 or IL-4. When responding to LPS, IL-33-primed macrophages expressed increased levels of MD2 and TLR-4, thereby elevating its pro-inflammatory effect (16). Macrophages pre-stimulated with IL-33 have been reported to act in the Th1 direction (17). Classical information was that IL-33 was a cytokine that had effects on Th2 immunity. In recent studies, IL-33 had emerged as a cytokine with too many pleiotropic properties. It had been reported that IL-33 did not only increase Th2 immunity but also induced Th1 immunity, depending on the immune cells targeted by IL-33 (3,18). In our study IL-33 cytokine increased neopterin production in cells to which it was administered 18 hours ago. In addition, we found that IL-33 influenced the Type I immune response and that this effect was time-dependent. In our experimental results, it was revealed that IL-33 influenced the macrophages in favor of Type I immunoreactivity and stimulated IFN-γ induced neopterin production.

It had been shown in experimental autoimmune encephalitis that cells in IL-33-treated mice produced less IFN-γ and IL-17, but more IL-5 and IL-13, than cells in control mice. In the same study, cytokines such as IL-10, TNF-α, IL-6, and IL-4 and chemokines such as IP-10, MCP-1, MIG, and VEGF were detected at low levels and no significant difference was found between the two groups (19). The response to treatment with different concentrations of IL-33 in serum and Fibroblast-like synoviocytes of patients with RA was evaluated *in vitro*. In the synovium of patients with RA, a decrease in IL-6, IL-8, IL-1β, MIG, and IP-10 cytokines and chemokines was observed as the dose of IL-33 increased, but not in the serum (20). Although IL-33 had effects altering according to the cytokine environment it is also a cytokine with effects in Th2 direction in macrophages. These results suggested that IL-33 did not affect IP-10 production among Th1 chemokines in naive macrophages. IP-10 values were not affected depending on treatments with IL-33 made at different times. In humans, IL-33 might potentially act as a critical regulator of innate immune responses. However, IL-33 might not have the same effects in mouse macrophages.

Pre-polarized M1 cells have been reported to exhibit the M2 phenotype after exposure to IL-13. Similarly, cells previously in the M2 phenotype were reported to exhibit the M1 phenotype after LPS + IFN-γ treatment (21). Another study found that IL-33 strongly increased mannose receptor (MR) expression, but not TLR2, in the presence of IL-13. It has been shown that IL-33 and IL-4 have a similar synergistic effect on MR expression (22). The plasticity of macrophages indicated that different phenotypes could synthesize products different from their phenotypic characteristics (23). The findings showed that macrophages polarized in the M2a direction were forced from the M2a direction towards the M1 direction when they encounter IL-33. Our study also showed that the synthesis of neopterin produced by M1 macrophages was also stimulated by a cytokine in the M2 phenotype.

Study Limitations

The main limitation of our study, in which we evaluated the effects of IL-33 on neopterin and IP-10 production in macrophages, was that it was performed only in the J7741 macrophage cell line. It would be appropriate to perform the same experiments on RAW 264 and THP-1 macrophages.

Conclusion

Our study demonstrated that IL-33 potentiated alternative-activated macrophage development during innate and adaptive immune responses and could achieve classically activated macrophage activation in different immune conditions. IL-33 caused a significant increase in neopterin production in J774.1 cells. No correlation was observed between neopterin levels and IP-10 inflammatory markers induced by IFN-γ in J774.1 macrophages. Future *in vivo* studies are needed to elucidate the immunological significance of the effects of IL-33 on neopterin and IP-10 production in macrophages.

Ethics

Ethics Committee Approval: The study was performed using a cell line. Does not include human and animal testing. Therefore, ethical committee approval is not required.

Informed Consent: Patient samples were not used in this study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: R.A., C.A., H.T., V.B., Design: R.A., C.A., H.T., V.B., Data Collection or Processing: R.A., C.A., V.B., Analysis or Interpretation: R.A., H.T., V.B., Literature Search: R.A., V.B., Writing: R.A.

Conflict of Interest: There is no conflicts of interest with respect to the authorship and/or publication of this article.

Financial Disclosure: Our study was supported by the Transplantation Immunology and Genetics Association.

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RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ

DOI: 10.4274/atfm.galenos.2023.80958
Ankara Üniversitesi Tıp Fakültesi Mecmuası 2023;76(1):37-44

MEDICAL SCIENCES / DAHİLİ TIP BİLİMLERİ

Duodenogastric Gadoxetic Acid Reflux on Routine Liver Magnetic Resonance Imaging and Upper Endoscopy Findings

Rutin Karaciğer MR Görüntülemede Duodenogastrik Gadoksetik Asit Reflüsü ve Üst Gastrointestinal Sistem Endoskopisi Bulguları

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Abstract

Objectives: Bile reflux gastritis is an underdiagnosed but common type of chemical gastritis (CG) caused by bile induced irritation of gastric mucosa. Diagnosis is based on endoscopic and histologic findings. However, if bile reflux is not observed, diagnosis of bile gastritis might be challenging. We aimed to evaluate duodenogastric gadoxetic acid (GA) reflux in routine liver magnetic resonance imaging (MRI) and to compare this finding with bile reflux and gastritis findings in upper endoscopy.

Materials and Methods: A total of consecutive 45 patients who underwent GA-enhanced liver MRI with at least one delayed phase image (≥40 minutes delay) and upper endoscopy were retrospectively included. Images were reviewed by two radiologists regarding the presence and extent of gastric GA. Endoscopic and histologic results were noted.

Results: Duodenogastric GA reflux was detected in 8 patients (17.8%). In 2 of them endoscopy revealed bile reflux. In one of these two patients gastritis was also noted,whereas gastric mucosa was considered as normal in the other patient. In 6 out of 8 patients with contrast reflux gastric bile stain was not reported. In each of these 6 patients erosive/erythematous gastritis was detected. Histopathology confirmed CG in 4 out of them.

Conclusion: It might be challenging to differentiate bile induced gastritits from other types of CG, in particular, if gastric bile stain is not observed during upper endoscopy. Thus, radiologists should be aware of duodenogastric GA reflux on delayed phase MRI and report this finding. We suggest, that diagnostic performance of MRI regarding bile reflux and bile gastritis should be investigated with a multidisciplinary prospective study design.

Key Words: Magnetic Resonance Imaging, Gadoxetic Acid, Bile Reflux, Bile Gastritis

Öz

Amaç: Safra gastriti, yaygın bir kimyasal gastrit (KG) türü olup gastrik mukozanın safra kaynaklı hasarı ile ortaya çıkmaktadır. Tanı endoskopik ve histolojik bulgulara dayanmakla birlikte safra reflüsünün saptanmadığı durumlarda safra gastritini belirlemek zor olabilmektedir. Çalışmamızda, rutin karaciğer manyetik rezonans (MR) görüntülemede duodenogastrik gadoksetik asit (GA) reflüsünü değerlendirmeyi ve bu bulguyu üst endoskopide saptanan safra reflüsü ve gastrit bulguları ile karşılaştırmayı amaçladık.

Gereç ve Yöntem: Temmuz 2011-Mayıs 2021 tarihleri arasında GA ile gerçekleştirilen toplam 1543 rutin dinamik karaciğer MR inceleme retrospektif olarak tarandı. Safra kanallarında kontrast maddenin izlendiği, en az bir geç faz (≥40 dakika) MR görüntüsü bulunan ve MR çekimi ile üst GİS endoskopi arasında 1 yıldan az süre olan 45 hasta çalışmaya dahil edildi. MR görüntüleri, kontrast madde reflüsü varlığı ve uzanımı açısından iki radyolog tarafından konsensus ile değerlendirildi. Hastaların endoskopik ve histopatolojik bulguları hastane kayıt sisteminden not edildi.

Bulgular: Kırk beş hastanın 8'inde (%17,8) duodenogastrik GA reflü saptandı. Duodenogastrik GA reflü saptanan 8 hastanın 2'sinde endoskopide safra reflüsü mevcuttu. Endoskopik olarak da safra reflüsü kanıtlanan 2 hastanın birinde gastrit mevcutken, diğer hastada mide mukozası normal

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Received/Geliş Tarihi: 30.08.2022 **Accepted/Kabul Tarihi:** 02.05.2023

Öz

olarak değerlendirilmişti. Kontrast reflüsü olan 8 hastanın 6'sında endoskopik olarak midede safra rapor edilmemişti. Bu hastaların hepsinde (N=6) eroziv/eritemli gastrit ve 4'ünde histopatolojik olarak kanıtlanmış KG mevcuttu.

Sonuç: Endoskopi sırasında midede safra gözlenmediği durumlarda safra kaynaklı gastritleri, diğer KG tiplerinden ayırt etmek güçtür. Duodenogastrik GA reflünün safra gastritinin göstergesi olabileceği radyologlar tarafından az bilinen bir durumdur. Klinik olarak da diğer KG tiplerinden ayırt edilmesi zor olabilen bu durumun, geç faz MRG'de saptanabileceğine dair farkındalığının artması gerekmektedir. Ön çalışma olan bulgularımızın, MRG'nin safra reflüsü ve gastritinde tanısal performansının ve klinik katkısının değerlendirileceği, multidisipliner prospektif çalışmalar ile destekleneceğine inanmaktayız.

Anahtar Kelimeler: Manyetik Rezonans Görüntüleme, Gadoksetik Asit, Safra Reflüsü, Safra Gastriti

Introduction

Bile reflux is the duodenogastric backflow of alkaline duodenal and pancreatic secretions, acids, and bile salts (1). A small amount of bile reflux into the stomach is considered physiologic and might occur post-prandial or in the early morning, whereas pathologic bile reflux tends to be more excessive and more prolonged. Pathologic reflux can develop either due to an underlying pyloric sphincter dysfunction, impaired gastroduodenal motility (primary reflux), or after gallbladder removal and gastric surgery (secondary reflux) (2-5).

Diagnosis of bile reflux is made by upper gastrointestinal endoscopy, other intubation methods such as gastric pH monitoring through the nasogastric tube or measurement of bile acids in gastric aspirates and hepatobiliary scintigraphy (6,7). Depending on the amount, concentration, and duration of pathologic bile reflux, this condition may cause chemical irritation of the gastric mucosa leading to chemical gastropathy/gastritis and increasing the risk of gastric malignancy (8,9).

The presence of gastric bile stain accompanied by gastritis findings, including mucosal erythema, erosions, or ulcers, support the diagnosis of bile-induced chemical gastritis via upper endoscopy (9,10). However, this diagnosis should be confirmed by histopathologic findings such as foveolar hyperplasia, reactive glandular changes, edema, chronic inflammation, intestinal metaplasia, and gastric polyps (11,12). In this context, it has to be noted that histopathologic findings are not specific to bile-induced chemical gastritis but can also be seen in other types of chemical gastritis (10,11). Therefore, endoscopic or radiologic proof of gastric bile stain is essential for a more specific diagnosis.

Gadoxetic acid is a hepatocyte-specific contrast agent routinely used in hepatobiliary magnetic resonance imaging (MRI). Since it is taken up by hepatocytes and is biliary excreted, it provides both functional and morphologic information. In addition, due to its biliary excretion, it is expected to be seen in the biliary tree and duodenal lumen 15-20 minutes after intravenous injection (13).

In this study, we aimed to emphasize that duodenal contrast reflux into the stomach might occur during routine gadoxetic-acid enhanced hepatic MRI and to compare the presence of contrast reflux with upper endoscopy findings.

Materials and Methods

This retrospective observational study was conducted according to the Declaration of Helsinki principles and was approved by Ankara University Human Research Ethics Committee (approval no: İ10-644-21, date: 02.12.2021).

Study Population

Overall, 1543 consecutive gadoxetic acid-enhanced dynamic liver MRI examinations of adult patients obtained between July 2011 and May 2021 were retrospectively reviewed.

Considering the total MR examinations (n=1543), the prevalance of duodenogastric gadoxetic acid reflux was 2.9% (n=44). The inclusion criterion was the presence of at least one delayed phase image (≥40 minutes delay). This was met in 196 out of the total 1543 examinations.

Of the 196 studies, 151 were excluded due to the following reasons: (a) gadoxetic acid not present in the biliary system or duodenum in at least one delayed phase image (n=4); (b) upper GI endoscopy not performed (n=116); the time interval between MRI and upper GI endoscopy was more than one year (n=31). Thus, 45 patients with each MRI study were included in this study (Figure 1).

MRI Protocol

MRI was performed by using a 1.5-T system (Aera, Siemens, Erlangen, Germany; Optima 450w, GE Healthcare, Milwaukee, WI) and a 3-T system (MAGNETOM® Verio, Siemens, Erlangen, Germany; Signa PET MR, GE Healthcare, Milwaukee, WI) device with a phased array torso coil. The sequences consisted of coronal T2w single-shot fast spin echo, axial T2w fat-suppressed FSE, axial diffusion-weighted images (b=50, 400, 800), axial T1w dual-echo gradient echo (GRE) and post-contrast fat-suppressed 3D GRE T1w sequences.

Post-contrast T1w images were obtained after intravenous injection (1 mL/sec) of 0.025 mmol/kg gadoxetic acid disodium (Gd-EOB-DTPA, Primovist®) followed by a saline chaser (10 mL) using an automatic injector.

MRI Analysis

Pre- and post-contrast axial and coronal T1w images retrieved from a picture archiving and communication system (RIS/PACS; Centricity 5.0 RIS-i, GE Healthcare, Milwaukee, WI, USA) were retrospectively evaluated by two radiologists (M.K., D.K.Ö.) in consensus regarding the presence and extent (gastric antrum, corpus, fundus) of duodenogastric gadoxetic acid reflux.

Institutional electronic medical records were used to screen endoscopy results for gastric bile staining and gastritis findings. Diagnosis of bile gastritis was based on the presence of erythematous and/or exsudative gastric mucosa with gastric bile stain detected on upper endoscopy.

Statistical Analysis

Descriptive statistical analyses were performed using SPSS for Windows 11.5 (SPSS Inc., Chicago, IL, USA). Continuous variables with normal distribution were presented as mean (± standard deviation); non-normal variables were expressed as a median (minimum-maximum), and the categorical variables were summarized as counts (n) and percentages (%).

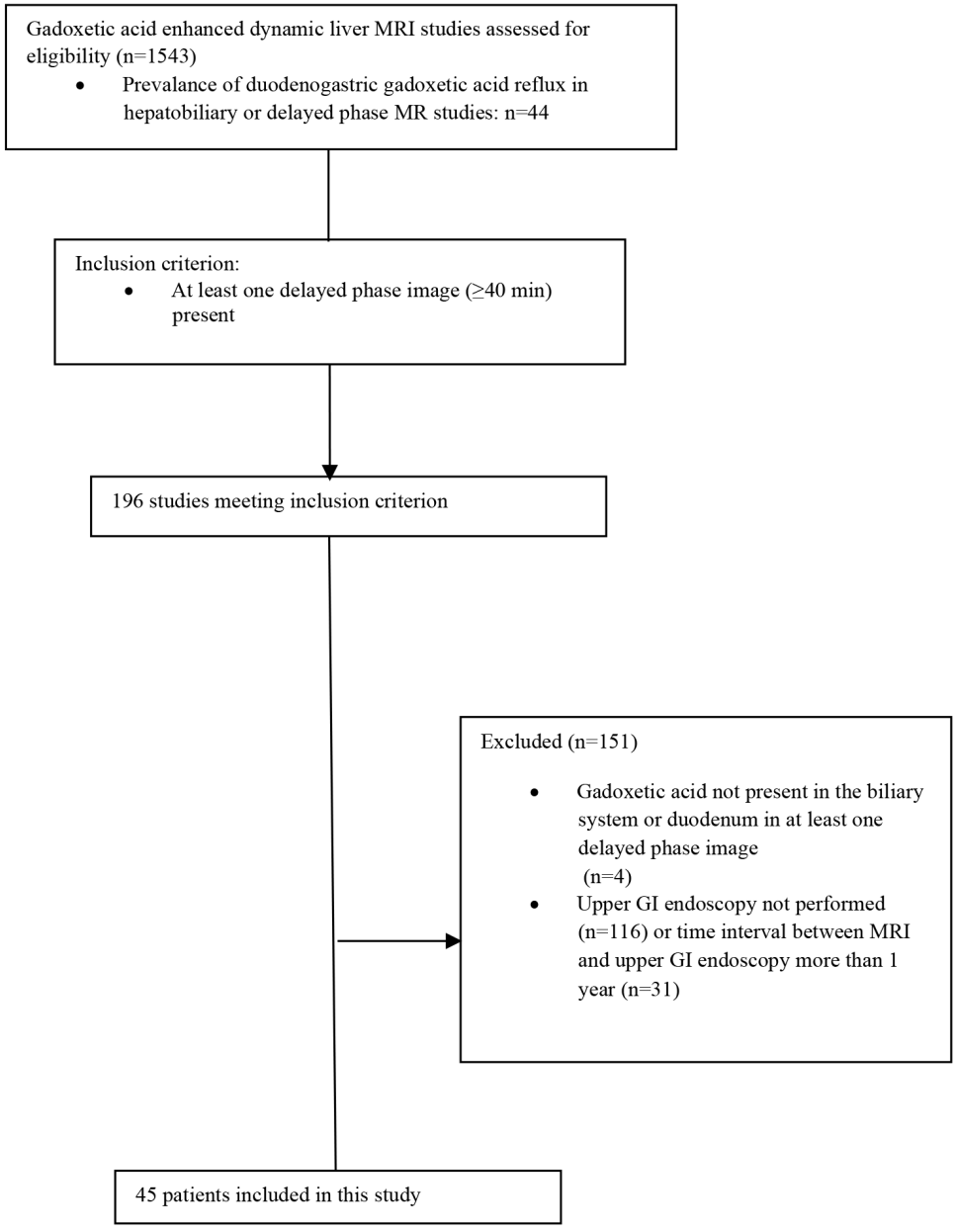

Figure 1: Flowchart

Results

Study Population

The study population consisted of 24 female (53.3%) and 21 male patients (46.6%) with a median age of 58 years (age range, 27-79 years).

While eight patients (17.7%) had a prior cholecystectomy, none of the patients had undergone gastric surgery.

Screening for early stage hepatocellular cancer in chronic hepatic parenchymal disease was the major indication for gadoxetic acid enhanced MRI (n=37, 82.2%), followed by evaluation of focal hepatic lesions (n=5, 11.1%) and cholangiocellular cancer (n=3, 6.6%).

MRI Findings

In 8 out of 45 patients (17.8%) gastric gadoxetic acid reflux was detected in delayed phase images with contrast medium extending into the gastric antrum (n=5), corpus (n=2) and fundus (n=1) (Figures 2-4). In 2 of these eight patients, reflux was also present in the hepatobiliary phase extending into the antrum and corpus, respectively (Table 1).

Imaging findings consistent with chronic liver disease were observed in each patient with and in 29 (%78.4) patients without duodenogastric contrast reflux.

Association of MRI Findings with Upper Endoscopy Results

In 2 out of 8 patients with duodenogastric gadoxetic acid reflux, gastric bile stain was also detected during upper endoscopy. While in 1 of them, endoscopic gastritis findings were present, in the other patient, endoscopy revealed normal mucosa. Gastritis findings were noted in each of the six patients without reported gastric bile staining. Biopsy revealed chemical gastritis in 4 of these 6 patients (Table 2).

Bile reflux was neither detected in the remaining 37 (82.2%) patients without contrast reflux. In 35 patients, gastritis

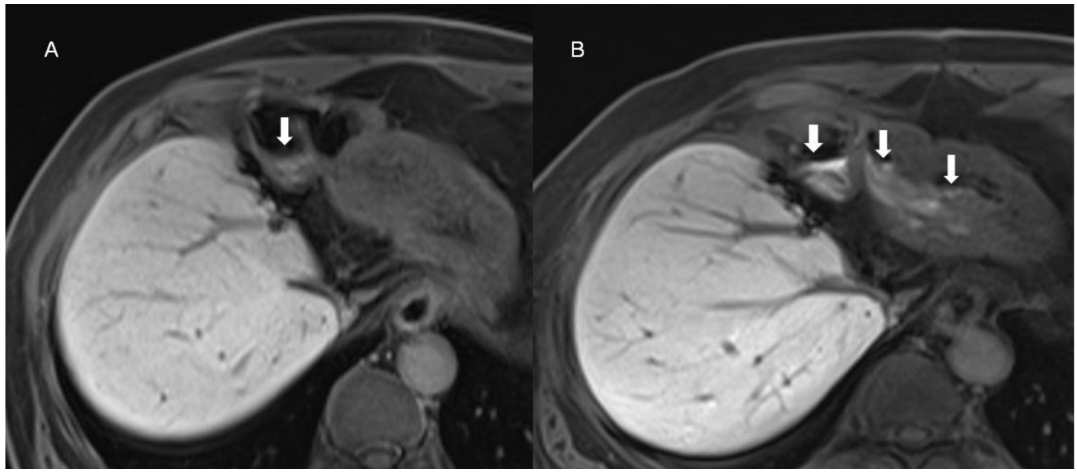

Figure 2: Axial gadoxetic-acid enhanced T1-weighted MR images of a 58-years-old male patient. Duodenogastric reflux (arrows) into the gastric antrum in the hepatobiliary phase **(A)** and into the gastric corpus (in the delayed phase (70 min after injection) **(B)**. Upper endoscopy revealed erosive gastritis but no bile reflux. Biopsy proved chemical gastritis

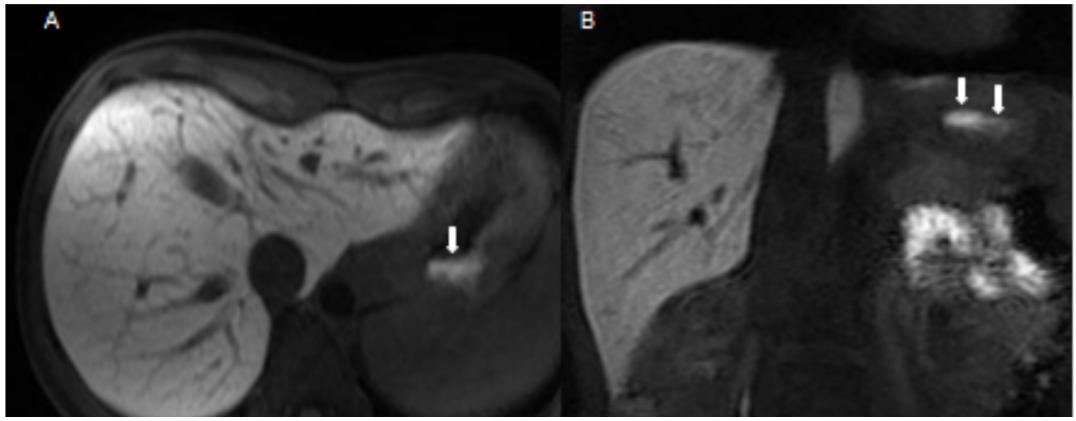

Figure 3: Axial **(A)** and coronal **(B)** gadoxetic-acid enhanced T1-weighted MR images of a 24-years-old female patient. Duodenogastric reflux into the gastric fundus in the delayed phase (75 min after injection). Upper endoscopy revealed erythematous gastritis but no bile reflux. Biopsy proved chemical gastritis

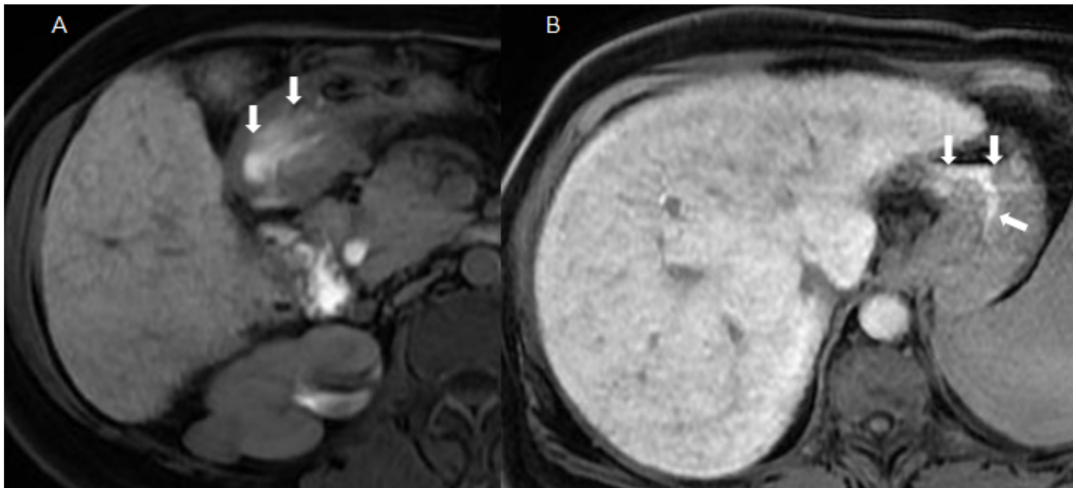

Figure 4: Axial gadoxetic-acid enhanced T1-weighted MR images of a 66-years-old female patient. Duodenogastric reflux into the gastric antrum in the hepatobiliary phase **(A)** and into the gastric corpus in the delayed phase (60 min after injection) **(B)**. Upper endoscopy revealed bile reflux but no gastritis. Biopsy was not performed

Table 1: Delay times of contrast reflux on MRI after gadoxetic acid injection

Patient	Presence of contrast reflux	
	Hepatobiliary phase (min)	Delayed phase (min)
1	-	75
2	-	50
3	20	60
4	-	60
5	20	70
6	-	120
7	-	75
8	-	60

MRI: Magnetic resonance imaging

Table 2: Endoscopic and histologic findings in patients with contrast reflux on MRI

Patient	Endoscopic findings	Histologic results
1	Erythematous gastritis Acute hemorrhagic gastritis	-
2	Erosive antral gastritis Atrophic gastritis of the gastric fundus and corpus	Chemical gastritis Chronic gastritis
3	Gastric bile stain No gastritis	-
4	Pangastritis with antral erosions	Normal antral mucosa
5	Erosive pangastritis	Chemical gastritis
6	Extensive gastric bile stain Erosive pangastritis	-
7	Mucosal hyperaemia of the gastric corpus and antrum	Chemical gastritis
8	Erythematous pangastritis Antral polyp	Chemical gastritis Antral hyperplastic polyp

MRI: Magnetic resonance imaging

findings (with concomitant portal gastropathy in 7 patients) were reported. In 22 of these 35 patients, biopsy results could be obtained and yielded chemical gastritis in 15 cases.

In 1 of the 8 patients with cholecystectomy, reflux was detected both in upper endoscopy and MRI. In another patient with cholecystectomy duodenogastric contrast reflux was present while gastric bile staining was not reported.

Discussion

Due to its alkaline ingredients, bile reflux might cause chemical gastritis, gastric ulcers, intestinal metaplasia, and gastric malignancy (6,7). Chemical gastritis results from the mucosal irritation of the gastric mucosa and may also be induced by exogenous substances such as non-steroidal anti-inflammatory drugs (NSAID) and chemotherapeutic agents (14,15).

Bile gastritis is generally diagnosed based on mucosal erosions, erythema, swelling, and gastric bile stain on upper endoscopy. These findings should be supported by a histopathologically proven chemical gastritis pattern (10). However, if gastric bile stain is not observed during endoscopy, it is not possible to distinguish bile gastritis from other forms of chemical gastritis histopathologically.

Previous studies indicated that the severity of chemical gastritis depends on the amount of bile reflux and that chronic inflammation is more severe in bile-induced gastritis than in other forms of gastritis (6,16,17). Additionally, bile gastritis has been identified as an independent risk factor for developing precancerous gastric lesions and gastric malignancy (18).

Medical treatment of bile gastritis is similar to that of the other chemical gastritis types with an additional option of prokinetic drugs and ursodeoxycholic acid administration, which might reduce the clinical symptoms due to bile reflux (19,20). Furthermore, Roux-en-Y diversion is often the only succeeding treatment in patients with bile reflux after gastric surgery (21). Thus, due to the higher risk of malignancy and therapeutical distinctions, the diagnosis of bile reflux as the underlying cause of chemical gastritis might be of clinical relevance.

Although histopathologically confirmed chemical gastritis was common in our study population, gastric bile reflux was reported in only one patient after endoscopy. This might be either since, despite the presence of bile reflux, it was considered as not clinically significant and thus was not mentioned in the endoscopy report, or was not observed during endoscopy owing to the intermittent nature of bile reflux, or else chemical gastritis was caused by exogenous substances but not by bile.

While upper endoscopy is routinely used to diagnose bile gastritis, a recent study revealed a lower accuracy and predictive value regarding the diagnosis of bile reflux compared to gastric pH monitoring and hepatobiliary scintigraphy (11). Previously, it was also concluded that accurate diagnosis of duodenogastric bile reflux is not possible with upper endoscopy and histopathology but should include the latter techniques, particularly if surgical treatment is planned (11). However, these techniques are either invasive or release radiation and, thus, are often performed with a more specific preliminary diagnosis.

To the best of our knowledge, there is only one study assessing the diagnostic utility of MRI in duodenogastric gadoxetic acid reflux (22). This study showed that duodenogastric contrast was an indication for bile reflux, and thus, gadoxetic acid-enhanced MRI might play a potential role in the diagnosis of bile reflux. The authors also stated that in several patients, MRI revealed contrast reflux in the stomach in delayed images, whereas no bile reflux was observed during endoscopy (22). In our study, out of the 8 patients with gastric contrast reflux in delayed phase MR images, gastric bile stain was noted in only 2 patients. Thus, in 75% of patients with contrast reflux into the stomach on MRI, endoscopy revealed no bile reflux or was not mentioned. In the study by Hyun et al. (22), this was the case in 53.8% of patients with positive MRI findings. This might be due to the fact, that on MR studies with delayed phase imaging, the overall examination period revealing functional information is longer than is the case with upper endoscopic studies that would rather provide a "snapshot" at the time of the study (22). With regard to the diagnosis of duodenogastric reflux, this might be listed as an additional advantage of gadoxetic acid enhanced MRI, which provides more detailed anatomic information, is a non-invasive technique, and does not use ionizing radiation. The higher rate of discrepancies between MRI and upper endoscopy findings in our study compared to the results of Hyun et al. (22) might be due to the possibility, that despite gastric bile being noticed during upper endoscopy, it was probably assumed to be physiologic and thus was not reported owing to the retrospective study design.

Hyun et al. (22) stated that duodenogastric contrast reflux corresponded to bile reflux, however, in 2/3 of cases, it was not associated with bile gastritis findings on endoscopy. In our study, in only 1 of 8 patients with duodenogastric contrast reflux, gastritis findings accompanied by gastric bile staining were present. However, it is noticeable, that in 50% of the patients with duodenogastric reflux chemical gastritis was present.

We suppose that even though bile gastritis was not diagnosed via upper endoscopy, bile might be the underlying cause of diagnosed chemical gastritis in at least some patients with duodenogastric contrast reflux. However, since bile reflux and, thus, contrast reflux into the stomach can occur physiologically, other causes of chemical gastritis could not be excluded at this point, in particular, since agents such as NSAID are widely used in the population. In addition, there was also a high prevalance of chemical gastritis among the

reflux, negative patients. Moreover, it was beyond the scope of this study to assess the diagnostic performance of MRI in bile gastritis but rather to raise awareness of duodenogastric contrast reflux which previously has been shown to be indicative of bile reflux (22).

Previous studies revealed a prevalence of 10% for bile reflux during upper endoscopy with much higher ratios of up to 80-90% after cholecystectomy (6,23). This could be both associated with increased bile flow to the duodenum owing to the lack of bile reservoir after cholecystectomy and the impairment of gastroduodenal motility (23,24). While none of our patients had undergone gastric surgery, 8 patients had cholecystectomy. In 1 of the 8 patients (12.5%) with cholecystectomy, bile reflux was detected during upper endoscopy, and in 2 patients (25%) duodenogastric contrast reflux was observed on MRI.

In our study, each patient with duodenogastric contrast reflux had chronic liver disease. Chronic liver disease is known to cause gastric paresis, and thus, duodenogastric contrast reflux might also be due to duodenogastric motility impairment (25). However, we could not presume any reliable potential association, since chronic liver disease was the most frequent diagnosis also in patients without contrast reflux.

Study Limitations

Our study has several limitations. First, this was a retrospective study. Thus, the endoscopic procedure was not standardized, and with respect to MRI, time delay after contrast injection in delayed phase imaging varied between patients. However, we included only MRI studies with a minimum delay of 40 minutes for delayed phase imaging, considering that gadoxetic acid is expected to extend to the distal common hepatic duct during the hepatobiliary phase with a progressive filling of the duodenum in delayed phases (>30 min). Consistently, we observed that duodenogastric contrast reflux occurred more frequently in delayed phase images without being apparent in the hepatobiliary phase. Second, the sample size was small. Third, we could not obtain sufficient information regarding the clinical symptoms and current or previous medication of the patients which might have induced chemical gastritis. Forth, since hepatobiliary scintigraphy or other diagnostic methods for bile reflux were not performed in our patients, diagnosis of bile reflux was limited to upper endoscopy results. Thus, we could not assess the diagnostic utility of MRI in bile reflux nor the patient-specific clinical relevance of contrast reflux. However, we could prove the existence of this finding, which, if it is being reported, might prompt a more detailed investigation for bile reflux and gastritis.

Conclusion

It might be challenging to differentiate bile-induced gastritis from other types of chemical gastritis, particularly if gastric bile stain is not observed during upper endoscopy. Thus, radiologists should be aware of duodenogastric gadoxetic acid reflux on delayed phase MRI and report this finding to support the gastroenterologist in the search for the underlying cause of gastritis. We suggest that the diagnostic performance of MRI regarding bile reflux and bile gastritis should be investigated with a multidisciplinary prospective study design.

Ethics

Ethics Committee Approval: Ethics committee approval was obtained from Ankara University Human Research Ethics Committee (approval no: İ10-644-21, date: 02.12.2021).

Informed Consent: Retrospective observational study.

Peer-reviewed: Externally peer-reviewed.

Authorship Contributions

Concept: M.K., A.E., Design: M.K., A.E., Data Collection and Processing: M.K., D.K.Ö., O.A., A.E., Analysis or Interpretation: M.K., D.K.Ö., Literature Search: M.K., Writing: M.K., D.K.Ö.

Conflict of Interest: The authors declared that there was no conflict of interest during the preparation and publication of this article.

Financial Disclosure: The authors declared that they did not receive any financial support during the research and authoring of this article.

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RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ

DOI: 10.4274/atfm.galenos.2023.05924
Ankara Üniversitesi Tıp Fakültesi Mecmuası 2023;76(1):45-49

MEDICAL SCIENCES / DAHİLİ TIP BİLİMLERİ

Left Subdiaphragmatic Echogenic Focus in the Fetus and Its Effect on Prognosis

Fetüste Sol Subdiyafragmatik Ekojen Odak ve Prognoza Etkisi

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Abstract

Objectives: The aim of this study was to determine the effect of fetal left subdiaphragmatic echogenic foci (LSEF) on prognosis and to research its clinical significance.

Materials and Methods: In this retrospective study, obstetric ultrasound was performed for 20142 pregnant women, at 16-40 gestational weeks. The fetuses who were diagnosed with LSEF incidentally were included in the study. The number and size of echogenic foci, associated anomalies and serological test results were recorded. The fetuses with LSEF were followed by ultrasound every 4 weeks until delivery, and postnatal ultrasound on the second month of life was performed for the cases who didn't exhibite resolution.

Results: During the study period, 285 fetuses were found to have 315 LSEFs with a prevalence of 1.4%. Twelve (4.2%) of the fetuses had minor anomalies (7 intracardiac echogenic foci, 4 minimal pyelectasis, 1 hyperechogenic bowel) and one of them (0.3%) had aneuploidy+major anomaly (1 atrioventricular septal defect+Trisomy 21). Intrauterine 274 fetuses (n=286 LSEF) were able to be followed, 242 LSEF (84.6%) disappeared antenatally, 18 LSEF (6.2%) showed regression. In the second month of neonatal period, 6 LSEF (2%) persisted including the case with aneuploidy.

Conclusion: LCEF had no clinically significant effect on the prognosis of the cases without aneuploidy, and postnatal follow up was not recommended for these cases.

Key Words: Echogenicity, Fetus, Isolated, Calcification, Subdiaphragmatic

Öz

Amaç: Bu çalışma, fetüste sol subdiyafragmatik ekojen odakların (SSEO) prognoza etkisi ve klinik öneminin araştırılması amacı ile planlanmıştır.

Gereç ve Yöntem: Bu retrospektif çalışmada, 16-40 gebelik haftaları arasındaki 20142 gebeye obstetrik ultrason incelemesi yapılmış olup sol subdiyafragmatik alanda tesadüfen ekojen odak saptanan fetüsler çalışmaya dahil edildi. Ekojen odakların sayısı, boyutu, eşlik eden anomaliler ve serolojik testlerin sonuçları kaydedildi. Doğuma kadar 4 hafta aralıklarla ultrason takibi yapılmış olup rezolüsyon göstermeyen olgular için postnatal 2. ayda ultrason kontrolü yapıldı.

Bulgular: Çalışmaya dahil olan 285 fetüste 315 SSEO saptanmış olup SSEO prevelansı %1,4 bulunmuştur. Olguların 12'sinde (%4,2) eşlik eden minör anomaliler (7 intrakardiyak ekojen odak, 4 hafif pelviektazi, 1 ekojen barsak) ve birinde (%0,3) anöploidi+majör anomali (1 atrioventriküler septal defekt+Trizomi 21) izlendi. Antenatal takip edilebilen 274 fetüste saptanan 286 SSEO'nun 242'si (%84,6) intrauterin dönemde kayboldu, 18'i (%6,2) spontan küçülme gösterdi. Neonatal 2. ayda, anöploidi olgusu dahil 6 SSEO (%2) sebat etti.

Sonuç: Kromozomal anomali olmayan olgularda, SSEO prognozda klinik olarak anlamlı bir değişikliğe yol açmamaktadır. Bu olgularda postnatal takibe gerek yoktur.

Anahtar Kelimeler: Ekojenite, Fetüs, İzole, Kalsifikasyon, Subdiyafragmatik

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Received/Geliş Tarihi: 27.02.2023 **Accepted/Kabul Tarihi:** 24.04.2023

Introduction

Echogenic foci (EF) detected in the abdomen of the fetus may appear as sonographic markers indicating infectious pathologies or anomalies. These EF are not specific and it may not always be easy to diagnose accompanying pathologies. Differential diagnoses include meconium peritonitis, hyperechoic bowel, toxoplasma, rubella, cytomegalovirus, herpes simplex (TORCH) infections, neoplasms, and portal vein thromboembolisms. The location and distribution of EF in the abdomen should be carefully evaluated (1). Small calcifications commonly found in the abdomen and pelvis are mostly evaluated in favor of meconium peritonitis, and calcifications in the liver are evaluated in favor of infection, tumor, or vascular pathology (2,3). Determining the number, location, and distribution of EF in the abdomen gives very important clues for making diagnoses and predicting the prognosis of many diseases.

EF observed in the right upper quadrant of the abdomen suggest calcifications in the liver but left subdiaphragmatic EF (LSEF) have not been sufficiently clarified in the literature. This study aimed to investigate the prognosis and clinical significance of LSEF detected in antenatal ultrasound.

Materials and Methods

This retrospective cohort study was conducted at a secondary referral center specialized in maternal health care between January 2015 and November 2021. Participants were selected from a cohort of 20,142 pregnant women who were referred for an obstetric ultrasound examination between 16-40 weeks of gestation. Electronic health records and the electronically stored radiologic images were retrospectively analyzed. Fetuses with an incidental finding of having an EF in the subdiaphragmatic area of the left upper quadrant of the abdomen were included in the analysis. All sonographic examinations were performed by an experienced radiologist on obstetric ultrasound using a high-resolution ultrasound device with a convex 6-1.9-MHz probe (Toshiba Aplio 500, Japan). The same machine was used throughout the study.

EF was defined as hyperechoic image which is 1-6 mm in length with no obvious acoustic shadowing (Figure 1). The fetuses who had LSEF in at least two imaging planes were included in the study, whereas fetuses who had LSEF in a single imaging plane and multiple pregnancies were excluded from the study. In the case of identification of LSEF, the location, number, and size of the EF in the longest plane were stated in the written report and digital images were electronically recorded along with other abnormal findings of fetuses. First trimester screening tests and serologic tests were performed for all fetuses, and amniocentesis was performed in the case of indication. Informed consent was obtained from all pregnant women to participate in the study. The research was conducted ethically in accordance with the guidelines for human studies and World Medical Association Declaration of Helsinki. The study was approved by the local research ethics committee of İstinye University Clinical Research Ethics Committee (3/2022.F-52)

Fetuses with LCEF were followed every 4 weeks until birth through sonographic examinations. Postnatal sonography was performed in the 2nd month of life for all cases with LSEF. The clinical data about the babies with LSEF were retrieved from the electronic health records of the pediatric clinic of the study center.

Statistical Analysis

The SPSS 22.0 statistical package was used for statistical analysis. The Shapiro-Wilk test was used to test the normality of data distribution. Descriptive statistics were expressed as mean, standard deviation, number, and frequency.

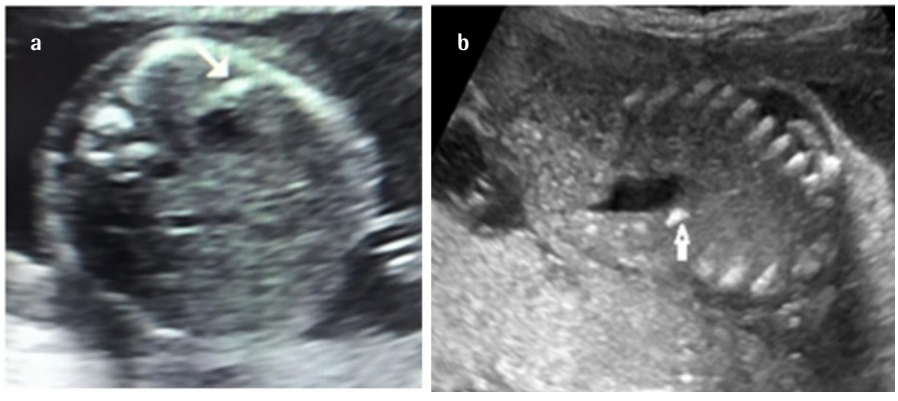

Figure 1: Left subdiaphragmatic echogenic focus (white arrow) in a 24-weeks fetus. **a.** Axial section, **b.** Sagittal section

Results

Routine obstetric ultrasound examinations revealed that 285 fetuses (1.4%) had LSEF (315 EF) out of 20,142 fetuses. The mean ± standard deviation gestational age of fetuses at the time of diagnosis was 18.5±2 weeks of gestation and ranged between 11 to 25 weeks. Maternal and fetal demographic characteristics and concomitant anomalies are presented in the Table 1.

During the study, 261 (91.5%) of 285 fetuses had a single EF, 19 (6.6%) had two EF, and 5 (1.7%) had three or more EF (Figure 2).

The diameters of the EF ranged from 2 to 6 mm (mean, 2.7±0.9 mm). LSEF were oval, rod, or round in shape, and posterior acoustic shadowing was not observed in any. Concomitant minor anomalies were observed in 12 (4.2%) fetuses (intracardiac EF n=7, mild pelviectasis n=4, echogenic bowel n=1), and one (0.3%) had a major anomaly [atrioventricular septal defect (AVSD)]. Amniocentesis of the fetus with AVSD revealed the presence of trisomy 21. Five of the fetuses with intracardiac EF had single EF, two of them had 2 EF in the left subdiaphragmatic area. The fetuses with mild pelviectasis, echogenic bowel and AVSD had single EF.

Antenatal follow-up could not be performed for 11 (3.8%) pregnant women included in the study because of not attending the ultrasound controls. Two hundred eighty-six LSEF were detected in the remaining 274 fetuses; 242 (84.6%) disappeared in the intrauterine period during antenatal follow-up and 18 (6.2%) showed spontaneous regression. Twenty-one (7.3%) that did not show intrauterine resolution could be evaluated in the postnatal period, and only six (2%) persisted in the neonatal 2nd month (Figure 3). In persistent fetuses, calcification foci were observed in the subcapsular area of the left liver lobe or the area adjacent to the spleen in the postnatal ultrasound examination. One of the persistent fetuses had trisomy 21 and no accompanying anomalies were detected in the others. The fetuses with minor anomalies were also followed up in the postnatal period and none of them had any abnormal finding. In serologic tests, eight pregnant women had immunoglobulin (Ig) G positivity in terms of TORCH infections, but postpartum infection was not observed in any of them.

Table 1: Maternal and fetal demographic characteristics and concomitant anomalies

Maternal-Fetal demographic characteristics	**Value (minumum-maximum)**	
Maternal age		27±3 (20-39)
Gestational age at diagnosis		18.5±2 (17-25) **N (%)**
Fetus gender	Male Female	151 (52.2%) 134 (47.0%)
Concomitant minor anomalies	IEF*	7 (2.4%)
	Pelviectasis	4 (1.4%)
	Echogenic bowel	1 (0.3%)
Concomitant major anomalies/aneuplody	AVSD**/Trisomy 21	1 (0.3%)

*Intracardiac echogenic foci, **Atrioventricular septal defect

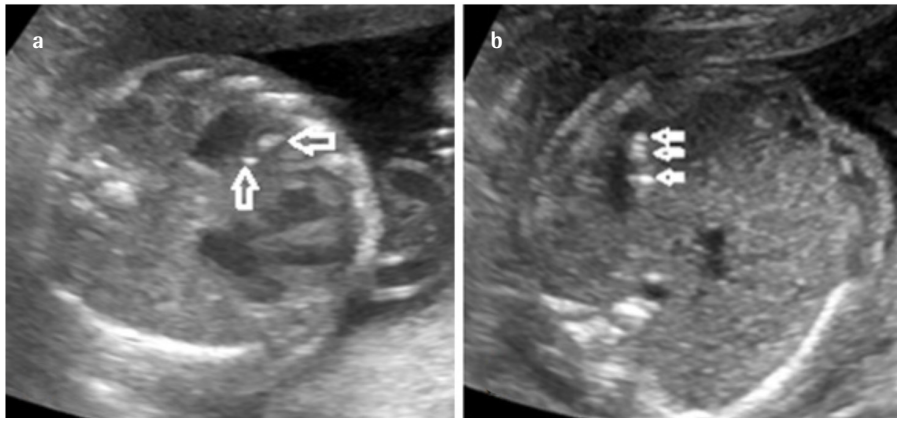

Figure 2: Axial section of the fetal abdomen. **a.** Two left subdiaphragmatic echogenic foci (white arrows) are seen in a 22-weeks pregnant. **b.** More than two echogenic foci (white arrows) are detected adjacent to the stomach in a 23-weeks pregnant

Discussion

In this study, the prevalence of LSEF detected during the antenatal period was found as 14 per 1000 pregnancies (1.4%, n=285/20,142). Approximately 85% of those disappeared in the intrauterine period, and 6% showed spontaneous regression. The rate of accompanying minor anomalies was 4.2%, and the rate of chromosomal anomalies was 0.3%. Chromosomal anomalies were found in one of six persistent cases. No relationship was observed between TORCH infections and LSEF. This study is the largest study on this subject in the literature.

Echogenicity observed in fetal tissues, called calcification, is used for echoes that are the same as adjacent bone echogenicity (4). In previous studies in the literature, the incidence of intrauterine hepatic calcifications was found to vary between 0.05% and 0.38% (5,6). In this study, the prevalence of LSEF was found as 1.4%, much higher than in previous studies. Isolated calcifications detected in the intrauterine period generally have a good prognosis (5,7). However, the outcome worsens when accompanied by other anomalies. Previous studies found a relationship between intra-abdominal calcifications and chromosomal abnormalities (7,8), intrauterine infections (7,9,10), and circulatory disorders (11). Sahlin et al. (12) emphasized that the probability of chromosomal anomalies in fetuses with intrauterine calcification and accompanying malformation was more than two times that of fetuses with only malformations. In our study, the rate of chromosomal anomalies was found as 3 per 1000 pregnant women, and the frequency of chromosomal anomalies increased compared with the normal population. However, studies with a larger number of cases are needed to determine the prevalence of chromosomal anomalies in fetuses with LSEF.

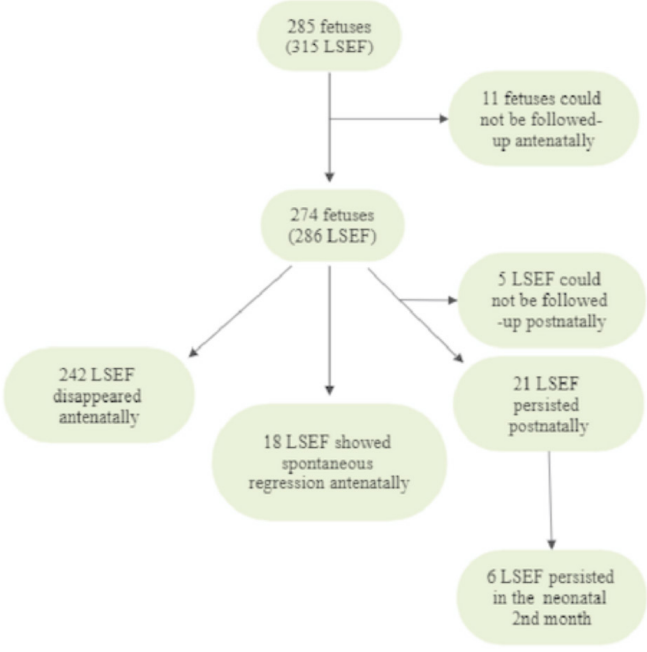

Figure 3: Flow chart of the recruitment and follow-up process

The fetuses with concomitant anomalies had single or two EF and no relationship was found between the number of EF and the concomitant anomalies. Approximately 90% of the LSEF included in the study either disappeared completely during the intrauterine period or reduced in size. Trisomy 21 was found in one of six persistent fetuses in the postnatal period; no chromosomal anomalies were found in any of the fetuses that showed resolution. Although no direct relationship was found between aneuploidy and the persistence of LSEF. LSEF did not lead to a significant change in prognosis in fetuses without accompanying chromosomal anomalies.

Studies in the literature focused more on intrauterine calcifications in the liver, heart, and peritoneum (2,9,12). Heart calcifications have been associated with chromosomal anomalies (12), peritoneal calcifications with meconium peritonitis and intrauterine infections (2), and hepatic calcifications with vascular etiology (13). The etiology of LSEF has not been sufficiently clarified in the literature. In this study, because persistent LSEF were detected in the subcapsular area of the left liver lobe and adjacent to the spleen in postnatal examinations, etiologies related to liver and spleen calcifications could be considered initially. In the literature, calcifications detected in the subcapsular area of the liver have been found to be associated with portal vein thrombosis (1,14). In our fetuses, small thrombi in the portal vein may have played a role in the etiology. It can be predicted that regressed LSEFs in the intrauterine period may be associated with left liver lobe calcifications because liver calcifications tend to regress spontaneously (15).

TORCH infections are one of the other causes of calcifications observed in the abdomen of the fetus and are generally encountered as scattered millimetric echogenicities accompanied by multiple organ anomalies (16). They can be distinguished from LSEF by the scattered location of calcifications and accompanying anomalies. In our study, IgG positivity in terms of TORCH infections was observed in eight pregnant women, and no accompanying infection findings were observed in any of them in the periuterine period. Ji et al. (1) found no significant relationship between LSEF and transplacental infections, as in our study. Another cause of multiple calcifications in the abdomen is meconium peritonitis, which occurs as a result of intestinal obstruction (17). Meconium peritonitis is mostly encountered as calcifications located on the peripheral surfaces of the liver and in the peritoneum. The location of calcifications, accompanying intestinal anomalies, and the presence of ascites are guides in the differential diagnosis of LSEF of more than two in number.

Study Limitations

Among the limitations of the study, first, there was the inability to elucidate the etiopathogenesis of LSEF that showed resolution in the antenatal period. Secondly, the relationship between aneuploidy and the persistence of LSEF could not be evaluated due to the insufficient number of fetuses. Aneuploidy was observed in only one fetus with persistent LSEF in our study. Studies with a larger number of fetuses are needed to evaluate the relationship between chromosomal anomalies and LSEF.

Conclusion

LSEF are encountered more frequently than other calcifications observed in the abdomen with a prevalence of 1.4% and mostly disappear in the intrauterine period. Their etiology has not been fully elucidated and no relation with transplacental infections has been observed. LSEF does not cause a clinically significant change in prognosis of the fetuses without chromosomal anomalies. Postnatal follow-up is not required for the cases without aneuploidy.

Ethics

Ethics Committee Approval: The study was approved by the local research ethics committee of İstinye University Clinical Research Ethics Committee (3/2022.F-52).

Informed Consent: Informed consent was obtained from all pregnant women to participate in the study.

Peer-reviewed: Externally peer-reviewed.

Financial Disclosure: The author declared that they did not receive any financial support during the research and authoring of this article.

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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

DOI: 10.4274/atfm.galenos.2023.16870
Ankara Üniversitesi Tıp Fakültesi Mecmuası 2023;76(1):50-55

DAHİLİ TIP BİLİMLERİ / MEDICAL SCIENCES

Kuduz Riskli Temaslarda Uygulanan Proflaksilerin ve Aşılamaya Uyumun Gözden Geçirilmesi

An Overview of Prophylaxis and Compliance with Vaccination in Contacts with Rabies Risk

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Öz

Amaç: Türkiye, önlenebilen bir hastalık olan kuduz yönünden halen endemik bir bölgedir. Bunun için aşılama önemlidir. Gelişmiş ülkelerde genellikle yabani hayvan kuduzu, ülkemiz gibi gelişmekte olan ülkelerde ise evcil hayvan, özellikle de köpek kuduzu daha çok görülmektedir. Çalışmamızın amacı, merkezimize başvuran kuduz riskli temas (KRT) olgularını ve aşı programına uyumlarını değerlendirmektir.

Gereç ve Yöntem: Çalışmamıza Ocak 2019 ile Haziran 2022 arasında KRT nedeni ile başvuran hastalar dahil edildi. Hasta verileri kayıtlardan sağlanmıştır. Tam aşılama/aşılamaya uyum; önerilen aşılama programının tamamlanması olarak tanımlandı.

Bulgular: KRT nedeni ile profilaksi alan ve yaş ortalaması 36,4 (11-90) olan toplam 2355 hastanın 1489'u erkek ve 866 kadındı. Hastaların 1299'una (%55,2) immünoglobulin (Ig) de verildi. Temasların 1445'i köpek, 872'si kedi ile idi. Sahipli hayvanların %80'i aşısızdı. Temaslar ilkbahar ve kış aylarında daha fazlaydı. Tam aşılamaya uyum oranı %65,7 idi. Kadın cinsiyet, Ig yapılanlar, ileri yaş, 4 doz aşı önerilenler, Ankara içerisinden başvuranlar tam aşılamaya daha uyumlu bulundu. Kış aylarında uyum anlamlı olarak artarken ilkbahar aylarında anlamlı olarak azaldı. Sahipli- aşılı hayvan temaslarında uyum az, kaçtı ya da öldürüldü ise anlamlı olarak uyum yüksek bulundu.

Sonuç: Çalışmamızda; tam aşılamaya uyum oranlarının düşük olduğu ve sahipli-aşısız hayvan grubu ile KRT'nin fazla olduğu bulunmuştur. Uyumsuzluk nedenleri aşıların yan etkilerinden ziyade sosyal nedenler olduğu görülmektedir. Halkımız evcil hayvan aşılanması ve KRT durumunda önerilen aşı şemasının tam olarak uygulanmasının önemi konusunda bilinçlendirilmelidir.

Anahtar Kelimeler: Kuduz, Aşılama, Risk, Uyum

Abstract

Objectives: Turkey is still an endemic region in terms of rabies which is a preventable disease. So, vaccination is important. Rabies is derived from wild animals in developed countries, whereas it is caused by domestic animals, especially dogs in developing countries. The aim of this study is to evaulate the patıents contact with rabies risk (CRR) who applied to our center and their compliance with the vaccination program.

Materials and Methods: We included the the patients that applied to our Rabies Vaccine Center with a CRR between January 2019 and June 2022. The patient data were obtained from patıent information record. Complete vaccination/compliance with vaccination was defined as completion of vaccination program.

Results: Of the 2355 patients [mean age; 36.4 (11-90) yrs] who received prophylaxis for CRR 1489 were men, and 866 were women. One-thousand two-hundred ninety-nine (55.2%) of the patients received also immunoglobulin (Ig). One-thousand four-hundred and eighty-nine of the contacts were with dogs and 872 were with cats. Eighty percent of the domestic animals were unvaccinated. Contacts were more frequent at Winter and Spring seasons. Compliance with complete vaccination rate was 65.7%. Women sex, Ig application, advanced age, recommendation of 4-doses of vaccination, being inhabitant of Ankara were associated with compliance with complete vaccination. Compliance with vaccination rates was

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Geliş Tarihi/Received: 20.10.2022 **Kabul Tarihi/Accepted:** 17.04.2023

Abstract

higher in winter months whereas it was less in Spring. Also compliance rates were less in domestic animal contacts, whereas it was higher in non-domestic contacts.

Conclusion: In this study, we found that CRR with domestic unvaccinated animal group was higher and compliance with complete vaccination rates were low. The reasons for non-compliance appear to be social reasons rather than side effects of vaccines. Our people should be made aware of the importance of pet vaccination and the full implementation of the recommended vaccination scheme in the case of CRR.

Key Words: Rabies, Vaccination, Risk, Compliance

Giriş

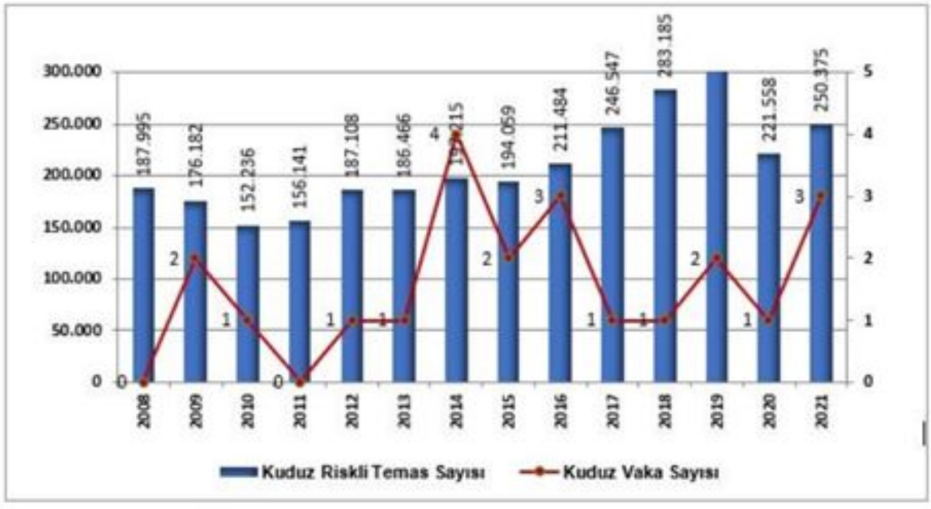
En eski zoonotik hastalıklardan biri olan kuduz halen dünyanın birçok yerinde endemiktir. Genellikle hayvan ısırıkları ile bulaşmakta olup hastalık geliştiğinde ölümle sonuçlanmaktadır. Dünyadaki epidemiyolojik değişimler de göz önüne alındığında yabani hayvanlardan ve kontrolsüz evcil hayvanlardan bulaşma riski yüksektir. İnsanlara ve diğer hayvanlara, enfekte hayvanların tükürüğü ile yakın teması, ısırık, hasarlı derinin ve mukozaların yalanması yoluyla bulaşır (1). Türkiye, kuduz yönünden hala endemik bir bölgedir. Türkiye'de her yıl yaklaşık 180.000 kuduz riskli temas (KRT) bildirimi yapılmakta olup her yıl 1 ila 4 kuduza bağlı insan ölümü gerçekleşmektedir (2).

Türkiye'deki riskli temas ve kuduz olgu bildirimleri Şekil 1'de verilmiştir. Kuduz önlenebilir bir hastalıktır. Bunun için aşılama önemlidir. Gelişmiş ülkelerde genellikle yabani hayvan kuduzu, ülkemiz gibi gelişmekte olan ülkelerde ise evcil hayvan, özellikle de köpek kuduzu daha çok görülmektedir. Kuduz, ülkemiz gibi endemik bölgelerde halen önemli bir halk sağlığı sorunudur (1).

Ankara'da birçok merkezde kuduz aşılaması yapılmakta olup bu merkezlerden birisi olan hastanemizde de erişkin kuduz aşılaması 2015 yılından beri yapılmaktadır. Çalışmamızın amacı; merkezimize başvuran KRT olgularını ve aşı programına uyumlarını değerlendirmektir.

Gereç ve Yöntem

Çalışmamıza Ocak 2019- Haziran 2022 yılları arasında Sağlık Bilimleri Üniversitesi, Dışkapı Yıldırım Beyazıt Eğitim ve Araştırma Hastanesi Kuduz Aşı Merkezi'ne KRT nedeni ile başvuran erişkin yaş grubu ve idame dozları için kabul edilen çocuk hastalar (11-18 yaş) dahil edildi. Köpek veya kedinin en az 10 gün sağlıklı ve canlı kaldığı ve bu nedenle aşılaması tamamlanmayan maruziyetler dahil edilmedi. Hasta verileri kayıtlardan taranarak elde edilmiştir. Bu kayıtlar sağlık bakanlığı önerisi ile ve kuduz saha rehberi doğrultusunda hazırlanmıştır (1). Bu kayıtlarda; riskli teması olan kişilerin yaş, cinsiyet, tarih, bulunduğu semt, şehir, temas ettiği hayvan cinsi (kedi, köpek, diğer, yabani hayvan), hayvanın durumu (sahipli-aşılı, sahipli-aşısız, sahipsiz-aşısız, kaçtı bilinmiyor, öldü-öldürüldü), temas edilen bölge sayısı ve kategorisi, kaç doz aşı önerildiği, immünoglobulin (Ig) uygulanıp uygulanmadığı, profilaksi durumu ile ilgili bilgiler bulunmaktadır. Aşılama için kullanılan aşı; Abhayrab 2,5 IU/0,5 mL IM/ID (inaktive edilmiş ve saflaştırılmış kuduz antijeni konsantresi) ve kullanılan Ig; Eguirab (rabies antiserum-equine) 1000 IU/5 mL'dir (40 IU/kg). Aşılama tüm hastalara intramüsküler olarak yapılmıştır. Hayvan temasları saha rehberine göre 4 kategoriye ayrıldı (1); bu kategoriler hayvanın aşılanma durumu ve gözlemlenebilirliği ile değerlendirilir. Buna göre kategori 1= Hayvana dokunma



Şekil 1: Türkiye Halk Sağlığı Kurumu kuduz istatistik verileri-2021 (https://hsgm.saglik.gov.tr/tr)

veya besleme, sağlam derinin yalanması, kategori 2= Çıplak derinin hafifçe sıyrılması (deri altına geçmeyen yaralanmalar), Kanama olmadan küçük tırmalama veya zedeleme, kategori 3= Deriyi zedeleyen tek veya çok sayıda ısırma ve tırmalamalar, Mukozaların, açık deri yaralarının hayvanın salyası ile temas etmesi, Lezyonun kafa, boyun, parmak uçları gibi sinir uçlarının yoğun olduğu bölgelerde olması, kategori 4= Kuduza yakalanma ihtimali olan yabani hayvan türleri ile riskli temas. Aşılama, temas öncesi profilakside 0 ve 7. günlerde bir doz olmak üzere toplam iki doz aşı IM olarak uygulandı. Temas sonrası, 4 doz (0., 3., 7. günlerde birer doz ve 14 ile 28. günler arasında dördüncü doz uygulama) veya 2.1.1. (0. gün 2 doz, 7. ve 21. günlerde birer doz) aşı şeması ile rehbere uygun olarak yapıldı. Tam aşılama/aşılamaya uyum; aşı karnesine yazılan aşılama programının tamamlanmış olması olarak değerlendirildi.

Çalışmamızın etik kurul kararı Yıldırım Beyazıt Üniversitesi, Yenimahalle Eğitim ve Araştırma Hastanesi Klinik Araştırmalar Etik Kurulu Başkanlığı'ndan 21.12.2022 tarihinde alınmıştır (karar no: E-2022-70).

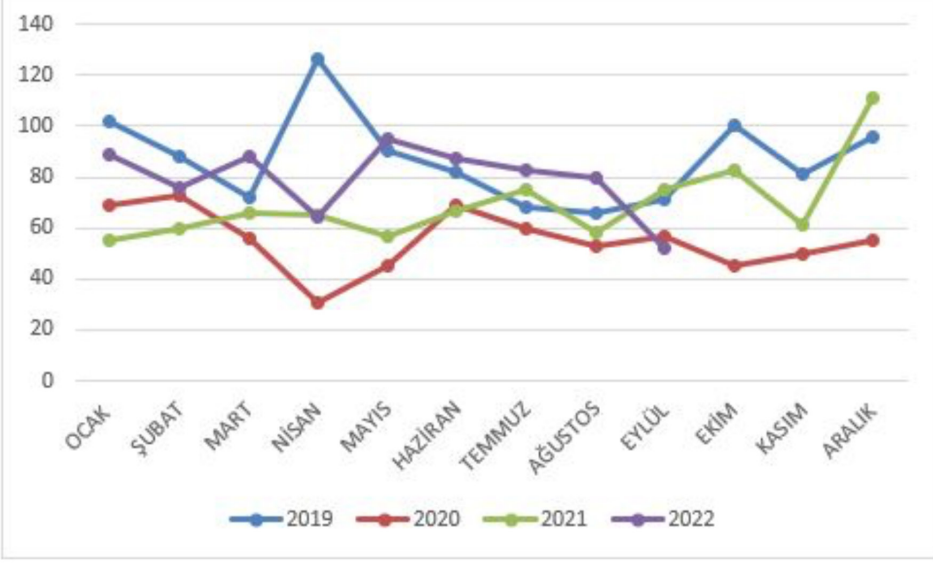
İstatistiksel Analiz

Tanımlayıcı istatistikler; kategorik değişkenler için frekans (%), sürekli değişkenler için ortalama ± standart sapma ve ortanca (minimum-maksimum) olarak verilmiştir. Kategorik değişkenlerin karşılaştırılmasında ki-kare testi veya Fisher'ın Exact testi kullanılmıştır. Tam aşılı olan ve olmayan grupların yaşları arasında istatistiksel olarak anlamlı bir farklılık olup olmadığına bakılırken normal Student's t-testi kullanılmıştır. İstatistiksel anlamlılık düzeyi $p<0,05$ olarak kabul edilmiştir. Verilerin değerlendirilmesi SPSS 11.5 for Windows programı kullanılarak yapılmıştır.

Bulgular

KRT nedeni ile profilaksi alan toplam 2355 hastanın 1489'u erkek ve 866'sı kadındı. Yaş ortalaması 36,4'tü (11-90). Hastaların 88'ine (%3,7) daha önce profilaksi uygulanmıştı. Bin iki yüz doksan dokuzuna Ig (%55,2) de yapıldı. Profilaksi uygulananlar arasında kuduz kliniği gelişen olmadı. Temas edilen hayvanlar evcil hayvan ağırlıklı olup, en sık köpek teması görülürken kedi teması da bunu takip etti. Toplam 1445 köpek, 872 kedi, 8 at, 6 tilki, 2 dana ve birer tane maymun kirpi, aslan, yarasa, hamster teması olmuştur. Kaçtı-tanınmıyor grubundaki hayvan sayısı 1887, sahipli-aşılı 86, sahipli-aşısız 347, öldü-öldürüldü 34 idi. Mevsimlere göre olgu sayılarındaki değişimler Şekil 2'de verilmiştir.

Temas eden kişilerin cinsiyet, yaş, adres, temas kategorisi ve temas edilen bölge sayısı, hayvanın özellikleri, kaç doz aşı yapıldığı, Ig uygulanma durumu ve profilaksi durumu ve mevsimsel dağılımına göre tam aşılanmaya uyumları incelendi. Tam aşılamaya uyum oran %65,7 olarak bulundu. Bunların arasında kadın cinsiyet, Ig yapılanlar, ileri yaş, 4 doz aşı önerilenler, Ankara içerisinden başvuranlar tam aşılamaya daha uyumlu bulundu. Mevsimlere göre uyumda anlamlı farklılıklar bulundu, kış aylarında uyum anlamlı olarak artarken ilkbahar aylarında anlamlı olarak azaldı, diğer mevsimlerde farklılık yoktu ($p=0,02$). Hayvanın özelliklerine göre bakıldığında uyumda anlamlı farklılık vardı ($p<0,001$), sahipli aşılı hayvan temaslarında anlamlı olarak uyum az ($p\leq0,05$), kaçtı ya da öldürüldü ise anlamlı olarak uyum yüksek bulundu ($p\leq0,05$) (Tablo 1).



Şekil 2: Aylara ve yıllara göre kuduz riskli temas sayıları

Tablo 1: Tam aşılama durumu ile diğer değişkenlerin karşılaştırılması

	Tam aşılı değil	Tam aşılı	p-değeri
Cinsiyet, n (%)			
Erkek	508 (78,2)	858 (68,9)	<0,001
Kadın	142 (21,8)	387 (31,1)	
Yaş, ort ± SS	33,89±13,05	37,78±15,23	<0,001
Ortanca (min.-maks.)	30,00 (17,00-80,00)	35,00 (11,00-90,00)	
Şehir, n (%)			
Ankara	615 (94,6)	1211 (97,3)	0,003
Ankara dışı	35 (5,4)	34 (2,7)	
Temas kategorisi, n (%)			
0	3 (0,5)	0 (0,0)	0,126
1	0 (0,0)	1 (0,1)	
2	192 (29,5)	372 (29,9)	
3	455 (70,0)	870 (69,9)	
4	0 (0,0)	2 (0,2)	
Temas bölge bilgisi, n (%)			
1	633 (97,4)[a]	1213 (97,4)[b]	0,026
2	11 (1,7)[a]	30 (2,4)[a]	
3	3 (0,5)[a]	1 (0,1)[a]	
4	0 (0,0)[a]	1 (0,1)[a]	
Hayvanın özellikleri, n (%)			
Kaçtı	479 (73,7)[a]	1014 (81,4)[b]	<0,001
Sahipli aşılı	50 (7,7)[a]	20 (1,6)[b]	
Sahipli aşısız	118 (18,2)[a]	184 (14,8)[a]	
Öldü	3 (0,5)[a]	27 (2,2)[b]	
Doz, n (%)			
2	24 (3,7)[a]	52 (4,2)[a]	<0,001
3	14 (2,2)[a]	19 (1,5)[a]	
4	588 (90,5)[a]	1173 (94,2)[b]	
Ig, n (%)			
Yok	312 (48,1)	539 (43,3)	0,047
Var	337 (51,9)	706 (56,7)	
Profilaxi, n (%)			
Yok	639 (98,3)	1184 (95,1)	0,001
Var	11 (1,7)	61 (4,9)	
Mevsim			
S	118 (18,2)[a]	252 (20,2)[a]	0,002
K	174 (26,8)[a]	411 (33,0)[b]	
İ	224 (34,5)[a]	337 (27,1)[b]	
Y	134 (20,6)[a]	245 (19,7)[a]	

[a], [b]: Farklı üst indisler istatistiksel olarak farklılığı göstermektedir (p<0,05)
Ort: Ortalama, SS: Standart sapma, min.: Minimum, maks.: Maksimum, Ig: İmmünoglobulin

Tartışma

Çalışmamızın sonuçlarına göre tam aşılamaya uyum %65,7 bulundu. Tedaviye uyum oranları farklı ülkelerde yapılan çalışmalarda da benzer şekilde düşük bulunmuş olup %60-65 uyum tespit edilmektedir (3,4). Ülkemizde yapılan kuduz aşısına uyum ile ilgili çalışmalar sınırlı sayıdadır. Van ilindeki bir merkezden 813 kişinin dahil edildiği çalışmada tam doz aşılama 5 doz olarak kabul edilmiş olup uyum %30 olarak bildirilmiştir (5).

Kuduz tedavisi günümüzde halen temas sonrası proflaksi şeklinde yapılmaktadır. Tam ve zamanında aşılama bu nedenle önemlidir. Birçok çalışmada uyum kısıtlılığı nedenlerinin hastaların tedavilerini unutmaları, vakit ayıramamaları, tedavinin maddi yükü gibi sosyal nedenler olduğu görülmüştür. Bu nedenle aşılaması başlatılan kişilerin motivasyonları önemlidir (3,4,6). Çalışmamızda uyumsuzluğun nedenini araştırmak amacı ile hastaların mevcut bilgileri ile uyum durumlarını karşılaştırdık. Ankara ilinden başvurmak, kadın cinsiyet, ileri yaş, kış mevsimi, Ig ve 4 doz aşı önerisi aşılamaya uyumu artıran faktörlerdi. Ankara ilinden başvuranların uyumlarının yüksek olması sağlık tesisine ulaşımlarının kolay olmasından kaynaklanıyor olabilir. Temaslılar arasında erkek sayısı fazla olmakla beraber kadın cinsiyetin tam aşılamaya uyumu daha yüksekti. Koronavirüs hastalığı-2019 (COVID-19) ve influenza aşılamaların da

kadınların daha az aşılanma oranları bildirilmiştir ancak tam aşılamaya uyumları değerlendirilmemiştir (7,8). Yaş ilerledikçe uyumun artması bizim verilerimizle uyumlu olarak influenza aşılamasında da gösterilmiştir (8). Ig yapılanların uyumunun yüksek olması ise yaralanmalarının daha ciddi olmasından ötürü olabilir. Hayvanların kaçtı ya da öldürüldü grubuna dahil olanlar ile temas edenlerin uyumunun fazla olması ise kuduz olma korkusunun daha yüksek olmasından kaynaklanıyor olabilir. Uyumun kışın en yüksek ilkbaharda en düşük olması, ilkbaharda evcil hayvanlar ile olan temasın artması kışın ise sahipsiz veya yabani hayvan teması artıyor olması ile açıklanabilir. Ancak COVID-19 pandemisinin etkisi ile yanıltıcı bir sonuçta bulunmuş olabilir.

Evcil hayvanlar özelliklede köpekler, hala dünyanın pek çok bölgesinde özellikle gelişmekte olan ülkelerde kuduzun bulaşmasındaki en önemli aracılardır (1,3,9,10). Hastanemiz aşı merkezine başvuranlar arasında da en sık köpek teması tespit edilmiştir. Çalışmamızda kaçtı-tanınmıyor olarak tanımlanan hayvan sayısı fazla olmakla beraber, sahipli olanlar arasında aşısız olan hayvan oranı da yüksek bulunmuştur. Aker ve Şahin (11), Canik/Samsun yöresinde yaptıkları çalışmalarında temas edilen hayvanlar arasında sahipli aşısız olan ile en sık karşılaşıldığını ve bu konuda gerekli mercilerin uyarılması gerektiğini bildirmişlerdir. Gelişmekte olan ülkemizde de evcil hayvan teması daha sık görülmektedir (5,9). Bu nedenle kuduzdan korunmanın en etkin yolu evcil hayvan aşılanmasıdır (1). Çalışmamızda KRT'de erkek sayısı daha fazlaydı. Akbulut ve ark. (12) da, Diyarbakır'da yaptıkları çalışmada erkek sayısının daha yüksek bulmuşlardır. Benzer diğer çalışmalarda da erkek sayısı daha fazla tespit edilmiştir (5,13,14). Mevsimsel olarak birçok çalışmada KRT sayılarının yaz aylarında arttığı kışın azaldığı belirtilmektedir (5,15-17). Bunun aksine çalışmamızda yıllara göre farklılıklar olmakla birlikte ilkbahar ve kış aylarında KRT sayısında artış vardı. Bu durum COVID-19 pandemisinin etkisi olabileceği gibi ilkbaharda insanların daha fazla doğa yürüyüşü, park bahçe gezileri gibi faaliyetlerde bulunması ile de ilişkili olabilir.

Sonuç

Çalışmamızda; tam aşılamaya uyum oranlarının düşük olduğu ve sahipli-aşısız hayvan grubu ile KRT'nin fazla olduğu bulunmuştur. Uyumsuzluk nedenleri aşıların yan etkilerinden ziyade sosyal nedenler olduğu görülmektedir. Halkımız evcil hayvan aşılanması ve KRT durumunda önerilen aşı şemasının tam olarak uygulanmasının önemi konusunda bilinçlendirilmelidir. Hayvan teması sonrası önerilen profilaksiler tam olarak yapılmalı, aşılama tamamlanmalıdır. Aşılarını tamamlamayan kişilerin kuduz olma ihtimalleri vardır. Ve şehirlerde gezen başı boş hayvanların kontrol altında olması da KRT nedeni ile başvuran hasta sayısının azalması için önerilmelidir.

Etik

Etik Kurul Onayı: Çalışmamızın etik kurul kararı Yıldırım Beyazıt Üniversitesi, Yenimahalle Eğitim ve Araştırma Hastanesi Klinik Araştırmalar Etik Kurulu Başkanlığı'ndan 21-12-2022 tarihinde alınmıştır (karar no: E-2022-70).

Hasta Onamı: Hasta verileri kayıtlardan taranarak elde edilmiştir.

Hakem Değerlendirmesi: Editörler kurulu dışında olan kişiler tarafından değerlendirildi.

Yazarlık Katkıları

Konsept: N.A., M.A., T.Ü.U., S.K., İ.Ş., Dizayn: N.A., M.A., T.Ü.U., S.K., İ.Ş., Analiz veya Yorumlama: N.A., M.A., T.Ü.U., S.K., İ.Ş., Literatür Arama: N.A., M.A., T.Ü.U., S.K., İ.Ş., Yazan: N.A., M.A., T.Ü.U., S.K., İ.Ş.

Çıkar Çatışması: Yazarlar arasında herhangi bir çıkar çatışması bulunmamaktadır.

Finansal Destek: Çalışma için doğrudan veya dolaylı mali destek alınmamıştır.

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RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ

DOI: 10.4274/atfm.galenos.2023.93824
Ankara Üniversitesi Tıp Fakültesi Mecmuası 2023;76(1):56-65

BASIC MEDICAL SCIENCES / TEMEL TIP BİLİMLERİ

Healthcare Team in Ward Round: Roles and Responsibilities from the Perspective of Pediatric Surgical Residency Teachers

Klinik Vizitlerde Sağlık Ekibi: Çocuk Cerrahisi Eğiticilerinin Perspektifinden Roller ve Sorumluluklar

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**Interleukin-33 effects on Neopterine and IP-10 products in Macrophages. Mollecules Symposium in Immunology, İzmir, Turkey, 18-21 October 2018.*

Abstract

Objectives: The aim of this study is to determine the roles and responsibilities of healthcare team members in ward rounds from the view of pediatric surgery teachers.

Materials and Methods: A qualitative design was used with semi-structured interviews were conducted with six pediatric surgery teachers.

Results: The consultant physician on ward rounds has a critical role and responsibilities. Concerning the duties of residents/senior residents and other surgeons, the importance of complying with hierarchical communication, patient management, and preparation before and during ward rounds were stressed. The roles and responsibilities of the supervisor nurse were defined as specifying medical information and observations during ward rounds and ensuring the flow of information among team members. The teachers described the nurses' roles as passive-listening, completing the resident deficiencies and fulfilling the doctor's instructions. Effective communication, teamwork and continuing professional development are among the important roles of the healthcare team.

Conclusion: The roles and responsibilities of residents differed by their experiences and competencies. It was determined that nurses are not expected to actively participate in the decision-making process regarding patient care and have a passive role as an observer. It should be noted that it is expected of doctors in the ward round team to maintain "hierarchical communication".

Key Words: Ward Rounds, Improving Health Quality, Qualitative Research

Öz

Amaç: Bu çalışmanın amacı, çocuk cerrahisi eğiticilerin gözünden klinik vizitlerde sağlık ekibi üyelerinin rol ve sorumluklarını belirlemektir

Gereç ve Yöntem: Araştırma niteliksel desene sahip olup, altı çocuk cerrahisi eğitici ile yarı-yapılandırılmış görüşmeler yapılmıştır

Bulgular: Klinik vizitlerde vizit sorumlu doktorunun kritik rol ve sorumlukları vardır. Asistan/kıdemli asistan ve diğer cerrahi doktorlarının hasta hakimiyeti başta olmak üzere, vizit öncesinde hazırlık ve vizit sırasında hiyerarşik iletişime uymalarının önemi öne çıkmıştır. Sorumlu hemşirenin rol ve sorumlukları ise vizit sırasında tıbbi bilgi ve gözlemlerini belirtme, ekip üyeleri arasında bilgi aktarımını sağlama olarak tanımlanmıştır. Eğiticilerin hemşirenin vizit sürecine aktif kalımına ilişkin görüş farklıkları olduğu dinleme asistan eksiklerini tamamlama ve hekim direktiflerini yerine getirilmesi pasif rollerinin olduğu görülmüştür. Vizit sürecinde yapıcı iletişimde bulunma, ekip işbirliği içinde çalışma ve sürekli mesleki gelişim sağlık ekibinin önemli rolleri arasındadır.

Sonuç: Asistanların rol ve sorumlulukları deneyim ve düzeylerine göre farklılık gösterdiği görülmüştür. Hemşirenin hasta bakımı ile ilgili karar verme sürecine aktif olarak katılımının beklenmediği, pasif izleyici rolünün tanımlandığı belirlenmiştir. Değişik deneyimlere sahip olan vizit ekibi içindeki doktorların "hiyerarşik iletişimi" sürdürme beklentisinin öne çıktığı dikkat çekmektedir.

Anahtar Kelimeler: Klinik Vizit, Sağlık Kalitesinin İyileştirilmesi, Nitel Araştırma

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Received/Geliş Tarihi: 23.04.2022 **Accepted/Kabul Tarihi:** 24.04.2023

Introduction

Cooperation and coordination of different professionals play an important role in ensuring quality and patient safety in healthcare services (1-3). Ward rounds are an integral part of healthcare services due to their impact on collaboration, coordination and patient safety in clinics. In the United States, it is estimated that five rounds, on average, take place per day for an inpatient (4). In all professional groups providing healthcare services, it is key for team members to be aware of their duties and responsibilities in ensuring safe, high-quality, patient-oriented care during ward rounds (1,5-7). Studies conducted in this field emphasise the fact that team members are aware their roles and the expectations in these roles enable the ward round process to be carried out in a qualified manner with a collaborative approach, including patient treatment and care (2,5,8). In addition, determining trends in the patient's condition during ward rounds leads to situational awareness and standardisation of the process (5,9), as well as preventing negative interactions and bedside chaos among team members, thus, improving the quality of the process (10). However, despite the importance of collaboration, it is pointed out that uncertainties in the definition and perception of the roles and responsibilities of healthcare professionals may pose a great risk for patient safety (8,11).

Ward rounds is the process of information flow between healthcare professionals regarding patient care, management and workflow. More generally, it is "the temporary or permanent transfer of some or all aspects of care for a patient or patient group to another person or professional group with professional responsibility and accountability" (3,8,9). Ward rounds are seen as an integral part of medical education in providing patient-centered care, clinical learning and competencies as well as contributing to patient care and management (12). Since William Osler, it has been the basic strategy for clinical education and is still considered essential for the training of physicians and their acquisition of clinical competence (12,13).

Many different theories underpin learning from ward round, but Kolb's experiential learning theory offers the most theoretical support. Kolb's learning cycle, is a constructivist theory concerning how learners take experiences from the external world into their private worlds of thought and emotions. They interpret experiences, give them personal meaning, and plan new actions in response to their interpretation (14).

To meet the needs of the ever-changing healthcare system, different ward rounds are carried out, such as training, consultation and multidisciplinary, with varied processes and purposes (8). In other studies, the nurse's roles were identified as patient advocacy, providing patient care, and leadership during the rounds. The roles and responsibilities of team members may change to carry out different visits effectively (5,11,15). Research shows that the current definitions of the roles and responsibilities of the consultant physician (1,5,16,17), resident (1,5,16,17), senior resident (1,18,19), supervisor nurse (5,7,20), and nurses (1,5,7,8,11,15,21), during rounds are unclear, and different definitions can be made. One study found (8), that doctors used nurses to supplement information and provide extra detail on patient assessment during ward rounds. In other studies, the nurse's roles were identified as patient advocacy, providing patient care, and leadership during the rounds (15). The socio-cultural conditions in which research has been conducted have a significant effect on the emergence of such different definitions. Apart from variability in the definition of roles and responsibilities during ward rounds, other agreed-upon issues are listed as follows: i) the uncertainties in the roles negatively affect multidisciplinary rounds, ii) it is important to define open roles for each team member, and iii) a clear approach needs to be established for each team member (1,6,7).

Due to the prime role of rounds in the patient care/treatment process, conducting studies to examine ward rounds and the roles and responsibilities of the healthcare team in this process is indispensable for a service-based approach and patient safety. Addressing these studies in socio-cultural contexts such as institutional, department culture, specialties, team members and ward round atmosphere will contribute to a detailed explanation of the problem. This study aims to determine the roles and responsibilities of health professionals participating in pediatric surgery ward rounds through the experiences of pediatric surgery teachers. It is thought that the views of the teachers can contribute to structuring ward rounds and removing uncertainties. To this end, the study has sought an answer to the following research question:

- How do pediatric surgery teachers define the roles and responsibilities of health professionals participating in pediatric surgery ward rounds?

Materials and Methods

The phenomenological design, a qualitative research approach, was used in this study. Phenomenological studies are conducted with relatively few people and focus on individual experiences, leaving aside the researcher's own perspective and prejudices (22).

Participants

The study group was composed of six pediatric surgery teachers - two pediatric surgeons, two associate professors of pediatric surgery and two pediatric surgery professors - working in the Clinic of Pediatric Surgery of University of Health Sciences Turkey, Ankara Child Health and Diseases Hematology Oncology Training and Research Hospital. A purposeful sampling method

was used, which is designed to select situations that are rich in knowledge and experience to maximise the effectiveness of limited resources (22). The pediatric surgery teachers were selected according to the following criteria: i) their ability to share rich experiences, ii) the specific information they have about ward rounds, and iii) their routine participation in pediatric surgery ward rounds. Participation in the research was voluntary. Other healthcare team members (pediatric surgical residents and nurses) were not included in the study because they did not have sufficient knowledge and experience about the ward rounds process and management and did not routinely participate in the ward rounds.

Data Collection

The interviews took place between April and May 2019 in the teachers' offices. Only the researcher and teachers were present during the interviews, which lasted 29-60 minutes. The interviews were done "face to face" by the female researcher (NC), and voice recordings were taken. A semi-structured interview form was used. The form included questions that examine the roles and responsibilities of team members during ward rounds generated by considering previous studies in line with the purpose of the research. To evaluate the appropriateness and understandability of the interview questions, two experienced general surgery specialists were consulted. The consent of the teachers was obtained by giving more detailed information about the purpose and scope of the study on the day of the interviews. Before starting the study, no relationship was established between the participants and the researcher. Field notes were taken by the researcher during the interview.

Statistical Analysis

The thematic analysis method was used. Verbatim transcriptions were made from the recorded interviews. A qualitative data set was composed by combining transcripts of all interview texts. This data set was read several times and an in-depth perspective on the interviews was formed. After repeated readings, similar and different data segments were marked, and codes were determined. General codes were determined by researchers within the framework of the first established codes and an approved code list was generated. To ensure the reliability of the data, a researcher experienced in qualitative research and the researcher (NC) coded over two randomly selected interview texts. Both researchers have experienced in qualitative research and attended a qualitative research workshop. Inter-coder reliability was calculated as 80% by dividing the number of agreed codes by the total number of both agreed and disagreed codes (23). The codes, sub-themes and themes were reviewed by the researchers and thematic schemes were set up. Coding was done using the Microsoft® Excel program. The findings of the study are presented with direct quotations to reflect the experiences of the surgery teachers. The study complied with the Consolidated Criteria for Reporting Qualitative Research (COREQ) checklist (See Supporting Information S1).

Results

The surgery teachers interviewed in the study were 36-54 years old and had been working 12-30 years as a doctor and 9-25 years as a pediatric surgeon. Two of the teachers had participated in the "Trainer-Training" programme, which includes bedside training, adult education, asking questions, peer teaching and development of educational programmes. The teachers defined the roles and responsibilities of healthcare professionals in ward rounds differently according to their position in the team (Figure 1). Findings on roles and responsibilities are summarised below.

Consultant Physician*

The teachers defined the duties and responsibilities of the consultant physician on ward rounds within a broad framework including clinical management, patient management, teaching, role modeling, monitoring and supporting the resident, compentency in non-technical skills and continuous professional development. The teachers stated that they and other team members obtained up-to-date information about the patient treatment/care process through ward rounds and that they should organise patient-centred clinical management by evaluating this information. It seems important to give feedback to team members in the clinical management process:

"What is the function of service? The thing to be applied to patients is service management, and routine. Which patient will be treated? what is planned? So to say, whether he needs to be hospitalized or in intensive care? these are all things to be evaluated during the ward rounds (Teacher 3, Female)".

"It cannot be a regular operation. Because in the morning ward round, after the patients have been toured together, a decision is made on what to do for patients, that is, "let's demand these patient examinations -patient-based assessments-", "let's demand X-ray", "let's do this...". You know, a supervisor nurse also exists during the visits.." (Teacher 1, Male)".

The teachers emphasised that they conducted ward rounds to obtain information about the general condition of patients, to evaluate patients and to manage their treatment/care plan. The consultant physician on ward rounds has an important role in patient management:

"To have information about the clinical course of the patient, to discharge if the patient needs to be discharged, to adjust his treatment, to give or reduce drugs if it is needed to his treatment... we do it to follow the process in that way (Teacher 4, Male)".

The teachers stated that ward rounds are valuable learning environments, and the consultant physician on the round contributes to the education and training of residents. Education and training activities are carried out through discussions, question-answer and demonstrating or observing clinical practices:

"For sure! we would like to contribute to the self-development of the resident and make them qualified on the patients/disease treatments by monitoring them during the patient evaluation process, by discussing with them on the patient, asking questions about the disease when necessary, or discussing things about the distinctive diagnosis of the disease, examinations, or other physical examination findings, etc. (Teacher 1, Male)".

It was emphasised that the consultant physician performs as a role model that shapes the management, evaluation and formation of professionalism among the residents:

"They see our management or our capacity to assess. I mean, how is the teaching done? How does a medical specialist stand? Or what should be the position of an residentt, and so on? Everyone observes their own positions. A discipline is being formed. We are role models. Our colleagues apply another variation of what they see in us or what they see in their fellows. They watch us all and draw a conclusion (Teacher 2, Male)".

The teachers defined the monitoring of residents' professional development and their individual support among the roles and responsibilities of the consultant physician on rounds. As part of these roles and responsibilities, they noted that residents consider patient dominance, patient treatment/care process management and identification of personal problems:

"Residens' control... The term "control" refers to: how did they evaluate the patient? How to say, how did they make the progress of the day? Have they done the necessary examinations? Have they already started the necessary treatments for patients' diseases after checking them? One of the shortest ways we use the most to understand whether they manage the patient's treatment processes is to carry out ward rounds. It is the thing that is most quickly and easily understood whether they have control over the patient or not (Teacher 1, Male)".

The teachers stated that the consultant physician of the ward round should have non-technical skills such as teamwork and the capacity for decision-making. In this context, it was mentioned that a moderate ward round atmosphere should be provided for team members to express their opinions freely, and decisions should be made after receiving the opinions of the team members:

"The person conducting the round should be open to every opinion. As I said, everyone should express their opinion after all. Right or wrong! Ultimately a decision should be made about the patient. There is also the following point. There is mainly a primary doctor following the patient, a staff specialist. Otherwise, he must make the final decision. The person conducting the rounds must approve or disapprove of it, and a decision must be made after all parties have expressed their opinions (Teacher 6, Male)".

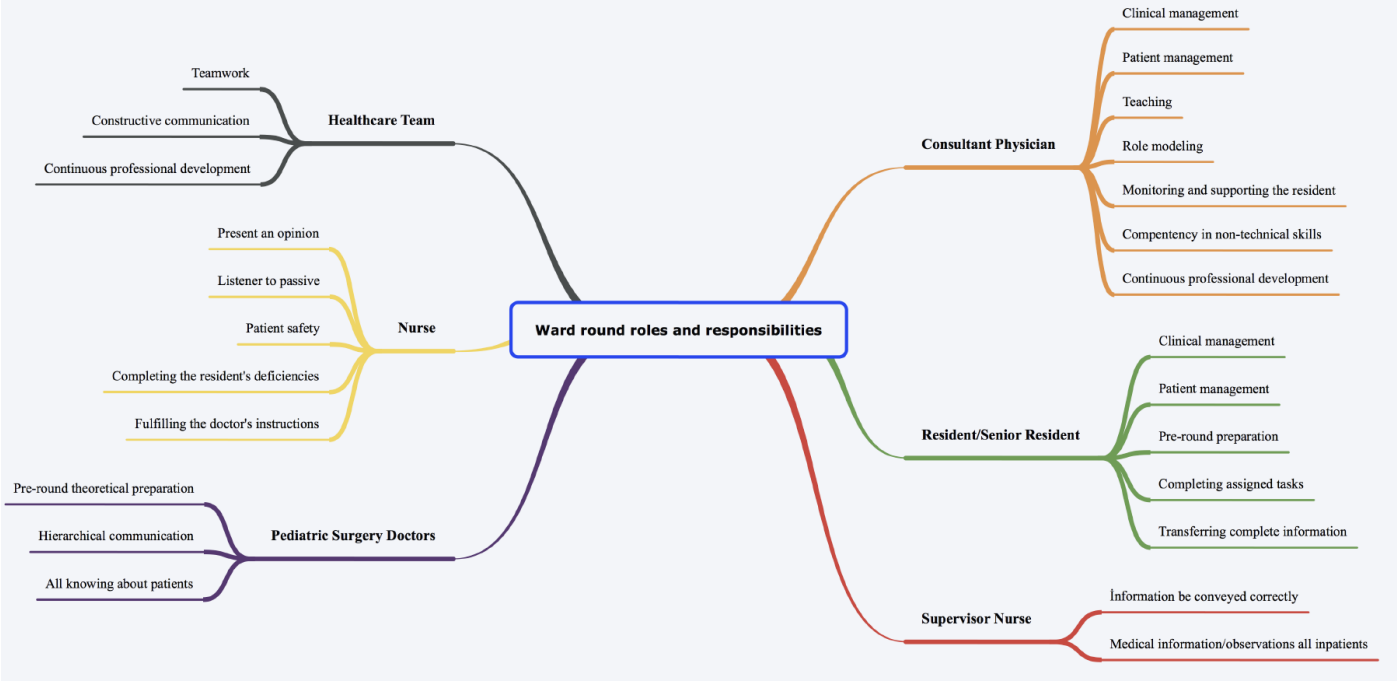

Figure 1: Roles and responsibilities of the healthcare team in the ward round

In addition to the training of healthcare team members, it has been stressed that ward rounds are also a training process for the consultant physician, and the consultant physician should also develop themselves professionally in this process:

"Now, for example, when the subject of the round is determined, I try to read it myself. So I'm looking at the latest literature. You need to be able to add something. You have to be superior to them. In this way, you encourage them to say something on the matter. They should say: "Well, this is the case, and I should take a look at it or something". You know, I think you need to be able to renew yourself (Teacher 5, Female)".

Resident/Senior Resident

The teachers consider patient management, clinical management, pre-visit preparation, completing assigned tasks and transferring complete information as the roles and responsibilities of the resident/senior resident during ward rounds. The teachers stated that pediatric surgery residents should have the treatment/care process from the day of hospitalisation, not just daily updates, of all patients in the clinic:

"Now, first of all, I would like a pediatric surgery resident to know absolutely everything about the patient. He must know the story of the patient, the results of the treatment methods applied to the child from the beginning of the monitoring, and everything else. After that, if he is on duty, he should be in control of the vital signs of the patient, and everything that happens to the patient so long as he stands by patients during the night (Teacher 3, Female)".

In addition to patient domination, the treachers stressed that the senior resident especially should control patient management, functioning of the clinic, complete the deficiencies and ensure clinical management:

"The surgical hierarchy starts right here. Before the professor's round, the most senior resident should supervise the round among the residents. Because it is thought that he is close to expertise and his level of education is better. Actually, he is expected to manage everything. He is required to ask: "What did you do to this now? What did you do to that? Tell me about all...". He should complete the deficiencies in this way (Teacher 4, Male)".

During rounds, the teachers stated that all residents should have a background related to the treatment/care process of the patients. In this context, they stated that it is important to be prepared for rounds and that residents should organise rounds among themselves. They emphasized that the senior resident is responsible for this preparation:

"New juniors! There is such a hierarchy. You know, their seniors are responsible for completing the resident round before, right? Everyone should get an idea about the night shift, even those who have not been participated in the night shift (Teacher 5, Male)".

"The residents need to know how the overnight progresses of the inpatients before the round was. We want them to know everything about the new inpatient during the rounds, such as where did the anamnesis come from? how was the patient? why was the patient hospitalized? (Trainer 2, Male)".

Some teachers stressed that residents should update information connected to patients on rounds, then search and read the points highlighted within the scope of the training activities:

"We expect the residents to run immediately to do the work spoken there and the duties that fall into their fields. Or he has to say: "let me read this". Because we say there: "read these criteria, look! we did such a thing there ..." those criteria... Because his seniority must be sufficient, and he needs to know every relevant thing. You say: "Tell it to him, and then we'll talk about it once you have read it". He needs to take his time and study what we gave him, as well as his duties (Teacher 2, Male)".

It is important to provide complete information about the patient to make the right decisions during rounds. One of the teachers defined the complete realisation of the information transfer process among the roles and responsibilities of the residents:

"The main duties and responsibilities of the residents are, here, to fully convey the patient's daily watch-out processes (Teacher 1, Male)".

Nurse

The teachers defined the roles and responsibilities of nurses during the round process as providing opinions, ensuring patient safety, listening to the patient, completing the resident's deficiencies and fulfilling the doctor's instructions. Some teachers stated that there may be information the healthcare team has overlooked or is incomplete, and it is important for the nurses participating in rounds to convey their medical information/observations to the patient during the ward round:

"That is why everyone needs to participate in the ward round. The attention of the team might be elsewhere. For example, the doctor may not notice some things. But, here, the nurse who monitors the process should remind, for example, "He has tachypnea. Are you going to do anything?" It's good to make such reminders (Teacher 6, Male)".

Contrary to the above view, another view stated by the teachers is that the nurse does not need to actively participate in ward rounds but should be a passive listener. According to this view, it is sufficient for nurses to attend and listen only to the patient's visit under his/her responsibility instead of attending

all ward rounds. One of the teachers further expanded this view and stated that fulfilling the doctor's directives is among the roles and responsibilities of the nurse during the ward round process:

"Everything we warned during the roundt, and everyone should listen to what is being said about their patient, including the nurses, we think of her as an auxiliary health staff (Teacher 2, Male)".

"Only at the round? I think there are also other things that are under the nurse's duties and responsibilities, such as to care for patients properly, or to follow orders (Teacher 4, Male)".

For nurses, extra roles and responsibilities might be added during the round, such as supporting residents in terms of completing their observations and eliminating their deficiencies, defining the obstacles related to patient care and conveying these obstacles:

"At the beginning, the nurse will supplement his (resident's) checks, observations, and his deficiencies. She will convey the defects that she noticed, and provide medication where required (Teacher 3, Male)".

"For example, we check the patient and we say: "mobilize him". The nurse in the team that will mobilize him, who is looking after him ... If the patient cannot be mobilized, this needs to be communicated, and shared, for example, "his mobilization is not possible, or Why can't he be mobilized?..." To look for the reasons lied under non-mobilization, such as, is it because of the pain? Is there a contracture? Or is there something else? What is it? We need to think about all possibilities (Teacher 1, Male)".

Patient safety requires teamwork. In this context, the nurse should work in cooperation with the residents and give warnings when necessary:

"There is a double check of the work done from all sides. Namely, there are aspects of the work done by the residents and the specialty students that concern the doctor. They are controlled by the seniors. There are also things they share with the nurses in the treatment process. Like orders, and things to do about the patient. Indeed, they should be monitored by senior nurses (Teacher 1, Male)".

Supervisor Nurse

Concerning nursing care, it is important that information be conveyed correctly during the ward round. It is emphasised that the person who will exchange information should be the right person:

"Accordingly, this is important. I mean, as I said, some things are not always possible. Our wish is for the supervisor nurses to do the morning visit with all their fellow nurses there, in that thing, and monitor the patients. Has the patient eaten? drank? whether moved or not? lung massage done? cough? all these need to be conveyed to us... this is relatively easy to do, but there must be the right people... (Teacher 1, Male)".

The teachers stated they can consult the supervisor nurse about his/her medical information/observations of all inpatients in the clinic when necessary during ward rounds. According to the teachers' opinions, the supervisor nurse should be capable of implementing the treatment/care process of all patients in the clinic, be professionally equipped and participate in primary patient care:

"The supervisor nurse should also listen to everyone, listen to all patients. There will also be questions that we can ask her during the ward round. That is, the assistant tells something but she is the one who observes something. We can get her opinion. How was this patient compared to yesterday? "I think it was unpleasant, or he has diarrhea..." the doctor might not have seen it, but it was green, watery, and so on... so we need her medical knowledge and observations (Teacher 2, Male)".

Pediatric Surgery Doctors

In the views of the teachers, specialist and resident pediatric surgeons should participate in the visit by making theoretical preparations for patients and diseases to carry out the visit efficiently:

"He must definitely read the patient, read from the previous night, do not come to the ward round by saying that he knows everything anyway, and he should review something that he can add to them according to each seniority levels, he must be prepared, and each team must be prepared for themselves. If the resident studies for himself, if the specialist doctor looked for himself, if the trainer had scanned the literature according to himself, if it was known what was in the hospital, its efficiency would be higher (Teacher 2, Male)".

The teachers stated that pediatric surgery specialists and residents should be able to master the patient treatment/care process during the round, although there are differences in institutional and clinical operations. It has been observed that patient dominance has positive contributions to the round:

"One who will attend the round, as I said before, can be residents, maybe a specialist, or a doctor, the institution is different, the functioning is different... Whoever might be... he must have control over the patient in every sense (Teacher 6, Male)".

It was emphasised that the round should be conducted within a certain hierarchical communication framework and that both residents and experts should act in accordance with this hierarchical communication chain during the ward round:

"Everyone acts according to their seniority level during rounds, only responsible one talks, anyone who talks about the patient, he might also be the junior, no one will interfere

until he finishes his word... if he is asked something, he should answer... after the junior, the service supervisor talks, and after that, if anything, the service specialist talks... everything works in that hierarchy and discipline (Teacher 2, Male)".

Healthcare Team

In this theme, the characteristics that the whole team should have - except for the responsibilities of individual team members - are defined. The teachers stated that the patient treatment/care process requires teamwork, and in some cases, it is important for the team members to support each other. In the teacher's comments, it was stressed that it is essential for team members to work in harmony and show support for each other:

"It is significant in terms of teamwork of course... so you rounds the wards together, and you want the nurses, at least the supervisor nurse, to be there. All in all, if you have someone else in your ward, for example, if you have a physiotherapist specialized in burns, you need to have a physical therapist... (Teacher 1, Male)".

"Therefore, teamwork is also like the organs of a body in this sense. It is necessary for everyone to work in harmony, to understand each other's situation, they must not hide something (Teacher 2, Male)".

The views of all team members during rounds are valuable, and it is critical for team members to express their opinions using constructive communication techniques during rounds:

"I mean, the education continues for all of us, from the most senior to the junior, from the expert to the professor. There are new things you can learn from every patient. Everyone's opinion is also very important. What comes to your mind may not come to my mind. If it is said in an appropriate condition, in an appropriate way, if everyone can be involved in scientific discussion, this shows the wealth of the team (Teacher 5, Female)".

Emphasising the educational aspect of rounds, visits contribute to the continuous professional development of healthcare team members. In this process, it has been observed that healthcare team members must make inferences to ensure their continuous professional development:

"Every round is an education of course. Namely, in the end, the purpose of the ward roundt is to discuss the patient. In this sense, at the bedside, I think it is useful that everyone participating in the visit should do some inferences, whether it is auxiliary health personnel or from other health branches (Teacher 6, Male)".

Discussion

A clear definition of roles is vital for patient-centred, effective, efficient and structured ward rounds. Understanding this process is a priority in terms of structuring specialty training and clinical training. Using a qualitative data set, this study attempted to define the roles and responsibilities of healthcare professionals who participate in visits, and it was carried out with a relatively small sample, considering the socio-cultural differences.

The teachers brought a multidimensional perspective to the roles and responsibilities of the consultant physician; the importance of patient and clinical management, education, role modeling, monitoring and supporting residents, compentency in non-technical skills and individual continuation of professional development were emphasised. Earlier studies also examined the roles and responsibilities of the consultant physician and described the different aspects of the ward round, such as clinical review, agreeing on a clinical plan, including clinical criteria for discharge and an expected date of discharge (1,8,16-18). Reece and Klaber (18), list the consultant physician's roles and responsibilities on rounds as helping residents, interpreting information by using their expertise in decision-making and ensuring inter-team communication. Manias and Street (17), stress the discussions on planning patient care, and management of these discussions are within the responsibilities of the ward round's consultant physician. Parallel to these study findings, earlier studies reported that patient and clinical management are predominant within the framework of roles and responsibilities. However, the teachers interviewed in this study highlighted the educational role of the consultant physician on rounds. Role modeling, providing observations and support and continuing professional development are defined among the roles and responsibilities of the consultant physician.

According to the teachers, there were differences in the roles and responsibilities of the senior residents and residents during ward rounds. These differences can guide the levelling of competencies, especially in residency education. Teachers have stated that pre-round preparation, patient control, completing assigned tasks, full information and clinical management are among the roles and responsibilities of the residents/senior residents. The responsibilities of the senior pediatric surgery resident were separated and new responsibilities were added, such as the control of patient management and clinical operation, completing deficiencies and ensuring clinical management. In the study, the roles and responsibilities defined for the specialist doctors in the team are the same as those specified for the senior residents. The roles and responsibilities defined for these two groups are similar because the senior resident is at the last stage of training before transitioning to specialist. In other studies, giving an overview of patient treatment and care, summarising the patient to the ward round team, keeping written records, making the clinical decisions for patients and leading the ward round are among the basic roles and responsibilities of the student resident (8,18,19). Falco and

Balmer (19), state that the roles and responsibilities of senior residents during rounds are to efficiently organise patient assignments for interns and medical students, review their studies, answer questions and manage the treatment and care process. As stated above, studies predominantly define some of the roles and responsibilities of the consultant physician on rounds, such as leading the round process, making decisions and being a teacher. Unlike Falco and Balmer's (19) study, this study does not address the educational and leadership role of residents, whether senior or not. It is thought that these roles and responsibilities are seen as competencies at the level of expertise rather than the resident level. However, it is known that peer learning is widely used in clinical education in practice (24). If these roles and responsibilities are ignored in the trainers' definitions, the residents' contributions to education may not be fully seen. Besides, if the responsibility of peer learning do not describe clearly, solidarity within professional groups may decrease and induce missed opportunities to become a qualified clinical teacher in the future. It is essential to study these perceptions extensively when reviewing residency training programmes and processes.

In this study, the emphasis on the importance of "hierarchical communication" by teachers, specialists and resident doctors during ward rounds was highlighted in the interviews. Here, two situations in hierarchical communication exist: first, communication between those at different stages in the same occupational group, such as between junior residents, senior residents and trainers; second, communication between different health professionals. There is a clear expectation from the teachers regarding the need to observe "hierarchical communication" among those in the same profession during rounds for doctors. This expectation is also seen in the descriptions of other healthcare team members, such as nurses. Defining nurses as "auxiliary health staff" and their duties and responsibilities as applying ordered treatment and obeying orders indicates hierarchical perceptions among professionals. It is thought that the traditional ward round culture and the perception of the health team are effective on the hierarchical views of the teachers. It is difficult to communicate with healthcare professionals who are generally perceived in a higher hierarchical rank during the ward round. This may affect the participation and roles in the round (15,16,25). Rice et al. (25) studied in the internal medicine clinic and found that the inter-professional hierarchy has negative effects on communication and cooperation. Similarly, Bould et al. (26), reported that negative hierarchical culture has negative effects on patient safety, residency teaching and team functionality. Improving and teaching professional teamwork and communication are increasingly recognised as a higher priority for patient safety (27). In this line, it has been observed that the development of training programmes is important in providing safe care to the patient, increasing the perception of inter-professional learning and improving communication and cooperation between team members (28).

In ward rounds, which are among the central activities of the patient treatment/care process, a collaborative approach between doctors and nurses affects patient care positively (16,29). However, studies have shown that doctors and nurses have little interaction with each other and are not inclined to cooperate during ward rounds. They have also shown that nurses take part in ward rounds as a supplementary object to remove incomplete information and to answer raised questions during rounds, apart from the decision-making process regarding the patient (6,11,17,30-32). Manias and Street (17), found that supporting doctors' knowledge about the patient and providing extra details during ward rounds seem to be among the roles and responsibilities of nurses. Similar research has shown the following: i) nurses' contributions to rounds are not appreciated and tolerated, ii) (32) ward rounds are not conducted in a democratic way, iii) doctors dominate the process, iv) (21) nurses have little participation, and v) it is among the roles and responsibilities of nurses to answer questions asked during rounds (11). From the teachers' standpoints, although doctors and nurses have the same aim, it is obvious there are different opinions about the participation of nurses in the patient care process. Although some of the teachers touched upon the importance of participation and cooperation, here, they gave nurses the role of "transferring and completing information". Other teachers said that they (nurses) should be listeners with passive participation during rounds rather than being professional members of the healthcare team.

During the ward round, the roles and responsibilities of all healthcare team members are specified as having a discharge plan, medication management, making acute decisions regarding patient care, teamwork and ensuring constructive communication security (1,8). Besides, it is essential that all healthcare professionals establish a learning culture individually or as a team during the ward round (1). The teachers stated that ward rounds are valuable learning areas for all healthcare team members as this process contributes to the continuous professional development of the team. Constructive communication and working in collaboration are among the roles and responsibilities of the healthcare team. The results of this study show that the roles defined by the teachers regarding hierarchical communication and nurses' participation in rounds conflict with other roles and responsibilities including communication and cooperation, which the healthcare team should undertake during the ward round. Collaborative practice environments have a positive impact on patient safety and quality of care by enabling the sharing of knowledge, different expertise and perspectives, and establishing equal status among team members (32). In addition, the collaborative approach

is recognised as an integral part of workplace learning for healthcare professionals. The perception of hierarchy can lay negative ground in workplace learning by affecting question asking, feedback and critical thinking (33). It is thought that the reasons such as lack of inter-professional training, experience and being unfamiliar with each other's roles may affect cooperation and communication during rounds. One of the most important findings of this study is that, although the importance of inter-professional collaboration has been stated by many studies at the pre- and post-graduate education level in recent years, it is necessary to work more to reflect this awareness in the field and to improve the understanding and competencies of it.

Study Limitations

The study is limited in generalisability because of its qualitative nature. The data were obtained from pediatric surgery teachers working in the same institution. However, it is expected that the results will contribute to similar studies carried out in different centres.

Conclusion

In this study, the expertise of healthcare team members participating in ward rounds as well as their roles and responsibilities at the individual and team level were defined from their teachers' perspectives. The consultant physician on rounds has a critical role in terms of learner, healthcare team, patient and clinical functioning. The roles and responsibilities of the residents are defined differently according to their level of experience. Although the importance of teamwork and communication was emphasised during the ward round, it was determined that the active participation of nurses in the decision-making process of patient care was limited, and the view that he/she has a passive audience role in the team continues. Despite its limitations on communication, it has been determined that hierarchical communication is among the roles and responsibilities that the team should pursue during rounds. To provide qualified and reliable patient care, it is important to conduct studies in different areas of expertise and with team members that reflect the perceptions of the roles and barriers to these roles during rounds. Although it is not possible to quickly change the established culture and perceptions that support it, action research examining the reflections of teamwork and inter-professional collaboration on patients must be carried out.

Acknowledgments: The authors would like to acknowledge the participants who took time from their busy schedules to contribute their thoughts and ideas to this work. We thank associate professors Senay Sarmasoglu for his contribution to the data coding phase.

Ethics

Ethics Committee Approval: Ethical approval was obtained from Ankara University Clinical Research Ethic Committee (approval no: 2019/024, date: 18.02.2019).

Informed Consent: The participants were verbally informed about the anonymisation of the data and voluntary participation, and their written consent was taken. To ensure the confidentiality of participants' identities, the interview data were presented in coding.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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

Financial Disclosure: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ

DOI: 10.4274/atfm.galenos.2023.38278
Ankara Üniversitesi Tıp Fakültesi Mecmuası 2023;76(1):66-69

SURGICAL MEDICAL SCIENCES / CERRAHİ TIP BİLİMLERİ

Management of Renal Traumas and Follow-up Results: Single Center Experience

Renal Travmaya Yaklaşım ve Takip Sonuçları: Tek Merkez Deneyimi

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Abstract

Objectives: Kidney traumas constitute a small part of all abdominal traumas. There is debate about whether to choose a conservative or surgical approach. Nephrectomy is the most common surgical treatment for renal trauma. Conservative treatment may be considered for preservation of renal function. Fourth and fifth degree injuries are associated with poor functional outcomes.

Materials and Methods: Kidney trauma admitted to a third-degree reference center was evaluated. Renal trauma assessment was performed with the help of computed tomography. Age, gender, laterality, and hemoglobin levels were evaluated. cases that were followed conservatively and underwent surgery were recorded.

Results: The median age of the patients was 25 years (18-44 years). 71.4% (n=15) of the cases were conservatively managed. Surgical repair of the injured kidney was preferred for 1 of the cases (4.8%). Nephrectomy was performed for 2 cases (9.5%) and embolization was preferred 2 cases (9.5%). Surgical repair and removal of foreign body was performed for only 1 case with penetrating renal trauma (4.8%). All grade I and II traumas were treated conservatively. One of grade III traumas underwent surgical repair, 1 of them underwent surgical repair and removal of foreign body and the other 4 patients were treated conservatively. One of grade IV traumas was performed nephrectomy, 1 of them underwent embolization and the other 4 were conservatively treated. For the patients with grade V traumas, 1 underwent nephrectomy and 1 was treated with embolization.

Conclusion: Different success rates have been reported for treatment approaches according to the literature. Both treatments have advantages and disadvantages. Conservative treatment is at the forefront in many patients, albeit at a high level. Interventions should be planned for hemodynamically unstable patients. This approach seems beneficial given its efficacy and safety. Thus, unnecessary kidney loss will be prevented.

Key Words: Renal Trauma, Nephrectomy, Conservative

Öz

Amaç: Böbrek travmaları tüm karın travmalarının küçük bir kısmını oluşturur. Konservatif mi yoksa cerrahi yaklaşım mı tercih edileceği konusunda tartışmalar vardır. Nefrektomi, renal travmanın en yaygın cerrahi tedavisidir. Konservatif tedavi böbrek fonksiyonunun korunması için düşünülebilir. Dört ve beşinci derece yaralanmalar kötü fonksiyonel sonuçlarla birlikte beraberliği mevcuttur.

Gereç ve Yöntem: Üçüncü derece referans merkezine kabul edilen böbrek travmaları değerlendirildi. Renal travma değerlendirmesi bilgisayarlı tomografi yardımı ile yapıldı. Yaş, cinsiyet, lateralite, hemoglobin seviyeleri değerlendirildi. Konservatif takip edilen ve cerrahi uygulanan olgular kaydedildi.

Bulgular: Hastaların medyan yaşı 25 (18-44 yıl) olarak saptandı. Olguların %71,4'ü (n=15) konservatif olarak takip edildi. Yaralanan böbreğin cerrahi onarımı olguların 1'inde (%4,8) tercih edildi. İki olguya (%9,5) nefrektomi yapıldı ve 2 olguya (%9,5) embolizasyon tercih edildi. Penetran renal travmalı sadece 1 olguda (%4,8) cerrahi onarım ve yabancı cisim çıkarıldı.

Sonuç: Literatüre göre tedavi yaklaşımları için farklı başarı oranları bildirilmiştir. Her iki tedavinin de avantajları ve dezavantajları vardır. Konservatif tedavi, yüksek dereceli de olsa birçok hastada ön plandadır. Hemodinamik açıdan stabil olmayan hastalar için müdahaleler planlanmalıdır. Bu yaklaşım, etkinliği ve güvenliği göz önüne alındığında faydalı görünmektedir. Böylelikle gereksiz böbrek kaybı önlenmiş olacaktır.

Anahtar Kelimeler: Renal Travma, Nefrektomi, İzlem

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Received/Geliş Tarihi: 05.07.2022 **Accepted/Kabul Tarihi:** 22.04.2023

Introduction

Renal traumas are not common trauma types that consititutes 1-5% of all traumas and 10% of abdominal traumas (1). There is a controversy of preferring conservative or surgical approach for the treatment of renal traumas (2). Experiences revealed lower nephrectomy and complication rates (2). Conservative management is a standart approach for low-grade traumas (1). During the last decade conservative approach replaced the surgical interventions (3). In case presentations, it has been shown that conservative approach would be preferred for also grade V renal traumas (4). During the conservative management some cases would be undergone for nephrectomy. Studies revealed that conservative approach would be also preferred for every grade of renal trauma for pediatric patients (5). Surgical exploration of renal traumas mostly result with nephrectomy (6). Trauma management depends on the parenchymal injury and risk of bleeding (7). Urinary extravasation accounts for 30% of all complications and immediate or delayed ureteral stenting is the main treatment of this condition (7).

Urinary extravasation would be managed by both observation and ureteral stenting (8). Time to excretory phase is an important point for the accurate diagnosis of collecting system injury (9). The percentage of the need to image for surgical intervention is approximately 12.5% in asymptomatic patients with high-grade renal trauma (10). It was found that most of the practitioners were intended to preserve renal functions while managing high-grade renal traumas (11). They were observed as comfortable with renorraphy and they have access to interventional radiology (12). Conservative management would be considered for renal function preservation and while assessing the functional outcomes, poor functional outcomes were found for grade V and specific subtypes of grade IV injuries (12). Hypotension at hospital admission, hematoma size greater than 3.5 cm, blood transfusion during the follow-up and worsening of injuries with computerised tomography are the predictive factors of conservative approach failure (6). In this study, we aimed to evaluate the patients that have been evaluated and treated in our clinic. Also we aimed to show the efficacy of conservative treatment for especially stable patients.

Materials and Methods

Renal traumas that were admitted to University of Health Sciences Turkey, Gülhane Training and Research Hospital, Clinic of Urology between January 2016 and June 2020 have been included in our study. Age, gender, laterality and grade of renal trauma, presence of foreign body, presence of macroscopic hematuria, complete blood count and biochemistry results during admission, on first and seventh day of hospital stay, presence of accompanying disease and treatment approaches were noted. Grades of renal traumas were evaluated with computerised tomography. Patients were hospitalised and observed with bed rest. Routine vital signs were observed during the hospital stay. Conservative and surgical approaches were chosen for the patients and both of the treatment modalities and the results of treatments were evaluated in detail.

Statistical Analysis

Statistical analysis was conducted with using Statistical Package for Social Sciences 22.0 software (SPSS 22.0 for MAC). Descriptive statistics of nominal samples were expressed with numbers and percentiles. Descriptive statistics of scale samples were expressed as mean or median according to their normalization. The frequency of the collected data has been also analyzed in detail. Shapiro-Wilk test was used to assess the variables' normalization. Spearman's correlation analysis was used to assess the correlation between the trauma grade and some of the noted variables that are the important factors for renal trauma. The value of $p<0.05$ was accepted as statistically significant.

Results

The study is a retrospective study and ethical approval was obtained from University of Health Sciences Turkey, Gülhane Training and Research Hospital (approval no: 2021-70, date: 11.02.2021). Twenty one patients have been admitted to our clinic with renal trauma between January 2016 and June 2020. All of these patients were male. The median age of the patients was 25 years (18-44 years). While assessing the laterality, 42.9% of the traumas were observed in left kidney (n=9) and 57.1% of them were in right kidney (n=12). Foreign bodies were observed in 57.1% of the renal traumas (n=12) and among these patients 4.8% of them were seen in left kidney (n=1), 28.6% of them were seen in right kidney (n=6), 9.5% were in right retroperitoneal area (n=2), 9.5% of them were in left retroperitoneal area (n=2) and 4.8% of them were in both right kidney and right retroperitoneal area (n=1). The number of foreign bodies were noted as; 1:38.1%, 2:9.5%, 3:4.8% and 4:4.8%. Mean size of these foreign bodies was 6.75 mm. While assessing the grades of renal traumas; grade 1: 5 patients (23.8%), grade 2: 2 patients (9.5%), grade 3: 6 patients (28.6%), grade 4: 6 patients (28.6%) and grade 5: 2 patients (9.5%). Macroscopic hematuria was observed in 57.1% of the patients and most of them were observed in high-grade renal traumas ($p<0.05$). There was strong positive correlation between the increase in trauma grade and the presence of macroscopic hematuria (Rho=0.696; $p<0.01$). Mean hemoglobin level at admission was 14.1 g/dL, it was 11.6 g/dL on first day 12.3 d/dL on 7th day and 12.9 g/dL on 30th day. Mean hemoglobin drop between the first day of stay and admission was 2.3 g/dL (0.3-6.8 g/dL). It was 1.8 g/dL

between the 7th day of hospital stay and admission and 1.04 g/dL between 30th day and admission. The differences between the creatinine values were not statistically significant and the values were similar between admission, first, 7th and 30th days. While assessing the correlation between trauma grade and hemoglobin drop at first, 7th and 30th day, it was found that there was not a statistically significant correlation (Rho=-0.224; p>0.05, Rho=-0.074; p>0.05 and Rho=0.029; p>0.05 respectively). The treatment approaches were also evaluated in detail. 71.4% of the cases were conservatively managed (n=15). Surgical repair of the injured kidney was preferred for 1 of the cases (4.8%). Nephrectomy was performed for 2 cases (9.5%) and embolization was preferred 2 cases (9.5%). Surgical repair and removal of foreign body was performed for only 1 case with penetrating renal trauma (4.8%). All grade I and II traumas were treated conservatively. One of grade III traumas underwent surgical repair, 1 of them underwent surgical repair and removal of foreign body and the other 4 patients were treated conservatively. One of grade IV traumas was performed nephrectomy, 1 of them underwent embolization and the other 4 were conservatively treated. For the patients with grade V traumas, 1 underwent nephrectomy and 1 was treated with embolization (Table 1).

Discussion

Renal traumas would be seen as blunt or penterating traumas and they could be managed with conservative or surgical approaches. Many studies in the literature showed dfferent success rates for these treatment approaches. Studies have shown that even high grade traumas of pediatric age group would be treated with conservative option (13). Regardless of injury details, nephrectomy rates have decreased consistently (14). There are some complications that would be seen with conservative approach. There is a risk of urinoma and the treatment of urinoma is still controversial as both percutanous drainage and ureteral stent placement options would be preferred (15). Delayed surgical exploration would also cause secondary hemorrhage and urinary extravasation (16). On the other hand early surgical approachs would result with high renal loss (16). When evaluating the prefferred treatment options, it is obvious to see that even in the high grade traumas, initial conservative approach is advocated (17). Studies reported the successful outcomes of conservative approaches even for grade V traumas (4).

Studies also showed the efficacy of percutanous embolization for hemodinamically unstable patients with grade V trauma (18). Results show this technique as a safe option with no intermediate-term adverse events (18). A study with large number of cases showed that clinical findings were the best guides for management and when managing the traumas it was not recommended to consider only the radiologic findings (19). While evaluating the cases with renal traumas that were treated in our center, it is clearly seen that most of the cases were treated conservatively. The cases in our center were all male. All the cases with grade I and II renal traumas were treated conservatively. Six patients with higher grades needed intervention and only 2 patients underwent nephrectomy. Our study results were compatible with the literature and as the studies reported in the literature, tendency for conservative approach is increasing recently. When considering the manuscripts in the literature, it is obvious that guidelines for renal trauma treatment must be revised and the conservative approach indications would be widened. Especially hemodynamically stable patients would be followed with close observation and monitorization. As it was seen in our study, hemoglobin drop was decreased with the days of observation and there was not significant creatinine change. These findings also support the efficacy of conservative approach for avoiding unnecessary renal loss.

Study Limitations

Our study has been conducted with limited number of cases. More cases and multicentric studies would contribute the literature and also they would be necessary for updating the guidelines.

Conclusion

Most of the patients with renal traumas, even if they are high grades, would be treated conservatively. Interventions must be considered for only hemodinamically unstable patients. This approach would be useful when considering the efficacy and safety. Also unnecessary renal loss would be avoided.

Table 1: Renal trauma grades and treatment options

Renal trauma grade	Conservative treatment	Embolization	Surgical repair	Surgical repair+foreign body removal	Nephrectomy
I	5 (23.8%)				
II	2 (9.5%)				
III	4 (19%)		1 (4.8%)	1 (4.8%)	
IV	4 (19%)	1 (4.8%)			1 (4.8%)
V		1 (4.8%)			1 (4.8%)

Ethics

Ethics Committee Approval: Ethical approval was obtained from University of Health Sciences Turkey, Gülhane Training and Research Hospital (2021-70).

Informed Consent: The study is a retrospective study.

Peer-reviewed: Externally peer-reviewed.

Authorship Contributions

Concept: C.Ö., S.S., C.A., B.T., S.B., Design: C.Ö., S.S., C.A., B.T., S.B., Data Collection and Processing: C.Ö., S.S., C.A., B.T., S.B., Analysis or Interpretation: C.Ö., S.S., C.A., B.T., S.B., Literature Search: C.Ö., S.S., C.A., B.T., S.B., Writing: C.Ö., S.S., C.A., B.T., S.B.

Conflict of Interest: The authors declared that there was no conflict of interest during the preparation and publication of this article.

Financial Disclosure: The authors declared that they did not receive any financial support during the research and authoring of this article.

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RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ

DOI: 10.4274/atfm.galenos.2023.79926
Ankara Üniversitesi Tıp Fakültesi Mecmuası 2023;76(1):70-74

SURGICAL MEDICAL SCIENCES / CERRAHİ TIP BİLİMLERİ

Safe Retrosigmoid Oblique Craniotomy Technique: A Retrospective Single-Centre Experience

Güvenli Retrosigmoid Oblik Kraniyotomi Tekniği: Retrospektif Tek Merkezli Deneyim

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Abstract

Objectives: In neurosurgical practice, retrosigmoid craniotomy (RC) is mostly performed to gain access of the posterolateral skull base area. The close relationship of the sigmoid sinus (SS) and transverse sinus (TS) is critical for craniotomy related sinus injury. Previous studies have evaluated preoperative identification of TS and SS to provide appropriate surgical location and achieve a safe approach. We aimed to perform the safest and simplest craniotomy technique to avoid sinus injury and minimise bone removal. In this article, we described our modified RC technique.

Materials and Methods: Data of 27 patients who underwent a safe RC were retrospectively evaluated. All procedures were performed by the same senior and junior surgeons. Patient with recurrent surgery and craniofacial bone anomaly were excluded from the study.

Results: The mean age of the patients was 57.41±8.75 years, 15 (55.56%) patients were male and 12 (44.44%) were female. Moreover, 15 patients had vestibular schwannoma, nine had petrous ridge meningioma and three had an epidermoid tumour. The procedure was performed in 25 patients with cerebellopontine angle (CPA) lesions and in two patients with brainstem lesions. Cerebrospinal fluid leakage was found in 2 (7.41%) patients postoperatively, and all of them were treated conservatively. None of the patients had sinus injury, bone flattening or cosmetic failure related to RC technique.

Conclusion: We described our experience with the modified safe RC technique for CPA tumours. A 3×3 cm size safe oblique RC technique with exposure of the 1/4 medial border of the SS and transverse SS junction is satisfactory enough for all of our cases. Our modified RC technique is a safe and easily performed surgical approach that can be used in CPA, brainstem lesions and vascular pathologies. Nevertheless, this technique minimizes unnecessary bone removal and extra cerebellar retraction are not needed because of the small craniotomy sizes.

Key Words: Retrosigmoid Approach, Sigmoid Sinus, Landmark, Posterolateral Cranial Base Approaches, Cerebellopontine Angle

Öz

Amaç: Nöroşirürji pratiğinde retrosigmoid kraniyotomi (RK) posterolateral kafa tabanı bölgesine erişim sağlamak için yapılmaktadır. Sigmoid sinüs (SS) ve transvers sinüsün (TS) yakın ilişkisi nedeniyle cerrahi esnasında sinüs yaralanma ihtimali vardır. Önceki çalışmalarda, güvenli bir yaklaşım sağlamak için cerrahi öncesi TS ve SS'nin preoperatif tanımlaması yapıldı. Bizim amacımız, sinüs yaralanmasını önlemek ve kraniektomi en aza indirmek için en güvenli ve basit kraniyotomi tekniğini gerçekleştirmektir. Bu yazımızda modifiye RK tekniğimizi anlattık.

Gereç ve Yöntem: Yapılan retrospektif çalışmamızda güvenli kraniyotomi tekniği uygulanan 27 hasta verisi değerlendirildi. Hepsi aynı kıdemli ve uzman cerrah tarafından ameliyat edildi. Tekrarlayan cerrahisi ve kraniyofasiyal kemik anomalisi olan hastalar çalışma dışı bırakıldı.

Bulgular: Hastaların ortalama 57,41±8,75 yaş aralığında idi. Bu hastalardan 15'i erkek (%55,56), 12'si (%44,44) kadındı. Hastaların 15'inde vestibüler schwannom, 9 hastada petröz menenjiom ve 3 hastada epidermoid tümör tanısı kondu. Hastaların 25'inde bu yaklaşım serebellopontin açı alanı için ve 2 hastada beyin sapı lezyonları için uygulandı. Hastaların 2'sinde (%7,41) beyin omurilik sıvısı kaçağı görüldü. Hastaların hiçbirinde RK tekniğine bağlı sinüs yaralanması veya kozmetik şikayet olmadı.

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Received/Geliş Tarihi: 10.09.2021 **Accepted/Kabul Tarihi:** 17.03.2023

Öz

Sonuç: Bu yazıda köşe tümörleri ve beyin sapı lezyonlarında uyguladığımız modifiye güvenli retrosigmoid kraniotomi tekniğimiz ile ilgili deneyimimizi anlattık. SS 1/4 medial sınırını ve TSS bileşkesini cerrahi olarak hakim olmayı sağlayan 3x3 cm boyutunda güvenli bir oblik RK tekniğimiz, köşe tümörü ve beyin sapı lezyonları olan tüm olgularımızda yeterince güvenli ve daha fazla görüş alanı sağladı. Modifiye RK tekniğimiz, köşe tümörlerinde, beyin sapı lezyonlarında ve vasküler patolojilerde kullanılabilen hızlı, güvenli ve kolay uygulanabilen bir cerrahi yaklaşımdır. Bununla birlikte, bu teknik ile gereksiz kemik çıkarmayı en aza indiriyor, sınırlı kraniyotomi nedeniyle aşırı serebellar retraksiyona gerek kalmıyor.

Anahtar Kelimeler: Retrosigmoid Yaklaşım, Sigmoid Sinüs, Landmark, Posterolateral Kafa Kaidesi Yaklaşımları, Köşe

Introduction

In neurosurgical practice, retrosigmoid craniotomy (RC) is mostly performed to gain access of the posterolateral skull base area. This approach enables high exposure of the lateral cerebellomedullary cisterns and cerebellopontine area (CPA), which contain essential neurovascular structures including the facial and vestibulocochlear nerves, lower cranial nerves, anterior inferior cerebellar artery and posterior inferior cerebellar artery (1).

The close relationship of the sigmoid sinus (SS) and transverse sinus (TS) is critical in preventing sinus injury (2). Previous studies have evaluated the relationship within the sinus and anatomical landmarks to provide appropriate surgical location and achieve a safe approach (2,3). Our main surgical aim is to perform the safest and simplest craniotomy technique to avoid sinus injury and minimise bone removal. In this paper, we described our modified RC technique.

Materials and Methods

This retrospective study analysed data of 27 patients who underwent a modified RC technique in Ankara University Faculty of Medicine, İbni Sina Hospital between 2018 and 2020 years. All procedures were performed by the same senior and junior surgeons. Patients with recurrent surgery and craniofacial bone anomaly were excluded from the study. The mean age of the patients was 60±12 years, 55.56% were male and 44.44% were female. Of the patients, 15 had vestibular schwannoma, nine had petrous ridge meningioma and three had epidermoid tumours. The technique was performed in 25 patients with CPA lesions and in two patients with brainstem lesions. Intraoperative neuromonitoring was used in all patients. None of the patients had sinus injury. The craniectomy defect was minimal. None of the patients have bone flattening and cosmetic complaints. No mortality was recorded.

Surgical Technique

After positioning the patient, the TS and SS junction and SS are identified with the guidance of a navigation system. The approximate projection of these structures was marked by using anatomical landmarks. The ligamentum nuchae and trapezius muscle are attached to the midline bony prominence located in the occipital bone. This line connects the zygomatic arch to the inion, and it is usually located below the lower border of the TS. The posterior edge of the mastoid process could establish the trace of the SS. However, it could not be applied in all patients because of the anatomical variability of the bony prominence.

Several skin incisions are used in performing RC. We use a post-auricular C-shaped incision that included the transverse SS junction (TSSJ). The incision is located nearly 2 cm behind the pinna. The incision at the level of the pinna is extended beneath the mastoid tip. This incision prevents tension in the flap. To decrease bleeding from the subcutaneous tissue, the incision is infiltrated with bupivacaine. After the cutaneous flap was elevated, the suboccipital muscles are detached from their attachments and with electrocautery stripped from underneath the bone. The use of surface landmarks could localise the junction of the TS and SS. The most important landmark is the asterion, the junction of the parieto-mastoid, lambdoid and occipito-mastoid sutures. This landmark determined the TSSJ during craniotomy. However, it is not a safe and permanent landmark on the cranial surface because of the unstable location of the asterion relative to the sinuses (4). Its localisation has been reported in 60-78% of the cases (5). Additionally, it could be difficult to determine the asterion correctly during surgery (6).

After localising the TSSJ, a burr hole is made beneath the TS and SS junction. After unroofing the SS, the dura is meticulously stripped from the overlying bone. Then, a 3×3 cm RC was performed. After placing the burr hole to the TSSJ, the first 2/3 of craniotomy was performed until the SS inferior border. At this stage, the footing attached was replaced with a bone cutter, and the remaining 1/3 of the craniotomy was crossed obliquely with a bone cutter (Figures 1-3). This craniotomy technique exposes the TSSJ and about 1/4 of the medial border of the SS. If mastoid air cells are involved, which can be opened during drilling to prevent cerebrospinal fluid (CSF) leakage after surgery, they are carefully sealed with bone wax.

After surgery, the craniotomy defect is covered with 3×3 cm bone flap. Then, the suboccipital muscle, fascia and galea are closed in layers. The skin is closed by surgical titanium staples. Finally, a sterile dressing was applied on the wound.

Results

RC was performed in 25 patients with CPA lesions and in two patients with brainstem lesions. On histopathological examination, 15 (55.56%) patients had schwannoma, 9 (33.33%) had meningioma and 3 (11.11%) had epidermoid tumours.

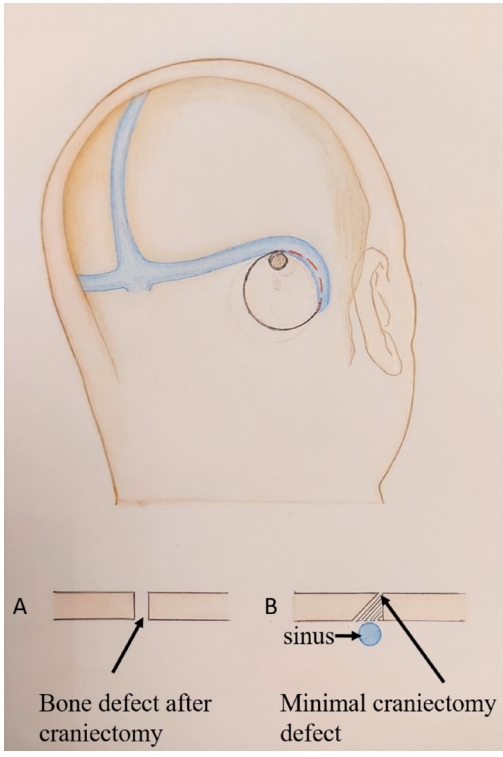

Figure 1. Schematic illustration of our safe retrosigmoid craniotomy. **A)** Defect between the bones after the standard craniectomy above the sinus, **B)** Minimal craniectomy defect between the bones after the safe retrosigmoid craniotomy

Red dotted lines - area of craniotomy performed with a bone cutter

Figure 2. Intraoperative illustration of our safe retrosigmoid craniotomy

Complications

CSF leakage was found in 2 (7.41%) of patients, and the patient was treated conservatively. Moreover, 16 (59.25%) patients experienced headache postoperatively. All patients respond well to non-steroidal anti-inflammatory drugs. No patients had bone flap mobility due to a large craniectomy defect. None of the patients had cosmetic complaints/failure (Figure 4). Sinus injury and mortality were recorded. All complications related with posterior fossa surgery and craniotomy are shown in Table 1.

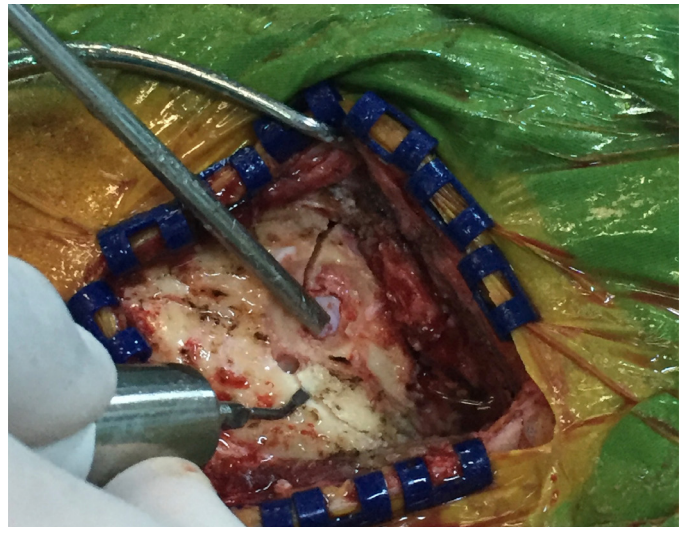

Figure 3. Remaining 13 of craniotomy (above sinus) crossed obliquely with a bone cutter

Figure 4. Postoperative image of a patient after suture removal. No sunken skin and no bone flattening due to the bone defect were noted

Table 1: All complications related with posterior fossa surgery and craniotomy

No	Age	Sex	Histopathology	Complication, (non related with craniotomy)	Bone flattening/cosmetic failure/sinus injury
1	60	M	Vestibular schwannoma	Headache	-
2	45	M	Epidermoid tumour		-
3	47	M	Vestibular schwannoma		-
4	65	F	Vestibular schwannoma	Headache	-
5	72	F	Petrous ridge meningioma	Headache	-
6	58	M	Vestibular schwannoma		-
7	56	F	Vestibular schwannoma	Headache	-
8	49	M	Petrous ridge meningioma	CSF leakage	-
9	40	F	Petrous ridge meningioma		-
10	55	F	Vestibular schwannoma		-
11	58	M	Petrous ridge meningioma	Headache	-
12	63	M	Petrous ridge meningioma	Headache	-
13	60	F	Vestibular schwannoma		-
14	67	F	Epidermoid tumour	Headache	-
15	68	M	Vestibular schwannoma		-
16	54	F	Vestibular schwannoma	Headache	-
17	48	F	Vestibular schwannoma		-
18	69	M	Petrous ridge meningioma	Headache	-
19	51	F	Petrous ridge meningioma	Headache	-
20	54	M	Vestibular schwannoma	Headache	-
21	72	M	Vestibular schwannoma		-
22	47	M	Petrous ridge meningioma	Headache	-
23	53	F	Petrous ridge meningioma		-
24	51	M	Vestibular schwannoma	Headache	-
25	64	M	Epidermoid tumour	CSF leakage, headache	-
26	68	F	Vestibular schwannoma	Headache	-
27	56	M	Vestibular schwannoma	Headache	-

Discussion

Our modified RC technique is a quick, safe and easily performed surgical approach that can be used in CPA lesions, brainstem lesions and vascular pathologies. In the traditional craniotomy technique, SS and bleeding-related complications are carefully prevented. Limited exposure of the SS resulted in the narrowed entry into the surgical corridor of the CPA. This condition causes a limited surgical area and thus requires additional cerebellar retraction. In the traditional method, after the craniotomy flap was elevated to expose the edge of the SS, the residual rim of the bone was removed, thereby increasing the free area between the bones (7). In our modified RC technique, initially, the burr hole was placed to the TSSJ, after the first 2/3 of the craniotomy was performed until the SS inferior border. At this stage, the footing attached was replaced with a bone cutter. To avoid sinus injury, oblique craniotomy is performed over the SS with a 45° inclination to the most medial dural surface near the SS. The remaining 1/3 of the craniotomy was performed by this technique. Compared with the traditional method, the modified technique allows for an extended craniotomy, provides increased exposure and requires minimal bone removal. Moreover, this technique reduces the gap between the nearby bones and bone surfaces become closer to each other. As a result, the gap between the adjacent bones was reduced, and the bone surfaces immobilised the bone flap, thereby contributing to the union (fusion) of bones postoperatively.

Our experience with 27 patients demonstrated that craniotomy performed above the "dangerous places" such as the SS and TSSJ with a bone cutter is safe. No haemorrhagic complications and sinus injuries occurred. This approach could avoid sinus injury, minimise bone removal and reach the CPA and brainstem lesions.

One of the patients (3.70%) had CSF leakage. In the literature, the incidence of CSF leakage was reported between

2% and 30% after surgery, and this rate depends on the closure method (8).

Headache is the most common complication of surgery to the CPA, with an incidence as high as 65% (9). Headache was related to discomfort caused by the incision, muscle spasms, dural irritation and decreased CSF pressure. In our retrospective study, 16 (59.25%) patients experienced headache postoperatively.

Strengths and Limitations

This retrospective study included a comparatively homogenous clinical series of successive brainstem and CPA lesions that were treated with our modified RC technique by a single surgeon. Data completeness was satisfactory. Clinical outcomes (bone flattening, cosmetic failure and SS damage) were assessed with varying postoperative follow-up periods.

Despite these strengths, the study has some limitations. Our technique should be used in a large group of patients. In addition, the use of a bone cutter (which sometimes could be missed in some clinics) in finishing the second part of the craniotomy is a relative disadvantage of this technique. However, prospective randomised controlled trials are warranted to confirm the encouraging results of this preliminary study.

Conclusion

We described our experience with the modified safe RC technique for CPA tumours. Moreover, this technique can be safely applied for vascular lesions of the posterior fossa and in vascular compression syndromes. Our modified RC technique is a simple and safe technique and increases the exposure of CPA lesions. Nevertheless, bone removal and extra cerebellar retraction are not needed because of the small craniotomy sizes.

Acknowledgements

The authors would like to thank Elnur Safarov and Nuray Abasova for the wonderful illustrations.

Preparation for publication of this article is partly supported by the Turkish Neurosurgical Society.

Ethics

Ethics Committee Approval: Ethical approval has not been obtained due to retrospective study.

Informed Consent: Informed consent was obtained from the patient.

Authorship Contributions

Concept: O.M., İ.D., Design: O.M., İ.D., Data Collection and Processing: O.M., İ.D., Analysis or Interpretation: O.M., İ.D., Literature Search: O.M., İ.D., Writing: O.M., İ.D.

Peer-review: Externally peer-reviewed.

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

Financial Disclosure: Preparation for publication of this article is partly supported by the Turkish Neurosurgical Society.

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RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ

DOI: 10.4274/atfm.galenos.2023.35492
Ankara Üniversitesi Tıp Fakültesi Mecmuası 2023;76(1):75-83

SURGICAL MEDICAL SCIENCES / CERRAHİ TIP BİLİMLERİ

Early Clinical Results of Arthroscopic Bone Grafting and Percutaneous K-Wire Fixation for the Treatment of Scaphoid Non-Union

Skafoid Kaynamama Tedavisi için Artroskopik Kemik Greftleme ve Perkütan K-Teli Tespitinin Erken Klinik Sonuçları

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Abstract

Objectives: The aim of this study was to examine the clinical results of patients treated with arthroscopic autologous bone grafting and K-wire fixation in surgery for scaphoid non-union, and the factors affecting the success of this method.

Materials and Methods: This single-centre retrospective study included 9 patients (7 males, 2 females) with a mean age of 31 years (range, 20-45 years). Radiological evaluations of the patients were made from direct radiographs and computed tomography images. The disability of the arm shoulder and hand (DASH), Mayo wrist score (MWS), and visual analog scale (VAS) scores were used in the evaluation of functional results. The range of flexion -extension of the wrist was evaluated pre and postoperatively. Grip strength of the operated hand was measured postoperatively and was compared with the non-operated contralateral hand.

Results: The mean time to union of the scaphoid bone was 8.5 weeks. Non-union was observed in 3 patients. The VAS score of the patients decreased from 6.5 preoperatvely to 1.7 postoperatively, and the wrist range of movement increased from 81° to 101°. These values were determined to be 75% of those of the non-operated side. The DASH score and MWS improved postoperatively.

Conclusion: The main reasons for failure are not observing bleeding points in the fracture ends with debridement of the non-union area and early removal at the end of 8 weeks of the fixation made with K-wires in a second operation. The use of headless screws instead of K-wires in this technique can reduce the rates of surgical failure.

Key Words: Scaphoid Non-Union, Arthroscopic, Bone Grafting

Öz

Amaç: Bu çalışmada skafoid kaynamama cerrahisinde artroskopik otolog kemik grefti ve K-teli fiksasyonunu ameliyatı yapılan hastaların klinik sonuçlarını incelemek ve bu yöntemin başarısını etkileyen faktörlere dikkat çekmek amaçlanmaktadır.

Gereç ve Yöntem: Tek merkezli retrospektif yapılan çalışmaya, ortalama yaşı 31 olan (aralık, 20-45 yaş) 9 hasta (7 erkek, 2 kadın) dahil edildi. Hastaları radyolojik olarak değerlendirmek için direkt skafoid seri grafileri ve bilgisayarlı tomografi kullanıldı. Fonksiyonel sonuçları değerlendirmede el bileği fonksiyonunu değerlendirmek için kol omuz ve el yetersizliği (DASH), Mayo bilek skoru (MWS), görsel analog skala (VAS) skorları kullanıldı. Hastaların ameliyat öncesi ve sonrası el bilek fleksiyon-ekstansiyon aralığı değerlendirildi. Ameliyat sonrası ameliyat olan el ile karşı etkilenmemiş elin kavrama kuvveti ölçüldü.

Bulgular: Ortalama skafoid kemiğin kaynama süresi 8,5 haftadır. Dokuz hastanın 3'ünde kaynama izlenmemiştir. Hastaların VAS skoru 6,5'ten 1,7'ye düştü. El bilek hareket açıklığı 81 derecen 101 dereceye çıktı. Bu değer karşı tarafın %75'i kadardır. DASH skoru ve MWS ameliyat sonrası iyileşti. Son takiplerinde, elde kavrama kuvveti karşı tarafın %78'i kadardı.

Sonuç: Skafoid kaynamama alanının debridmanı ile kırık uçlarında noktasal kanama gözlenmemesi ve K-teliyle yapılan tespitin ikinci bir ameliyatla 8 hafta sonunda erken çıkarılması başarısızlığın en önemli sebepleridir. Bu teknikte K-telleri yerine başsız vidaların kullanımı cerrahi başarısızlığı azaltabilir.

Anahtar Kelimeler: Skafoif Kaynamama, Artroskopi, Kemik Greft

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Received/Geliş Tarihi: 04.01.2023 **Accepted/Kabul Tarihi:** 02.03.2023

Introduction

The scaphoid is the carpal bone of the wrist which is the most frequently fractured (1). Healing of the scaphoid bone is not easy because of both the cartilage coverage adjacent to the carpal bones and the anatomy of the vascular structures providing nourishment (1,2). In literature, the treatment of scaphoid non-union has been attempted with several surgical techniques such as cancellous, corticocancellous, vascularised and non-vascularised bone grafting (3). Failure rates of up to 45% have been reported in these studies (4). More minimally invasive methods have been developed for fractures of this bone because of the risk of damage to the capsule and vascular structures in open surgery, and delayed union and joint stiffness associated with open surgery in the postoperative period (5).

The arthroscopic repair and percutaneous grafting method was first described in literature by Slade and Dodds (6) in 2008, and a success rate of 98% was reported in scaphoid non-union. However, the arthroscopy technique characteristics were explained more than the clinical results in that study. Without performing open capsulotomy and without altering the vascularity of the scaphoid bone, better functional results and less joint stiffness are expected in this technique (6).

The aim of this study was to examine the postoperative clinical and radiological results of 9 patients applied with percutaneous K-wire fixation using arthroscopic cancellous bone grafting because of scaphoid non-union, and to evaluate the factors affecting the success of this method.

Materials and Methods

The study was approved by Ankara University Faculty of Medicine, Human Research Ethics Committee (date: 19.12.2022, approval no: İ11-675-22). The study initially enrolled 13 patients who presented at the hand surgery polyclinic of our hospital between January 2020 and November 2022 because of non-union of the proximal third or mid-third of the scaphoid. Four of these patients were excluded; 2 patients who had undergone an operation on the wrist for another reason, one patient with Scaphoid Non-union Advanced Collapse grade ≥2 arthrosis, and one patient with carpal deformity. The study continued with 9 patients. The patient information was retrieved retrospectively from the hospital electronic records system, patient files, and from the Ministry of Health Public Health Institute Death Registration System. The patient data were evaluated in a 24-month period postoperatively.

The demographic characteristics of the patients (age, gender, fractured side) were recorded (Table 1). The mechanism of injury, and the time from injury to surgery were also recorded.

In the physical examination of the patients for preoperative clinical evaluation, sensitivity in the snuffbox region was examined. In the radiological examination, anterior-posterior, lateral, 45° semipronation and semisupination oblique and ulnar deviation direct radiographs were examined. The cases were separated into two groups as stable and unstable non-union. Displacement of 1mm, poor alignment or humpback deformity (lateral intrascaphoid angle >45°) and dorsal intercalar segment instability (radiolunate angle >15°) were used as markers of unstable non-union (Table 2) (7).

As computed tomography (CT) scans showing increased proximal fragment radiodensity are strongly associated with a histological diagnosis of osteonecrosis, CT scans were taken to determine osteonecrosis in proximal non-union (8). As every patient did not undergo magnetic resonance imaging (MRI), the MRI scans were not included in the study. Fractures in which trabecular bone bridging did not form in the fracture line after surgical treatment, or sclerosis seen in the fracture ends together with cystic changes forming a permanent space, were accepted as non-union. For the classification of scaphoid non-union, the Alnot, Slade and Geissler classification systems were used (Table 3a, b). Intracarpal ligament injuries determined during arthroscopy were classified according to Geissler, and TFCC lesions according to Palmer. Poor prognostic factors which could affect postoperative union were scored (Table 4) (9).

All the operations were performed by the senior author. The percutaneous minimally invasive technique with arthroscopic bone grafting, as described by PC Wong and Ho (10) was used as the surgical technique. In the intracarpal ligament evaluation during arthroscopy, dorsal scapholunate (SL) ligament lesion was determined in all 9 (100%) patients. According to the Geissler classification, type 2 lesion was present in 6 patients and type 1 lesion in 3. Debridement and a thermal shrinkage process with radiofrequency were applied to patients observed with type 2 ligament lesion. Palmer type 1A TFCC lesion was observed in 2 patients and debridement was performed in these 2 cases. During arthroscopic surgery, fibrous tissue in the fracture ends was debrided extensively until points of bleeding were obtained from the bone.

The spongious graft harvested from the iliac bone was placed in the non-union line with the help of a cannula (3 mm) and a trocar (2.7 mm), and the K-wires were advanced (Figure 1). The patients were followed up for 8 weeks postoperatively with a thumb-supporting short-arm plaster cast. At the end of 8 weeks, the plaster cast was removed, the K-wires were removed, and the patients were admitted to the rehabilitation program. Bone healing was evaluated both clinically and radiologically in the postoperative period. Healing was evaluated clinically as the absence of sensitivity in the snuffbox region, and radiologically as the formation of bone trabeculae in the pseudo-arthrosis line on wrist posteroanterior, lateral, and scaphoid series (Figure 2).

Table 1: Demographic characteristics of patient

Patient no	Gender	Age	Occupation	Trauma mechanism	Time since injury (Month)	Side	Dominant hand	Alnot's Fracture Classification	Slade and Geisler Classification	Ligament injury	Location	Point bleeding
1	M	45	Plumber	Fall	252	Left	Right	3a	Grade 3	SL Grade 2*	Proximal 1/3	D+/P-
2	M	33	Topographical engineer	Fall	36	Left	Right	2a	Grade 2	SL Grade 1*	Proximal 1/3	D+/P+
3	M	33	Officer	Fall	24	Right	Right	2b	Grade 3	SL Grade 2* TFCC 1A**	Proximal 1/3	D+/P-
4	M	28	Student	Fall	30	Left	Right	2b	Grade 3	SL Grade 2* TFCC 1A**	Proximal 1/3	D+/P-
5	M	31	Tailor	Fall	120	Right	Right	2a	Grade 2	SL Grade 2*	Middle 1/3	D+/P+
6	M	20	Student	Fall	10	Right	Right	2b	Grade 2	SL Grade 2*	Middle 1/3	D+/P+
7	F	31	Lawyer	Fall	18	Left	Right	2a	Grade 2	SL Grade 2*	Proximal 1/3	D+/P+
8	F	37	Worker	Fall	12	Left	Right	2a	Grade 2	SL Grade 1*	Middle 1/3	D+/P+
9	M	23	Salesman	Direct blow	6	Left	Right	3a	Grade 2	SL Grade 1*	Proximal 1/3	D+/P+

M: Male, F: Female, SL: Scapholunate ligament, TFCC: Triangular fibrocartilage complex
*Geissler Classfication, **Palmer Classification

Table 2: Stable and unstable non-union criteria

Patient no	Fractured disreplacement (mm)	Lateral intrascaphoid angle	Radiolunat angle
1	<1 mm	52°	8°
2	<1 mm	50°	14°
3	<1 mm	48°	8°
4	<1 mm	46°	12°
5	<1 mm	48°	14°
6	<1 mm	52°	11°
7	<1 mm	46°	12°
8	<1 mm	48°	14°
9	<1 mm	54°	12°

Table 3a: Alnot's Scaphoid Non-union Classification

Stage 1	**Linear pseudarthrosis**
Stage 2	
2A	Slight bone resorption no displacement
2B	Unstable Pseudoarthrosis, Palmar flexion and adaptive DISI, Palmar bone loss
Stage 3	
3A	Unstable pseudarthrosis, Palmar bone loss, Radioscaphoid arthritis
3B	Radiocarpal arthritis

Table 3b: Scaphoid nonunion classification according to Slade and Geissler

Grade 1	Late diagnosed acute fractures
Grade 2	Non-union with fibrous union
Grade 3	Non-unions that do not develop deformity but have a gap <1 mm
Grade 4	Non-unions with cystic changes (1-5 mm)
Grade 5	The type in which deformity develops and structural bone graft is required

Scaphoid serial radiographs and CT images were used for evaluation of the postoperative time to union. In the clinical measurement evaluation, the pre and postoperative visual analog scale (VAS) (0=no pain, 10= the worst pain), Mayo wrist score (MWS), and the disability of the arm shoulder and hand (DASH) scores were used. The total wrist flexion-extension range was measured with a goniometer preoperatively and in the postoperative follow-up period in both the operated wrist and the non-operated wrist, and the values were recorded. The grip strength of both hands was measured with a JAMA hydraulic dynamometer (Asimov Engineering, Los Angeles, CA, USA).

Statistical Analysis

Data obtained in the study were analyzed statistically using SPSS for Windows vn. 20.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were stated as mean ± standard deviation values for continuous data showing normal distribution and as median (minimum-maximum) values for data not showing normal distribution. Categorical variables were stated as number (n) and percentage (%).

Table 4: Bad prognostic factors

Patient no	Proximal pole fracture	Proximal pole AVN	>5 year non-union	Story of failed surgery	Smoking	Chronic disease	Bad prognostic factors	Union time
1	Yes	Yes	Yes	No	Yes	No	+4	Non-union
2	Yes	No	No	No	No	No	+1	5 weeks
3	Yes	Yes	No	No	Yes	No	+3	Non-union
4	Yes	Yes	No	No	Yes	No	+3	Non-union
5	No	No	Yes	No	Yes	No	+2	10 weeks
6	No	No	No	No	No	No	0	9 weeks
7	Yes	No	No	No	No	No	+1	8 weeks
8	No	No	No	No	Yes	No	+1	8 weeks
9	Yes	No	No	No	Yes	No	+2	11 weeks

AVN: Avascular necrosis

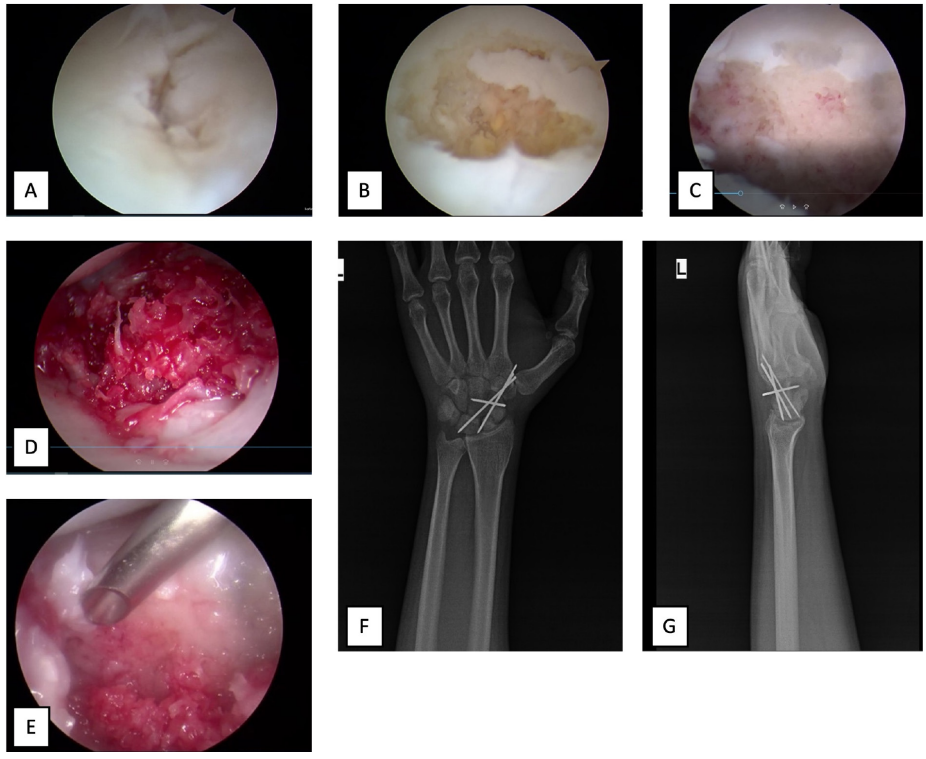

Figure 1: A) Arthroscopic image of scaphoid non-union, B) Debridement of fibrous tissue at the non-union line, C) Spot hemorrhages showing that we have reached the living bone tissue in the debridement procedure, D) Placing the cancellous graft taken from the iliac orest into the cavity, E) Tisseel [(Greenplast Kit, Green Cross) on the graft, Yongin Korea] tightening procedure, F) AP direct X-ray showing the stabilization of the fracture fragments and midcarpal (scacocapitate) with 3 K-wires, G) lateral direct X-ray

Results

Evaluation was made of 9 patients, comprising 7 males and 2 females with a mean age of 31 years (range, 20-45 years). When the cause of trauma was questioned retrospectively, the fracture was caused by a fall in 8 patients and as a result of assault in 1 patient. The mean time from trauma to operation was 56.5 months (range, 6-252 months). The postoperative follow-up period in our clinic was mean 22.5 months (range, 17-30 months). According to the Alnot fracture classification system, type 2A non-union was observed in 4 patients, type 2B in 3, and type 3A in 2. Union in the scaphoid was observed in 6 patients and this method was not successful in 3 patients. No complications related to arthroscopy were seen in any patient.

Radiologically, the time to union was mean 8.5 weeks (range, 5-10 weeks). The mean VAS score decreased from preoperative mean 6.5 (range, 1-10) to 1.7 (range, 1-3) at the final follow-up examination. In the scoring applied for the functional

Figure 2: A) Radiographic images showing the bone bridges in the 10th week postoperatively in the middle 1/3 of the scaphoid bone A) AP radiograph B) AP radiograph in ulnar deviation, C) Lateral X-ray, D) CT image in the coronal plane, E) CT image in the sagittal plane, F) CT image in the axial plane

CT: Computed tomography

Table 5: Postoperative clinical and radiological results of the patients

Patient no	Preop VAS	Postop VAS	DASH	MWS	Grip strength (kg)	Functional hand grip (kg)	Preoperative flexion-extension arc (degree)	postoperative flexion-extension arc (degree)	Contralateral flexion-extension arc (degree)	Preop SL angle	Postop SL angle
1	3	2	0 (Perfect)	95 (Perfect)	36.5	45.5	80	95	140	51	43
2	9	1	0 (Perfect)	85 (Good)	33	42	75	110	135	37	46
3	6	2	6.25 (Good)	85 (Good)	34.5	44	85	100	135	50	57
4	7	3	6.25 (Good)	85 (Good)	33.5	40	80	105	130	28	48
5	9	1	25 (Satisfactory)	85 (Good)	34	38.5	85	100	135	35	44
6	7	1	10 (Good)	95 (Perfect)	35	48	80	105	140	70	57
7	7	1	33.3 (Satisfactory)	70 (Satisfactory)	37	47.5	90	110	135	35	40
8	10	3	32.5 (Satisfactory)	75 (Satisfactory)	36.5	44.5	80	95	130	51	55
9	1	2	20 (Satisfactory)	75 (Satisfactory)	35.5	45	75	90	140	52	50

DASH: Disabilities of the arm, shoulder and hand, MWS: Mayo modified wrist score, SL: Scapholunate ligament, VAS: Visual analogue scale

evaluation, the mean MWS was 83.5 (range, 75-95), with 2 patients evaluated as excellent, 4 as good, and 3 as satisfactory. In the DASH evaluation, the mean value of all the patients was 15 (range, 0-33.5), with the results evaluated as excellent in 2 patients, good in 3, and satisfactory in 4 (Table 5).

The wrist flexion-extension range of the patients was measured as mean 81° (range, 75-90°) preoperatively, and mean 101° (range, 90-110°) postoperatively. A 20° increase was obtained in the wrist flexion-extension range. This range of movement was up to 75% of the contralateral side, which was measured as mean 135° (range, 130-140°). The grip strength value of the patients was measured as mean 35 kg (range, 33-37 kg). This value was 78% of the contralateral, non-operated side value, which was measured as 45 kg (range, 42-48 kg). The SL angle measured on direct radiographs was mean 45° (range, 28-70°) preoperatively, and 49° (range, 40-57°) in the early postoperative period (Table 5).

Discussion

Many surgical treatment methods have been described for scaphoid non-union, but as yet no ideal surgical method has been developed for all non-unions (11). The advantage of open surgery methods is that reduction is easily obtained with direct visualisation of the region of non-union. However, opening the joint capsule causes disruption to the blood flow of the scaphoid. This method lost popularity because of the decrease in scaphoid vascularity with open reduction and this has driven surgeons to develop new methods (6). One of these is the arthroscopy-assisted percutaneous minimally invasive surgery method, which has become increasingly used in recent years (12). The advantages of this surgical technique are that open capsulotomy is not performed, the weak blood flow of the scaphoid is preserved, and the carpal ligaments are not damaged (13,14). Preservation of the scaphoid vascularity provides more rapid and better bone healing compared to open surgery (4,10). In literature, this method has been reported to have been applied in cases of non-union of distal third, mid-third, and proximal third fractures together with acute fractures (15,16). In the current study, the factors affecting the success of this method were examined by evaluating the postoperative clinical results of 9 patients applied with percutaneous K-wire fixation using arthrosccopy-assisted cancellous bone grafting.

In a study by Slade and Gillon (4) arthroscopy-assisted percutaneous minimally invasive surgery was performed on 234 patients, and the time to union in acute scaphoid fractures was seen to be shorter than in cases operated on because of non-union. It has been reported that there is a higher risk of avascular necrosis development in proximal third fractures in particular and there could be non-union (2,16,17). Inoue et al. (18) examined the factors affecting prognosis in scaphoid non-union and showed that vascularisation of the proximal fragment, instability, and delayed surgery were associated with non-union. In the current study, the time to union was mean 8.5 weeks, which was consistent with the literature (19). In the 3 patients of the current study where union was not obtained, the fracture region was in the proximal third. In one case the operation was 21 years after the time of the fracture. Vascularised bone graft can be considered a good alternative in delayed operations for scaphoid non-union with impaired vascularity. Microvascular bone grafts both transferred free and with pedicle have been used successfully in the treatment of scaphoid non-union (20). In cases where points of bleeding cannot be obtained from the proximal bone, it may be logical to prefer vascularised bone operations.

In the arthroscopy-assisted percutaneous minimally invasive surgery technique, fixation of the scaphoid bone is extremely important. This fixation is usually made with headless screws and sometimes with K-wires (21). The tightening force of a headless screw on the fracture line is significantly greater than that of a K-wire. Moreover, it is not necessary to remove a headless screw and it can remain in the bone for longer than a K-wire (17). Close to 100% bone healing has been reported in literature in stabilisations made with K-wires using corticocancellous bone graft (5,22). The use of these wires has been shown not to affect the blood flow of the bone and to have a positive effect on healing (23). In addition, K-wires cause less bone loss than screws and preserve the biology (23). However, it has also been shown that compared with screws, K-wires can only create tightening of the fracture line of up to a third of the force exerted by a screw (24). Placement of K-wires passing through the centre of the scaphoid reduces the bending and shear forces loaded onto the scaphoid. If it is wished to reduce the effects of these forces on the fracture line, the K-wire can be placed to pass from the scaphoid to the adjacent carpal bones (lunate, capitate) (Figure 3) (25). In this way, the fixation also provides better stabilisation (5,25). Especially in proximal third fractures of the scaphoid, the tension over the K-wires increases greatly and the fracture line is exposed to greater bending and shear forces. The fixation of these fractures is more difficult compared to other regions. Therefore, in these types of fractures, by adding scaphocapitate fixation with K-wires, the midcarpal joint is locked, and these wires are embedded subcutaneously, then removed at 6-8 weeks (Figure 4) (17,26). In the current study, the K-wires were removed from all the patients in the 8th week. This 8-week period may be shorter than the period needed for healing of the scaphoid bone. In a study by Wong and Ho (27), a union rate of 91% (62/68) was obtained with this method, and the mean radiographic time to union was reported to be 12 weeks. In 2 of the 3 patients where union was not obtained in the current study, the midcarpal locking procedure with the K-wire was not applied. This can be considered to have caused increased tension

over the K-wires and have exposed the fracture line to strong bending and shear forces. In the cases where healing was not obtained, micro-movement formed in the fracture line had a negative effect on scaphoid union (Figure 5).

Other poor prognostic signs of scaphoid non-union include the presence of the fracture in the proximal region, the observation of avascular necrosis during surgery in the proximal region, a history of unsuccessful surgery, non-union lasting for longer than 5 years, smoking, and an accompanying chronic disease (9).

When the functional scores of the current study patients were compared with those of open surgery in literature, it

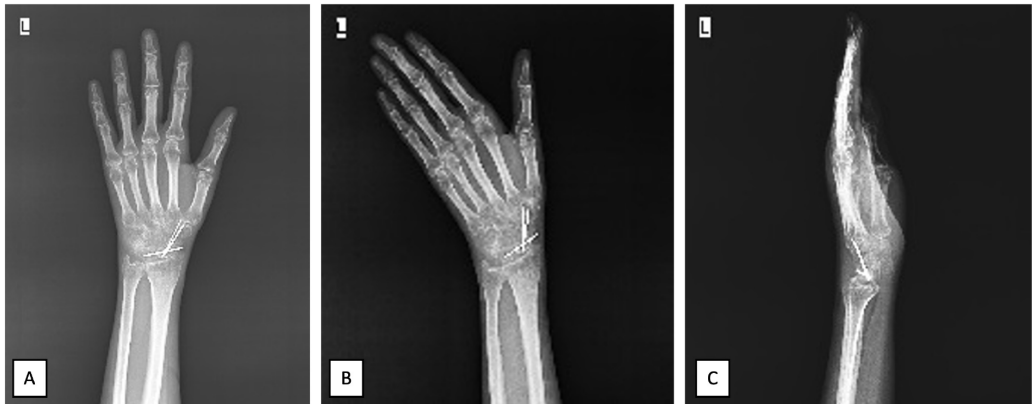

Figure 3: K-wire fixation radiographs between the scaphoid fracture ends and the scapholunate joint in the early postoperative period of the patient who was operated for non-union in the middle 1/3 of the scaphoid bone A) AP X-ray, B) AP X-ray in the ulnar deviation, C) Lateral X-ray

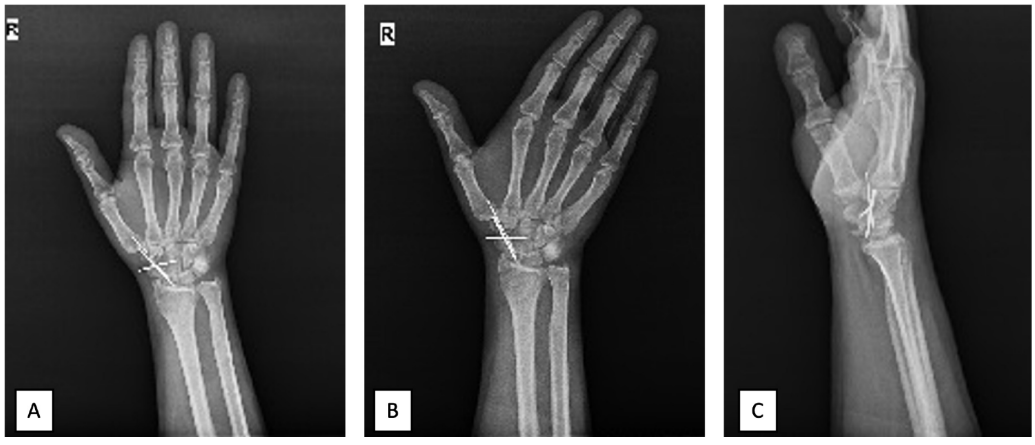

Figure 4: Early postoperative radiographs of the patient who was operated for non-union in the proximal 1/3 of the scaphoid bone. Fixation with K-wire between the scaphoid fracture ends and in the scacocapitate joint A) AP X-ray, B) AP X-ray in ulnar deviation, C) Lateral X-ray

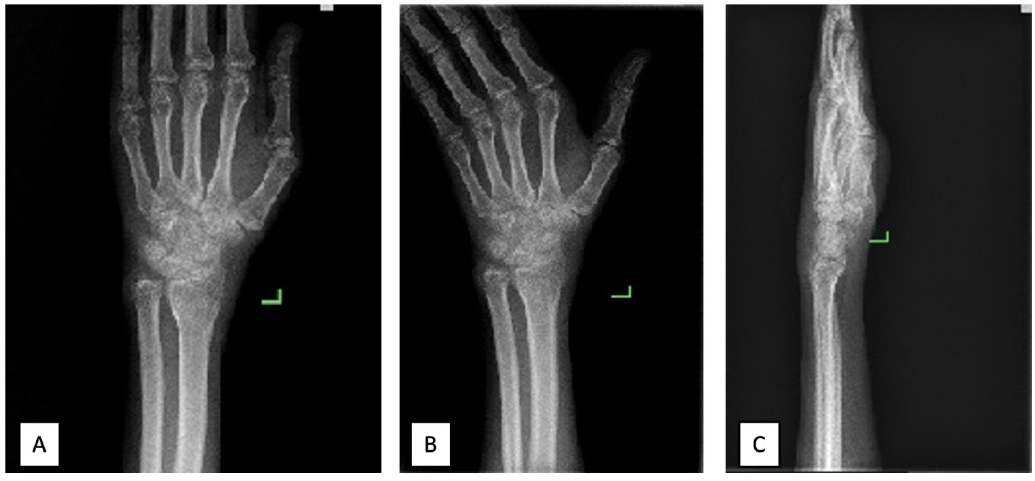

Figure 5: Non-union in the proximal 1/3 of the scaphoid bone A) AP X-ray, B) AP X-ray in ulnar deviation, C) Lateral X-ray

was seen that better wrist function was obtained with arthroscopy-assisted repair of scaphoid non-union than with open surgery (3,28). In a study by Lamon et al. (29), the pain scores fell from 7 preoperatively to 1 postoperatively, and postoperative grip strength was seen to have reached 83% of the non-operated side. Similarly in the current study, the pain scores of the patients significantly decreased compared to the values in the preoperative period, and the mean VAS score was 1.7 postoperatively. The wrist flexion-extenson range of the patients showed a 20° increase after surgery, and the postoperative grip strength reached 79% of the non-operated side. These results suggest that with minimally invasive surgery, less joint stiffness developed and rapid bone healing was due to better preservation of the scaphoid blood flow compared to open surgery.

Study Limitations

There were some limitations to this study, primarily that it was retrospective in design and was conducted with a small patient group. In addition, the MWS and DASH scores were not evaluated preoperatively. Finally, only direct radiographs and CT images were used for the measurement of avascular necrosis, and MRIs, which are more sensitive and specific, could not be included as they were not available for all the patients. Nevertheless, this study can be considered of value in drawing attention to the factors for success in the surgical technique of arthroscopy-assisted percutaneous K-wire fixation using bone grafting in the treatment of scaphoid non-union.

Conclusion

Arthroscopy-assisted percutaneous K-wire fixation using autologous bone graft is an extremely effective treatment method for scaphoid non-union. However, the main reasons for failure are not observing bleeding points in the fracture ends with debridement of the non-union area and removal of the fixation made with K-wires after 8 weeks with a second operation. The use of headless screws instead of K-wires in this technique may increase the rates of surgical success.

Ethics

Ethics Committee Approval: The study was approved by Ankara University Faculty of Medicine, Human Research Ethics Committee (date: 19.12.2022, approval no: İ11-675-22).

Informed Consent: Single-center retrospective study.

Peer-reviewed: Externally peer-reviewed.

Authorship Contributions

Concept: U.B., M.C.G., Design: M.A., Y.Y., Data Collection and Processing: M.C.G., Y.Y., Analysis or Interpretation: U.B., Y.Y., Literature Search: M.C.G., Y.Y., Writing: U.B., Y.Y., M.C.G., M.A.

Conflict of Interest: The authors declared that there was no conflict of interest during the preparation and publication of this article.

Financial Disclosure: The authors declared that they did not receive any financial support during the research and authoring of this article.

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